ABSTRACT

Title of Dissertation: THE IMPACT OF PRIVATE DRINKING WATER WELLS AND ANIMAL FEEDING OPERATIONS ON THE INCIDENCE OF ACUTE GASTROENTERITIS IN MARYLAND: A MIXED METHODS APPROACH

Rianna Teresa Frederika Murray, Doctor of Philosophy, 2019

Dissertation directed by: Dr. Sacoby M. Wilson, Maryland Institute for Applied Environmental Health

More than 44.5 million people in the United States (approximately 14% of the population) rely on private domestic wells as their primary source of drinking water. The water quality of private wells is not regulated at the state or federal level, leaving homeowners with wells responsible for the safety of their water. Meanwhile, each year, an estimated 48 million people in the U.S. are sickened, 128,000 are hospitalized, and 3,000 die of gastrointestinal (GI) illnesses caused by microorganisms that are typically transmitted through contaminated food.

Given that the potential of private wells as a non-foodborne transmission pathway for these microorganisms is not well understood, my dissertation evaluated these relationships, as well as characterized the quality of private well water in
Maryland. My objectives were to: 1.) Investigate the water quality of private wells in Maryland and the effect of animal feeding operations on this water quality using fecal indicator bacteria; 2.) Investigate associations between the prevalence of private wells and the incidence of campylobacteriosis in Maryland at the zip code level using data from the FoodNet active surveillance System; and 3.) Investigate associations between the prevalence of private wells and the incidence of salmonellosis in Maryland at the zip code level using FoodNet data.

My findings demonstrated that 43.2% of private wells tested in Maryland did not meet at least one federal health-based drinking water standard. Additionally, my data showed that the prevalence of private wells in Maryland is a risk factor for the incidence of campylobacteriosis and salmonellosis in the coastal counties of the state. The presence of broiler chicken operations in a zip code is also a risk factor for campylobacteriosis and salmonellosis in coastal counties. These findings provide evidence for the strengthening of private well water regulations and for improving education and outreach to private well owners on proper maintenance and testing for their wells.
THE IMPACT OF PRIVATE DRINKING WATER WELLS AND ANIMAL FEEDING OPERATIONS ON THE INCIDENCE OF ACUTE GASTROENTERITIS IN MARYLAND: A MIXED METHODS APPROACH

by

Rianna Teresa Frederika Murray

Dissertation submitted to the Faculty of the Graduate School of the University of Maryland, College Park, in partial fulfillment of the requirements for the degree of Doctor of Philosophy

2019

Advisory Committee:
Dr. Sacoby M. Wilson, Chair
Dr. Xin He
Dr. Devon Payne-Sturges
Dr. Robin Puett
Dr. Amy R. Sapkota
Dedication

I dedicate this dissertation to my family, Russell, Delmar and Chelsie Murray, whose love, support, encouragement and understanding were invaluable in completing my PhD journey. I could not have done this without you.
Acknowledgements

Thank you Dr. Sacoby M. Wilson for the journey of the last seven years under your guidance as my Mater of Public Health advisor and then as my PhD advisor. Thank you to my committee members, Dr. Xin He, Dr. Devon Payne-Sturges, Dr. Robin Puett and Dr. Amy R. Sapkota for your support and encouragement. A very special thank you to Dr. Amy R. Sapkota for your suggestion to investigate private wells in Maryland and for your guidance into this field of research. I could not have completed this dissertation without your unwavering support. Thank you to Maurice Rocque for your friendship, for being the rock of our department, and for helping me to navigate through confusing times and all the red tape. To my manuscript co-authors Dr. Raul Cruz-Cano and Dr. Daniel Nasko, thank you for your invaluable help during the data analysis process.

I was very fortunate to have a large support system during my graduate school journey, both in Maryland and overseas. Thank you to my friends Laura Dalemarre, Mandy Mitchell, Jennifer Guida, Jenni Young, Prachi Kulkarni, Kelsey Babik, Jessica Montressor-Lopez, Kristen Burwell-Naney, Stephanie Cork, Laura Drew and Katrina Bibb, for our many conversations, allowing me to vent, and the words of positivity when I needed them. Thank you to my Sapkota Lab mates, especially Sarah Allard, Hillary Craddock, Jessica Chopyk and Suhana Chattopadhyay. Thank you to Dr. Wendy Carter-Veale and the PROMISE team at the University of Maryland Baltimore County for providing excellent programming to support USM graduate students. To my extended Murray family in Maryland, David, Evva, Christian and Aiden, thank you for your support and for being my home away from home. To
Sheldon Browne, thank you for your love, help, and patience, and for making the last few months of my dissertation journey a little less crazy and a bit more bearable.

To my support system in my home of Trinidad and Tobago, thank you for always checking on me and being just a call or text message away when I needed it. To my best friend Alicia Montrose-Scheer, thank you for virtually walking this journey with me for the last seven years. Tamika Chung-Davidson, Erin Camps, Nkese Felix, Delia Pereira Andrews, Michelle Allum and my extended Murray, Assam and Cherrie families in Trinidad, your support was essential to my success. Finally, to the best parents in the world, Russell and Delmar Murray, and my wonderful sister, Chelsie Murray, although you could not be physically present during this journey, I felt your love, prayers, and support across the many miles. Thank you for always believing in me, even before I believed in myself.
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<th>Description</th>
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<tbody>
<tr>
<td>95% CI</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>ACS</td>
<td>American Community Survey</td>
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<tr>
<td>AFO</td>
<td>Animal Feeding Operations</td>
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<tr>
<td>API</td>
<td>Application programming interface</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>CWA</td>
<td>Clean Water Act</td>
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<tr>
<td>CAFO</td>
<td>Concentrated Animal Feeding Operation</td>
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<tr>
<td>CSTE</td>
<td>Council of State and Territorial Epidemiologists</td>
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<tr>
<td>FIB</td>
<td>Fecal Indicator Bacteria</td>
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<tr>
<td>FoodNet</td>
<td>Foodborne Diseases Active Surveillance Network</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<td>GBS</td>
<td>Guillain-Barré syndrome</td>
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<tr>
<td>HUS</td>
<td>Hemolytic Uremic Syndrome</td>
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<tr>
<td>IRR</td>
<td>Incidence Rate Ratio</td>
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<tr>
<td>MDNR</td>
<td>Maryland Department of Natural Resources</td>
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<td>MDH</td>
<td>Maryland Department of Health</td>
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<tr>
<td>MDE</td>
<td>Maryland Department of the Environment</td>
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<tr>
<td>MCL</td>
<td>Maximum Contaminant Level</td>
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<td>MST</td>
<td>Microbial Source Tracking</td>
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<tr>
<td>NASS</td>
<td>National Agricultural Statistics Service</td>
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<tr>
<td>NARMS</td>
<td>National Antimicrobial Resistance Monitoring System</td>
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<tr>
<td>NNDSS</td>
<td>National Notifiable Diseases Surveillance System</td>
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<td>NORS</td>
<td>National Outbreak Reporting System</td>
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<td>NPDES</td>
<td>National Pollutant Discharge Elimination System</td>
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<td>NPDWR</td>
<td>National Primary Drinking Water Regulations</td>
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<td>NAWQA</td>
<td>National Water-Quality Assessment Program</td>
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<td>NJDEP</td>
<td>New Jersey Department of Environmental Protection</td>
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<td>NLVs</td>
<td>Norwalk-like viruses</td>
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<td></td>
<td>Definition</td>
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<tr>
<td>NMP</td>
<td>Nutrient Management Plan</td>
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<tr>
<td>OR</td>
<td>Odds Ratio</td>
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<tr>
<td>ppb</td>
<td>Parts per billion</td>
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<tr>
<td>RR</td>
<td>Rate Ratio</td>
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<tr>
<td>RT-PCR</td>
<td>Reverse transcription polymerase chain reaction</td>
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<td>SDWA</td>
<td>Safe Drinking Water Act</td>
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<tr>
<td>STEC</td>
<td>Shiga toxin-producing <em>Escherichia coli</em></td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<tr>
<td>US EPA</td>
<td>United States Environmental Protection Agency</td>
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<tr>
<td>USGS</td>
<td>United States Geological Survey</td>
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<tr>
<td>FDA</td>
<td>United States Food and Drug Administration</td>
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<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
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<tr>
<td>WBDOSS</td>
<td>Waterborne Disease and Outbreak Surveillance System</td>
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<tr>
<td>WBDO</td>
<td>Waterborne Disease Outbreak</td>
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<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>ZCTA</td>
<td>Zip Code Tabulation Area</td>
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Chapter 1: Introduction

Water is essential to life. Water makes up 60% of our body weight and is needed in virtually all bodily functions, from cellular chemical processes, to movement of joints, to removal of waste (Kleiner, 1999; U.S. Geological Survey, 2016). As such, health professionals recommend that we drink 8 glasses of water per day for optimal health. However, consumption of the recommended amount of water per day can be a serious risk if the water is contaminated. The provision of safe drinking water is regarded as one of the most successful public health interventions in history and is often regarded as a hallmark of a developed country (CDC, 2011).

While the World Health organization (WHO) guidelines for drinking water place the greatest need on ensuring the safety of drinking water with respect to microbiological contamination (WHO, 2011), the WHO also estimates that about 1.1 billion people globally drink unsafe water (Kindhauser, 2003).

The recent water crisis in Flint, Michigan renewed national attention on the consequences of unsafe drinking water (Hanna-Attisha, LaChance, Sadler, & Champney Schnepp, 2015). This crisis underscored that we cannot take the quality of our drinking water for granted, regardless of whether its source is public or private. In the United States, the U.S. Environmental Protection Agency (US EPA) developed and enforces the National Primary Drinking Water Regulations (NPDWR) under the Safe Drinking Water Act (SDWA), which is the federal law that protects public drinking water supplies throughout the nation (US EPA, 1974). The NPDWR are primary standards and treatment techniques that protect public health by limiting the levels of over 90 contaminants in drinking water supplied by public water systems.
(US EPA, 2009). Although most of the U.S. population receives its water from public water systems, there are many communities, particularly those located in rural regions that rely on water from private domestic wells. An estimated 60 million people, or one in five Americans, live in areas that are designated as rural by the U.S. Census (U.S. Census Bureau, 2017), and approximately 44.5 million Americans supply their own water through groundwater from private wells (Maupin et al., 2014).

The quality and safety of water from these private domestic wells are not regulated by the SDWA or by state laws, with few states as an exception. Rather, individual homeowners are responsible for maintaining their domestic well systems and for monitoring water quality (US EPA, 1974). A U.S. Geological Survey (USGS) study conducted in 2009 estimated that 23% of U.S. wells have at least one contaminant at a level of potential health concern, including contaminants such as radon, arsenic, uranium, manganese, nitrate, total coliform and *Escherichia coli* (DeSimone, 2009). Other studies determined that many homeowners with private wells do not regularly assess their well water quality per the US EPA drinking water guidelines (Knobeloch, 2010; Krometis, 2014).

Worldwide, reliance on groundwater as an important source of water is increasing, making the security of water quality for groundwater a major issue (Levantesi et al., 2012). The work conducted in this dissertation assesses the quality of groundwater used in private wells in Maryland, and investigates the influence of animal feeding operations on private well water quality. This dissertation also evaluates private wells, animal feeding operations and certain socioeconomic variables as risk factors for two of the most common gastrointestinal illnesses in
Maryland, campylobacteriosis and salmonellosis, which are typically thought to be due to foodborne transmission. Given the increasing body of evidence of non-foodborne risk factors of *Campylobacter* and *Salmonella*, it is important to investigate drinking water as an alternative transmission source for these pathogens.

There are three primary research objectives of this dissertation as follows:

1. To investigate the water quality of private wells in Maryland and the effect of animal feeding operations on this water quality using fecal indicator bacteria.
2. To investigate associations between private wells as a drinking water source and the incidence of campylobacteriosis in Maryland at the zip code level using data from the FoodNet Surveillance System.
3. To investigate associations between private wells as a drinking water source and the incidence of salmonellosis in Maryland at the zip code level using data from the FoodNet Surveillance System.

Each of these three research objectives is addressed in a separate manuscript included in this document, and the overall dissertation consists of six chapters that are described below.

Chapter 2 provides background information on drinking water in the U.S., private well water quality and regulations, existing studies on private well water, the Foodborne Diseases Active Surveillance Network (FoodNet) and the gastrointestinal diseases campylobacteriosis and salmonellosis.

Chapter 3 is a manuscript entitled “Prevalence of Microbiological and Chemical Contaminants in Private Drinking Water Wells in Maryland, USA” that evaluates the water quality of private wells across four counties in Maryland. This
manuscript also examines associations between the presence of animal feeding operations and well water quality at the zip code level using logistic regression. This manuscript was published in a special issue of the *International Journal of Environmental Research and Public Health (IJERPH)* on Drinking Water Quality and Human Health in August 2018 (10.3390/ijerph15081686).

Chapter 4 is a manuscript entitled “Association between Private Drinking Water Wells and the Incidence of Campylobacteriosis in Maryland: An Ecological Analysis Using Foodborne Diseases Active Surveillance Network (FoodNet) Data (2007-2016)” that uses an ecological approach to examine the association between the prevalence of private drinking water wells and the incidence of campylobacteriosis by physiographic province in Maryland.

Chapter 5 is a manuscript entitled “Prevalence of Private Drinking Water Wells Impacts Salmonellosis Incidence in Maryland, USA: An Ecological Analysis Using Foodborne Diseases Active Surveillance Network (FoodNet) Data (2007-2016)”. This manuscript also uses an ecological study design and negative binomial regression models to explore the association between private well prevalence, proximity to animal feeding operations and salmonellosis incidence in coastal and non-coastal communities in Maryland.

Finally, Chapter 6 provides the conclusions of my work, strengths and limitations of all three studies, information on the public health significance of my findings, and directions for future research.

Not only can the results of this dissertation be used to educate homeowners with private wells, but they can also be used to inform policies around private well
protection, and hopefully provide evidence of the need for improved well regulation practices.
Chapter 2: Background

*Drinking Water in the United States*

1.1 Public Water Systems

Public drinking water systems are those that provide water for human consumption through pipes or other constructed conveyances to at least 15 service connections or serves an average of at least 25 people for at least 60 days a year (US EPA, 2015). There are over 151,000 public water systems in the United States (US EPA, 2015), which can be publicly or privately owned. Approximately 268 million Americans, about 86% of the population, drink water that has been delivered to their homes by a public water system (Maupin et al., 2014).

All public water systems are regulated by the US EPA under the Safe Drinking Water Act (SDWA) (US EPA, 1974, 2009). The SDWA sets legally enforceable limits on over 90 contaminants in drinking water in the National Primary Drinking Water Regulations (NPDWR)(US EPA, 2009). The legal limit for a contaminant reflects the level that protects human health and that water systems can achieve using the best available technology. The SDWA also gives individual states the opportunity to set and enforce their own drinking water standards if the standards are at a minimum as stringent as EPA's national standards.

The US EPA classifies three types of public water systems according to the number of people they serve, the source of their water, and whether they serve the same customers year-round or on an occasional basis (US EPA, 2015). A Community Water System (CWS) supplies water to the same population year-round. A Non-
Transient Non-Community Water System (NTNCWS) regularly supplies water to at least 25 of the same people at least six months per year, such as schools, factories, office buildings, and hospitals that have their own water systems. A Transient Non-Community Water System (TNCWS) provides water in a place such as a campground where people do not remain for long periods of time (US EPA, 2015).

1.2 Private Domestic Wells

Although the majority of the U.S. population receives water from a public water system, the 2015 American Housing Survey published by the U.S. Census Bureau estimated that over 13 million occupied households in the United States rely on private domestic wells as a primary source of their drinking water (U.S. Census Bureau, 2015). The U.S. Geological Survey (USGS) equates this to 44.5 million people, approximately 14% of the U.S. population, that rely on domestic wells as their source of drinking water (Maupin et al., 2014). These wells are the sole source of drinking water and water for other household needs such as cooking, cleaning, bathing and flushing toilets for most people in many rural areas. The USGS estimates that domestic wells withdraw 3,600 million gallons per day (4,040 thousand acre-ft/year) of groundwater for household use (Maupin et al., 2014). Additionally, more than 90,000 new domestic wells are installed each year across the United States (CDC, 1998). A cross-sectional diagram of a typical private well is shown in Figure 1 (Pennsylvania Department of Conservation and Natural Resources, 2007).

States with the largest populations that rely on private wells are Pennsylvania, North Carolina, and Michigan, while the states with the largest percentages of their
population that rely on wells are Maine, Alaska, and the U.S. Virgin Islands (Maupin et al., 2014). Figure 2 indicates the spatial distribution of U.S. population that relies on private domestic wells as a water supply.

2. Private Well Water Quality

2.1 Private Well Monitoring and Regulations

Most groundwater in the United States is generally considered of good quality and safe to drink (US EPA, 2002a, 2009c). The majority (> 98%) of domestic well water supplies are from groundwater sources (Maupin et al., 2014), that are vulnerable to a range of natural and anthropogenic contaminants. The large number and widespread presence of potential sources of contaminants make water quality monitoring necessary to determining the safety of consumption of water from domestic wells. Unlike public water systems, private domestic wells are not subject to federal regulations and are minimally regulated, if at all, by states (US EPA, 1974). State and local regulations regarding domestic wells are rare, and when they do exist, they require homeowners to conduct minimal testing of water from domestic wells. Without systematic monitoring the extent of contamination of private wells and true risk to public health is unknown.

Direct monitoring for waterborne human pathogens is often impractical due to their presence in low concentrations, the wide variety of pathogens that can be targeted (viruses, bacteria, parasitic protozoa, and fungi), and the cost of laboratory analyses (Savichtcheva & Okabe, 2006). Instead, monitoring protocols generally detect fecal indicator bacteria (FIB), which are chosen based on their presence in the
feces of animals, their inability to multiply outside the intestinal tract, their association with the presence of human pathogenic microorganisms, and their detection through simple laboratory methodology (Savichtcheva & Okabe, 2006; Yates, Nakatsu, Miller, & Pillai, 2016). The presence of FIB in private wells used for drinking water indicates contamination of water by human or animal waste and has been linked to gastrointestinal illnesses in humans in previous studies (Gruber, Ercumen, & al, 2014; Leclerc, Mossel, Edberg, & Struijk, 2001; Macler & Merkle, 2000; Raina, Pollari, Teare, Goss, & al, 1999).

Under the NPDWR, the US EPA currently requires that public water systems maintain a maximum contaminant level (MCL) of zero for *Escherichia coli* and contain no more than 5% of samples testing positive for total coliform in a month (US EPA, 2009d). For water systems that collect fewer than 40 routine samples per month, no more than one sample can be total coliform-positive per month (US EPA, 2009d). While these standards are not legally enforceable for domestic wells, the NPDWR for public water systems are useful as guidelines when assessing water quality in private supplies.

The US EPA and most states provide guidelines for private well construction, maintenance and testing. The US EPA recommends that homeowners test their private wells annually for a number of parameters including total coliform bacteria, nitrates, total dissolved solids and pH (US EPA, 2002a). Since this testing is voluntary, little is known about the level or frequency of annual testing (if any) that is performed by private well owners, or their knowledge and literacy regarding proper well maintenance, testing and test results. The microbiological and chemical quality
of drinking water consumed from private wells on local, regional and national scales is also unknown. Additionally, many households that utilize private water wells live in rural regions where there is generally a lack of access to the educational and/or financial resources necessary to address water quality issues associated with private water systems (Gasteyer & Vaswani, 2004; Wescoat, Headington, & Theobald, 2007). While the lack of monitoring of private wells may suggest a public health issue, there is a lack of necessary data on private well water quality and management, making the issue difficult to address.

Existing private well regulations apply primarily at the time of well installation and are limited in scope. Fewer than half of the states require testing of new domestic wells, typically for bacteria and nitrate only (DeSimone, 2009). County or other local testing requirements for new wells also exist in some states, however majority of private wells in US have never faced a testing requirement (Flanagan & Zheng, 2018). Well water quality testing at the time of home sales is a condition of some home loans and also is required by a few states (NJDEP, 2017; Oregon Health Authority, n.d.; U.S. Department of Veterans Affairs, n.d.). Some states require that a well be dug or drilled by a certified contractor and that the water from the well be tested at least once for nitrate and coliform bacteria. However after initial installation, well owners are not required to inspect the well or test the water quality. New Jersey is the only state that requires additional well testing, at the time of home resale, and periodically in rental properties (Atherholt, Louis, Shevlin, Fell, & Krietzman, 2009; NJDEP, 2017). As such, private well owners are responsible for maintaining the water quality of their own wells and for any routine monitoring.
A few states such as Utah and California conduct free voluntary testing programs or test high-risk wells (California State Water Resources Control Board, 2010; Quilter & Riding, 2007). Many public health agencies, environmental protection agencies, and non-profit organizations advise homeowners on testing procedures and recommend annual testing (National Groundwater Association, n.d.; US EPA, 2015). These programs, regulations, and recommendations all contribute to the safety of drinking water supplied by domestic wells. However, the limited number of contaminants assessed, the small numbers of wells tested, and the voluntary nature of monitoring do not provide domestic well owners the same level of protection afforded to customers of public water systems.

2.2 Previous Studies on Private Well Water Quality

Comprehensive and reliable information on the occurrence of contaminants in domestic wells is essential for the protection of public health. However, there are few studies that have assessed the quality of private domestic wells in the United States outside of a waterborne disease outbreak. In 1991, the USGS implemented the National Water-Quality Assessment (NAWQA) program to support national, regional, state, and local information needs and decisions related to water-quality management and policy (DeSimone, 2009). The NAWQA program addresses where, when, why, and how the nation's water quality has changed, or is likely to change in the future, in response to human activities and natural factors (DeSimone, 2009).

In 2009, the NAWQA program conducted the first comprehensive study to assess the water quality of over 2,100 private wells to measure the existence and
extent of contamination. Physical properties and the concentrations of major ions, trace elements, nutrients, radon, and organic compounds (pesticides and volatile organic compounds) were measured in as many as 2,167 wells, while fecal indicator bacteria and radionuclides also were measured in approximately 400 wells (DeSimone, 2009). Wells were sampled across 48 states and included 30 of the 62 principal aquifers of the United States. The study found that approximately 23% of wells had at least one contaminant at a level of potential health concern (DeSimone, 2009). Total coliform and *E. coli* were detected in about 34% and 8% respectively of sampled wells (DeSimone, 2009). Detections occurred in samples from at least one well from each of 15 principal aquifers for which bacteria were measured. Wells in which total coliform and *E. coli* were detected also tended to have open intervals that were closer to land surface than wells in which these bacteria were not detected. Wells in which total coliform bacteria were detected were typically older and had higher percentages of agricultural land surrounding them than wells without total coliform detections (DeSimone, 2009).

Domestic wells, cisterns, or natural springs supply drinking water to 18% of the households in the nine upper Midwestern states (CDC, 1998). In 1994, state departments of health of these nine states collaborated to undertake the Midwest Well Water Survey to assess the quality of wells. Water samples were collected from 5520 households with domestic wells and interviews were conducted with homeowners in Illinois, Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, and Wisconsin. Total coliform bacteria were present in 41.3% of samples while *E.*
coli was detected in 11.1% of the samples (CDC, 1998). Nitrate was also detected in 65.4% of the samples (CDC, 1998).

Wells in southern Illinois, western Iowa, northern Missouri, and eastern Kansas had a greater proportion of samples with microbial contaminants. Water samples from households with wells older than 25 years, shallower than 100 feet, or greater than 6 inches in diameter were more likely to have contaminants than samples from households with a newer, deeper, and smaller-diameter drilled or driven well (CDC, 1998). Water samples from households with bored or dug wells were 10 to 15 times more likely to contain coliform bacteria or E.coli than were samples from households with drilled or driven wells (CDC, 1998).

There are a limited number of peer-reviewed studies that provide an analysis of private well water quality that can be used as measure of potential human exposure. Some studies only enumerated the FIB content of wells, others analyzed inorganic contaminants such as arsenic and nitrates, while others directly investigated the potential effects of homeowner well maintenance, environmental, agricultural and weather factors on microbial contamination.

Sworobuk et al. (1987) analyzed 155 private groundwater wells in Preston County, West Virginia and found that 105 of these wells (68%) were contaminated with total coliform bacteria (Sworobuk, Law, & Bissonnette, 1987). This study also determined that wells that were shallower, older, and lacking adequate casing (i.e., grout around the casing or well cap seal) characteristically were more likely to be contaminated with FIB than wells which were deeper, of more recent construction, and had sufficient casing (Sworobuk et al., 1987). Bifulco et al. (1989) repeated an
analysis of 70 wells that were previously included in the study by Sworobuk et al. (1987). Total coliforms were detected in approximately 58% of the samples analyzed, and fecal coliforms were detected in 30% of samples (Bifulco, Shirey, & Bissonnette, 1989).

This study also investigated the presence of *Acinetobacter* spp., a pathogen that poses very little risk to healthy people but can be debilitating in immunocompromised individuals, causing diseases such as septicemia, pneumonia and meningitis (CDC, 2010). *Acinetobacter* spp. were detected in 38% of the well samples at an arithmetic mean density of 8 CFU/100 ml, and were also present in 16% of the water supplies in the absence of total coliforms (Bifulco et al., 1989). The EPA NPDWR does not currently specify a limit for *Acinetobacter* spp in drinking water, however the MCL for every other biological contaminant under the NPDWR is 0mg/L (US EPA 2009).

Following an analysis of 78 private wells in Oregon, Lamka et al. (1980) also determined that improper placement of wells, lack of sanitary seals, proximity of grazing animals to the well, and lack of knowledge of the significance of contaminated water all contributed to a higher incidence of FIB in these wells (Lamka, LeChevallier, & Seidler, 1980). This study also found that FIB contamination was higher after periods of rainfall, suggesting leakage of surface water into improperly sealed wells or aquifer contamination (Lamka et al., 1980).

In 1988, researchers in Minnesota sampled water from 18 private wells monthly for a period of 16 months (Amundson, Lindholm, Goyal, & Robinson, 1988). Total coliforms were detected at least once in 17 wells (94%) over the
sampling period, and fecal coliforms were detected at least once in 13 wells (72%) (Amundson et al., 1988). Samples from 10 wells yielded drug-resistant indicator bacteria, with 65.8% of total coliforms and 33.3% of fecal coliforms isolated found to be drug resistant (Amundson et al., 1988). The highest levels of indicator bacteria were detected immediately after rainfall of 0.25 inches or greater (Amundson et al., 1988). The karst topography of the soil in the study location of southeastern Minnesota was also thought by the authors to contribute to water contamination, with sinkholes and subsurface cracks in soil acting as pathways for contaminants to reach groundwater (Amundson et al., 1988). Well construction features, particularly well depth and well casing, are therefore important in preventing contamination in regions composed of primarily karst soil.

The Montana Extension Service conducted a statewide water quality education program between 1989-1990 that was primarily centered on voluntary private well water testing. Approximately 1,300 private well samples were tested across 70% of Montana’s 56 counties for total coliform bacteria, nitrate-nitrogen, total dissolved solids, sodium, and pH. Almost 40% of all well samples from counties across the state tested positive for total coliform bacteria (Bauder, White, & Inskeep, 1991). The authors attributed the coliform contamination to factors such as faulty well construction, contamination at the time of initial concentration, well casing entry due to flooding, surface runoff from animal farming operations and improper septic tank sighting (close proximity to well). However, none of these factors were examined in the study as statistical predictors of microbial contamination.
A similar statewide rural well water survey was conducted in Iowa between 1988-1989 to determine the proportion of private, rural wells and rural Iowa residents affected by various environmental contaminants (Kross, Hallberg, Bruner, Cherryholmes, & Johnson, 1993). A total of 686 wells were sampled in the study which found that approximately 18% of Iowa's private, rural drinking-water wells contain nitrate above the EPA MCL of 10 mg/L or 10 ppm (Kross et al., 1993). Total coliform bacteria were detected in 27% of samples analyzed. Well depth was found to be the best predictor of well-water contamination, with 35% of wells less than 15 meters deep exceeding the limits for nitrate and total coliform (Kross et al., 1993).

In Nebraska approximately 17% of the housing units in the state utilize private domestic well water supplies (Gosselin, Headrick, Tremblay, Chen, & Summerside, 1997). Approximately 1808 wells were sampled across the state in a 1997 study that sought to assess rural domestic water quality. Gosselin et al. (1997) found that the average statewide incidence of total coliform contamination was approximately 15% (Gosselin et al., 1997). Bacterial contamination varied by region in the state, with most contamination occurring in the northeastern and southeastern glacial drift areas (Gosselin et al., 1997). Wells constructed with open-jointed casings (concrete, brick or tile) were more likely to be contaminated, and 43% of wells with this construction type were found to have total coliform bacteria (Gosselin et al., 1997).

An estimated 940,000 Wisconsin households obtain their drinking water from a privately owned well (Knobeloch, Gorski, Christenson, & Anderson, 2013). In 2002, researchers analyzed 50 private wells for not only total and fecal coliforms, but also several viruses, including enteroviruses, rotavirus, hepatitis A virus (HAV), and
Norwalk-like viruses (NLVs) (Borchardt, Bertz, Spencer, & Battigelli, 2003). Among the 50 wells, a total of four (8%) were positive for viruses by RT-PCR. Three wells were positive for HAV, and the fourth well was positive for rotavirus, NLV and enterovirus (Borchardt et al., 2003). Total coliforms were detected in 28% of wells, while fecal enterococci were detected in 10% of wells analyzed (Borchardt et al., 2003). FIB incidence was not statistically associated with virus occurrence in the study.

A later study in Wisconsin reviewed the results of samples from 3,868 rural private wells that were analyzed for coliform bacteria, nitrate, fluoride, and 13 metals from 2007 – 2010 as part of a state-funded program that provides assistance to low-income families (Knobeloch et al., 2013). Approximately 47% of these wells exceeded one or more health-based water quality standards, with coliform bacteria detected in 18% of wells tested (Knobeloch et al., 2013). Contamination of wells with coliform bacteria in this study was found to be seasonal, peaking in late summer and being least frequent in early spring (Knobeloch et al., 2013).

Approximately 12% of New Jersey’s 8.9 million residents obtain their drinking water from an estimated 400,000 private domestic wells (NJDEP, 2008). The New Jersey Private Well Testing Act (PWTA) requires the analysis of untreated source water from residential private wells at the time of a sale, and periodically in rental properties. The untreated water is sampled and analyzed by state-certified laboratories for 30 chemical and microbial parameters, including coliform bacteria, pH and nitrate (Atherholt et al., 2009; NJDEP, 2017). Using data from 50,800 domestic wells analyzed in NJ by state-certified laboratories from September 2002 –
April 2007, researchers investigated the impact of the geologic setting of the wells on the likelihood that its water contains coliform bacteria (Atherholt et al., 2013). The authors found that total coliform bacteria were detected in 6615 (13.0%) wells and fecal coliform or *E. coli* bacteria were detected in 1052 (2.1%) wells. The rate of coliform detections in the water samples was influenced by the geology, with total coliform and fecal coliform or *E. coli* detected in wells located in bedrock 3 and 3.7 times more frequently, respectively, than in wells located in the Coastal Plain (Atherholt et al., 2013). In bedrock, coliform bacteria were detected more often in wells with a pH of 3 to 6 than in wells with a pH of 7 to 10 whereas conversely, for Coastal Plain wells with pH 3 to 6, detection rates were 4.4% for total coliform and 0.6% for fecal coliform or *E. coli* (Atherholt et al., 2013).

In Pennsylvania over three million residents rely on private wells for drinking water and approximately 10,000 new water wells are drilled annually throughout the state (Swistock, Clemens, Sharpe, & Rummel, 2013). A 2001 USGS study analyzed 78 private domestic wells in southern Pennsylvania that were located in close proximity to agricultural land uses (Zimmerman, Zimmerman, Lindsey, & others, 2001). The study also compared the presence of bacterial contamination in sanitary wells (constructed with a sealed well cap and grout installed along the casing) and non-sanitary wells (constructed without grout and a loose-fitting well cap) (Zimmerman et al., 2001). Total coliform bacteria were found in 48 of the 78 wells (62%), and bacteria were just as likely to be found in sanitary wells as in non-sanitary wells (Zimmerman et al., 2001). Areas of carbonate bedrock had the highest percentages of total coliform detected (75%). *E. coli* was detected in 10% of the
wells, and 17% of the samples that were positive for total coliform also were positive for *E. coli* (Zimmerman et al., 2001). The presence of *E. coli* was more likely in non-sanitary wells and in wells located in carbonate bedrock (Zimmerman et al., 2001).

In addition to seasonality, weather, well construction characteristics, and soil composition, septic tanks have also been implicated in private well contamination. Domestic wells are commonly used in communities that are served by onsite wastewater treatment systems such as septic systems and cesspools, which can be sources of pathogens and chemical contaminants to groundwater (Schaider, Ackerman, & Rudel, 2016). Sandhu et al (1979) analyzed 460 private wells in a rural community in South Carolina and detected total coliforms in 92.5% of wells, while *E.coli* was observed in 43% of wells (Sandhu, Warren, & Nelson, 1979). FIB concentrations, particularly that of *E. coli*, were found to be statistically associated with the depth of the well and its distance from the septic tank. *E. coli* concentrations decreased in relation to total coliforms as the distance between septic tank and the well increased, and as the depth of the well increased. Total coliform counts were also weakly correlated with the pH of the well water, with total coliform counts increasing as pH increased (Sandhu et al., 1979).

Septic tanks have also been associated with private well contamination by non-microbial contaminants. Wallender et al. (2014) evaluated data from the CDC’s Waterborne Disease and Outbreak Surveillance System (WBD OSS) and found that improper design, maintenance, or location of private wells and septic systems contributed to 67% of reported outbreaks from groundwater contamination from 1971 and 2008 (Wallender, Ailes, Yoder, Roberts, & Brunkard, 2014). In Florida, 60
private wells closer to septic tanks were found to contain higher nitrate and phosphate concentrations during the wet season (Arnade, 1999). A study of 20 domestic wells in Cape Cod, Massachusetts, found 27 organic wastewater compounds, including 12 pharmaceuticals, five perfluoroalkyl and polyfluoroalkyl substances, and four organophosphate flame-retardants in wells located in areas served exclusively by onsite wastewater treatment systems (Schaider et al., 2016).

Although previous studies regarding well water quality are limited, all studies described here suggest that microbial contamination of private water wells is common throughout the country, indicating a need for studies such as this one.

2.3 Private Well Water Quality in Maryland

An estimated 1,070,000 Maryland residents (19% of the population) rely on groundwater from private domestic wells as their primary source for drinking water and other household needs (Maupin et al., 2014). Maryland ranks 16th among states in terms of number of residents who rely on private wells, and 20th among states in terms of percentage of the state population that relies on private wells (Maupin et al., 2014). To date, very few peer-reviewed studies and reports have investigated the quality of Maryland groundwater that services private wells. Additionally, most of the research into Maryland groundwater quality has focused on chemical contaminants such as arsenic and nitrates rather than microbial contamination (Hamilton, Shedlock, & Phillips, 1989; Haque, Ji, & Johannesson, 2008; Lichtenberg & Shapiro, 1997; Mose, Mushrush, & Chrosniak, 1990; Mose et al., 1990).
The Aquia aquifer, a confined aquifer located primarily on Maryland’s Eastern Shore, spans outward from Washington, DC towards Annapolis, MD and into northern Kent County (Andreasen, Staley, & Achmad, 2013). It is an important water source in Anne Arundel, Calvert, Queen Anne’s, Kent, St. Mary’s, and Talbot Counties (Andreasen et al., 2013). The Aquia aquifer has been shown to contain groundwater arsenic concentrations as high as 50 parts per billion (ppb) (Haque et al., 2008), a level that is five times higher than the current US EPA maximum contaminant level (MCL) for arsenic in drinking water (US EPA, 2009d).

Radon has also been detected in private wells in Maryland and Virginia at levels that greatly exceed the MCL for radon in drinking water (Mose et al., 1990; Mose, Mushrush, & Simoni, 2001). Elevated levels of nitrates have been detected in community water system wells in Maryland that depend on groundwater (Lichtenberg & Shapiro, 1997). Elevated concentrations of nitrate, iron, and manganese are prevalent in water sampled from surficial aquifers in Maryland and Delaware (Hamilton et al., 1989). Surficial aquifers in this region are important in recharging the underlying confined aquifer system, and in supplying drinking water to the majority of Delmarva Peninsula residents (Hamilton et al., 1989). Another study conducted in Maryland found that groundwater drawn from aquifers in the Monocacy River basin in Western Maryland are impacted by elevated levels of fecal indicator bacteria, including antibiotic-resistant Enterococcus spp. (Sapkota, Curriero, Gibson, & Schwab, 2007).

A USGS study of 146 private wells in the Lower Susquehanna River Basin between 1993-1995 included wells from 17 counties in Pennsylvania and two
counties in Maryland (Bickford, Lindsey, & Beaver, 1996). Approximately 70% of the samples were positive for total coliform, 25% for fecal coliform, and 30% for *E. coli*. The study also determined that bacteria were more likely to be found in groundwater from wells in agricultural areas, a higher percentage of contaminated wells were located in the Valley and Ridge physiographic province, and *E. coli* concentrations were higher in areas underlain by carbonate bedrock (Bickford et al., 1996). Only one study has previously solely investigated the water quality of private wells in Maryland. Using records obtained from the Health Department in Frederick County, Maryland, Tuthill et al. (1998) investigated the presence of coliform bacteria and nitrate in 832 private wells and the influence of factors of lot size and well construction on contamination (Tuthill, Meikle, & Alavanja, 1998). Septic systems were found to influence well contamination on smaller lots of land, and coliform bacteria contamination was highest when well casings were shortest (Tuthill et al., 1998).

2.4 Waterborne Disease Outbreaks Associated with Private Domestic Wells

An outbreak is defined as a Waterborne Disease Outbreak (WBDO) when two or more persons have experienced a similar illness after exposure to the same water source and can be epidemiologically linked by time and by location of exposure to water (CDC, n.d.). Since 1920, data on the occurrence and causes of WBDOs in the United States have been collected and reported (Gorman & Wolman, 1939; Weibel, Dixon, Weidner, & McCabe, 1964). In 1971, the CDC, the US EPA, and the Council of State and Territorial Epidemiologists (CSTE) initiated the Waterborne Disease and Outbreak Surveillance System (WBDOSS) that collects data and reports on...
waterborne disease and outbreaks associated with recreational water, drinking water,
and environmental and undetermined exposures to water (CDC, 2015c). The US EPA
also maintains a list of over 500 waterborne pathogens of potential concern in
drinking waters within several microbial groups (viruses, bacteria, parasitic protozoa,
and fungi) known as the Candidate Contaminant List (‘CCL 3 Universe’ list) (US
EPA, 2014).

The most recent report from WBDOSS indicated that from 2013–2014, a total
of 42 drinking water–associated outbreaks occurred, resulting in at least 1,006 cases
of illness, 124 hospitalizations, and 13 deaths (Benedict, 2017). Legionella was
responsible for 57% of outbreaks and 13% of illnesses, and chemicals/toxins and
parasites together accounted for 29% of outbreaks and 79% of illnesses (Benedict,
2017). Eight outbreaks caused by parasites resulted in 289 (29%) cases, among which
279 cases (97%) were caused by Cryptosporidium and the remaining 10 cases (3%)
were caused by Giardia. Chemicals or toxins were implicated in four outbreaks
involving 499 cases, with 13 hospitalizations, including the first outbreaks associated
with algal toxins (Benedict, 2017). Ten outbreaks (23.8%) were attributed to private
or individual water systems with no form of water treatment. The etiologic agents
associated with the outbreaks from private well water sources included Legionella,
Cryptosporidium, Norovirus, Campylobacter, and E. coli (Benedict, 2017).

Since the recognition, investigation, and reporting of WBDOSS is voluntary
on behalf of state, territorial, and local health departments, the data are likely an
underestimate of the actual incidence of these outbreaks (Benedict, 2017). It should
also be noted that gastrointestinal illness associated with the consumption of domestic
well water is likely underreported, resulting in a further underestimation of the actual number of waterborne disease cases (Mead et al., 1999; Scallan et al., 2011a). Health risks associated with contaminants in domestic well water also have been noted to include exposure to elevated concentrations of nitrate, arsenic, radon, lead, and organic compounds (CDC, 2015b).

In a review of the causes of outbreaks reported to WBDOSs that were associated with drinking water in the U.S. from 1971 to 2006, Craun et al. (2010) reported a total of 833 outbreaks associated with drinking water, water not intended for drinking, and water of unknown intent, resulting in 577,991 cases of illness and 106 deaths in 48 states and 5 U.S. territories (Craun et al., 2010). Of these, 93.6% of outbreaks were attributed to drinking water, 5.6% were attributed to water not intended for drinking, and 0.7% were attributed to water of unknown intent (Craun et al., 2010). Craun also determined that while the number of outbreaks associated with public water supplies decreased considerably after 1980, the proportion of annual waterborne disease outbreaks associated with private groundwater supplies increased between 1976-2006 relative to the total number of outbreaks reported in all system types (Craun et al., 2010).

Outbreaks associated with untreated, private groundwater systems accounted for almost a third of the outbreaks associated with drinking water in the U.S. between 1971 and 2006 (Craun et al., 2010). In 87.8% of outbreaks, the health outcome resulting from the outbreak was acute gastrointestinal illness, followed by hepatitis (3.7%), acute respiratory illness (3.1%) due to Legionella, skin conditions (0.6%), and neurological illness (0.1%) (Craun et al., 2010). A more recent study in North
Carolina found that between 2007 and 2013, 99% of emergency department visits for acute gastrointestinal illness caused by microbial contamination of drinking water were associated with private wells (DeFelice, Johnston, & Gibson, 2016). Adverse health outcomes due to microbial contamination of private well water supplies are therefore an issue of concern in the U.S., especially since a large proportion of the population relies on private wells.

3. Animal Feeding Operations

3.1 Overview of Animal Feeding Operations

Over the last 50 years, the method of producing food animals in the U.S. has changed from the extensive system of small and medium-sized farms owned by a single family to a system of large, intensive operations where the animals are housed in large numbers in enclosed structures that resemble industrial buildings more than they do a traditional barn (Pew Charitable Trusts & Johns Hopkins Bloomberg School of Public Health, 2009; J. M. MacDonald & McBride, 2009; Burkholder et al., 2007a). Such farms are referred to as Animal Feeding Operations (AFOs), defined by the US EPA as agricultural operations where animals are kept and raised in confined situations (US EPA, 2015a). AFOs congregate animals, feed, manure and urine, dead animals, and production operations on a small land area, and feed is brought to the animals rather than the animals grazing or otherwise seeking feed in pastures or fields (USDA, n.d.). The U.S. Department of Agriculture (USDA) estimates that there are 450,000 AFOs in the United States (USDA, n.d.). Due to the high concentration of animals and the large amount of animal manure and wastewater generated, it is widely recognized that AFOs can pose risks to water quality and public health.
(Burkholder et al., 2007a; Bartelt-Hunt, Snow, Damon-Powell, & Miesbach, 2011; Greger & Koneswaran, 2010; Wing & Wolf, 2000).

When an AFO exceeds 1,000 animal units confined on site for more than 45 days during any 12-month period, it is referred to as a Concentrated Animal Feeding Operation (CAFO) (US EPA, 2015b). An animal unit is defined as an animal equivalent of 1,000 pounds live weight and equates to 1,000 head of beef cattle, 700 dairy cows, 2,500 swine weighing more than 55lbs, 125 thousand broiler chickens, or 82 thousand laying hens or pullets (US EPA, 2015b). Additionally, an AFO of any size that discharges manure or wastewater into a natural or man-made ditch, stream or other waterway is considered to be a CAFO, regardless of its size (USDA, n.d.).

The federal Clean Water Act (CWA) prohibits the discharge of pollution to the waters of the United States from any point source, unless the discharge is authorized by a National Pollutant Discharge Elimination System (NPDES) permit issued by the US EPA (or a state delegated by the US EPA) (US EPA, 1972). CAFOs are defined as point sources by the CWA Section 502(14), and in 2003 the EPA published the Concentrated Animal Feeding Operations Rule (amended in 2008) to ensure that manure and wastewater from CAFOs are properly managed to protect the environment and public health (68 FR 7175) (US EPA, 1999; USEPA, 1972). CAFOs are regulated under the NPDES permitting program which specifies the maximum allowable amounts of pollution that can be discharged by facilities (US EPA, 1972).
3.2 Impact of CAFOs on Public Health and the Environment

CAFOs are of increasing concern with regard to their impacts on public health and the environment, including microbiological quality of groundwater (Harter, Kourakos, & Lockhart, 2014; Kirkhorn, 2002; Lockhart, King, & Harter, 2013). Many of the adverse public health and environmental effects caused by CAFOs are a result of the volume of manure produced, which must be stored and disposed of when continuously confining so many animals exclusively or primarily indoors (Greger & Koneswaran, 2010). The amount of manure that a large CAFO can generate depends primarily on the types and numbers of animals confined on-site, but can range from more than 2,800 tons to more than 1.6 million tons per year (U.S. Government Accountability Office, 2008). CAFO manure management strategies include pumping liquefied manure onto spray fields, trucking it off-site, or storing it until it can be used or treated (Hribar & Schultz, 2010). Manure is stored in deep pits under the buildings that house animals, in clay or concrete pits, treatment lagoons, or holding ponds (Hribar & Schultz, 2010).

There is a plethora of contaminants present in livestock wastes, including nutrients, pathogens, veterinary pharmaceuticals, heavy metals and hormones (Barker & Zublena, 1995; Bartelt-Hunt et al., 2011; Campagnolo et al., 2002; Gerba & Smith, 2005; Kolodziej, Harter, & Sedlak, 2004; Nicholson, Chambers, Williams, & Unwin, 1999; Raman et al., 2004). Despite being regulated under the NPDES program, contaminants from animal wastes can enter the environment through pathways such as through leakage from poorly constructed manure lagoons, or during major
precipitation events resulting in overflow of lagoons and runoff from recent applications of waste to farm fields (Aneja, Nelson, Roelle, Walker, & Battye, 2003).

The presence of many contaminants from livestock waste has been documented in both surface water and groundwater supplies in agricultural areas within the U.S. (Campagnolo et al., 2002; Kolpin et al., 2002; Meyer, 2004; Barnes et al., 2008). *Salmonella*, a pathogen commonly found on animal farms and a leading cause of human disease, can persist for up to 405 days in soil after manure is applied to a field, thereby posing a risk of contamination of groundwater (You et al., 2006).

Li et al. (2015) investigated microbial contamination of 200 domestic and community supply wells in California’s Central Valley, a region with intensive animal agriculture. Approximately 5.9% and 10.3% of wells were positive for generic *E. coli* and *Enterococcus*, respectively, with concentrations of *Enterococcus* found to be significantly associated with proximity of wells to animal agricultural operations (X. Li et al., 2015). Monitoring of fecal indicator and pathogenic bacteria in groundwater is therefore important for assessing the risk of microbial contamination of groundwater, especially in regions influenced by CAFOs.

Studies have indicated that people residing near CAFOs may be at increased risk of developing respiratory illnesses, neurobehavioral symptoms, and psychological impairments because of exposure to contaminants released at the facilities. Residents living near CAFOs have reported experiencing increased rates of a number of interrelated symptoms, including headaches, respiratory problems, eye irritation, nausea, weakness, and chest tightness (K. Thu et al., 1997; K. M. Thu, 2002; Wing & Wolf, 2000). Another study conducted in North Carolina demonstrated
that communities located near hog CAFOs had higher all-cause and infant mortality, mortality due to anemia, kidney disease, tuberculosis, sepsis, and higher hospital admissions and emergency room visits of low birth weight infants compared to communities that are not located near a CAFO (Kravchenko, Rhew, Akushevich, Agarwal, & Lyerly, 2018). Given the potential for CAFOs to contaminate groundwater supplies, this study seeks to determine the risk that these facilities pose to the quality of private drinking water wells, and to determine if well water quality can impact the incidence of campylobacteriosis and salmonellosis in Maryland.

3.3 Animal Feeding Operations in Maryland

Agriculture is the largest commercial industry in Maryland, with a total of 12,200 registered farms in 2015 (Maryland State Archives, 2016). Agriculture is also the largest single land use in the state, with 2.02 million acres (approximately 32%) of total land area used for animal farming (Maryland State Archives, 2016). In 2014, Maryland ranked ninth among U.S. states in the number of broilers, or chickens raised for their meat, with 288 million birds produced (Maryland State Archives, 2016). Maryland also has a thriving dairy and livestock industry, with 49,000 milk-producing cows and another 190,000 beef cattle and calves (Maryland State Archives, 2016). The Eastern Shore of Maryland produces close to 300 million broiler chickens annually (USDA, 2106). Broiler chicken operations produce an estimated 5.5 tons (446 cubic feet) of waste per 1,000 birds (Carr, Brodie, & Miller, 1990), totaling over 1.6 million tons of waste produced by broiler operations in Maryland. This waste
typically applied to land and other agricultural fields, leading to potential contamination of nearby water supplies (Burkholder et al., 2007b).

The Maryland Department of the Environment (MDE) allows animal feeding operations within the state to discharge into surface waters of the state following the issuance of a permit (MDE, n.d.a). There are 610 broiler chicken farms (non-laying hens) within the state and an additional 5 farms that consist of laying hens, all of which have a permit to discharge wastewater into waters of the state (MDE, n.d.b). Permits are only required of farms which have 37,500 chickens or greater, meaning that broiler operations with less chickens exist in the state without permits, making them difficult to quantify and their location difficult to assess (MDE, n.d.a).

Additionally, the USDA does not disclose the number of animal feeding operations with sales at the state and/or county level so as not to identify individual farms within an area, further complicating the ability to assess the number of animal feeding operations and the number of animals they contain (The Pew Environment Group, 2011). All of the permitted broiler chicken farms and four of the farms with laying hens in the state of Maryland are located within the Eastern Shore (MDE, n.d.b). The Eastern Shore of Maryland is therefore at a greater risk of groundwater contamination compared to the rest of the state due to the high density of broiler chicken operations in this region. Chapters 4 and 5 of this study investigate the risk of gastrointestinal diseases associated with private drinking water supplies in the Eastern Shore region compared to the rest of the state.
4. The Foodborne Diseases Active Surveillance Network (FoodNet)

4.1 Overview of the FoodNet Surveillance System

FoodNet was established in July 1995 and is a collaborative program of the CDC, 10 state health departments, the USDA’s Food Safety and Inspection Service (USDA-FSIS), and the United States Food and Drug Administration (FDA) (CDC, n.d.). FoodNet conducts population-based active surveillance for laboratory-confirmed infections commonly transmitted through food that are caused by seven bacterial pathogens (Campylobacter, Listeria, Salmonella, Shiga toxin-producing Escherichia coli [STEC], Shigella, Vibrio, and Yersinia), two parasitic pathogens (Cyclospora and Cryptosporidium), and hemolytic uremic syndrome (HUS) (CDC, n.d.a). FoodNet contributes to food safety efforts by providing data used to estimate the burden of foodborne illness in the United States, monitoring changes in incidence of specific illnesses over time, attributing illnesses to specific sources and settings, and disseminating information on enteric diseases (CDC, n.d.b).

Initially starting with two states and 12 counties in 1996, FoodNet’s surveillance area has grown to include 15% of the United States population, approximately 48 million people (CDC, 2016; Henao, Jones, Vugia, Griffin, & for the Foodborne Diseases Active Surveillance Network (FoodNet) Workgroup, 2015). Since 2004, the states of Connecticut, Georgia, Maryland, Minnesota, New Mexico, Oregon, Tennessee, and selected counties in California, Colorado, and New York have been monitored under the surveillance program (Figure 3). FoodNet has the advantage of being an active surveillance network, whereby FoodNet staff at each site receive reports of every identification of a pathogen under surveillance from clinical
laboratories that conduct tests on patients’ specimens ordered by health care providers (Henao et al., 2015). There are now over 650 clinical laboratories in the 10 sites of the FoodNet surveillance area that test specimens from gastroenteritis cases (CDC, n.d.a).

In addition to reporting on foodborne illness surveillance data from the 10 sites in the program, FoodNet also conducts surveys and studies to better understand these illnesses. Laboratory surveys are conducted to understand testing practices for foodborne pathogens and in methods they use, as differences may contribute to variation in the incidence rate of reported infections between FoodNet sites (CDC, 2015a). To understand current knowledge, attitudes, and practices of physicians, FoodNet conducts periodic surveys of physicians practicing within the surveillance area since identification of a foodborne illness case depends upon accurate diagnosis by a healthcare provider as well as laboratory testing (CDC, 2015a). Since the laboratory-confirmed infections reported to FoodNet only a subset of the true number of cases of diarrheal illness that occur in the community, FoodNet conducts population-based telephone surveys of people residing in the surveillance area (CDC, 2015a). To more precisely estimate the burden of acute diarrheal illness, FoodNet also conducts studies to examine the importance of various risk factors, such as specific foods, and practices for specific pathogens (CDC, 2015a).

4.2 Limitations of the FoodNet System

Although FoodNet’s laboratory-based surveillance provides very useful trend information, one disadvantage of the system is that it underestimates the true burden
of gastrointestinal illness (Mead et al., 1999; Scallan et al., 2011a). In order to be included in the surveillance system, a potential case must seek medical care, submit a specimen (usually stool), the specimen must be tested by a laboratory for a pathogen, the lab must report a positive finding, and the laboratory-confirmed infection must be ascertained by public health authorities (Majowicz et al., 2010). Another problem facing surveillance systems is that many patients with acute gastroenteritis do not visit a health care provider or do not submit a specimen for laboratory testing (de Wit et al., 2001; Majowicz et al., 2005; J. G. Wheeler et al., 1999), further contributing to the underestimation of the burden of foodborne illness. Cases identified through FoodNet therefore represent a fraction of the total cases within the community. Additionally, FoodNet surveillance is conducted in a geographic area that covers only 15% of the U.S. population (Scallan & Mahon, 2012), thereby excluding trends and data for the majority of the county.

4.3 FoodNet in Maryland

The FoodNet surveillance system is housed under the Emerging Infections Program (EIP) in the Maryland Department of Health (MDH) (MDH, n.d.). Maryland began performing surveillance for the pathogens monitored by FoodNet in 1998. The initial catchment area was limited to the Baltimore Metropolitan Area, which consisted of Baltimore City, Baltimore County, Anne Arundel County, Carroll County, Harford County, and Howard County. In 2000, Montgomery County and Prince George’s County were added and in 2002, FoodNet surveillance expanded to
the entire state (MDH, n.d.). Currently, Maryland represents approximately 12.5% of the total FoodNet surveillance area (roughly 41.5 million U.S. residents) (MDH, n.d.).

The pathogens that are responsible for the majority of foodborne illness in Maryland are Salmonella, Campylobacter and Shigella (Centers for Disease Control and Prevention (CDC), 2018a). From 1998 to 2017, there were 32,348 cases of foodborne illnesses reported to the Maryland FoodNet, with an average annual incidence rate of 30.98 per 100,000 people (CDC, 2018a). With an average annual incidence rate of 15.33 per 100,000 people between 1998-2017, Salmonella was responsible for 49% of foodborne illness annually in Maryland (CDC, 2018a). Campylobacter has an average annual incidence rate of 8.7 per 100,000 people and is responsible for 28% of foodborne illnesses, while Shigella is responsible for 13% of foodborne illnesses with an average annual incidence rate of 3.89 per 100,000 people (CDC, 2018a). Salmonella and Campylobacter were selected for investigation in this study due to their burden as the pathogens most responsible for foodborne illness in Maryland. These pathogens will be discussed in further detail in section 5 and 6.

5. Campylobacteriosis

5.1 Overview of Campylobacter

Campylobacter is a genus of Gram-negative bacteria that is a leading cause of bacterial gastroenteritis in the United States, as well as in much of the developed and developing world (Butzler, 2004; Kaakoush, Castaño-Rodríguez, Mitchell, & Man, 2015). Currently, there are 17 species and 6 subspecies assigned to the genus Campylobacter, of which the most frequently reported in human diseases
are *C. jejuni* and *C. coli* (Ryan, Plorde, Ahmad, Peterson, & George, 2010; WHO, 2018). The most common clinical effect seen in humans infected with *Campylobacter* is acute gastroenteritis, referred to as campylobacteriosis (Ketley & Konkel, 2005). Other subspecies such as *C. lari* and *C. upsaliensis* have also been isolated from patients with gastroenteritis, but are reported less frequently (WHO, 2018). In most individuals, gastroenteritis associated with *Campylobacter* infection is self-limiting and will resolve within a week unless the individual is immunocompromised or an underlying illness is present, such as human immunodeficiency virus (HIV) infection (Ketley & Konkel, 2005). Following an incubation period of typically 24-72 hours, an acute diarrheal illness develops, followed by fever, chills, myalgia, headache and abdominal cramping (Ketley & Konkel, 2005).

*Campylobacter* has also been implicated in more serious sequelae, such as Guillain-Barré syndrome (GBS), reactive arthritis, post-infectious irritable bowel syndrome and inflammatory bowel disease (Allos, 1998; Keithlin, Sargeant, Thomas, & Fazil, 2014; Riddle, Gutierrez, Verdu, & Porter, 2012). GBS is a form of acute neuromuscular paralysis with an annual incidence of 1 to 2 cases per 100,000 population in most developed countries (Ketley & Konkel, 2005), with 4,250 new cases of GBS diagnosed in the U.S. each year (Allos, 1998). While the majority of patients recover completely, 15-20% of GBS patients are left with severe neurological impairments, while 5-10% of GBS patients die as a direct consequence of this disease (Allos, 1998; Ketley & Konkel, 2005). Almost 25%-40% of GBS patients worldwide experience a *C. jejuni* infection 1–3 weeks prior to the illness (Nyati & Nyati, 2013). Although several pathways have been proposed, the exact
mechanism by which *Campylobacter* infections trigger GBS is unknown (Nyati & Nyati, 2013).

5.2 Campylobacteriosis Incidence Rates

*Campylobacter* is 1 of 4 key global causes of diarrheal diseases and is considered to be the most common bacterial cause of human gastroenteritis in the world (WHO, 2018). There is evidence to suggest that there has been a rise in the global incidence of campylobacteriosis in the past decade, with an increase in the number of cases of campylobacteriosis in North America, Europe, and Australia (Kaakoush et al., 2015). While the CDC estimates the campylobacteriosis incidence rate in the U.S. to be 14.3 per 100,000 population through data from FoodNet (CDC, n.d.), campylobacteriosis incidence rates vary widely in different countries across the world (Kaakoush et al., 2015). Poland, Mexico and the U.S. have some of the lowest reported campylobacteriosis incidence rates in the world, while incidence rates can climb as high as 1,512 per 100,000 population in Japan (Kaakoush et al., 2015).

Since the FoodNet surveillance area represents only 15% of the U.S. population (Scallan & Mahon, 2012), a recent study investigated the campylobacteriosis incidence rate in the U.S. using multiple data sources between 2004–2012 from 4 surveillance systems: the National Notifiable Diseases Surveillance System (NNDSS), the National Outbreak Reporting System (NORS) the National Antimicrobial Resistance Monitoring System (NARMS) and FoodNet (Geissler et al., 2017). This study found that during 2004–2012, a total of 303,520 culture-confirmed campylobacteriosis cases were reported (Geissler et al., 2017). The
average annual incidence rate was 11.4 cases per 100,000 population, with substantial variation by state (range, 3.1–47.6 cases per 100,000 population). This nationwide campylobacteriosis incidence rate was slightly lower than the most recent estimate provided by FoodNet for 2017 of 12.23 per 100,000 population (CDC, 2018a).

In Maryland, *Campylobacter* infection was responsible for 28% of the foodborne illnesses that occurred between 1996-2017, the duration of FoodNet surveillance in the state (CDC, 2018a). There were 9,182 cases of campylobacteriosis reported during this time period, resulting in an average annual incidence rate of 8.7 per 100,000 population. Campylobacteriosis incidence rates in Maryland were lowest in 2004 with 5.23 cases per 100,000 population, while 2016 saw the highest incidence rates of 11.60 cases per 100,000 population (CDC, 2018a). In 2017, there were 656 cases of *Campylobacter* infection reported to FoodNet in Maryland, with an average incidence rate that year of 10.90 per 100,000 cases (CDC, 2018a).

5.3 Risk Factors of Campylobacteriosis

*Campylobacter* are naturally present in digestive tracts of animals such as cattle, swine and poultry (Abley, Wittum, Funk, & Gebreyes, 2012; Boes et al., 2005; Hermans et al., 2011; Møller Nielsen, Engberg, & Madsen, 1997; Newell & Fearnley, 2003), thereby presenting the opportunity for the bacteria to enter the food system. Poultry farms, particularly broiler chicken farms, are recognized as environmental reservoirs of *Campylobacter* since broiler chickens are frequently colonized with *Campylobacter* (Beery, Hugdahl, & Doyle, 1988; Hardy, Lackey, Cannon, Price, & Silbergeld, 2011; Harris, Weiss, & Nolan, 1986; Stanley & Jones, 2003). Cattle and
sheep also shed *Campylobacter*, and dairy farms have also been implicated as environmental sources of *Campylobacter* (Stanley & Jones, 2003). Common food-related risk factors for *Campylobacter* include consumption of undercooked poultry, unpasteurized milk, cheese, eggs, ground beef and pork (Batz, Hoffmann, & Morris, 2012; Domingues, Pires, Halasa, & Hald, 2012; Friedman et al., 2004; Møller Nielsen et al., 1997). The prevalence of *Campylobacter* in the environment, including in wild and domesticated animals, animal waste, soil and water is related to the ultimate contamination of food with *Campylobacter*. The majority of reported *Campylobacter* cases are sporadic (Friedman et al., 2004), however *Campylobacter* has been associated with large outbreaks that affect thousands of individuals (Ketley & Konkel, 2005).

While most cases of *Campylobacter* infection are considered to be foodborne, recent studies have identified non-foodborne transmission sources of this pathogen. *Campylobacter* transmission has been linked with handling of raw poultry, consumption of contaminated water, direct contact with infected animals, and person-to-person transmission both sporadically and in outbreaks (Domingues et al., 2012; Friedman et al., 2004; Kaakoush et al., 2015; Taylor et al., 2013). International travel has also been found to play a significant role in campylobacteriosis diagnosed in the United States, with as many as 40% U.S. travelers to developing countries experiencing diarrheal illness (Kendall et al., 2012; Ricotta et al., 2014).

Water has been found to be a reservoir of *Campylobacter* (Thomas C., Hill D. J., & Mabey M., 2001), and under experimental conditions, *Campylobacter* can be transported through contaminated soil to groundwater (Close, Noonan, Hector, &
Groundwater can therefore be contaminated by land surface activities, particularly when it is in close proximity to animal farms (K. Jones, 2001; Stanley, Cunningham, & Jones, n.d.; Thomas C. et al., 2001). Stanley et al. (1998) determined that the source of contamination of groundwater was *Campylobacter* when identical strains of *C. jejuni* were isolated from groundwater and a dairy herd situated in the same hydrological catchment area (Stanley et al., n.d.). Additionally, it has been demonstrated that *Campylobacter* can be transported through contaminated soil to groundwater under experimental conditions (Close et al., 2010). Entry of *Campylobacter* into surface water is mainly due to runoff from agricultural and residential land, especially during periods of heavy rainfall and flooding (Bolton, Coates, Hutchinson, & Godfree, 1987; Daczewska-Kozon & Brzostek-Nowakowska, 2001), waste water from poultry houses and processing facilities (Koenraad, Jacobs-Reitsma, Van der Laan, Beumer, & Rombouts, 1995), and releases of municipal sewage (Bolton et al., 1987; Koenraad, Ayling, Hazeleger, Rombouts, & Newell, 1995).

*Campylobacter* infection has been previously associated with consumption of contaminated, untreated water, in both sporadic cases and outbreaks in community water systems (Furtado et al., 1998; Sacks et al., 1986). In May 2000, a waterborne outbreak of *E. coli* O157:H7 and *Campylobacter* that occurred in Walkerton, Ontario was attributed to a community water well serving the town (Hrudey, Payment, Huck, Gillham, & Hrudey, 2003). Heavy rainfall flushed cattle manure from an adjacent into the well, resulting in contamination of the municipal water supply (Clark et al., 2003).
Previous studies have indicated that socioeconomic factors influence the risk of campylobacteriosis. In a study conducted in Maryland, researchers from our group determined that living in a rural zip code was a risk factor for campylobacteriosis, and that overall, male rates of infection were higher than female rates in both rural and urban zip codes (Zappe Pasturel et al., 2013). Incidence of campylobacteriosis in zip codes with the highest median incomes (≥ $66,500) was twice that in zip codes with the lowest median incomes (<$40,000) (Incidence Rate Ratio [IRR]= 2.09; 95% CI 1.79, 2.44) (Zappe Pasturel et al., 2013). Additionally, zip codes with a higher percentage of African American and Hispanic residents exhibited a protective effect against campylobacteriosis, with higher rates of illness observed in zip codes whose populations were predominantly White (Zappe Pasturel et al., 2013).

Rosenberg Goldstein et al. (2016) found that similar SES factors at the zip code level influenced campylobacteriosis rates across all FoodNet sites (Rosenberg Goldstein et al., 2016). However, this study found that overall campylobacteriosis incidence was higher in zip codes with higher percentages of Hispanic residents (IRR= 1.005; 95% CI 1.002, 1.007) (Rosenberg Goldstein et al., 2016). In six of the 10 FoodNet sites, campylobacteriosis incidence rates were lower in zip codes with higher percentages of African Americans. Samuel et al. (2004) observed a similar finding of reduced campylobacteriosis risk in zip codes characterized by higher percentages of African American individuals in an investigation of trends in Campylobacter infection across all FoodNet sites from 1996-1999 (Samuel et al., 2004).
Factors of weather and climate, including seasonality, have also been found to be important risk factors of *Campylobacter* infection. Campylobacteriosis incidence rates display a marked seasonal pattern, and increased incidence rates have been found to be correlated with temperature (Louis et al., 2005). The seasonal rise typically begins at the beginning of May and peaks between mid-June and mid-July, thereby producing the most cases during the summer season (Louis et al., 2005). Other studies have also noted a rise in the rate of *Campylobacter*-positive samples of meat and poultry during the spring and summer, as compared to winter months (Kärenlampi, Rautelin, Schönberg-Norio, Paulin, & Hänninen, 2007; Meldrum, Griffiths, Smith, & Evans, 2005; Williams, Golden, Ebel, Crarey, & Tate, 2015).

A similar summer increase in human cases of campylobacteriosis has been observed and some of these cases have been linked to increases in *Campylobacter* contamination of surface water in addition to contamination of food (Sopwith et al., 2008). A recent study in Maryland found that a one-day increase in exposure to extreme precipitation events was associated with a 3% increase in risk of campylobacteriosis in coastal areas of Maryland (IRR=1.03, 95% CI= 1.01, 1.05) (Soneja et al., 2016). The risk associated with extreme precipitation events and extreme heat events was considerably higher during La Niña (naturally occurring phase of cold temperatures) periods (IRR=1.09, 95% CI= 1.05, 1.13 and IRR=1.04, 95% CI= 1.01, 1.08, respectively) (Soneja et al., 2016).
6. Salmonellosis

6.1 Overview of Salmonella

Salmonella is a genus of rod-shaped (bacillus) Gram-negative bacteria of the family Enterobacteriaceae and comprises a large, closely related population of pathogens (Su & Chiu, 2007). There are two species of Salmonella: Salmonella enterica and Salmonella bongori. S. enterica is further divided into six subspecies that include over 2,600 serotypes (Gal-Mor, Boyle, & Grassl, 2014). S. enterica has long been associated with a wide spectrum of infectious diseases, including typhoid fever and nontyphoid salmonellosis, which cause public health problems worldwide (Su & Chiu, 2007).

Infection with Salmonella can follow two very different disease courses, depending on whether the host is infected with a typhoidal or nontyphoidal Salmonella serotype. Infection with nontyphoidal serotypes often causes mild self-limited illness known as salmonellosis that can last between 4-7 days, including diarrhea, fever and abdominal cramping 12–72 hours after infection (Dekker & Frank, 2015). However, much more serious sequelae including osteomyelitis, pneumonia, meningitis and death may occur, especially among immunocompromised individuals or those with underlying medical conditions such as sickle cell anemia (Crump, Sjölund-Karlsson, Gordon, & Parry, 2015; Dekker & Frank, 2015; Pond, 2005). In contrast to infection with non-typhoidal Salmonella, infection with typhoidal strains (primarily serotypes Typhi and Paratyphi) presents as a systemic, often serious, disease. Some individuals will develop typhoid fever, which involves high temperature (>39° C), vomiting, and headache, sometimes with complications
that include neurologic effects, intestinal perforation and death (Dougan & Baker, 2014).

6.2 Salmonellosis Incidence Rates

*Salmonella* is a leading cause of gastroenteritis worldwide (Kirk et al., 2015). Globally, it is estimated that there are over 78 million cases of salmonellosis annually, resulting in over 28,600 deaths (Kirk et al., 2015). In the U.S., over 1 million cases of acute gastroenteritis caused by infection with nontyphoidal *Salmonella* spp. occur annually, including an estimated 19,500 hospitalizations and more than 375 deaths (Scallan et al., 2011b). Though relatively uncommon in the U.S., typhoid, paratyphoid, and enteric fever constitute a very serious global public health problem, with 25 million new infections and >200,000 deaths occurring annually (Dougan & Baker, 2014).

Based on FoodNet surveillance data, there were 135,115 cases of *Salmonella* infection across all 10 FoodNet sites between 1996-2017, with an average annual incidence rate of 15.18 per 100,000 population (CDC, 2018a). An estimated 26% of cases were hospitalized, and less than 1% of cases died (CDC, 2018a). International travel was reported in 8% of cases. Approximately 35% of *Salmonella* infections were attributed to unknown *Salmonella* species, while *S. Enteritidis* (17%), *S. Typhimurium* (15%) and *S. Newport* (10%) were responsible for the majority of remaining infections. Compared with other pathogens monitored by FoodNet, *Salmonella* was responsible for the most cases of foodborne illness, with 135,115 cases of illness attributed to Salmonella of the total 354,360 cases of foodborne
illness that occurred from 1996-2017 (CDC, 2018a). *Campylobacter* was responsible for 121,099 cases between the same time period, followed by *Shigella* with 48,520 (CDC, 2018a).

In Maryland, *Salmonella* infection was responsible for 49% of the foodborne illnesses that occurred between 1996-2017 (CDC, 2018a). There were 15,771 cases of salmonellosis reported during this time period, resulting in an average annual incidence rate of 15.33 per 100,000 population. Salmonellosis incidence rates in Maryland were lowest in 2009 with 13.19 cases per 100,000 population, while 1998 saw the highest incidence rates of this time period with 17.94 cases per 100,000 population (CDC, 2018a). In 2017, 847 cases of *Salmonella* infection were reported to FoodNet in Maryland, with an average incidence rate that year of 14.08 per 100,000 cases (CDC, 2018a).

6.3 Risk Factors of Salmonellosis

*Salmonella* infection has been most commonly been associated with food. Nontyphoidal *Salmonella* is transmitted predominantly by commercially-produced food contaminated by animal feces, such as meat, eggs, poultry products and fresh produce (Batz et al., 2012; Braden, 2006; Hanning, Nutt, & Ricke, 2009; Painter et al., 2013; Patrick et al., 2004). *Salmonella* are frequently detected in broiler chickens (Clemente et al., 2014; Madden, Moran, Scates, Mcbride, & Kelly, 2011), eggs (Van Hoorebeke, Van Immerseel, Haesebrouck, Ducatelle, & Dewulf, 2011), hogs (Arnold, Gosling, Martelli, Mueller-Doblies, & Davies, 2015; A. F. A. Pires, Funk, & Bolin, 2014) and cows (Hanson et al., 2016); as such the consumption of contaminated
broilers, eggs, pork and beef have been identified as significant exposure pathways for both sporadic cases and outbreaks (Mughini-Gras et al., 2014; S. M. Pires, Vieira, Hald, & Cole, 2014).

Several environmental sources of exposure to *Salmonella* have also been identified. Transmission of *Salmonella* to humans has occurred through direct contact with animals, particularly reptiles (Hoelzer, Moreno Switt, & Wiedmann, 2011), and contact with commercial animal production and processing environments, such as broiler chicken houses (S. M. Pires et al., 2014). Exposure to fecally-contaminated water, such as surface water contaminated by runoff events of untreated poultry litter from broiler chicken farms, has also been shown to be a possible environmental exposure pathway to humans (Haley, Cole, & Lipp, 2009; S. M. Pires et al., 2014; Vereen Jr. et al., 2013). Additionally, infection with *Salmonella* was associated with the use of private wells as sources of drinking water (Odds Ratio [OR]=6.5; 95% CI=1.4, 29.7), and the use of residential septic systems (OR=3.2, 95% CI=1.3–7.8) in a study conducted in Washington State (Denno et al., 2009). International travel is also associated with an increase in salmonellosis risk, particularly to countries in the Caribbean, India and China (Johnson, Gould, Dunn, Berkelman, & Mahon, 2011).

Recent studies have also shown that additional environmental and community socioeconomic factors can impact rates of salmonellosis. In an county-level ecological analysis of the sociodemographic factors associated with the incidence of salmonellosis across the U.S., Chang et al. (2009) found that age, race, ethnicity, urbanization, poverty level, crime rate, and physician rate per 100,000 population (a surrogate measure of access to health care) were positively associated with the
incidence of salmonellosis (Chang, Groseclose, Zaidi, & Braden, 2009). In a similar ecological study, Younus et al. (2007) found that indicators of high socioeconomic status were generally associated with increasing salmonellosis incidence at the block group level in Michigan (Younus et al., 2007). Block groups with a lower educational attainment had a lower incidence of salmonellosis compared to block groups with higher education levels (< high school degree vs. ≥ college degree: Rate Ratio (RR) = 0.79, 95% CI =0.63-0.99; ≥ and high school degree, but no college degree vs. ≥ college degree: RR = 0.84; 95% CI = 0.76-0.92). Levels of education also showed a dose-response relationship with the outcome variable (i.e., decreasing years of education was associated with a decrease in Salmonella infections incidence at the block group level) (Younus et al., 2007).

Using nationwide data from FoodNet, Shaw et al. (2016) determined that multiple agricultural factors were associated with salmonellosis incidence rates, and these relationships varied by state (Shaw et al., 2016). The presence of broiler chicken operations, dairy operations and cattle operations in a zip code was associated with significantly higher rates of infection with at least one serotype in states that are leading producers of these animal products. In Georgia, Maryland and Tennessee, all of which are leading broiler chicken producing states, rates of Salmonella infection were 48%, 58% and 46% higher respectively in zip codes with broiler chicken operations compared to those without these operations (Shaw et al., 2016). Similarly, in New Mexico, a state with one of the highest volumes of milk sales out of all ten FoodNet sites, the presence of dairy operations in a zip code was associated with
significantly higher rates of salmonellosis caused by at least one serotype (Shaw et al., 2016).

Seasonal and extreme weather variations have also been identified in salmonellosis incidence. *Salmonella* infection is typically higher in the spring and summer months compared to fall and winter, exhibiting a distinct seasonal pattern (Amin, 2002; Varga et al., 2013). A recent study in Maryland identified differences in salmonellosis risk in coastal counties of the state compared to non-coastal counties due to extremes in weather patterns (Jiang et al., 2015). The authors detected a 4.1% increase in salmonellosis risk associated with a 1-unit increase in extreme temperature events (IRR=1.041; 95% CI=1.013, 1.069). This increase in risk was more pronounced in coastal versus non-coastal areas (5.1% vs 1.5%) (Jiang et al., 2015). The authors also observed a 5.6% increase in salmonellosis risk (IRR=1.056; 95% CI=1.035, 1.078) associated with a 1 unit increase in extreme precipitation events, with the impact disproportionately felt in coastal areas (7.1% vs 3.6%) (Jiang et al., 2015).
7. Figures

**Figure 1.** Cross section of a groundwater well located in bedrock (Pennsylvania Department of Conservation and Natural Resources, 2007).
**Figure 2.** United States population (1995) supplied by domestic wells, in percent of total county population (DeSimone, 2014).
Figure 3. The Foodborne Diseases Active Surveillance Network Surveillance Area, United States, 2004–present. (Henao et al., 2015)
Chapter 3: Prevalence of Microbiological and Chemical Contaminants in Private Drinking Water Wells in Maryland, USA


Abstract

Although many U.S. homes rely on private wells, few studies have investigated the quality of these water sources. This cross-sectional study evaluated private well water quality in Maryland, and explored possible environmental sources that could impact water quality. Well water samples (n = 118) were collected in four Maryland counties and were analyzed for microbiological and chemical contaminants. Data from the U.S. Census of Agriculture were used to evaluate associations between the presence of animal feeding operations and well water quality at the zip code level using logistic regression.

Overall, 43.2% of tested wells did not meet at least one federal health-based drinking water standard. Total coliforms, fecal coliforms, enterococci, and *Escherichia coli* were detected in 25.4%, 15.3%, 5.1%, and 3.4% of tested wells, respectively. Approximately 26%, 3.4%, and <1% of wells did not meet standards for pH, nitrate-N, and total dissolved solids, respectively. There were no statistically significant associations between the presence of cattle, dairy, broiler, turkey, or aquaculture operations and the detection of fecal indicator bacteria in tested wells. In conclusion, nearly half of tested wells did not meet federal health-based drinking
water standards, and additional research is needed to evaluate factors that impact well water quality. However, homeowner education on well water testing and well maintenance could be important for public health.

1. Introduction

An estimated 44.5 million people in 13 million households across the United States, 14% of the nation’s population, rely on private domestic wells as their primary drinking water source (Maupin et al., 2014; U.S. Census Bureau, 2015). The Safe Drinking Water Act (SDWA) was originally passed by Congress in 1974 to protect public health by regulating the nation’s public drinking water supply and its sources, including rivers, lakes, reservoirs, springs, and groundwater wells (US EPA, 1974). However, private wells that serve less than 25 people or have less than 15 service connections are neither regulated by the SDWA nor monitored by local regulatory agencies for contaminants that may be associated with adverse human health outcomes (US EPA, 1974).

The US EPA and the National Groundwater Association provide guidance to homeowners and recommend testing private wells annually for a number of parameters including total coliform bacteria, nitrates, total dissolved solids (TDS), and pH (National Groundwater Association (NGA), n.d.; US EPA, 2015\textsuperscript{c}). As this testing is voluntary, little is known about the level or frequency of testing that is performed by private well owners, or about their knowledge and literacy regarding proper well maintenance, testing, and test results. Data on the microbiological and chemical quality of well water are also scarce. Additionally, many homeowners who utilize private water wells may lack the educational and/or financial resources
necessary to address water quality issues associated with private water systems (Gasteyer & Vaswani, 2004; Wescoat et al., 2007). The CDC recently reported a significant decrease in the annual proportion of reported waterborne disease outbreaks between 1971 and 2006 in public drinking water systems; however, an increase was observed in the annual proportion of outbreaks associated with individual (private) water systems over the same time period (Craun et al., 2010). More recently, a study in North Carolina found that between 2007 and 2013, 99% of emergency department visits for acute gastrointestinal illness caused by microbial contamination of drinking water were associated with private wells (DeFelice et al., 2016). While the CDC report and the North Carolina study suggest a potential public health issue regarding private wells, the lack of information on private well water quality and monitoring makes it difficult to determine the specific contaminants causing these observed illnesses.

Recent studies conducted in Pennsylvania, Virginia, and Wisconsin reported that 40–50% of private wells exceed at least one SDWA health-based standard, most often for coliform bacteria (Knobeloch et al., 2013; Pieper, Krometis, Gallagher, Benham, & Edwards, 2015; Smith et al., 2014; Stillo & MacDonald Gibson, 2016; Swistock et al., 2013). These studies and others have demonstrated the influence of factors such as well construction characteristics, local geology, and climatic conditions on private well water quality (DeSimone, 2009; Knobeloch et al., 2013; Pieper, Krometis, Benham, & Gallagher, 2016; Swistock et al., 2013; Swistock, Sharpe, & Robillard, 1993). Wallender et al. (2014) evaluated data from the CDC’s Waterborne Disease and Outbreak Surveillance System (WBDOSS) and found that
improper design, maintenance, or location of private wells and septic systems contributed to 67% of reported outbreaks from groundwater contamination from 1971 and 2008 (Wallender et al., 2014).

In Maryland, approximately 19% of the population relies on private wells (Maupin et al., 2014); however, only one previous study has investigated private well water quality in the state (Tuthill et al., 1998). Additionally, previous studies have indicated that homeowners generally do not regularly test their private wells or seek technical assistance unless they perceive a water quality problem at the point of use (Knobeloch, 2010; Knobeloch et al., 2013; Krometis, 2014), illustrating a need to educate well owners on the importance of monitoring their wells. To address this need, we developed safe drinking water clinics in several Maryland counties. The goals of the clinics were as follows: (1) to educate well owners on proper well maintenance practices and health risks of contaminated wells; (2) to provide well water quality testing in accordance with EPA guidelines; and (3) to characterize the prevalence of microbiological and chemical contaminants in tested wells.

After the clinics were completed, we recognized a need to evaluate potential environmental factors that could influence well water quality in Maryland. Recently, Li et al. (2015) investigated microbiological contamination of domestic and community supply wells in California’s Central Valley, a region with intensive animal agriculture (X. Li et al., 2015). Approximately 5.9% and 10.3% of wells were positive for generic *E. coli* and *Enterococcus* spp., respectively, with significant associations observed between concentrations of enterococci and proximity of wells to animal feeding operations (X. Li et al., 2015). In Maryland, there are 12,200
registered farms, including a number of animal feeding operations (Maryland State Archives, 2016). In 2014, the state ranked ninth among U.S. states in broiler chicken production (Maryland State Archives, 2016). Maryland also has dairy and livestock farms, with 49,000 milk-producing cows and another 190,000 beef cattle and calves (Maryland State Archives, 2016). If wells are not properly constructed or maintained, there is potential for surface contaminants from agricultural operations to influence well water quality. As such, we leveraged the well water data collected during the safe drinking water clinics to investigate the possible association between the presence of animal feeding operations and well water quality.

2. Materials and Methods

2.1 Safe Drinking Water Clinics

Between 2012 and 2014, five safe drinking water clinics were held in four Maryland counties: Cecil (two clinics), Kent, Montgomery, and Queen Anne’s (Figure 1, Table 1). Cecil, Kent, and Queen Anne’s counties are located on Maryland’s Eastern Shore (Figure 1), where a large number of homes rely on private wells. The Eastern Shore is highly agricultural and has the highest concentration of animal feeding operations (particularly broiler chicken operations) in the state (Dance, 2016; Environmental Integrity Project, 2015; T. Wheeler, 2015). Montgomery County is also characterized by a large number of homes that rely on private wells; however, there are fewer animal feeding operations in this county.

Clinic participants \( n = 150 \) were recruited at county health fairs, farmers’ markets, and through promotional material on community email listservs and local
newspapers. Participants were limited to homeowners in the aforementioned counties with private wells who were interested in participating in the clinics. The safe drinking water clinics were a multi-stage process (Figure 2) that began with a kick-off meeting where registered participants were told of the purpose and significance of the project, provided with water sampling instructions and kits (gloves, two 1 L sterile, polypropylene, wide-mouth Nalgene environmental sampling bottles (Nalgene, Lima, OH, USA) and a large Ziploc bag), and taught how to sample their well water from kitchen or bathroom faucets in accordance with standard protocols. A paper-based survey that was developed by our research and extension teams, and approved by the University of Maryland College Park Institutional Review Board (IRB), was also given to participants at the kick-off meetings. The survey included questions on well characteristics, homeowner well management practices, prior testing conducted (if any), demographic questions (age, sex, race/ethnicity, and income level), and general health-related questions, including, “In the past month, have you experienced diarrhea?” and “In the past month, have you experienced vomiting?”

Participants returned their water samples and completed surveys to their local University of Maryland (UMD) extension office. Samples were kept on ice and transported to the lab within 12 h. Following completion of laboratory analyses (described below), a second follow-up clinic was held where water quality results were returned to participants who provided water samples. The results were individually and confidentially interpreted for participants and potential solutions for wells that did not meet federal standards were discussed where necessary. A follow-up survey was sent to all participants within 12 months after the clinics were
conducted to document actions taken by well owners to solve water quality problems or improve the management of their water supply as a result of attending our clinics (data not shown).

2.2 Laboratory Analyses

Water samples were analyzed within 24 h of collection for total coliforms, fecal coliforms, *E. coli*, *Enterococcus* spp., and *Salmonella* spp., according to standard US EPA membrane filtration methods (US EPA, 2002\(^b\); US EPA, 2005; US EPA, 2009\(^a\), 2009\(^b\)). Briefly, 100 mL of each sample was filtered through 0.45-µm, 47-mm mixed cellulose ester filters. The filters were then placed on the appropriate selective media for each microorganism. Membrane-*Enterococcus* Indoxyl-β-D-Glucoside Agar (mEI) was used for the isolation and enumeration of *Enterococcus* spp.; MI Agar was used for the isolation and enumeration of both total coliforms and *E. coli*; and mFC was used for the isolation and enumeration of fecal coliforms. The mEI plates were incubated at 41 °C for 24 h, mFC plates were incubated at 44.5 °C for 24 h and MI plates were incubated at 37 °C for 24 h. For *Salmonella* detection, membranes were placed in lactose broth, vortexed vigorously for 3 min, and incubated for 24 h at 37 °C. An aliquot of this enrichment was transferred to TT (tetrathionate) broth base, Hajna; incubated at 37 °C for 24 h; plated on XLT4; and incubated at 37 °C for 24 h. Positive and negative controls were used during each test, and plate counts were performed immediately after incubations.

TDS (mg/L) and pH were analyzed using the Pocket Pal TDS Tester and the Stream Survey Test Kit, respectively (Hach Company, Loveland, CO, USA) (Hach
USA, 2017\textsuperscript{b}, 2017\textsuperscript{a}). For nitrate testing, 1 L of each sample was placed into a sterile 1 L polypropylene Nalgene environmental sampling bottle (Nalgene, Lima, OH, USA), 2 mL sulfuric acid solution was added, and the pH was adjusted to <2. For total arsenic testing, 1 L of each sample was placed into a sterile 1 L polypropylene Nalgene environmental sampling bottle (Nalgene, Lima, OH, USA), 2–3 mL of nitric acid solution was added, and the pH was adjusted to <2. The remainder of each water sample was used for sulfate testing. Nitrate and sulfate testing were completed at the Maryland Department of Health (MDH) Labs using an Agilent (Santa Clara, CA, USA) gas chromatograph-mass spectrometer. Nitrate analyses were performed according to US EPA Method 353.2, while sulfate analyses were performed according to US EPA Method 375.2 (US EPA, 1993\textsuperscript{a}, 1993\textsuperscript{b}). Total arsenic testing was also completed at the MDH Labs using an Agilent (Santa Clara, CA, USA) inductively-coupled plasma-mass spectrometer per US EPA Method 200.8 (US EPA, 1994). All quality control/quality assurance approaches recommended by the US EPA methods were employed, including analyses of quality control samples, as well as laboratory reagent blanks and fortified blanks (US EPA, 1993\textsuperscript{a}, 1993\textsuperscript{b}, 1994).

2.3 Animal Feeding Operations Data

We obtained animal feeding operations data from the 2007 U.S. Census of Agriculture, National Agricultural Statistics Service (USDA, n.d.). Specifically, we obtained data on the number of animal feeding operations with sales by zip code for the following animal types: 1) broiler chickens, 2) turkeys, 3) aquaculture, 4) sheep,
5) goats, 6) hogs, 7) dairy cattle, or beef cattle. The 2007 Census was used because it is the most recent U.S. Census of Agriculture that provides data at the zip code level.

2.4 Statistical Analyses

We performed descriptive statistics on all well water data. We also linked well water data and animal feeding operation data by zip code and used univariate logistic regression models to evaluate associations between the presence of each type of animal feeding operation and detection of indicator bacteria in well water samples. The presence of total coliform bacteria and fecal coliform bacteria were the dichotomous (presence/absence) outcome variables of interest. All statistical analyses were performed in SAS 9.4 (Cary, NC, USA) (SAS Institute, 2014).

3. Results

3.1 Characteristics of Safe Drinking Water Clinic Participants

A total of 150 homeowners attended our safe drinking water clinics. However, only 118 participants returned both a water sample and a completed survey (Table 2). Only the 118 participants who returned both a water sample and a completed survey were included in this study’s analyses. The Queen Anne’s County clinic drew the most participants ($n = 28; 23.1\%$), followed by the first clinic conducted in Cecil County ($n = 25; 21.4\%$). A vast majority of participants were white (87.3\%) and most were in the 60–69 age group (33.9\%). Participants were also well-educated: 29.7\% had obtained a Bachelor’s degree and 39.8\% had obtained a graduate degree. At the time of the clinics, a large number of participants had lived at their current residence
for at least 10–20 years (39%). Twenty-nine (24.6%) participants indicated that they had never tested their well water quality, and 58 (49.2%) participants had only tested their water once. Approximately 12% and 0% of participants experienced diarrhea and vomiting, respectively, within 30 days prior to completing the survey.

3.2 Well Water Quality

Overall, 43.2% of wells tested in this study did not meet at least one US EPA health-based drinking water standard (Figure 3). Total coliform bacteria were the most common (25.4%) microbiological contaminant detected. Fecal coliforms (15.3%), *Enterococcus* spp. (5.1%), and *E. coli* (3.4%) were also detected. *Salmonella* was not detected in any of the private wells analyzed in this study.

Regarding chemical contaminants, 26% of tested wells did not meet the recommended drinking water standard for pH (Figure 3), with most of these (83.8%) having a pH below the lower limit of 6.5. There were a few wells (16.2%) with a high pH above the recommended limit of 8.5. Nitrate occurred above the 10 mg/L drinking water standard in 3.4% of tested wells, and less than 1% of wells exceeded the recommended limit for total dissolved solids (TDS) of 500 mg/L. None of the wells had an arsenic level above the US EPA maximum contaminant level (MCL) for arsenic (10 mg/L). Similarly, none of the wells tested exceeded the US EPA MCL for sulfate of 250 mg/L. Although there were individual wells in each county that exceeded the EPA MCLs for some of the chemical water quality parameters investigated, the mean levels in each county were within US EPA specifications (Figure 4).
Kent County had the highest percentage of wells that tested positive for fecal indicator bacteria, with 52.4% of wells testing positive for at least one type of indicator bacteria (Figure 5). *E. coli* was detected in wells sampled in every county with the exception of Cecil County. *Enterococcus* was detected in samples from all counties; however, it was not detected during the first clinic conducted in Cecil County.

3.3 Influence of Animal Feeding Operations on Well Water Quality

Our zip code-level analysis found no evidence that the presence of animal feeding operations influenced the occurrence of fecal indicator bacteria in tested wells (Table 3). In zip codes that contained cattle operations, the contamination of wells by total coliform bacteria was 1.23 times greater than in zip codes that did not contain cattle operations; however, this finding was not statistically significant (Odds Ratio (OR) = 1.23; 95% Confidence Interval (CI) = 0.89, 1.68). In zip codes that contained dairy and aquaculture operations, the contamination of wells by total coliform bacteria was more likely than in zip codes that did not contain one of these operations (dairy operations: OR = 1.12; 95% CI = 0.96, 1.31; aquaculture operations: OR = 1.32; 95% CI = 0.59, 2.93). However, these associations were not statistically significant (Table 3).

Similarly, in zip codes that contained broiler, cattle, dairy, turkey, and aquaculture operations, the contamination of wells by fecal coliform bacteria was more likely than in zip codes that did not contain one of these operations; however, none of these associations were significant. The presence of broiler, hog, and turkey
operations in zip codes was slightly protective for total coliform bacteria, and the presence of hog operations in zip codes was slightly protective for fecal coliform bacteria (Table 3). However, these findings were not significant for any type of operation with either indicator bacterium.

4. Discussion

Our data demonstrate that a majority of private wells included in this study are contaminated with fecal indicator bacteria and/or chemical contaminants at levels that exceed the SDWA drinking water quality guidelines set forth by the US EPA. These findings are consistent with previous studies of private water wells that have been conducted in other states. A recent study of private wells in Pennsylvania found that 41% of wells failed to meet at least one drinking water standard (Swistock et al., 2013), comparable with the 43% of wells that failed to meet one or more standards in our study. Similarly, in Wisconsin, an analysis of private water wells in rural areas found that 47% of these wells exceeded one or more health-based water quality standards (Knobeloch et al., 2013). Total coliform bacteria was also the most common microbiological contaminant in the Pennsylvania study and was detected in 33% of wells (Swistock et al., 2013), comparable with the 25% of tested wells contaminated with total coliforms in our study. A recent study of private wells in Virginia found that 46% tested positive for total coliform bacteria, with 10% testing positive for E. coli (Pieper et al., 2015). Meanwhile, a North Carolina study of private wells found that 49% tested positive for total coliform bacteria and 6.4% tested positive for E. coli (Stillo & MacDonald Gibson, 2016).
Previous studies have also indicated that seasonality may play a role in well water quality (Knobeloch et al., 2013; H. Y. Richardson, Nichols, Lane, Lake, & Hunter, 2009). In our study, the county with the highest percentage of wells that tested positive for fecal indicator bacteria was Kent County, which was sampled in the Fall (Table 1). However, because our study was cross-sectional, we did not collect samples over multiple seasons and, therefore, we cannot evaluate whether seasonal trends influenced our results. Nevertheless, our study adds to the growing body of research nationwide on the water quality of private wells that illustrates the need for improved monitoring of these wells.

Monitoring of fecal indicator bacteria in private well water is important for assessing the potential health risks associated with these water sources. To improve understanding of environmental factors that may impact private well water quality, we also investigated whether proximity to animal feeding operations was associated with microbial contamination of wells. Our data showed that there were no statistically significant associations between the presence of an animal feeding operation within a zip code and microbial contamination of private wells within the same zip code; however, this may be due to the small number of well water samples obtained during this initial study. Given that exposure to well water has been shown as an important risk factor for gastrointestinal illnesses (Carrique-Mas et al., 2005; Kapperud et al., 2003; E. MacDonald et al., 2015), such as campylobacteriosis, exploration of this potential association deserves further study involving a larger number of private wells.
In a case-control study conducted in Sweden, Carrique-Mas et al. (2005) demonstrated that living in a household with a private well was a risk factor for *Campylobacter* infection (OR = 2.6; 95% CI = 0.9–7.4), although that association was not statistically significant (Carrique-Mas et al., 2005). Another case-control study conducted in Norway also found that the risk of campylobacteriosis was higher for those who obtained their water from a private household well compared with those receiving water from a public system (OR = 2.0; 95% CI = 1.2, 3.2) (Kapperud et al., 2003). Consumption of water from a private well was also identified as a significant risk factor for sporadic campylobacteriosis (OR = 1.92; 95% CI = 1.46, 2.53) in a second Norwegian study (E. MacDonald et al., 2015). The potential for private wells to influence gastrointestinal illnesses such as campylobacteriosis (that are traditionally thought to be foodborne) remains understudied in the U.S. and deserves further attention.

One major challenge of improving private well water quality and reducing the risk of adverse health outcomes associated with this water source is that the numbers and locations of U.S. private wells are poorly characterized. Neither individual counties nor states have a complete database with addresses and other contact information for private well homeowners. As such, regular communications to homeowners reminding them to test their wells annually and delivering interventions where necessary is challenging. While the USGS developed a nationwide inventory on the private well population (Maupin et al., 2014), it was created using data on the population served by public water supply systems by county in each state and lacks the specific geographic locations of private wells. Creating a nationwide database of
private well owners that is regularly updated by states could allow for improved
evaluation of the factors that may influence well contamination, enhanced
communication with well owners, and potential improvements in levels of waterborne illness.

In this study, we demonstrated the presence of fecal indicator bacteria in private
drinking water wells in Maryland. As the presence of these indicator bacteria suggests
a potential human health risk, well owners are often left to mitigate these risks
through system repair, enhancement, or decontamination. However, knowledge of the
contamination source of the well would be helpful in selecting an appropriate
remediation method. Microbial source tracking (MST) is a collection of methods used
to determine the likely source of contamination associated with the presence of fecal
indicator bacteria (Simpson, Santo Domingo, & Reasoner, 2002). MST has been
previously used in a variety of applications, including in the management of surface
water contamination and watershed remediation (Bradshaw et al., 2016; Simpson et
al., 2002). Allevi et al. (2013) utilized MST techniques to characterize the magnitude
and incidence of microbial contamination in private wells in Virginia, and to identify
the likely sources of this contamination (Allevi et al., 2013). Similarly, Krolik et al.
(2014, 2016) analyzed well water samples from southeastern Ontario using MST to
elucidate whether human or bovine sources were responsible for well contamination
(Krolik et al., 2014; Krolik, Maier, Thompson, & Majury, 2016). Future work relating
to our study could include the application of MST methods to help identify the source
of microbial contamination in Maryland wells, and to elucidate potential relationships
between microbial contamination and environmental characteristics, particularly those relating to land use.

Given the small, cross-sectional nature of our study, there are several limitations to be considered. Our sample size of 118 households was relatively small, representing only a small fraction of the estimated 1,070,000 people who rely on private wells in Maryland (Maupin et al., 2014). Another limitation is the possibility that study participants may have improperly collected the water sample in their homes, which could then influence our ability to accurately determine their water quality parameters. We sought to minimize this potential problem by training participants on water sampling techniques during the safe drinking water clinic kickoff meetings, and by providing instruction sheets (along with the water sampling kits) on how participants should collect their water samples. Another limitation of this study is the use of U.S. Census of Agriculture data from 2007 with results from well water samples that were collected between 2013 and 2014. As noted above, the Census of Agriculture data were only available at the zip code level for the 2007 Census, and not for subsequent years. However, it is unlikely that the number of animal feeding operations in Maryland changed significantly between 2007 and 2013.

Despite these limitations, this is the first study to assess the water quality of private wells across multiple counties in Maryland, and to investigate the influence of animal feeding operations on well water quality, thereby addressing an important research gap in the state. This study also demonstrated the value in partnerships between land grant university research faculty and county-based extension faculty. Finally, the study highlighted the need for more educational outreach to private well
owners in Maryland in order to improve private drinking water quality in the state. Additional studies are needed to identify and confirm potential factors that can influence private well water quality in Maryland, such as animal feeding operations, septic tanks, well construction characteristics, soil geology, and climatic conditions.

5. Conclusions

Our findings suggest that there are a significant number of private domestic wells in Maryland that do not meet the guidelines for well water quality set forth by the SDWA. This finding is similar to studies conducted in other states, including the nearby states of Virginia and Pennsylvania. In addition, while other studies have reported associations between proximity to animal feeding operations and microbial contamination of private wells, this association was not observed in this cross-sectional study and may have been influenced by our limited sample size. Further studies are needed to identify and confirm possible sources of contamination of private wells in Maryland. The lack of regular monitoring of private wells makes periodic assessments at national, regional, and local scales important sources of information about this key source of drinking water throughout the U.S. The presence of microbial contaminants at levels greater than human health-based standards in 43.2% of private wells tested in this study highlights the importance of education and routine monitoring regarding the water quality of domestic wells to protect public health.
6. Acknowledgements

The authors wish to thank Emma Claye for her help processing the water samples in the lab; Kate K. Manchisi who helped with data entry of surveys; and Andrew Lazur, Extension Specialist—Water Quality, for his review of the manuscript and helpful insights.
7. References


Hach USA. (2017a). Pocket Pro Low Range TDS Tester - Hach USA. Retrieved July 12, 2018, from https://www.hach.com/pocket-pro-low-range-tds-tester/product?id=17990686204&_bt=218809546514&_bk=&_bm=b&_bn=g&gclid=EAIaIQobChMIlN_F6Jua3AIVjYvlCh1clwGYEAAYASAAEgIJ9_D_BwE


Infections in Norway 2010–2011: A National Prospective Case-Control Study. PLOS ONE, 10(10), e0139636. https://doi.org/10.1371/journal.pone.0139636


8. Tables

**Table 1.** Dates on which the safe drinking water clinics were held

<table>
<thead>
<tr>
<th>Maryland County</th>
<th>Kick-off Meeting</th>
<th>Interpretation Meeting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecil County I</td>
<td>March 2012</td>
<td>May 2012</td>
</tr>
<tr>
<td>Kent County</td>
<td>October 2012</td>
<td>December 2012</td>
</tr>
<tr>
<td>Montgomery</td>
<td>February 2013</td>
<td>March 2013</td>
</tr>
<tr>
<td>Cecil II</td>
<td>September 2013</td>
<td>November 2013</td>
</tr>
<tr>
<td>Queen Anne’s</td>
<td>February 2014</td>
<td>March 2014</td>
</tr>
<tr>
<td>Characteristic</td>
<td>Category</td>
<td>Number (%) (n = 118)</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>----------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>County</td>
<td>Cecil (1)</td>
<td>25 (21.2)</td>
</tr>
<tr>
<td></td>
<td>Kent</td>
<td>21 (17.8)</td>
</tr>
<tr>
<td></td>
<td>Montgomery</td>
<td>25 (21.2)</td>
</tr>
<tr>
<td></td>
<td>Cecil (2)</td>
<td>19 (16.1)</td>
</tr>
<tr>
<td></td>
<td>Queen Anne’s</td>
<td>28 (23.7)</td>
</tr>
<tr>
<td>Age</td>
<td>18–49</td>
<td>17 (14.4)</td>
</tr>
<tr>
<td></td>
<td>50–59</td>
<td>29 (24.6)</td>
</tr>
<tr>
<td></td>
<td>60–69</td>
<td>40 (33.9)</td>
</tr>
<tr>
<td></td>
<td>70–79</td>
<td>23 (19.5)</td>
</tr>
<tr>
<td></td>
<td>≥80</td>
<td>9 (7.6)</td>
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<td></td>
<td>Hispanic</td>
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<tr>
<td></td>
<td>White</td>
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<td></td>
<td>Other or Unspecified</td>
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</tr>
<tr>
<td>Level of formal education</td>
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</tr>
<tr>
<td></td>
<td>High School</td>
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<td></td>
<td>High school and some college</td>
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<td>Associate’s degree</td>
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<td>Graduate degree</td>
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<td>Number of years living at current home</td>
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<td>10–20 years</td>
<td>46 (39.0)</td>
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<td>Unknown</td>
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<tr>
<td>Previous testing of well water quality</td>
<td>Never</td>
<td>29 (24.6)</td>
</tr>
<tr>
<td></td>
<td>Once</td>
<td>58 (49.2)</td>
</tr>
<tr>
<td></td>
<td>Every few years</td>
<td>11 (9.3)</td>
</tr>
<tr>
<td></td>
<td>Every year</td>
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</tr>
<tr>
<td></td>
<td>≥Once per year</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td></td>
<td>Other or Unsure</td>
<td>12 (10.2)</td>
</tr>
<tr>
<td>Experienced diarrhea within the last 30 days</td>
<td>Yes</td>
<td>14 (11.9)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>104 (88.1)</td>
</tr>
<tr>
<td>Experienced vomiting within the last 30 days</td>
<td>Yes</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>118 (100%)</td>
</tr>
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</table>
Table 3. Zip code-level analysis of the association between the presence of animal feeding operations and the occurrence of total and fecal coliforms in tested wells.

<table>
<thead>
<tr>
<th>Zip Code Variable</th>
<th>Total Coliforms</th>
<th>Fecal Coliforms</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio (95% CI)</td>
<td>Odds Ratio (95% CI)</td>
</tr>
<tr>
<td>Cattle operations</td>
<td>1.23 (0.89, 1.68)</td>
<td>1.19 (0.82, 1.73)</td>
</tr>
<tr>
<td>Broiler operations</td>
<td>0.93 (0.84, 1.03)</td>
<td>1.10 (0.41, 3.00)</td>
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<td>Hog operations</td>
<td>0.76 (0.49, 1.17)</td>
<td>0.81 (0.48, 1.37)</td>
</tr>
<tr>
<td>Dairy operations</td>
<td>1.12 (0.96, 1.31)</td>
<td>1.11 (0.93, 1.33)</td>
</tr>
<tr>
<td>Turkey operations</td>
<td>0.92 (0.68, 1.24)</td>
<td>1.24 (0.44, 3.47)</td>
</tr>
<tr>
<td>Aquaculture operations</td>
<td>1.32 (0.59, 2.93)</td>
<td>1.33 (0.52, 3.40)</td>
</tr>
</tbody>
</table>
Figure 1. Maryland counties where safe drinking water clinics were held

Figure 2. University of Maryland safe drinking water clinic approach. (TDS—total dissolved solids)
Figure 3. Percentage of tested private wells that did not meet U.S. Environmental Protection Agency (US EPA) drinking water standards.
Figure 4. Mean levels of nitrate (Panel A), sulfate (Panel B), pH (Panel C) and total dissolved solids (TDS) (Panel D) detected in tested private wells by county. (US EPA, n.d.) MCL—maximum contaminant level.
Figure 5. Percentage of tested private wells that were positive for fecal indicator bacteria by county.

The US EPA National Primary Drinking Water Regulations for public water systems specify a Maximum Contaminant Level (MCL) of 0 for all microbiological contaminants.
Chapter 4: Association between Private Drinking Water Wells and the Incidence of Campylobacteriosis in Maryland: An Ecological Analysis Using Foodborne Diseases Active Surveillance Network (FoodNet) Data (2007-2016)

Rianna Murray, Raul Cruz-Cano, Daniel Nasko, David Blythe, Patricia Ryan, Michelle Boyle, Sacoby Wilson and Amy R. Sapkota.

Abstract

Campylobacter is a leading cause of foodborne illness in the United States. Campylobacter infections have most often been associated with food-related risk factors, such as the consumption of poultry and raw milk. Socioeconomic, agricultural and environmental factors can also influence the risk of campylobacteriosis. Approximately 19% of Maryland residents rely on private wells as their sole source of water. Given that the federal Safe Drinking Water Act does not regulate the water quality of private wells, these could be important non-foodborne transmission pathways for Campylobacter. Using an ecological approach, we examined the association between the prevalence of private drinking water wells and the incidence of campylobacteriosis.

Data on the number of culture-confirmed cases of Campylobacter infection in Maryland between 2007-2016 were obtained from the Foodborne Diseases Active Surveillance Network (FoodNet). Cases were linked by zip code with data from the Maryland well permits registry, the 2010 U.S. Census, the 2016 American Community Survey, and the USDA Agricultural Census. Campylobacteriosis incidence rates and well prevalence were calculated by zip code. Negative binomial
regression models were then used to evaluate the association between the prevalence of private wells, proximity to animal feeding operations and the incidence of campylobacteriosis across the physiographic provinces in Maryland.

From 2007-2016, a total of 5,746 cases of campylobacteriosis were reported in Maryland, and annual incidence rates ranged from 6.65 to 11.59 per 100,000 people. In our statewide analysis, a significant positive association was detected between well prevalence and increased campylobacteriosis incidence at the zip code level (Incidence Rate Ratio [IRR]=1.35, 95% Confidence Interval [CI] = 1.11, 1.63). A significant positive association was also observed between well prevalence and increased campylobacteriosis incidence in the Appalachian and Coastal provinces of Maryland (IRR=2.94, 95% CI=1.11, 7.76 and IRR=1.70, 95% CI=1.25, 2.31 respectively). The presence of broiler chicken operations, increasing median age and percentage of residents living in poverty were also significantly associated with campylobacteriosis incidence at the zip code level in some physiographic provinces in Maryland.

To our knowledge, these are the first US data to demonstrate an association between prevalence of private wells and campylobacteriosis incidence at the zip code level. Our findings suggest that increased outreach and education to private well owners regarding water quality testing and remediation could positively impact public health.
1. Introduction

_Campylobacter_ is a leading cause of bacterial gastroenteritis in the United States, as well as in much of the developed and developing world (Butzler, 2004; Kaakoush et al., 2015). Each year in the U.S., _Campylobacter_ spp. is responsible for an estimated 800,000 illnesses, accounting for 9% of the 9.4 million annual cases of foodborne illness (Scallan et al., 2011b). _Campylobacter_ spp. is also responsible for 15% of the 55,961 annual hospitalizations caused by microorganisms commonly associated with contaminated food in the United States (Scallan et al., 2011b). The annual worldwide costs associated with _Campylobacter_ infection are also high. For example in the U.S., the estimated annual cost of campylobacteriosis is $1.7 billion, while in the Netherlands, it is estimated to be $23.8 million (Batz et al., 2012; Havelaar et al., 2005). Other than the acute gastrointestinal illness symptoms of diarrhea and vomiting that typically result from _Campylobacter_ infection, the illness has also been associated with more serious sequelae, such as Guillain-Barré syndrome, reactive arthritis, post-infectious irritable bowel syndrome and inflammatory bowel disease (Keithlin et al., 2014; Riddle et al., 2012).

The majority of reported _Campylobacter_ cases are sporadic (Friedman et al., 2004). The most common species of _Campylobacter_ associated with human illnesses are _Campylobacter jejuni_ and _Campylobacter coli_ (Epps et al., 2013). _Campylobacter_ are naturally present in digestive tracts of animals such as cattle, swine and poultry (Abley et al., 2012; Boes et al., 2005; Hermans et al., 2011; Møller Nielsen et al., 1997; Newell & Fearnley, 2003), thereby presenting the opportunity for the bacteria to enter the food system. Cattle and sheep also shed
*Campylobacter*, and dairy farms have been implicated as environmental reservoirs of *Campylobacter* (Stanley & Jones, 2003). Common food-related risk factors for *Campylobacter* include consumption of undercooked poultry, unpasteurized milk, cheese, and ground beef and pork (Batz et al., 2012; Domingues et al., 2012; Friedman et al., 2004; Møller Nielsen et al., 1997). In addition to foodborne infections, *Campylobacter* transmission has been linked with international travel, handling of raw poultry, consumption of contaminated water, direct contact with animals, and person-to-person transmission both sporadically and in outbreaks (Domingues et al., 2012; Friedman et al., 2004; Kaakoush et al., 2015; Taylor et al., 2013).

Groundwater is generally considered to be clean and safe, and is often used for drinking without treatment by homeowners with private wells (Gallay et al., 2006). However, groundwater can be influenced by contaminants originating from land use activities, particularly those associated with food animal production (K. Jones, 2001; Stanley et al., n.d.; Thomas C. et al., 2001). Stanley et al. (1998) demonstrated that the source of *Campylobacter* contamination of groundwater was a dairy herd when identical strains of *C. jejuni* were isolated from groundwater and a herd situated in the same hydrological catchment area (Stanley et al., n.d.). Additionally, it has been demonstrated that *Campylobacter* can be transported through contaminated soil to groundwater under experimental conditions (Close et al., 2010).

In Maryland, approximately 19% of the population relies on private wells for home consumption (Maupin et al., 2014), which includes water for drinking, cooking,
bathing, washing, toilet flushing and other needs. While public water systems are regulated by the US EPA (under the Safe Drinking Water Act) to ensure safe drinking water, private wells are not regulated (US EPA, 1974). Instead, private well users are responsible for ensuring the safety of their own drinking water.

*Campylobacter* infection has been previously associated with consumption of contaminated, untreated water, among both sporadic cases and outbreaks linked to community water systems (Furtado et al., 1998; Sacks et al., 1986). However, there have been very few studies that have investigated private domestic well water sources as risk factors for *Campylobacter* infection. Carrique-Mas et al. (2005) detected an increased risk of campylobacteriosis among children in Sweden who consumed water from a private well (Odds Ratio [OR]=2.6; 95% CI= 0.9, 7.4) (Carrique-Mas et al., 2005), although that association was not statistically significant. In a study conducted in British Columbia, Canada, Galanis et al (2014) found that the odds of campylobacteriosis were higher for individuals serviced by private wells than municipal water systems (OR= 1.4, 95% CI= 1.1,1.8) (Galanis et al., 2014). Additional studies in Canada and Europe have suggested that individuals who consume private well water may be at a higher risk of campylobacteriosis (Kapperud et al., 2003; NygåRd et al., 2004; Uhlmann et al., 2009).

Recent studies have also shown that additional environmental and community socioeconomic factors can impact rates of campylobacteriosis. In Maryland, researchers from our group found that increased rurality and presence of broiler chicken operations in a zip code were associated with higher campylobacteriosis rates (Zappe Pasturel et al., 2013). Another study that investigated trends in *Campylobacter*
infection across multiple surveillance sites in the U.S. found that incidence was slightly higher in zip codes with higher percentages of housing units within rural areas, and in zip codes with turkey operations (Rosenberg Goldstein et al., 2016). Both studies indicate that rurality is an important factor in predicting the risk of campylobacteriosis. Additionally, a recent study estimated the impact of extreme heat and precipitation events on the risk of campylobacteriosis and observed that a one-day increase in exposure to extreme precipitation events was associated with a 3% increase in risk of campylobacteriosis in coastal areas of Maryland (Soneja et al., 2016). However, such an association was not observed in noncoastal areas, suggesting a potential waterborne risk factor for campylobacteriosis.

Since many rural areas are characterized by a higher prevalence of private domestic drinking water wells (Waller, 1988), and Campylobacter can be transported through groundwater, we hypothesized that increased exposure to contaminated groundwater from private wells may affect the risk of campylobacteriosis in Maryland. Utilizing reported campylobacteriosis data for the state of Maryland from 2007 to 2016, we employed an ecological approach to investigate the association between the prevalence of wells and campylobacteriosis incidence at the zip code level.

2. Methods

2.1 Campylobacter case data

We obtained Campylobacter case data between 2007-2016 from the Maryland FoodNet. The Maryland FoodNet is one of 10 sites that participate in the CDC FoodNet program. The FoodNet program conducts active surveillance for infections
caused by *Campylobacter, Cryptosporidium, Cyclospora, Listeria, Salmonella*, Shiga
toxin-producing *Escherichia coli* (STEC) O157 and non-O157, *Shigella, Vibrio*, and
*Yersinia* (CDC, n.d.a). We restricted our analyses to culture-confirmed cases of
*Campylobacter* infection (including infections caused by *C. jejuni, C. coli* and
unknown *Campylobacter* spp.) that occurred in Maryland between 2007-2016. A
campylobacteriosis case was an individual whose biological specimen (stool, blood,
or other) was culture-confirmed for the presence of *Campylobacter*, regardless of
symptoms or date of onset. Both sporadic cases and those associated with outbreaks
were included. For each campylobacteriosis case, we also obtained limited
demographic data (e.g., age, gender, race/ethnicity, international travel) and each
case’s zip code at the time of diagnosis.

2.2 Socioeconomic data

We obtained population data from the 2010 U.S. Census of Population and
Housing by 5-digit zip code tabulation area (ZCTA) (U.S. Census Bureau, n.d.a). We
also obtained socioeconomic variables from the 2010-2016 American Community
Survey (5-year estimates) by 5-digit ZCTA using the American Fact Finder Service
(U.S. Census Bureau, 2014). We selected socioeconomic variables on the basis of
recommendations from previous studies (N. Krieger, Williams, & Moss, 1997;
Rosenberg Goldstein et al., 2016; Zappe Pasturel et al., 2013), including % of the
population that identifies as African American, % of the population that identifies as
Hispanic, % of houses occupied by their owners, % of the population living below the
poverty level, % of the population that is ≥25 years without high a school diploma,
and % of the population that is unemployed. In order to determine a prevalence rate of wells per zip code, we obtained data on the number of houses per zip code in Maryland from the 2012-2016 American Community Survey (5-year estimates) via the American Fact Finder Service (U. S. Census Bureau, 2014).

2.3 Animal Feeding Operations Data

We obtained animal feeding operation data in Maryland from the 2007 U.S. Census of Agriculture, National Agricultural Statistics Service (NASS) (USDA, n.d.). Specifically, we obtained data on the number of animal operations with sales by zip code for broiler chickens, turkey, aquaculture, sheep or goats, hogs, and dairy or beef cattle.

2.4 Private Well Data

The Maryland homeowner well permits registry was obtained from the Maryland Department of the Environment (MDE). The registry consisted of a tab-delimited file of 446,781 residential wells in Maryland, some of which had an associated latitude and longitude coordinate (362,075 wells), and for some of which the only locational information provided were the names of the town and street nearest to the property that contained the well (84,706 wells). Zip codes were retrieved for records with valid latitude and longitude coordinates or valid Maryland town and street addresses using Google’s Geocoding application programming interface (API) (Google Developers, 2018). Custom parsers were written to query the API for each record’s zip code using either the latitude and longitude coordinates or
the town and street name. Only wells with both a valid town and street name were queried to ensure accuracy (19,621 wells). The zip codes for each record were verified to be among the 619 registered Maryland zip codes. After completing the query process, valid zip codes were obtained for a total of 374,162 private wells in Maryland.

2.5 Maryland Physiographic Provinces

The United States Geological Survey (USGS) defines five distinct physiographic provinces in Maryland: the Appalachian Plateau, the Valley and Ridge, the Blue Ridge, the Piedmont and the Coastal Plain (Appendix 1, Figure A1) (USGS, 2017). Each physiographic province is a geographic area in which the geology (including lithology and structure) and climate history have resulted in landforms that are distinctly different from adjacent areas (Maryland Geological Survey, n.d.). For the purposes of this study, the Appalachian Plateau and the Valley and Ridge province were combined due to the small size of each individual province. The Piedmont and Blue Ridge provinces were also combined for similar reasons.

2.6 Descriptive Analyses

We calculated *Campylobacter* incidence rates per 100,000 population by year for the State of Maryland using population estimates from the U.S. Census Bureau (U.S. Census Bureau, n.d.a). Cases that were potentially associated with international travel were then excluded from further descriptive and inferential analysis (described in detail below). We then determined a prevalence rate of wells per zip code (the well
prevalence variable) using the Maryland homeowner well permits registry and data on the number of houses per zip code from the 2012-2016 American Community Survey via the American Fact Finder Service (U.S. Census Bureau, 2014). Well prevalence per zip code was calculated by dividing the number of wells per zip code by the number of houses per zip code. *Campylobacter* case count data (excluding cases associated with international travel) were then linked with the socioeconomic, animal feeding operation, and well prevalence data by zip code and 5-digit ZCTA. A choropleth map of campylobacteriosis incidence rates by zip code was created to illustrate our findings. We performed all mapping using ArcGIS version 10.3 (ESRI, 2016) (ESRI, Redlands, CA).

### 2.7 Statistical Models

We developed regression models to evaluate associations between well prevalence, socioeconomic, and agricultural factors and campylobacteriosis incidence at the zip code level. First, any campylobacteriosis cases for which zip codes were missing or incorrect were excluded from analysis, along with those cases associated with international travel. We then evaluated collinearity among all independent variables of well prevalence, SES factors, and agricultural factors using the variance inflation factor and excluded highly collinear variables. We also determined the Pearson correlation among all independent variables to avoid using highly correlated variables in the multivariate regression model.

We compared several regression models for count data and tested models with and without zero inflation. The negative binomial regression model provided the best fit for the dataset and the final model included the zip code level variables described
in Table 2. We ran a statewide regression model, a univariate regression model between well prevalence and incidence of campylobacteriosis stratified by county, and univariate and multivariate physiographic province-specific regression models. Only the well prevalence variable and the independent variables that were significantly associated with campylobacteriosis incidence (whether increase or decrease in incidence) at the zip code level for each individual province by univariate analysis were included in the province’s multivariate regression models. We performed all modeling using SAS version 9.4 (SAS Institute, Cary, NC), and used p-values of $\leq 0.05$ to assess statistical significance (SAS Institute, 2014). We used R version 1.0.153 (Vienna, Austria) (R Core Team, 2017) to create bar charts (Figure 1 and 3) and whisker plots of the multivariate analyses (Figure 5).

3. Results

3.1 Maryland Campylobacter cases, 2007-2016

A total of 5,746 cases of culture-confirmed Campylobacter infections were reported to the FoodNet active surveillance system in Maryland between 2007-2016. A total of 5,706 cases (99.3%) had valid zip codes and were included in this study.

The majority of cases were between the ages of 20-59 (57.08%), White (64.67%), and Non-Hispanic (72.34%) (Table 1). Most cases were sporadic infections (85.79%), while just 0.86% of cases were associated with outbreaks. A total of 9 unique species (i.e., C. jejuni, C. coli, C. fetus, C. gracilis, C. lari, C. curvus, C. rectus, C. showae, and C. upsaliensis) were identified among all cases of culture-confirmed Campylobacter infections. Approximately 62% of all cases were identified
as *C. jejuni*, 33% as unknown *Campylobacter* spp., 3.14% as *C. coli*, and the remaining unique species accounted for the remaining 1.86% of infections.

The average annual incidence rates of campylobacteriosis in Maryland have been on a steady increase during the study time period of 2007-2016 as illustrated in Figure 1. The lowest annual incidence rate for this 10-year period of 6.65 per 100,000 people was recorded in 2008, and the highest annual incidence rate of 11.6 per 100,000 people was recorded in 2016 (Figure 1). The choropleth map indicates the spatial distribution of campylobacteriosis by zip code in Maryland (Figure 2). *Campylobacter* infection rates are highest along the Eastern Shore, a region of Maryland characterized by the presence of high numbers of broiler chicken operations (Environmental Integrity Project, n.d.).

3.2 Campylobacteriosis incidence by zip code

We removed all cases that reported undertaking international travel within 7 days prior to experiencing symptoms of gastroenteritis, which amounted to 1,634 cases (28.6%). These cases were removed since travel outside of the United States was found to be a significant risk factor for campylobacteriosis in previous studies using data from FoodNet (Kendall et al., 2012; Ricotta et al., 2014). The final dataset used in our regression analyses was therefore composed of 4,072 campylobacteriosis cases. Using these counts, we then calculated campylobacteriosis by zip code (Figure 2) and used these values as the outcome of our multivariate models (described below).
3.3 Maryland Private Wells

The Maryland county with the most wells was Anne Arundel county with 53,192 wells, while the county with the least wells was Baltimore City with 153 wells (Figure 3). However, well prevalence, which is a measure of the number of wells per number of houses in each county, was highest in Somerset county (0.967) and lowest in Baltimore City (0.0006) (Figure 3). Most wells fell within the Coastal Plain province, a region characterized by limestone, sandstone and shale (Reger & Cleaves, 2008; Vokes, 1957).

3.4 Statewide Multivariate Analysis

In our statewide multivariate negative binomial regression model we observed a significant positive association between well prevalence and increased campylobacteriosis incidence at the zip code level (Incidence Rate Ratio [IRR]=1.35, 95% Confidence Interval [CI] = 1.11, 1.63) (Table 2). Agricultural and socioeconomic factors were also found to influence campylobacteriosis incidence at the zip code level. In zip codes that contain broiler chicken operations, the incidence rate of campylobacteriosis was 1.28 times that in zip codes that do not contain broiler chicken operations (IRR =1.28, 95% Confidence Interval [CI] = 1.12, 1.48). On the other hand, the risk of campylobacteriosis was lower in zip codes that contained turkey operations compared to those that did not (IRR =0.81, 95% CI = 0.67, 0.97).

In zip codes that are characterized by residents of a higher median age, the incidence rate of campylobacteriosis was higher compared to zip codes where the median age is lower (IRR= 1.03; 95% CI = 1.02, 1.03). Similarly, in zip codes where
a higher percentage of the population lives below the poverty line, the incidence rate of campylobacteriosis was higher compared to zip codes where a lower percentage of the population lives below the poverty line (IRR= 1.03; 95% CI = 1.01, 1.04).

However, we observed the opposite relationship with the variable of owner occupancy status, another indicator of socioeconomic status. Campylobacteriosis incidence rates were lower in zip codes characterized by a higher percentage of owner-occupied homes (IRR= 0.99; 95% CI = 0.98, 0.99). Additionally, zip codes in which a higher percentage of the population identified as African American experienced a lower risk of campylobacteriosis in the statewide model (IRR= 0.98; 95% CI = 0.97, 0.98).

3.5 Stratified Analysis

Given that well prevalence was a significant predictor of campylobacteriosis incidence at the zip code level in the statewide model, we decided to examine the relationship more closely by investigating the impact of well prevalence on campylobacteriosis incidence in each county. In a univariate analysis of well prevalence by county, we observed that well prevalence was a significant predictor of campylobacteriosis incidence in nine counties in Maryland (Table A1, Appendix 2). In this stratified analysis by county, we determined that seven of the counties where a significant relationship between well prevalence and campylobacteriosis incidence occurred were located mostly within the Coastal province of the state; however, one of the counties was located within the Piedmont province and the other was located
within the Appalachian province (Figure 4 and Figure A1, Appendix 1). We therefore decided to further analyze the relationship by physiographic province in Maryland.

By univariate analysis of well prevalence and incidence of campylobacteriosis stratified by physiographic province, we observed that well prevalence was a significant predictor of an increased incidence of campylobacteriosis in all three provinces of Maryland (Table A2, Appendix 3). We then created multivariate models stratified by physiographic province in Maryland to investigate whether the association between well prevalence and the incidence of campylobacteriosis differed by geographic region. Separate models were built for each province, and only the variables that were significantly associated with an increase in campylobacteriosis incidence at the zip code level by univariate analysis were included in the multivariate model for that province (results of the univariate analyses of agricultural and socioeconomic variables by province are provided in the Table A2, Appendix 3).

In the counties of the Appalachian province, we observed that well prevalence was significantly associated with campylobacteriosis incidence in a multivariate negative binomial regression model (IRR=2.94, 95% CI=1.11, 7.76) (Figure 5). None of the socioeconomic variables included in the multivariate model of the Appalachian province were found to be significantly associated with campylobacteriosis incidence.

In the Coastal province, well prevalence was also significantly associated with an increase in campylobacteriosis incidence at the zip code level (IRR=1.70, 95% CI=1.25, 2.31) (Figure 5). Zip codes in the coastal province that contained broiler chicken operations had significantly higher incidence rates of campylobacteriosis than zip codes without these operations (IRR=1.62; 95% CI=1.32, 1.98). However,
we observed a decrease in campylobacteriosis incidence in zip codes that contained cattle operations (IRR= 0.75; 95% CI= 0.58, 0.97). As the median age within a zip code increased, we observed a corresponding increase in the incidence of campylobacteriosis (IRR=1.03; 95% CI= 1.02, 1.05). We observed that the campylobacteriosis risk was reduced as the percentage of the population that identified as African American increased within a zip code in the Coastal province (IRR=0.98; 95% CI= 0.98, 0.99). As the percentage of the population within a zip code living in poverty increased, so did the risk of campylobacteriosis (IRR=1.02; 95% CI= 1.00, 1.04). However, we observed a decrease in campylobacteriosis risk as the percentage of owner occupied homes increased in a zip code (IRR=0.99; 95% CI= 0.98, 1.00).

We observed that well prevalence was associated with an increase in the risk of campylobacteriosis at the zip code level in the Piedmont province; however, this association was not statistically significant (IRR=1.29; 95% CI= 0.99, 1.67). None of the agricultural variables in the multivariate negative binomial model were significantly associated with campylobacteriosis risk; however, an association was observed with some of the socioeconomic variables in the Piedmont province. As the median age within a zip code increased, we also observed an increase in the incidence of campylobacteriosis (IRR=1.02; 95% CI= 1.01, 1.04). However, we observed a decrease in campylobacteriosis risk as the percentage of African American population and the percentage owner occupied homes increased in a zip code (IRR=0.99; 95% CI= 0.98, 0.99 and IRR=0.98; 95% CI= 0.98, 0.99, respectively).
4. Discussion

To our knowledge, this is the first U.S. study to evaluate the impact of private drinking water wells on the risk of campylobacteriosis, an illness that is typically viewed as foodborne, by linking active surveillance data with other environmental, agricultural and socioeconomic data at the zip code level. Our results add to the growing body of international research that has demonstrated an association between private drinking water supplies and the risk of *Campylobacter* infection.

From 2007-2016 a total of 62,941 cases of confirmed campylobacteriosis were reported to the CDC FoodNet program across all ten participating sites (CDC, 2018a). In comparison with other sites during the same ten-year period, the overall incidence rate of campylobacteriosis in Maryland is the third lowest (9.71 per 100,000 population), preceded by Georgia (6.83 per 100,000 population) and Tennessee (6.77 per 100,000 population). The site with the highest rate of *Campylobacter* infection during this period was California (27.63 per 100,000 population).

Our statewide multivariate analysis showed that campylobacteriosis rates were higher in zip codes that contain broiler chicken operations compared to zip codes that did not contain broiler chicken operations. This finding corroborates results of other zip code-level studies conducted in Maryland and across all FoodNet sites (Rosenberg Goldstein et al., 2016; Zappe Pasturel et al., 2013). In our province-specific analyses, campylobacteriosis rates were also higher in zip codes that contain broiler chicken operations in the Coastal Plain province, which is a region that is characterized by high numbers of broiler chicken operations (MDE, n.d.b). Broiler
chicken farms are known to be reservoirs of *Campylobacter* as broiler chickens are frequently colonized with *Campylobacter* (Beery et al., 1988; Hardy et al., 2011; Harris et al., 1986). Therefore, high numbers of chicken farms in the Coastal province may be contributing to groundwater contamination. Since the application of chicken farm waste to agricultural soil is considered an acceptable waste disposal method for broiler chicken operations (Burkholder et al., 2007b), it is possible that farm waste being applied to soils is contaminating groundwater and presenting a mode of transmission for *Campylobacter* to humans, thereby influencing the rates of campylobacteriosis in the Coastal province. Additionally, since a high proportion of the Coastal province depends on private well water, exposure to contaminated well water may be more frequent among individuals living in the Coastal province compared to the Appalachian and Piedmont provinces.

Our findings also showed that campylobacteriosis incidence rates were lower in zip codes characterized by higher percentages of African Americans both in our statewide model and in all individual models constructed for each physiographic province, although the association was not statistically significant in the Appalachian province. This association suggests a somewhat protective effect, and is similar to the findings of other ecological studies of campylobacteriosis incidence rates in Maryland, as well as studies that investigated *Campylobacter* infection across all ten FoodNet sites (Rosenberg Goldstein et al., 2016; Samuel et al., 2004; Zappe Pasturel et al., 2013). Previous studies have identified that African Americans were less likely to have health insurance and access to healthcare services (Manuel, 2018; L. D.
Richardson & Norris, 2010), which could result in underreporting of illnesses among
this group to surveillance programs such as FoodNet.

We also found that the risk of campylobacteriosis increased in zip codes with
populations characterized by a higher median age compared to zip codes where the
median age was lower in the statewide model and in the Coastal and Piedmont
provinces. This finding was expected since older persons are known to be a group
that is at risk for contracting campylobacteriosis (CDC, 2018b; Gillespie et al., 2002).
Higher owner occupancy rates were inversely associated with *Campylobacter*
infections in the statewide model and in the Piedmont province, and this may be
attributed to differences in illness reporting among populations living in different
housing conditions as previously detected in England (Tam, Rodrigues, & O’Brien,
2003).

Our results also showed that some zip codes characterized by lower
socioeconomic status (i.e., a greater percentage of the population living below
poverty) had higher incidence rates of campylobacteriosis in the statewide model and
in the Coastal province, suggesting that poverty might be associated with higher rates
of *Campylobacter* infection. However, another widely used indicator of poverty used
in this study, percentage owner occupancy rates, did not reflect the same result. The
owner-occupied housing unit rate is measured by the U.S. Census Bureau as the
number of owner-occupied housing units divided by the number of occupied housing
units or households (U.S. Census Bureau, n.d.), and is often used proxy measurement
of poverty (Krieger et al., 1997). In this study, higher percentages of owner
occupancy rates were associated with lower incidences of *Campylobacter* infection in
the statewide model and in the Coastal and Piedmont provinces. Previous literature has illustrated similarly contradicting associations between socioeconomic indicators of poverty and campylobacteriosis incidence (Bemis, Marcus, & Hadler, 2014; Simonsen, Frisch, & Ethelberg, 2008a; Whitney et al., 2015).

Well prevalence was a significant predictor of increased campylobacteriosis risk in the multivariate statewide model and in the multivariate models of the Appalachian and Coastal provinces (Figure 5). As described previously, a high density of broiler chicken operations in the Coastal province may be contributing to contamination of groundwater in this region, thereby influencing the association observed between well prevalence and campylobacteriosis. The Appalachian region of the United States, which spans 13 states, is mostly designated as rural and depends heavily on private wells as a water source (Hughes et al., 2005; Pollard & Jacobsen, 2018). The counties of the Appalachian province in Maryland, Allegany, Garrett, and Washington, have a total of 21,430 private wells across 109,778 houses in the province. A high reliance on wells in the Appalachian province of Maryland could explain why we observed a significant association between well prevalence and campylobacteriosis in this province.

A previous study in Michigan found that consumption of well water at home was independently associated with *Campylobacter* infection in rural areas at the county level (OR=5.4, 95%CI= 3.9, 7.4) (Cha, Henderson, Collins, & Manning, 2016). Counties with the highest incidence rates of campylobacteriosis were more likely to have a high cattle density (OR 2.5, 95% CI 2.1, 2.9); however, such a relationship was not observed for broiler chicken operations (Cha et al., 2016). In our
study, the presence of cattle operations was found to be significantly associated with campylobacteriosis incidence in the Piedmont province by univariate analysis, however a significant association did not occur in the multivariate analysis.

Our study has several limitations. We were limited by zip code being the smallest possible level of analysis due to the availability of the FoodNet data, agricultural census data and well permit registry data at the zip code level only. A census block or census tract level analysis may have provided finer resolution and more precisely captured the impact of private drinking water wells. Additionally, having exact addresses of both the private wells and the animal feeding operations would have allowed for us to employ geocoded buffer analyses and spatial regression techniques. Similarly, the use of our ecological study approach results in the issue of ecological fallacy, where associations between the risk factor of well prevalence and the outcomes of campylobacteriosis cannot be used to infer associations at the individual level. A final limitation is that performing the analysis at the zip code level required us to obtain data from the U.S. Census and the ACS by ZCTAs. However, zip codes and ZCTAs do not always correlate, resulting in some zip codes for which census data are unavailable. Grubesic and Matisziw (2006) and Krieger (2002) also highlight the discrepancies in matching ZCTA and zip code level data, indicating that using ZCTAs to link geographic data is convenient but can result in relatively large geographic zones with linkages that can lead to imprecise estimates (Grubesic & Matisziw, 2006; Nancy Krieger et al., 2002).

Despite these limitations, this study provides a good starting point for the evaluation of the role of private wells on the risk campylobacteriosis in the U.S.;
however, additional work is required that employs more complex analyses to enhance our present ecological study. Methods such as Microbial Source Tracking (MST) could be used to determine the likely source of *Campylobacter* or other pathogens detected in well water (Simpson et al., 2002). MST has been previously used in a variety of applications, including in the management of surface water contamination and watershed remediation (Bradshaw et al., 2016; Simpson et al., 2002). Allevi et al. (2013) utilized MST techniques to characterize the magnitude and incidence of microbial contamination in private wells in Virginia, and to identify the likely sources of this contamination (Allevi et al., 2013). Combined with studies that model groundwater flow and direct sampling of private wells, MST could be used to elucidate potential relationships between microbial contamination and environmental characteristics, particularly those relating to land use.

Spatial regression analysis techniques should also be employed to investigate whether the risk of campylobacteriosis is associated with private wells as a drinking water source. Researchers in Canada performed a cross-sectional analysis at the individual level to assess whether the risk of sporadic foodborne illness differed by drinking water source, whether municipal or private well (Uhlmann et al., 2009). Using surveillance data similar to FoodNet and geocoded addresses of campylobacteriosis cases in GIS software, the authors determined that the risk of disease was 8.1 times higher for individuals on private wells compared to cases on the municipal ground water system (Uhlmann et al., 2009).

Additionally, aquifer type can potentially influence the quality of private well water. Confined aquifers exist within layers of impermeable rock, while unconfined
aquifers are located closer to the earth’s surface than confined aquifers, and as such are impacted by external factors and contamination sources much more than confined aquifers (Heath, 1983; Waller, 1988). Given that different physiographic provinces can have different aquifer types based on the lithography and rock type in the province, whether a private well is drilled in a confined or unconfined aquifer may influence its potential to be contaminated by pathogens such as *Campylobacter*.

5. Conclusion

To our knowledge, this is the first study to examine the association between private well prevalence and the incidence of campylobacteriosis at the zip code level in the U.S. We observed that as well prevalence increased, so did the risk of campylobacteriosis at the zip code level in a statewide model, as well as in the Appalachian and Coastal provinces of Maryland. The presence of broiler chicken operations, increasing median age and percentage of residents living in poverty were also significantly associated with campylobacteriosis incidence at the zip code level in some physiographic provinces in Maryland. Studies such as this are important because improved understanding of the risk factors of *Campylobacter* infection can lead to improved knowledge of potential exposure pathways of campylobacteriosis, thereby informing the development of more effective prevention strategies for this illness.
6. Acknowledgements

The authors would like to acknowledge the Maryland Department of the Environment for providing information on permitted wells in the state. We would also like to acknowledge Michelle Boyle of the Emerging Infections Program at the Maryland Department of Health for compiling the foodborne diseases surveillance data used in this analysis. Additionally, we would like to acknowledge Jessica Chopyk for assistance with creating some of the figures in the manuscript.
7. References


Samuel, M. C., Vugia, D. J., Shallow, S., Marcus, R., Segler, S., McGivern, T., … Group, for the E. I. P. F. W. (2004). Epidemiology of Sporadic Campylobacter Infection in the United States and Declining Trend in


### 8. Tables

**Table 1.** Demographic characteristics of campylobacteriosis cases reported to the Maryland FoodNet program, 2007-2016

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>538 (9.43)</td>
</tr>
<tr>
<td>5-9</td>
<td>247 (4.33)</td>
</tr>
<tr>
<td>10-19</td>
<td>505 (8.85)</td>
</tr>
<tr>
<td>20-59</td>
<td>3257 (57.08)</td>
</tr>
<tr>
<td>≥ 60</td>
<td>1155 (20.24)</td>
</tr>
<tr>
<td>Unknown</td>
<td>4 (0.07)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>577 (10.11)</td>
</tr>
<tr>
<td>Other</td>
<td>1439 (25.22)</td>
</tr>
<tr>
<td>White</td>
<td>3690 (64.67)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>367 (6.43)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>4128 (72.34)</td>
</tr>
<tr>
<td>Unknown or Missing</td>
<td>1211 (21.22)</td>
</tr>
<tr>
<td><strong>International Travel</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1634 (28.64)</td>
</tr>
<tr>
<td>No</td>
<td>3314 (58.08)</td>
</tr>
<tr>
<td>Unknown</td>
<td>758 (13.28)</td>
</tr>
<tr>
<td><strong>Disease type</strong></td>
<td></td>
</tr>
<tr>
<td>Sporadic</td>
<td>4895 (85.79)</td>
</tr>
<tr>
<td>Outbreak</td>
<td>49 (0.86)</td>
</tr>
<tr>
<td>Unknown or missing</td>
<td>762 (13.35)</td>
</tr>
</tbody>
</table>
### Table 2. Incidence rate ratios (IRR) and 95% confidence interval (CI) for campylobacteriosis in Maryland (2007–2016)

<table>
<thead>
<tr>
<th>Variables</th>
<th>IRR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IRR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well Prevalence</td>
<td>1.35</td>
<td>(1.11, 1.63)</td>
</tr>
<tr>
<td>Cattle operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00</td>
<td>(Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>0.89</td>
<td>(0.78, 1.01)</td>
</tr>
<tr>
<td>Broiler chicken operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00</td>
<td>(Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>1.28</td>
<td>(1.12, 1.48)</td>
</tr>
<tr>
<td>Hog operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00</td>
<td>(Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>0.91</td>
<td>(0.70, 1.19)</td>
</tr>
<tr>
<td>Turkey Operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00</td>
<td>(Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>0.81</td>
<td>(0.67, 0.97)</td>
</tr>
<tr>
<td>Median age, years</td>
<td>1.03</td>
<td>(1.02, 1.04)</td>
</tr>
<tr>
<td>% African American</td>
<td>0.98</td>
<td>(0.97, 0.98)</td>
</tr>
<tr>
<td>% Hispanic</td>
<td>1.01</td>
<td>(0.99, 1.01)</td>
</tr>
<tr>
<td>% Owner occupancy</td>
<td>0.99</td>
<td>(0.98, 0.99)</td>
</tr>
<tr>
<td>% Residents below poverty</td>
<td>1.03</td>
<td>(1.01, 1.04)</td>
</tr>
<tr>
<td>% Population ≥25 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>without high school diploma</td>
<td>0.99</td>
<td>(0.98, 1.01)</td>
</tr>
<tr>
<td>% Unemployment</td>
<td>1.01</td>
<td>(0.98, 1.02)</td>
</tr>
</tbody>
</table>

Note: IRR = incident rate ratio; CI = confidence interval
<sup>a</sup> Adjusted after controlling for other independent variables in the model.
9. Figures

Figure 1. Incidence rates of campylobacteriosis per 100,000 population in MD, 2007-2016
Figure 2. Average annual campylobacteriosis incidence rates per 100,000 population in MD by zip code, 2007-2016
Figure 3. Number of wells (panel A) and well prevalence (panel B) by county and physiographic province in Maryland
Figure 4. Map of Maryland indicating the counties within each Physiographic Province
Figure 5. Incidence rate ratios (IRRs) and 95% confidence intervals (CIs) for the risk of campylobacteriosis by physiographic province, socioeconomic status, agricultural factors and well prevalence in Maryland.
Chapter 5: Prevalence of Private Drinking Water Wells Impacts Salmonellosis Incidence in Maryland, USA: An Ecological Analysis Using Foodborne Diseases Active Surveillance Network (FoodNet) Data (2007-2016)

Rianna T. Murray, Raul Cruz-Cano, Daniel Nasko, David Blythe, Michelle Boyle, Patricia Ryan, Sacoby M. Wilson and Amy R. Sapkota.

Abstract

Salmonellosis is a leading cause of foodborne illness worldwide. *Salmonella* infections have most often been associated with food-related risk factors, including the consumption of eggs, poultry and raw vegetables. Recently, socioeconomic, agricultural and environmental factors, such as drinking water source, have also been shown to influence the risk of salmonellosis. However, there are few data evaluating the association between consuming private well water and risk of *Salmonella* infections. Using an ecological approach, we examined the association between the prevalence of private drinking water wells and the incidence of salmonellosis in Maryland.

Data on the number of culture-confirmed cases of *Salmonella* infection in Maryland (2007-2016) were obtained from the Foodborne Diseases Active Surveillance Network (FoodNet). Cases were linked by zip code with data from the Maryland well permits registry, the 2010 U.S. Census, the 2016 American Community Survey, and the USDA Agricultural Census. Well prevalence and salmonellosis incidence rates were calculated by zip code. Negative binomial
regression models were used to explore the association between private well prevalence, proximity to animal feeding operations and salmonellosis incidence in coastal and non-coastal communities in Maryland.

From 2007-2016, a total of 8,850 salmonellosis cases were reported in Maryland, and annual incidence rates ranged from 12.98 to 17.25 per 100,000 people. Prevalence of private wells in a zip code was statistically significantly associated with salmonellosis incidence at a statewide level (Incidence Rate Ratio [IRR]=1.62; 95% Confidence Interval [CI]= 1.35, 1.93) and in the coastal zone of Maryland (IRR = 1.61; 95% CI = 1.31, 1.99). The presence of broiler chicken operations and the percentage of people living below the poverty level were also significantly associated with salmonellosis incidence at the zip code level in the coastal zone.

To our knowledge, these are the first U.S. data to characterize the relationship between private drinking water wells and the risk of salmonellosis using an ecological study design. Our findings suggest that strengthening private well water regulations and improving the education of private well owners on well maintenance could potentially reduce the burden of salmonellosis in Maryland.
1. Introduction

*Salmonella* is a leading cause of gastroenteritis worldwide (Kirk et al., 2015). Globally, it is estimated that there are over 78 million cases of salmonellosis annually, resulting in over 28,600 deaths (Kirk et al., 2015). In the U.S., over 1 million cases of acute gastroenteritis caused by infection with nontyphoidal *Salmonella* spp. occur annually, including an estimated 19,500 hospitalizations and more than 375 deaths (Scallan et al., 2011b). Infection with *Salmonella* can follow two very different disease courses, depending on whether the host is infected with a typhoidal or nontyphoidal *Salmonella* serotype. Infection with a nontyphoidal serotype often causes mild self-limited illness, including diarrhea, fever and abdominal cramping 12–72 hours after infection (Dekker & Frank, 2015). However, much more serious sequelae including osteomyelitis, pneumonia, meningitis and death may occur, especially among immunocompromised individuals or those with underlying medical conditions such as sickle cell anemia (Crump et al., 2015; Dekker & Frank, 2015; Pond, 2005). Infection with a typhoidal serotype can lead to typhoid fever, and complications that include neurologic effects, intestinal perforation and death (Dougan G & Baker S, 2014).

Given the high disease burden of salmonellosis, the economic consequences of this illness, in terms of both medical expenses and lost productivity, are considerable. Hoffman et al. (2015) estimated the economic burden of foodborne illnesses acquired in the U.S. and found that the medical costs, productivity losses and costs due to loss of life associated with nontyphoidal *Salmonella* totaled over $3.6 billion dollars in 2013 (Hoffman, Maculloch, & Batz, 2015). Overall, the costs
associated with *Salmonella* infections accounted for 24% of the total costs of all acquired foodborne illnesses in the U.S. from 15 leading pathogens (Hoffman et al., 2015).

Nontyphoidal *Salmonella* is transmitted predominantly by commercially-produced food contaminated by animal feces, such as meat, eggs, poultry products and fresh produce (Batz et al., 2012; Braden, 2006; Hanning et al., 2009; Painter et al., 2013; Patrick et al., 2004). Transmission to humans has also occurred through contact with animals, particularly reptiles, and contact with animal environments (Hoelzer et al., 2011). Recently, waterborne transmission of *Salmonella* to humans has also been demonstrated (Ashbolt, 2004; Leclerc, Schwartzbrod, & Dei-Cas, 2002). *Salmonella* spp. can enter the aquatic environment directly with feces of infected humans or animals or indirectly, such as through untreated sewage discharge or agricultural runoff (Levantesi et al., 2012). *Salmonella* has also been detected in different types of natural aquatic environments such as rivers, lakes, coastal waters, and in contaminated ground water (Haley et al., 2009; Martinez-Urtaza, Liebana, Garcia-Migura, Perez-Piñeiro, & Saco, 2004; Levantesi et al., 2010; Wilkes et al., 2009). Moreover, *Salmonella* has been demonstrated to remain viable for longer than many other enteric bacteria in freshwaters (Chao, Ding, & Chen, 1987), thereby increasing the probability of environmental exposure to humans.

Municipal drinking water and untreated spring water have also been associated with salmonellosis outbreaks in the U.S. (Berg, 2008; Farooqui, Khan, & Kazmi, 2009; Kozlica, Claudet, Solomon, Dunn, & Carpenter, 2010). In 2008, an untreated supply of spring water stored in a small unprotected reservoir was
recognized as the source of *Salmonella* infection in a rural community in Tennessee (Kozlica et al., 2010). An outbreak of salmonellosis was reported in Colorado when *Salmonella* in animal feces contaminated a storage reservoir in the public water system that supplies drinking water to the city of Alamosa (Berg, 2008). The outbreak resulted in 442 reported illnesses, 122 of which were laboratory-confirmed, and one death (Berg, 2008). Contaminated drinking water sourced from community wells also has been implicated in outbreaks of salmonellosis. Farooqui et al. (2009) reported that a community outbreak of typhoid fever in a Pakistani village was associated with a drinking water well contaminated with a multidrug resistant strain of *Salmonella enterica* serotype Typhi, claiming three lives and infecting more than 300 people (Farooqui et al., 2009).

Private well water remains the sole source of drinking water for approximately 14% of the U.S. population (Maupin et al., 2014). While the quality of municipal drinking water systems is protected under the Safe Drinking Water Act, homeowners with private wells are responsible for ensuring the safety of their own drinking water (US EPA, 1974). In Maryland, approximately 19% of the population (over 1 million people) relies on private wells for home consumption (Maupin et al., 2014), which includes water for drinking, cooking, bathing, washing, toilet flushing and other needs. Although *Salmonella* infections have been previously associated with consumption of contaminated and untreated water, very few studies have investigated private domestic well water sources as risk factors for salmonellosis.

A Turkish study of 40 groundwater samples from private wells detected *Salmonella* in 15% of the samples tested (Özler & Aydın, 2008). In Nigeria,
Salmonella serotype Typhi and Paratyphi were detected in private well water, and microbial water quality was found to be positively correlated with the waterborne diseases detected within the study area (Oguntoke, Aboderin, & Bankole, 2009). In a Taiwanese study, Li et al. (2009) determined that the consumption of groundwater from private wells was an independent risk factor for a number of confirmed cases of infection with Salmonella choleraesuis (Li et al., 2009). In the U.S., a matched case-control study of almost 300 children in Washington state found that infection with Salmonella was associated with the use of private wells as sources of drinking water (Odds Ratio [OR]= 6.5; 95% CI =1.4, 29.7), and with the use of residential septic systems (OR=3.2; 95% CI= 1.3, 7.8) (Denno et al., 2009).

Recent studies have also shown that agricultural and community-level socioeconomic factors can impact rates of salmonellosis. Shaw et al. (2016) determined that multiple agricultural factors were associated with salmonellosis incidence rates, and these relationships varied by state (Shaw et al., 2016). For example, the presence of broiler chicken operations, dairy operations and cattle operations in a zip code was associated with significantly higher rates of infection with at least one serotype in states that are leading producers of these animal products. In Georgia, Maryland, and Tennessee, all of which are leading broiler chicken producing states, rates of Salmonella infection were 48%, 58% and 46% higher respectively in zip codes with broiler chicken operations compared to those without these operations (Shaw et al., 2016). These same states also saw higher rates of Salmonella infections in zip codes characterized by greater rurality (Shaw et al., 2016).
Given that a significant proportion of the Maryland population relies on groundwater from private wells, and that *Salmonella* is known to persist in many different water sources, we hypothesized that increased exposure to contaminated groundwater from private wells may influence the risk of salmonellosis in Maryland. We utilized an ecological approach to investigate the association between the prevalence of wells and salmonellosis incidence at the zip code level using surveillance data on *Salmonella* infection from 2007 to 2016. Since previous studies have indicated that coastal communities face a higher risk of *Salmonella* infection (Jiang et al., 2015; Simental & Martinez-Urtaza, 2008), we examined the relationship between the prevalence of wells and salmonellosis incidence in coastal and non-coastal areas in Maryland.

2. Methods

2.1 Salmonella case data

We obtained *Salmonella* case data between 2007-2016 from the Maryland Foodborne Diseases Active Surveillance Network (FoodNet). The Maryland FoodNet is one of 10 sites that participate in the Centers for Disease Control and Prevention (CDC) FoodNet program. The FoodNet program conducts active, population-based surveillance for infections caused by nine bacterial and parasitic pathogens transmitted commonly through food, including *Salmonella* (CDC, n.d.⁹; Henao et al., 2015). We restricted our analyses to culture-confirmed cases of *Salmonella* infection that occurred in Maryland between 2007-2016. A salmonellosis case was an individual whose biological specimen (stool, blood, or other) was culture-confirmed for the presence of *Salmonella*, regardless of symptoms or date of onset. Both
sporadic cases and those associated with outbreaks were included. For each salmonellosis case, we also obtained limited demographic data (e.g., age, gender, race/ethnicity) and each case’s zip code at the time of diagnosis.

2.2 Socioeconomic Data

We obtained population data from the 2010 U.S. Census of Population and Housing by 5-digit zip code tabulation area (ZCTA) (U.S. Census Bureau, n.d.a). We also obtained socioeconomic variables from the 2010-2016 American Community Survey (5-year estimates) by 5-digit ZCTA using the American Fact Finder Service (U. S. Census Bureau, 2014). We selected socioeconomic variables based on previous research (Shaw et al., 2016; N. Krieger et al., 1997; Zappe Pasturel et al., 2013), including % of the population that identifies as African American, % of the population that identifies as Hispanic, % of houses occupied by their owners, % of the population living below the poverty level, % of the population that is ≥25 years without high a school diploma, and % of the population that is unemployed. In order to calculate well prevalence by zip code, data on the number of houses per zip code in Maryland was also obtained from the 2012-2016 American Community Survey (5-year estimates) via the American Fact Finder Service (U. S. Census Bureau, 2014).

2.3 Animal Feeding Operations Data

We obtained animal feeding operation data in Maryland from the 2007 U.S. Census of Agriculture, National Agricultural Statistics Service (NASS) (USDA, n.d.). Specifically, we obtained data on the number of animal operations with sales by zip
code for broiler chickens, turkey, aquaculture, sheep or goats, hogs, and dairy or beef cattle.

2.4 Private Well Data

The Maryland homeowner well permits registry was obtained from the Maryland Department of the Environment (MDE). The registry consisted of a tab-delimited file of 446,781 residential wells, some of which had an associated latitude and longitude coordinate (362,075 wells). The only locational information provided for the other permit records were the names of the town and street nearest to the property that contained the well (84,706 wells). Zip codes were retrieved for records with valid latitude and longitude coordinates or valid Maryland town and street addresses using Google’s Geocoding application programming interface (API) (Google Developers, 2018). Custom parsers were written to query the API for each record’s zip code using either the latitude and longitude coordinates or the town and street name. Only wells with both a valid town and street name were queried to ensure accuracy (19,621 wells). The zip codes for each record were verified to be among the 619 registered Maryland zip codes. After completing the query process and removing incorrect records, valid zip codes were obtained for a total of 374,162 private wells in Maryland.

2.5 Maryland Coastal and Non-coastal Zones

Maryland is located in the Mid-Atlantic region of the continental United States and is comprised of 24 counties (including Baltimore City) that are divided
into two distinct zones by the Chesapeake Bay. The counties in the Atlantic coastal plain lie to the east of the Bay where some border the Atlantic Ocean (known as the Eastern Shore), and counties to the west of the Bay comprise the Appalachian and Piedmont areas of the state (MDNR, n.d.; Maryland Geological Survey, n.d.). We used definitions from the Maryland Department of Natural Resources to classify the 24 counties as being a part of the coastal or non-coastal zone as shown in Figure 1 (MDNR), n.d.). The Maryland coastal zone extends from three miles out in the Atlantic Ocean to the inland boundaries of the 16 counties and Baltimore City that border the Atlantic Ocean, Chesapeake Bay and the Potomac River up to the District of Columbia. This zone encompasses two-thirds of the state’s land area and is home to almost 70% of Maryland’s residents (MDNR, n.d.). The remaining 7 counties are classified as the non-coastal zone.

2.6 Descriptive Analyses

We calculated Salmonella incidence rates per 100,000 population by year for the State of Maryland using population estimates from the 2010 U.S. Census (U.S. Census Bureau, n.d.a). Cases that were potentially associated with international travel (described in detail below) were then excluded from further descriptive and inferential analysis. We then determined a prevalence rate of wells per zip code (the well prevalence variable) using the Maryland homeowner well permits registry and data on the number of houses per zip code from the 2012-2016 ACS via the American Fact Finder Service (U.S. Census Bureau, 2014). Well prevalence per zip code was calculated by dividing the number of wells per zip code by the number of houses per
zip code. *Salmonella* case count data (excluding cases associated with international travel) were then linked with the socioeconomic, animal feeding operation, and well prevalence data by zip code and 5-digit ZCTA. A choropleth map of salmonellosis incidence rates by zip code was created to illustrate the distribution of salmonellosis across the state. We performed all mapping using ArcGIS version 10.3 (ESRI, 2016) (ESRI, Redlands, CA).

2.7 Statistical Models

We developed regression models to evaluate associations between well prevalence, socioeconomic and agricultural factors, and salmonellosis incidence at the zip code level. First, any salmonellosis cases for which zip codes were missing or incorrect were excluded from the analysis, along with those cases associated with international travel. We then evaluated collinearity among all independent variables of well prevalence, SES factors, and agricultural factors using the variance inflation factor. We excluded highly collinear variables. Following evaluation of collinearity, we also determined the Pearson correlation coefficients among all independent variables to avoid using highly correlated variables in the multivariate regression model.

Regression models typically employed for count data were compared and we also tested models with and without zero inflation. A negative binomial regression model provided the best fit for the dataset. The final model included well prevalence and the zip code level independent variables described in Table 2. We ran a statewide regression model, a univariate regression model between well prevalence and
incidence of salmonellosis stratified by county, and univariate and multivariate regression models by coastal/non-coastal zone. Only the well prevalence variable and the independent variables that were significantly associated with salmonellosis incidence at the zip code level for each zone by univariate analysis were included in the multivariate regression models of that zone. We performed all modeling using SAS version 9.4 (SAS Institute, Cary, NC) (SAS Institute, 2014), and used p-values of ≤ 0.05 to assess statistical significance. We used R version 1.0.153 (Vienna, Austria) (R Core Team, 2017) to create bar charts (Figure 2 and 4) and whisker plots of the multivariate analyses (Figure 5).

3. Results

3.1 Maryland Salmonellosis cases, 2007-2016

A total of 8,926 cases of culture-confirmed Salmonella infections were reported to the FoodNet active surveillance system in Maryland between 2007-2016. A total of 8,850 cases (99.1%) had valid zip codes. The majority of cases were between the ages of 20-59 (42.23%), White (50.47%), and Non-Hispanic (74.54%) (Table 1). Most cases were sporadic infections (81.36%), while 4.95% of cases were associated with outbreaks. Over 200 unique Salmonella serotypes were identified among all cases of infection. Approximately 21.28% were identified as Enteritidis, 6.25% as Typhimurium, 5.53% as Newport, and 3.57% as Javiana. An additional 23.68% were other unique serotypes, and 34.43% were unknown serotypes.
The average annual incidence rates of salmonellosis in Maryland between 2007-2016 are illustrated in Figure 2. The lowest annual incidence rate for this 10-year period of 13.19 per 100,000 people was recorded in 2009, and the highest annual incidence rate of 17.64 per 100,000 people was recorded in 2010 (Figure 2). The choropleth map (Figure 3) indicates the spatial distribution of salmonellosis by zip code in Maryland. *Salmonella* infection rates are highest within the coastal zone of Maryland, particularly along the Eastern Shore. The Eastern Shore is characterized by the presence of high numbers of broiler chicken operations that may play a role in the relationship between salmonellosis incidence rates and the risk factors investigated in this study.

3.2 Salmonellosis Incidence Rate by Zip Code

We removed all cases that reported undertaking international travel within 7 days prior to experiencing symptoms of gastroenteritis, which amounted to 1,550 cases (17.5%). These cases were removed since travel outside of the United States was found to be a significant risk factor for salmonellosis in previous studies (Johnson et al., 2011; Tighe et al., 2012). The final dataset used in regression analyses was therefore composed of 7,300 salmonellosis cases. Using these counts, we then calculated salmonellosis by zip code (Figure 3) and used these values as the outcome of our multivariate models (described below).
3.3 Maryland Private Wells

The Maryland county with the most wells was Anne Arundel county with 53,192 wells, while the county with the least number of wells was Baltimore City with 153 wells (Figure 4). However, well prevalence (the number of wells per number of houses in each county) was highest in Somerset county (0.967) and lowest in Baltimore City (0.0006) (Figure 4). Most wells in the state are located within the coastal zone, a region characterized by limestone, sandstone and shale (Reger & Cleaves, 2008; Vokes, 1957).

3.4 Statewide Multivariate Analysis

Overall, we observed a significant positive association between well prevalence and increased salmonellosis incidence at the zip code level (Incidence Rate Ratio [IRR]=1.62, 95% Confidence Interval [CI] = 1.35, 1.93) (Table 2). Agricultural and socioeconomic factors were also found to influence salmonellosis incidence at the zip code level. In zip codes that contain broiler chicken operations, the incidence rate of salmonellosis was 1.21 times that in zip codes that do not contain broiler chicken operations (IRR=1.21, 95% CI = 1.06, 1.38). In zip codes characterized by the presence of cattle operations, the incidence rate of salmonellosis was lower than that of zip codes that do not contain cattle operations (IRR= 0.84; 95%CI = 0.74, 0.95). Salmonellosis incidence was also higher in zip codes characterized by higher percentages of the population living below the poverty level (IRR= 1.03; 95%CI = 1.02, 1.04).
3.5 Stratified Analysis

Given that well prevalence was a significant predictor of salmonellosis incidence at the zip code level in the statewide model, we decided to examine the relationship by geographic region in Maryland, specifically in coastal vs. non-coastal counties. Additionally, a previous study indicated that coastal communities in Maryland face a higher risk of *Salmonella* infection due to extreme temperature and precipitation events (Jiang et al., 2015). Following a univariate analysis of well prevalence and other independent variables and incidence of salmonellosis stratified by coastal/non-coastal zone, we observed that well prevalence was a significant predictor of an increased incidence of salmonellosis in the coastal zone only (Table A3, Appendix 4). We then built a multivariate model for only the coastal zone to investigate the association between well prevalence and salmonellosis incidence, controlling for other significant factors (Table A3, Appendix 4).

In the coastal counties, we observed that well prevalence was significantly associated with an increase in salmonellosis incidence after controlling for agricultural and socioeconomic variables (IRR=1.61, 95% CI=1.31, 1.99) (Figure 5). Agricultural and socioeconomic factors at the zip code level were also associated with salmonellosis incidence in coastal counties (Figure 5). For instance, salmonellosis incidence was higher in zip codes that contained broiler chicken operations (IRR=1.58, 95% CI=1.36, 1.82). Zip codes characterized by a higher percentage of the population living below the poverty line also had increased incidence rates of salmonellosis (IRR=1.02; 95% CI=1.01, 1.04).
4. Discussion

Our findings suggest that the prevalence of private drinking water wells within a zip code is associated with an increased risk of salmonellosis in the coastal counties of Maryland (IRR=1.61, 95% CI=1.31, 1.99) (Figure 5). Our data also confirm that other environmental factors, including proximity to large-scale broiler chicken facilities, can also influence salmonellosis incidence. To our knowledge, these are among the first U.S. data to use an ecologic study design to characterize the relationship between private drinking water wells and the risk of salmonellosis, an illness that is typically viewed as foodborne.

From 2007-2016, a total of 75,304 cases of confirmed salmonellosis were reported to the CDC FoodNet program across all ten participating sites (CDC, 2018a). In comparison with other sites during the same ten-year period, the overall incidence rate of salmonellosis in Maryland is the fourth highest (15.3 per 100,000 population), followed by California (15.77 per 100,000 population) and New Mexico (17.44 per 100,000 population). The site with the highest rate of Salmonella infection during this period was Georgia (24.06 per 100,000 population).

The association between well prevalence and the risk of salmonellosis was observed in our statewide multivariate model and in the multivariate model for the coastal zone of Maryland (Figure 5). However, well prevalence was not found to be significantly associated with salmonellosis incidence in the non-coastal zone by univariate analysis (Table A3, Appendix 4), and as such a multivariate model for this zone was not performed. In addition to well prevalence, the presence of broiler chicken farms in a zip code was also significantly associated with an increase in the
risk of salmonellosis in the statewide model (IRR=1.21, 95% CI = 1.06, 1.38), and in the coastal counties of Maryland (IRR=1.58, 95% CI =1.36, 1.84). The Eastern Shore of Maryland, which is located within the coastal zone, produces close to 300 million broiler chickens annually (USDA, 2016). Broiler chicken operations produce an estimated 5.5 tons (446 cubic feet) of waste per 1,000 birds (Carr et al., 1990), totaling over 1.6 million tons of waste produced by broiler operations in Maryland. This waste is typically applied to land and other agricultural fields, leading to potential contamination of nearby water supplies (Burkholder et al., 2007b). You et al. (2006) demonstrated that *Salmonella* can persist for up to 405 days in soil after manure is applied to a field, thereby posing a risk of contamination of groundwater (You et al., 2006).

MDE allows animal feeding operations within the state to discharge into surface waters of the state following the issuance of a permit (MDE, n.d.). There are 610 broiler chicken farms (non-laying hens) within the state and an additional 5 farms that consist of laying hens, all of which have a permit to discharge wastewater into waters of the state (MDE, n.d.). Permits are only required of farms which have 37,500 chickens or greater, meaning that broiler operations with less chickens exist in the state without permits, making them difficult to quantify and their location difficult to assess (MDE, n.d.). Additionally, the USDA does not disclose the number of animal feeding operations with sales at the state and/or county level so as not to identify individual farms within an area, further complicating the ability to assess the number of animal feeding operations and the number of animals they contain (The Pew Environment Group, 2011). All of the permitted broiler chicken farms and four
of the farms with laying hens in the state of Maryland are located within the coastal counties (MDE, n.d.). Given that animal feeding operations have been previously implicated in contamination of surface water and groundwater with *Salmonella* (Haley et al., 2009; Jenkins, Endale, Schomberg, & Sharpe, 2006; Maurer et al., 2015), broiler facilities could play a role in the relationship between well prevalence and salmonellosis risk observed in this study.

Our multivariate regression model also provided evidence that socioeconomic factors are associated with an increase in salmonellosis incidence. We found that as the percentage of people living below the poverty line in a zip code increased, so did the risk of salmonellosis (IRR= 1.04; 95% CI=1.02, 1.05). A previous study using national FoodNet data from 2004-2010 also found that higher poverty levels in zip codes were associated with higher rates of *Salmonella* infection in Maryland, New Mexico and Tennessee (Shaw et al., 2016). Other research previously identified a positive association between poverty levels and salmonellosis incidence rates in a nationwide county-level study using data from the National Notifiable Diseases Surveillance System (Chang et al., 2009). However, this study also found that another variable commonly used to assess poverty, the percentage of the adult population that is unemployed, was negatively associated with salmonellosis incidence rates at the county level.

Our study found that the percentage of unemployed individuals within a zip code was positively associated with the risk of salmonellosis; however, it was not significant (IRR=1.010, 95% CI=0.99, 1.03). A recent Danish study found that that the risk of *Salmonella* infection was not primarily associated with poverty, but rather
with increasing socioeconomic status (Simonsen, Frisch, & Ethelberg, 2008b). Other studies have found similar contrasting results regarding the associations between indicators of socioeconomic status and incidence of enteric diseases (Newman, Leon, Rebollo, & Scallan, 2015; Rosenberg Goldstein et al., 2016; Zappe Pasturel et al., 2013).

There are notable strengths of our study. We used a decade of illness surveillance data from the Maryland FoodNet program to conduct our study, ensuring that we had a large number of salmonellosis cases from which to draw our conclusions. In addition, FoodNet has the advantage of being an active surveillance network, thereby avoiding some of the inconsistencies and heavy underreporting that can characterize passive national surveillance systems.

A limitation of our study is that it was performed on an ecological scale using community-level socioeconomic data at the zip code level rather than individual-level data of the cases. As such our findings cannot be used to infer associations between private wells and salmonellosis at the individual level. Performing the analysis with salmonellosis case data at the zip code level required us to pair these data with data from the U.S. Census and the American Community Survey by ZCTAs. However, zip codes and ZCTAs do not always correlate, resulting in some zip codes for which census data are unavailable. Grubesic and Matisziw (2006) also highlight the discrepancies in matching ZCTA and zip code level data, indicating that using ZCTAs to link geographic data is convenient but can result in relatively large geographic zones with linkages that can lead to imprecise estimates (Grubesic & Matisziw, 2006).
An additional limitation is that while the FoodNet active surveillance system provides reliable data on the cases that are entered into the system, it underestimates disease burden and typically represents a fraction of the total community cases (Majowicz et al., 2010; Mead et al., 1999). Underreporting of foodborne illnesses leading to underestimation of disease burden is also recognized as a problem of laboratory-based illness surveillance systems in other countries (de Wit et al., 2001; Flint et al., 2005; J. G. Wheeler et al., 1999). Finally, the Census of Agriculture data were only available at the zip code level for the 2007 Census, and not for subsequent years. It is possible that additional animal feeding operations could have been established in Maryland since 2007, rendering the census data used in this study an underestimate of the true number of operations (USDA, n.d.).

Nevertheless, this is the first study to use an ecologic study design to investigate the role of private wells on the risk of salmonellosis in the United States, and it provides a rationale for continuing to evaluate wells as a risk factor for gastrointestinal diseases. Future research in this area could include sampling of private well water for the detection of Salmonella, and employing techniques such as Microbial Source Tracking (MST) to determine the likely sources of Salmonella contamination, whether human or animal. MST has been previously used in similar studies to characterize the magnitude and incidence of microbial contamination in private wells, and to identify the likely sources of this contamination (Allevi et al., 2013). Aquifer type, whether confined or unconfined, can contribute to the quality of private well water since the potential for contamination changes with each type. Confined aquifers exist within layers of impermeable rock, while unconfined aquifers
are located closer to the earth’s surface than confined aquifers, and as such are
impacted by external factors and contamination sources much more than confined
aquifers (Heath, 1983; Waller, 1988). A spatial analysis of salmonellosis incidence
that incorporates aquifer type could illustrate the effect of drilling a well in an
unconfined vs. confined aquifer on this illness.

5. Conclusions

We observed an increased risk of salmonellosis associated with increasing
well prevalence in coastal counties of Maryland. Other risk factors, such as the
presence of broiler chicken operations and the percentage of the population living
below the poverty level were also found to be significantly associated with
salmonellosis incidence in the coastal zone of Maryland. Our results add to the
growing body of international research that has identified drinking water as a
potential risk factor for Salmonella infection. Our findings can provide evidence for
the strengthening of private well water regulations and for improving education and
outreach to private well owners on proper maintenance and testing for their wells.

6. Acknowledgements

The authors would like to acknowledge the Maryland Department of the Environment
for providing information on permitted wells in the state. We would also like to
acknowledge Michelle Boyle of the Emerging Infections Program at the Maryland
Department of Health for compiling the foodborne diseases surveillance data used in
this analysis. Additionally, we would like to acknowledge Jessica Chopyk for
assistance with creating some of the figures in the manuscript.
7. References


Samuel, M. C., Vugia, D. J., Shallow, S., Marcus, R., Segler, S., McGivern, T., … Group, for the E. I. P. F. W. (2004). Epidemiology of Sporadic Campylobacter Infection in the United States and Declining Trend in


### 8. Tables

**Table 1.** Demographic characteristics of salmonellosis cases reported to the Maryland FoodNet program, 2007-2016

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td></td>
</tr>
<tr>
<td>0-4</td>
<td>1939 (21.91)</td>
</tr>
<tr>
<td>5-9</td>
<td>714 (8.07)</td>
</tr>
<tr>
<td>10-19</td>
<td>928 (10.49)</td>
</tr>
<tr>
<td>20-59</td>
<td>3737 (42.23)</td>
</tr>
<tr>
<td>≥ 60</td>
<td>1468 (16.59)</td>
</tr>
<tr>
<td>Unknown</td>
<td>64 (0.72)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>2579 (29.14)</td>
</tr>
<tr>
<td>White</td>
<td>4467 (50.47)</td>
</tr>
<tr>
<td>Other Race</td>
<td>1804 (20.38)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>619 (6.99)</td>
</tr>
<tr>
<td>Non-Hispanic</td>
<td>6597 (74.54)</td>
</tr>
<tr>
<td>Unknown or Missing</td>
<td>1634 (18.46)</td>
</tr>
<tr>
<td><strong>International Travel</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1550 (17.51)</td>
</tr>
<tr>
<td>No</td>
<td>6123 (69.19)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1550 (17.51)</td>
</tr>
<tr>
<td><strong>Disease type</strong></td>
<td></td>
</tr>
<tr>
<td>Sporadic</td>
<td>7200 (81.36)</td>
</tr>
<tr>
<td>Outbreak</td>
<td>438 (4.95)</td>
</tr>
<tr>
<td>Unknown or missing</td>
<td>1212 (13.69)</td>
</tr>
</tbody>
</table>
Table 2. Incidence rate ratios (IRR) and 95% confidence interval (CI) for salmonellosis in Maryland (2007–2016)

<table>
<thead>
<tr>
<th>Variables</th>
<th>IRR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IRR 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well Prevalence</td>
<td>1.62</td>
<td>(1.35, 1.93)</td>
</tr>
<tr>
<td>Cattle operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0.84 (0.74, 0.95)</td>
<td></td>
</tr>
<tr>
<td>Broiler chicken operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>1.47 (1.29, 1.66)</td>
<td></td>
</tr>
<tr>
<td>Hog operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0.94 (0.73, 1.22)</td>
<td></td>
</tr>
<tr>
<td>Turkey Operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>0.88 (0.75, 1.03)</td>
<td></td>
</tr>
<tr>
<td>Median age, years</td>
<td>1.01</td>
<td>(0.99, 1.01)</td>
</tr>
<tr>
<td>% African American</td>
<td>1.00</td>
<td>(0.99, 1.00)</td>
</tr>
<tr>
<td>% Hispanic</td>
<td>0.99</td>
<td>(0.99, 1.01)</td>
</tr>
<tr>
<td>% Owner occupancy</td>
<td>0.99</td>
<td>(0.99, 1.00)</td>
</tr>
<tr>
<td>% Residents below poverty</td>
<td>1.03</td>
<td>(1.02, 1.04)</td>
</tr>
<tr>
<td>% Population ≥25 years</td>
<td>1.00</td>
<td>(0.99, 1.01)</td>
</tr>
<tr>
<td>without high school diploma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Unemployment</td>
<td>1.01</td>
<td>(1.00, 1.03)</td>
</tr>
</tbody>
</table>

Note: IRR = incident rate ratio; CI = confidence interval
<sup>a</sup> - Adjusted after controlling for other independent variables in the model.
9. Figures

Figure 1. Map of Maryland indicating Coastal and Non-coastal counties
Figure 2. Incidence rates of salmonellosis per 100,000 people in MD, 2007-2016
Figure 3. Average annual salmonellosis incidence rates in MD by zip code, 2007-2016
Figure 4. Number of Wells (panel A) and Well Prevalence (panel B) by County and Zone in Maryland.
**Figure 5.** Salmonellosis incidence rate ratios (IRRs) and 95% confidence intervals (CIs) in the coastal zone of Maryland, by socioeconomic variables, agricultural variables, and well prevalence
Chapter 6: Conclusions, Strengths and Limitations, Public Health Implications and Directions for Future Research

1. Conclusions

Groundwater continues to be an important source of water in many areas of the U.S. The USGS estimates that 82.3 billion gallons of fresh groundwater are withdrawn in the U.S. per day, an increase of 8% compared to 2010 (Dieter et al., 2018). Of this total, approximately 3.2 billion gallons per day are withdrawn by homeowners with private domestic wells (Dieter et al., 2018). Approximately 44.5 million people (14% of the U.S. population) rely on groundwater from domestic wells as their sole source of water for drinking and other household needs, such as cooking, bathing, cleaning, and flushing toilets (Maupin et al., 2014).

The majority of Americans consume water that has been treated by a water treatment facility to US EPA standards and is then piped to their homes. This water is closely regulated by the Safe Drinking Water Act (SDWA), the federal regulation that ensures the safety of water for public consumption and its sources, including rivers, lakes, reservoirs, springs, and groundwater aquifers (US EPA, 1974). However, homeowners who rely on private wells are not required to ensure that their water meets the requirements of the SDWA (US EPA, 1974), and many of these homeowners do not regularly monitor their wells for contaminants that may be harmful to human health (Knobeloch, 2010; Knobeloch, Gorski, Christenson, & Anderson, 2013). Therefore, individuals who consume water from private wells may face adverse health risks associated with well water quality due to the presence of
contaminants in their water at levels that exceed those set forth in the SDWA. Given the large proportion of the U.S. population that relies on private wells and the high volume of groundwater withdrawn for domestic usage, understanding the risks associated with groundwater use and consumption is imperative in protecting public health. Therefore, the overall goal of this dissertation was to evaluate the quality of private wells, and to examine the public health risks associated with private well water in Maryland.

Approximately 19% of the Maryland population relies on private wells (Maupin et al., 2014); however, research into the quality of wells in the state has been limited. The first study of my dissertation sought to bridge this gap by investigating well water samples in four counties of Maryland for microbiological and chemical contaminants that could have implications for human health. The second and third papers evaluated private wells as a risk factor for campylobacteriosis and salmonellosis respectively, both of which are leading causes of gastroenteritis in Maryland and across the globe. Campylobacteriosis and salmonellosis are also both considered to be foodborne illnesses, since their transmission is typically associated with consumption of food. However, this research aimed to assess the role of private wells as a non-foodborne transmission source of these illnesses in an effort to improve current knowledge on their risk factors.

My research demonstrated that there are private wells in Maryland that contain microbiological and chemical contaminants at levels that exceed those deemed acceptable for protection of human health by the SDWA. Overall, 43.2% of wells tested in my first study did not meet at least one EPA health-based drinking
water standard. Total coliform bacteria were the most common (25.4%) microbiological contaminant detected in the wells sampled, while fecal coliforms (15.3%), *Enterococcus* spp. (5.1%), and *E. coli* (3.4%) were also detected. Approximately 26% of tested wells did not meet the recommended drinking water standard for pH, and most of wells had a pH below the lower limit of 6.5. Nitrate occurred above the 10 mg/L drinking water standard in 3.4% of tested wells, and less than 1% of wells exceeded the recommended limit for total dissolved solids (TDS) of 500 mg/L. Nitrates in drinking water are particularly harmful to infants, and can lead to a potentially fatal condition known as methemoglobinemia (Knobeloch, Salna, Hogan, Postle, & Anderson, 2000).

None of the wells exceeded the EPA maximum contaminant level (MCL) for arsenic (10 mg/L) or sulfate (250 mg/L). Consumption of arsenic in drinking water can lead to acute effects such as nausea, vomiting and fatigue, while chronic arsenic exposure is also associated with an increased risk of skin, bladder, and lung cancer (Kumar, Adak, Gurian, & Lockwood, 2010). Although there were individual wells in each county that exceeded the EPA MCLs for some of the chemical water quality parameters investigated, the mean levels in each county were within EPA specifications. My findings were consistent with studies of private well water quality in other states such as Pennsylvania, Virginia, Wisconsin and North Carolina, where wells sampled were found to exceed one or more federal health-based water quality standards (Knobeloch et al., 2013; MacDonald Gibson & Pieper, 2017; Pieper, Krometis, Gallagher, Benham, & Edwards, 2015; Swistock, Clemens, Sharpe, & Rummel, 2013).
I also investigated whether the presence of animal feeding operations within a zip code influenced the water quality of wells in that zip code. Multivariate logistic regression analysis did not detect any statistically significant associations between the presence of six different types animal feeding operations (broiler chickens, hogs, dairy and beef cattle, turkey and aquaculture) within a zip code and the occurrence of microbial contamination in wells tested within the same zip code. However, given that previous studies have demonstrated an association between animal farms and contamination of private wells (Clark et al., 2003; Gallay et al., 2006; Li et al., 2015; Licence, Oates, Synge, & Reid, 2001), it is possible that I did not observe a similar relationship due to the small sample size of my study (n=118 private wells) and the ecological design of the study at the zip code level.

My second study evaluated private wells as a risk factor for campylobacteriosis across the different physiographic regions in Maryland. This relationship was investigated using the independent variable well prevalence, which was calculated by dividing the number of wells in a zip code by the number of houses in that zip code. Multivariate logistic regression models were used to evaluate the association between well prevalence and campylobacteriosis incidence at the zip code level, while controlling for several agricultural and socioeconomic variables. Well prevalence was significantly associated with campylobacteriosis incidence in the Appalachian and Coastal provinces of the state, indicating that private wells are an important risk factor for Campylobacter infection in these regions. The presence of broiler chicken farms in zip codes of the Coastal province was also significantly associated with campylobacteriosis incidence. This finding may be attributed to the
high density of broiler chicken farms in the coastal province of Maryland since broiler chickens are frequently colonized with *Campylobacter* (Beery, Hugdahl, & Doyle, 1988; Hardy, Lackey, Cannon, Price, & Silbergeld, 2011; Harris, Weiss, & Nolan, 1986), and chicken waste is often applied to nearby agricultural land as a form of waste disposal (Burkholder et al., 2007).

My third study investigated the association between well prevalence and another illness typically classified as foodborne, salmonellosis. This relationship was also investigated using an ecological study at the zip code level; however, in this instance the relationship was investigated by coastal and non-coastal zone in Maryland instead of by physiographic province. The rationale for this difference in classifying the regions of the state was due to the results of the univariate analysis of well prevalence and the incidence of each GI illness. In manuscript 2, univariate analysis of well prevalence and the incidence of campylobacteriosis by county revealed a statistically significant relationship in nine counties in Maryland (Table A1, Appendix 2). In this stratified analysis by county, seven of the counties where a significant relationship between well prevalence and campylobacteriosis incidence occurred were located within the Coastal province of the state; however, one of the counties was located within the Piedmont province and the other was located within the Appalachian province (Manuscript 2, Figure 4 and Figure A1, Appendix 1).

Further analyses of relationships between well prevalence and campylobacteriosis incidence were therefore performed by physiographic province in Maryland. However, in manuscript 3, a univariate analysis of well prevalence and incidence of salmonellosis resulted in a statistically significant relationship in 4 counties, all of
which are located within the coastal province of the state. It was therefore preferable to conduct additional analyses between well prevalence and incidence of salmonellosis by coastal and non-coastal zone rather than by physiographic province.

The findings manuscript 3 were similar to those of manuscript 2 – well prevalence is significantly associated with an increase in the incidence of salmonellosis at the zip code level in coastal counties of Maryland. As with campylobacteriosis, the presence of broiler chicken farms in a zip code was also significantly associated with an increase in the risk of salmonellosis in the coastal counties of Maryland. Private water wells and broiler chicken farms are therefore a risk factor for both campylobacteriosis and salmonellosis in the coastal counties of Maryland.

2. Strengths and Limitations

2.1 Strengths

There are several notable strengths to the studies conducted in this dissertation. Across the U.S., there is a dearth of research into private wells, despite the large proportion of the population that relies on wells as a water source. Manuscript 1 is the first study to assess the water quality of private wells across multiple counties in Maryland, and to investigate the influence of animal feeding operations on well water quality, thereby addressing an important research gap in the state. This study also highlights the need for more educational outreach to private well owners in Maryland in order to improve private drinking water quality in the state. This study can also form the basis for other states to undertake similar assessments of their private well water quality.
Another strength of this research is that manuscripts 2 and 3 are the first studies to investigate private wells as a risk factor for gastrointestinal illnesses in Maryland. These studies utilized a decade of illness surveillance data from the Maryland FoodNet program, ensuring a large number of campylobacteriosis and salmonellosis cases from which to draw reliable conclusions. In addition, FoodNet has the advantage of being an active surveillance network, thereby avoiding some of the inconsistencies and underreporting that can characterize passive national surveillance systems.

2.2 Limitations

There are also limitations to this research. The first study was cross-sectional and as such limits certain evaluations of the data, such as whether seasonal trends influenced our results. Previous studies have indicated that well water quality may be affected by seasonality (Knobeloch et al., 2013; Richardson, Nichols, Lane, Lake, & Hunter, 2009). Our data showed that there were no statistically significant associations between the presence of an animal feeding operation within a zip code and microbial contamination of private wells within the same zip code; however, this may be due to the small number of well water samples obtained during this initial study. Exploration of this potential association deserves further study involving a larger number of private wells.

There are also limitations associated with the well permit registry obtained from MDE used in manuscripts 2 and 3. The registry consisted of 446,781 residential wells in Maryland, some of which had associated latitude and longitude coordinates.
(362,075 wells), and for some of which the only locational information provided were the names of the town and street nearest to the property that contained the well (84,706 wells). Zip codes were retrieved for records with valid latitude and longitude coordinates or valid Maryland town and street addresses using Google’s geocoding application programming interface (API) (Google Developers, 2018). Following the cleaning and extraction process, there were 374,162 wells in the database, meaning that 72,619 wells (16.25% of the original registry) were excluded from the dataset. Reasons for this exclusion were missing and/or incorrect latitude and longitude coordinates, missing either the nearest street or nearest town information, or retrieving a zip code from the Google API parsing process that was incorrect. The incorrect zip codes were often located in other nearby states, such as Pennsylvania and Virginia. The problems experienced while working with the well permit registry highlight the need for a comprehensive, accurate database of the locations of private wells in Maryland and across the U.S.

Use of the MDE well permit registry posed another limitation to this study. Without collecting individual-level water consumption data on cases in the FoodNet database, I used information from the well permit registry as a proxy for the water use and consumption habits of individual residents in Maryland zip codes in manuscripts 2 and 3. This proxy may not accurately reflect each case’s drinking water exposure since individuals may consume water in other locations such as work or school, drink bottled water, or have point-of-use treatment such as filters in their homes (Jones et al., 2006; Shimokura, Savitz, & Symanski, 1998). This likely led to some amount of exposure misclassification of cases, a problem that is typical in ecological studies. An
additional limitation of manuscripts 2 and 3 is that while the FoodNet active surveillance system provides reliable data on the cases that are entered into the system, the problem of underreporting means that the system underestimates disease burden and typically represents a fraction of the total GI illness cases within a community (Majowicz et al., 2010; Mead et al., 1999). Underreporting of foodborne illnesses leading to underestimation of disease burden is also recognized as a problem of laboratory-based illness surveillance systems in other countries (de Wit et al., 2001; Flint et al., 2005; Wheeler et al., 1999).

A limitation of all three studies is that they were conducted at the ecological level, with the exception of the laboratory analysis of individual private well water samples in the first study. All studies employed the use of aggregate agricultural data at the zip code level, and manuscripts 2 and 3 also used socioeconomic data at the zip code level rather than individual-level data of the campylobacteriosis and salmonellosis cases. As such these findings cannot be used to infer associations between private wells and gastroenteritis cases at the individual level. The availability of the FoodNet data and well permit registry data at the zip code level also limited the study to zip code being the smallest possible unit of analysis. However, a census block or census tract level analysis may have provided finer resolution and more precisely captured the impact of private drinking water wells. Additionally, having exact addresses of both the homes with private wells and the animal feeding operations in all three studies would have allowed for geocoded buffer analyses and spatial regression techniques.
Performing analyses with illness data at the zip code level required us to combine these data with data from the U.S. Census and the American Community Survey by zip code tabulation areas (ZCTAs). However, zip codes and ZCTAs do not always correlate, resulting in some zip codes for which census data are unavailable. This is the case in Maryland, where there are 619 zip codes but 468 ZCTAs (U.S. Census Bureau, 2014). Previous research has also highlighted the discrepancies in matching ZCTA and zip code level data, indicating that using ZCTAs to link geographic data is convenient but can result in relatively large geographic zones with linkages that can lead to imprecise estimates (Grubesic & Matisziw, 2006). Another limitation across all three studies is the use of U.S. Census of Agriculture data from 2007 together with results from well water samples that were collected between 2012-2014 (manuscript 1), and with foodborne illness surveillance data from 2007-2016 (manuscripts 2 and 3). However, the Census of Agriculture data were available at the zip code level for the 2007 Census but not for subsequent years, limiting these analyses to the use of agricultural data from 2007.

Despite these limitations, this work provides a good starting point for determining the influence of animal feeding operations on the quality of private well water, and for the evaluation of the role of private wells on the risk campylobacteriosis and salmonellosis in the United States. However, additional work is required that employs more complex analyses to enhance my present ecological studies, as described below.
3. Public Health Implications

Overall, my findings are that wells in Maryland do not meet federal guidelines for safe drinking water and that private wells are a risk factor for diseases that are commonly classified as foodborne. These results add to the growing body of U.S. research that finds water from some private wells potentially unsuitable for human consumption. These findings also add to both U.S. and international research that has identified drinking water as risk factor for *Campylobacter* and *Salmonella* infection. Gastrointestinal illness caused by both *Campylobacter* and *Salmonella* remains a significant health problem worldwide, and improved understanding of the role of drinking water in the dissemination of these illnesses should be an ongoing priority. My studies can provide evidence for the strengthening of private well water regulations and for improving the education and outreach to private well owners on adequate maintenance and regular testing schedules for their wells. My research can also be applied to assist in developing future private well water regulations and practices that are protective of public health.

In addition, this research suggests that outreach to homeowners with private wells should be improved, particularly regarding the frequency of well testing. Currently, the US EPA guidelines suggest that private wells should be tested annually for total coliform bacteria, nitrates, total dissolved solids, and pH levels (US EPA, 2015), which were the components analyzed in the first manuscript of this dissertation. However, that first study also indicated that homeowners are not adhering to these guidelines, with 24.6% of participants reporting that they had never tested their well water quality, and 49.2% participants reporting only testing their
water once. A 2013 study of private well owners in Wisconsin found residents in that state reporting similar rates of well testing (Knobeloch et al., 2013). Many homeowners do not test their wells since they have not experienced any problems with their well water quality in the past, they are confused about how or where to get their water tested, and for fear of negative consequences should they decide to sell their homes (Knobeloch, 2010; Knobeloch et al., 2013). Individual departments of health or departments of the environment within counties should emphasize the importance of private well testing to their constituents, with annual reminders to private well owners. County officials could also partner with water testing companies to offer discounted well testing services, thereby encouraging well owners to test their wells. Improved education on the remediation options for homeowners with contaminated wells and ensuring confidentiality of homeowners with such issues would also improve their desire to test their wells more frequently.

Additionally, since the results of these studies are specific to certain geographic areas of Maryland, outreach to homeowners and review of well water policies can be focused on the areas of the state where well prevalence was found to be a risk factor for campylobacteriosis and salmonellosis. Extension agents may also find this study useful for rural homeowner education with a goal of reducing gastrointestinal illness. These results are not only helpful in informing polices around private well water, but also around the broiler chicken farming. Though additional studies are needed to determine exactly how land-applied chicken waste could contaminate groundwater, this study provides an impetus for future research in that direction.
The FoodNet active surveillance system provides a wealth of data that has been used to monitor and analyze trends in foodborne illnesses for several years. However, the FoodNet database suffers from underreporting due to the number of GI illness cases that are not captured by its system (Mead et al., 1999; Scallan et al., 2011). In order to be included in the surveillance system, a potential case must seek medical care, submit a specimen (usually stool), the specimen must be tested by a laboratory for a pathogen, the lab must report a positive finding, and the laboratory-confirmed infection must be reported to public health authorities (Majowicz et al., 2010). Thus, the database of illnesses captured by FoodNet is not as robust as it could be. Education of the public on the existence of FoodNet and its importance could encourage potential GI illness cases to seek medical care and provide a specimen for laboratory testing, a problem which plagues illness surveillance systems globally (Majowicz et al., 2005). Surveillance data are a key information source for determining the epidemiology of GI illnesses, underscoring the need for the public to seek medical care when experiencing a GI illness.

This research also demonstrates the need for improved polices around the management of agricultural feeding operations, particularly CAFOs, within Maryland. Currently, wastewater discharges from farms are regulated under the National Pollutant Discharge Elimination System (NPDES). Animal feeding operations are required to apply for a NPDES permit if they have 37,500 or more broiler chickens (non-laying hens); 25,000 or more laying hens; 200 or more dairy cattle; 300 or more beef cattle; 750 or more swine weighing greater than 55 pounds each; or 3,000 or more swine weighing less than 55 pounds each (MDE, n.d.a).
According to MDE, there are 660 farms in Maryland which have a permit to discharge wastewater into waters of the state (MDE, n.d.), however according to the 2012 USDA Census of Agriculture, there are 12,256 farms in the state (National Agricultural Statistics Service, 2016). Therefore, a large number of farms in Maryland do not have a NPDES permit, and as such their wastewater disposal practices are unregulated. This is problematic regardless of the number of animals present on the farm. NPDES regulations should be revised to include permitting requirements for smaller number of animals, thereby allowing for improved control of all farm discharges to waters of the state.

Improving waste management on farms would also have a significant impact on the health of residents who live in close proximity to CAFOs. Currently, most CAFOs dispose of their waste by storing it in large lagoons, trucking it to off-site holding facilities, and/or land application onto nearby fields (Hribar & Schultz, 2010). People who live near CAFOs have reported experiencing increased rates of a number of interrelated symptoms, including headaches, respiratory problems, eye irritation, nausea, weakness, and chest tightness, leading to a decrease in quality of life (K. Thu et al., 1997; K. M. Thu, 2002; Wing & Wolf, 2000). Meanwhile, contaminants from livestock waste have been detected in both surface water and groundwater supplies in agricultural areas within the U.S. (Campagnolo et al., 2002; Kolpin et al., 2002; Meyer, 2004; Barnes et al., 2008), demonstrating the negative consequences of CAFOs to human health and the environment. The US EPA requires CAFOs to have an approved nutrient management plan (NMP) under the NPDES permit system. The NMP is a document that sets rates for waste application to spray fields that meet the
water and nutrient requirements of the selected crops and soil types (Bradford, Segal, Zheng, Wang, & Hutchins, 2008). The NMP is meant to protect nearby surface water resources, however extreme weather events such as heavy rainfall, heavy snowfall and hurricanes could still result in the contamination of nearby surface and ground water by a CAFO despite having a NMP. Creating and enforcing more stringent NMP requirements for farms, including planning for extreme weather events, could reduce the potential for CAFOs to be a point source of contamination for nearby water bodies.

More innovative waste management technologies as alternatives to lagoons and spray fields could also improve the current adverse impacts of CAFOs. In North Carolina, the fourth largest hog producing state in the country, technologies are being investigated to convert hog waste into energy using digesters. A recent study from Duke University found that injecting biogas collected from an optimized network of farms into the natural gas pipeline could be a cost-effective approach to meeting the state’s Renewable Energy and Energy Efficiency Portfolio Standard (REPS) mandate (Prasodjo, Vujic, Cooley, Yeh, & Lee, 2013). Another innovative technology for hog waste is being explored on a farm in North Carolina through a partnership with Google, Duke University, and Duke Energy to create a sustainable farming operation that generates renewable energy and carbon offsets. The 8,600-head hog farm captures methane from its waste using an anaerobic digester, thereby providing enough fuel to run a micro-turbine that powers part of the farm and supports components that reduce odors, nutrients, pathogens, and heavy metals (Duke University, 2018). Although practices such as these are not yet commonplace in the
CAFO industry, these approaches could become more widespread with investments from the large corporations that often control the CAFO industry. As an alternative to digesters, wastewater treatment plants could be constructed to serve groups of CAFOs to treat animal waste in the same way that human waste is also treated and released into the environment. A caveat is that CAFOs in the U.S. are estimated to produce more than 40 times the amount of waste than human biosolids generated from wastewater treatment plants (Graham & Nachman, 2010), which could be problematic for this potential solution since the plants may not be able to keep up with the sheer volume of animal waste received.

Additionally, farmers who implement innovative waste management strategies and demonstrate reductions in discharges could be allowed incentives such as tax breaks and other financial concessions. Similarly, stricter fines on farmers who violate their NPDES permits could be placed into a fund for assisting homeowners whose private wells have become contaminated, and to help remediate polluted waterways that farm wastes have impacted. Large corporations that ultimately own the CAFOs could provide funding for grants specifically targeted at environmental enhancement projects in neighborhoods that host CAFOs and for additional remediation efforts.

4. Directions for Future Research

This research provides the foundation for future studies on the role of animal feeding operations on private well water quality, and for studies on private wells as a risk factor for gastrointestinal diseases. My first study did not detect an association
between microbiological contamination of private wells in zip codes of Maryland and the presence of animal feeding operations within the same zip codes. This may be due to the small sample size of the study, and exploration of this potential association deserves further analysis involving a larger number of private wells. Additional studies are also needed to identify and confirm other potential factors that can influence private well water quality in Maryland, such as animal feeding operations, well construction characteristics, soil geology, and seasonal variability. Septic tanks have also been implicated in the contamination of private wells in other states with both microbiological and chemical contaminants (Sandhu, Warren, & Nelson, 1979; Schaider, Ackerman, & Rudel, 2016; Wallender, Ailes, Yoder, Roberts, & Brunkard, 2014), since wells are commonly used in communities that are served by onsite wastewater treatment systems such as septic systems (Schaider et al., 2016). Over 420,000 septic systems exist in Maryland (Stoltzfus, 2009), demonstrating the need for additional research on proximity of private wells to septic tanks as a risk factor for well contamination in the state.

My first study demonstrated the presence of fecal indicator bacteria in private drinking water wells in Maryland. Knowledge of the contamination source of the well would be helpful to homeowners in selecting an appropriate remediation method. Microbial source tracking (MST) is a collection of methods used to determine the likely source of contamination associated with the presence of fecal indicator bacteria (Simpson, Santo Domingo, & Reasoner, 2002). MST has been previously used in a variety of applications, including in the management of surface water contamination and watershed remediation (Bradshaw et al., 2016; Simpson et al., 2002). Allevi et al.
(2013) utilized MST techniques to characterize the magnitude and incidence of microbial contamination in private wells in Virginia, and to identify the likely sources of this contamination (Allevi et al., 2013). Similarly, Krolik et al. (2014, 2016) analyzed well water samples from southeastern Ontario using MST to elucidate whether human or bovine sources were responsible for well contamination (Krolik et al., 2014; Krolik, Maier, Thompson, & Majury, 2016).

Future work could include the application of MST methods to help identify the source of microbial contamination in Maryland wells, and to elucidate potential relationships between microbial contamination and environmental characteristics, particularly those relating to land use. MST techniques could also be applied to the associations investigated in manuscripts 2 and 3, to determine the likely source of *Campylobacter* and *Salmonella* detected in well water (Simpson et al., 2002). Combined with studies that model groundwater flow and direct sampling of private wells, MST could be used to elucidate potential relationships between microbial contamination of private wells and environmental characteristics, particularly those relating to land use.

Some of the challenges and limitations encountered in this study highlight the need for an improved and reliable well registry database. Most U.S. states do not have a reliable, comprehensive database or tracking system for wells, and as such it is impossible to know exactly how many wells exist across the country. Future studies in this area would benefit from the creation of a de-identified, geocoded database of wells that could be made available to researchers to conduct studies similar to this work. A reliable well registry would also provide county officials with the ability to
contact homeowners with private wells for targeted outreach, well testing reminders, and alerts of potential contamination.

Similarly, a geocoded database of animal feeding operations or a database that includes the latitude and longitude coordinates of these operations would be useful to researchers. Such databases, including data on clustering of CAFOs, would allow for more sophisticated spatial analyses of the association between contaminated wells and their proximity to animal feeding operations, and the association between gastrointestinal illnesses and proximity of homeowner wells to animal farms. Future studies would also benefit from improved data on CAFO characteristics, such as farm size, number (head) of animals per farm, the farm’s steady state live weight, and the amount of waste produced by the farm annually. Additionally, the availability of this type of information and the location of waste application fields could greatly assist in mitigating the impacts of CAFOs. Environmental assessment tools, such as cumulative risk index analysis and use of GIS technology, are being developed and validated to provide a more systematic and reliable approach to assessing the impacts of CAFOs (Osowski et al., 2001).

I used information from a well permit registry as a proxy for the water use and consumption habits of individual residents in Maryland zip codes in manuscript 2 and 3. Use of this proxy was required because data on the actual individual water consumption habits of campylobacteriosis and salmonellosis cases was unavailable. This proxy may not adequately reflect each case’s drinking water exposure since individuals may consume water in other locations such as work or school, drink bottled water, or have point-of-use treatment such as filters in their homes (Jones et
al., 2006; Shimokura et al., 1998). This likely led to some amount of exposure misclassification, however it is expected to be non-differential. In 2015, the Maryland Department of Health included a series of questions on their Gastroenteritis Case Report Form that asks a case about their potential water-related exposures for disease, including if they primarily use water from a well for drinking water (Maryland Department of Health, 2015). While this data was not available for an adequate number of years to be used in my dissertation, it would be beneficial to repeat this study in five years or more after a sufficient amount of cases with answers to this question have accumulated. An investigation of the role of private wells in GI illnesses would be improved by having accurate, individual-level data on whether cases actually consumed water from a well.

Groundwater from private wells continues to be an important water source, and it is important to evaluate the human health risks associated with its use to adequately protect the health of those who rely on this source. Understanding pathogens that may be present in well water and their role in human illness is imperative in improving the quality of this water and controlling the risk factors for illness. It is my hope that the results of this dissertation research will encourage additional research and help inform policy makers about possible human health risks surrounding the use of private well water.
Appendices

Appendix 1

Figure A1. USGS map of physiographic provinces of Maryland and Delaware by county. (U.S. Geological Survey, 2017)
Appendix 2

**Table A1.** Campylobacteriosis in univariate association with private well prevalence at the zip code level by county in Maryland, 2007–2016.

<table>
<thead>
<tr>
<th>County</th>
<th>Univariate Negative Binomial Regression Incidence Rate Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allegany</td>
<td>4.30 (0.97, 9.19)</td>
</tr>
<tr>
<td>Anne Arundel</td>
<td>1.52 (0.95, 2.43)</td>
</tr>
<tr>
<td>Baltimore</td>
<td>1.76 (1.25, 2.47)</td>
</tr>
<tr>
<td>Baltimore City</td>
<td>2.80 (0.26, 2.92)</td>
</tr>
<tr>
<td>Calvert</td>
<td>0.59 (0.17, 2.06)</td>
</tr>
<tr>
<td>Caroline</td>
<td>2.71 (0.36, 2.57)</td>
</tr>
<tr>
<td>Carroll</td>
<td>2.05 (0.34, 2.25)</td>
</tr>
<tr>
<td>Cecil</td>
<td>1.19 (0.22, 6.59)</td>
</tr>
<tr>
<td>Charles</td>
<td>3.43 (1.79, 6.56)</td>
</tr>
<tr>
<td>Dorchester</td>
<td>1.70 (0.52, 5.58)</td>
</tr>
<tr>
<td>Frederick</td>
<td>1.30 (0.76, 2.21)</td>
</tr>
<tr>
<td>Garrett</td>
<td>0.25 (0.01, 4.47)</td>
</tr>
<tr>
<td>Harford</td>
<td>2.17 (1.32, 3.57)</td>
</tr>
<tr>
<td>Howard</td>
<td>1.81 (1.12, 2.91)</td>
</tr>
<tr>
<td>Kent</td>
<td>0.57 (0.04, 7.49)</td>
</tr>
<tr>
<td>Montgomery</td>
<td>1.64 (0.65, 4.11)</td>
</tr>
<tr>
<td>Prince George's</td>
<td>3.13 (0.47, 2.91)</td>
</tr>
<tr>
<td>Queen Anne's</td>
<td>1.49 (0.32, 7.02)</td>
</tr>
<tr>
<td>St. Mary's</td>
<td>3.54 (1.57, 7.97)</td>
</tr>
<tr>
<td>Somerset</td>
<td>4.55 (1.39, 4.92)</td>
</tr>
<tr>
<td>Talbot</td>
<td>2.45 (1.04, 5.74)</td>
</tr>
<tr>
<td>Washington</td>
<td>2.40 (1.15, 5.00)</td>
</tr>
<tr>
<td>Wicomico</td>
<td>1.12 (0.48, 2.57)</td>
</tr>
<tr>
<td>Worcester</td>
<td>1.17 (0.65, 2.12)</td>
</tr>
</tbody>
</table>
### Table A2. Campylobacteriosis in univariate association with private well prevalence, agricultural and socioeconomic factors by physiographic province in Maryland, 2007–2016

<table>
<thead>
<tr>
<th>Zip Code Variable</th>
<th>Appalachian Plateau &amp; Valley and Ridge</th>
<th>Coastal Plain</th>
<th>Piedmont &amp; Blue Ridge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well prevalence</td>
<td>3.28 (1.92, 5.58)</td>
<td>3.30 (2.52, 4.33)</td>
<td>1.67 (1.34, 2.07)</td>
</tr>
<tr>
<td>Cattle operations</td>
<td>Absent 1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>Present 1.22 (0.75, 1.99)</td>
<td>1.24 (0.85, 1.81)</td>
<td>1.20 (1.01, 1.42)</td>
</tr>
<tr>
<td>Broiler chicken operations</td>
<td>Absent 1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>Present 1.07 (0.67, 1.71)</td>
<td>2.24 (1.77, 2.83)</td>
<td>1.22 (0.98, 1.52)</td>
</tr>
<tr>
<td>Hog operations</td>
<td>Absent 1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>Present 0.64 (0.32, 1.28)</td>
<td>1.36 (0.70, 2.65)</td>
<td>1.57 (1.05, 2.33)</td>
</tr>
<tr>
<td>Dairy operations</td>
<td>Absent 1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>Present 1.03 (0.61, 1.72)</td>
<td>1.44 (1.03, 2.02)</td>
<td>1.23 (1.06 1.44)</td>
</tr>
<tr>
<td>Turkey operations</td>
<td>Absent 1.00 (Reference)</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td></td>
<td>Present 1.06 (0.64, 1.77)</td>
<td>0.85 (0.55, 1.32)</td>
<td>1.23 (0.98, 1.56)</td>
</tr>
<tr>
<td>% Owner occupancy</td>
<td>Absent 1.01 (1.00, 1.03)</td>
<td>1.01 (1.00, 1.01)</td>
<td>1.01 (1.00, 1.01)</td>
</tr>
<tr>
<td>% Residents below poverty</td>
<td>1.00 (0.95, 1.05)</td>
<td>1.03 (1.01, 1.04)</td>
<td>0.98 (0.97, 0.99)</td>
</tr>
<tr>
<td>% Population ≥25 years without high school diploma</td>
<td>1.01 (0.96, 1.06)</td>
<td>1.01 (1.00 1.03)</td>
<td>0.98 (0.98, 0.99)</td>
</tr>
<tr>
<td>% African American</td>
<td>0.96 (0.93, 1.00)</td>
<td>0.98 (0.97, 0.98)</td>
<td>0.99 (0.98, 0.99)</td>
</tr>
<tr>
<td>% Hispanic</td>
<td>0.84 (0.73, 0.97)</td>
<td>0.98 (0.97, 0.99)</td>
<td>0.99 (0.98, 1.00)</td>
</tr>
<tr>
<td>Median age, years</td>
<td>1.03 (0.99, 1.08)</td>
<td>1.05 (1.03, 1.06)</td>
<td>1.03 (1.02, 1.05)</td>
</tr>
</tbody>
</table>
Appendix 4

Table A3. Salmonellosis in univariate association with private well prevalence, agricultural and socioeconomic factors by coastal and non-coastal zone in Maryland, 2007–2016

<table>
<thead>
<tr>
<th>Zip Code Variable</th>
<th>Coastal</th>
<th>Non-coastal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Well prevalence</td>
<td>1.50 (1.24, 1.82)</td>
<td>1.04 (0.84, 1.29)</td>
</tr>
<tr>
<td>Cattle operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>0.97 (0.75, 1.25)</td>
<td>1.03 (0.93, 1.15)</td>
</tr>
<tr>
<td>Broiler chicken operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>2.01 (1.69, 2.38)</td>
<td>1.01 (0.90, 1.14)</td>
</tr>
<tr>
<td>Hog operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>1.38 (0.87, 2.19)</td>
<td>0.98 (0.79, 1.22)</td>
</tr>
<tr>
<td>Dairy operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>0.95 (0.76, 1.19)</td>
<td>1.02 (0.92, 1.13)</td>
</tr>
<tr>
<td>Turkey operations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>Present</td>
<td>0.99 (0.74, 1.35)</td>
<td>1.04 (0.89, 1.21)</td>
</tr>
<tr>
<td>Median age, years</td>
<td>1.01 (0.99, 1.02)</td>
<td>0.99 (0.98, 1.00)</td>
</tr>
<tr>
<td>% African American</td>
<td>1.00 (0.99, 1.00)</td>
<td>1.00 (0.99, 1.00)</td>
</tr>
<tr>
<td>% Hispanic</td>
<td>0.99 (0.98, 1.00)</td>
<td>1.01 (1.01, 1.02)</td>
</tr>
<tr>
<td>% Owner occupancy</td>
<td>0.99 (0.98, 0.99)</td>
<td>0.99 (0.99, 1.01)</td>
</tr>
<tr>
<td>% Residents below poverty</td>
<td>1.04 (1.03, 1.04)</td>
<td>0.99 (0.98, 1.01)</td>
</tr>
<tr>
<td>% Population ≥25 years without high school diploma</td>
<td>1.03 (1.02, 1.04)</td>
<td>1.01 (1.00, 1.01)</td>
</tr>
<tr>
<td>% Unemployment</td>
<td>1.02 (1.01, 1.03)</td>
<td>1.01 (0.98, 1.03)</td>
</tr>
</tbody>
</table>
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