# Associations between biogeoclimatic zones, aquifer type, agricultural land and five gastrointestinal illnesses in British Columbia from 2000-2013 and potential implications under projected climate change

**by**

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# Approval

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or

b. advance approval of the animal care protocol from the University Animal Care Committee of Simon Fraser University;

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# Abstract

Rates of five acute gastrointestinal illnesses (AGIs) were calculated across three environmental variables in British Columbia: biogeoclimatic zone, aquifer type and agricultural land. The three bacterial pathogens (campylobacteriosis, Verotoxin-producing *Escherichia coli* and salmonellosis) were strongly correlated with many temperature-related variables calculated at the biogeoclimatic zone level. Combined relative risk for the three bacterial AGIs was 1.11189 (p=0.006) for every degree Celsius increase in mean annual temperature. When amalgamated into two groups (bacteria and parasites) both groups had significantly higher proportions associated with unconsolidated aquifers than with bedrock aquifers. Verotoxin-producing *Escherichia coli* rates were significantly higher in watersheds with agricultural land than those with none. Conversely, rates of campylobacteriosis, salmonellosis and giardiasis were significantly lower in agricultural watersheds.



**Keywords**: gastrointestinal illness; British Columbia; ecohealth; climate change; projection; biogeoclimatic zone

## Dedication

*I dedicate this report to the hope that posterity will one day look back at this time and wonder what it was like to be in the presence of so many brilliant minds and yet such stupidity as a species*

## Acknowledgements

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# List of Acronyms

| | |
|---|---|
| AGI | Acute gastrointestinal illness |
| ALR | Agricultural land reserve |
| BC | British Columbia |
| BGC | Biogeoclimatic |
| CDWQ | Canadian drinking water quality |
| GCM | General circulation model |
| GHG | Greenhouse gas |
| GIS | Geographic information system |
| HA | Health Authority |
| MAUP | Modifiable areal unit problem |
| RCP | Representative concentration pathway |
| SFU | Simon Fraser University |
| VBNC | Viable but nonculturable |
| VTEC | Verotoxin-producing *Escherichia coli* |

# Glossary

| | |
|---|---|
| Biofilm | A group of microscopic organisms that together form a layer on a surface |
| Climate envelope modelling | A statistical approach that describes the climate within which a species or ecosystem most often occurs. |
| Clip | A geoprocessing tool that removes sections of one data layer that do not overlap with another data layer |
| Ecumene | A term used to describe inhabited land |
| Geocoding | The process of assigning X and Y geographic coordinates to addresses |
| Geoprocessing | The act of manipulating data with GIS |
| Hargreaves' Climatic moisture-deficit | Calculated by subtracting mean annual precipitation from reference evaporation |
| Hargreaves' reference evaporation | The amount of water that would evaporate if sufficient water is present |
| Microclimate | A localized region, typically ranging from one square meter to many square kilometers, with a climate differing from those of adjacent regions. |

# Chapter 1.

# Introduction

Food and waterborne pathogens continue to impact people of all ages living in developed and undeveloped nations, with 10% of global deaths among children less than age five caused by diarrheal diseases (Lozano et al., 2013). In Canada, the numbers are considerably lower, with 90 deaths and 90,000 illnesses attributed annually to contaminated drinking water (Christensen, 2006) with an additional one in eight acquiring a domestic foodborne illness .

Encouraging behavioural changes related to food, water and sanitation would work towards reducing incidence of these preventable diseases. However, gaining an understanding of how these organisms have evolved to exploit various elements of their environments can allow for further decreases in disease through facilitating effective intervention design and implementation. At the very least, it can provide information that may be used to help best allocate the resources and interventions currently available. This study is by no means implying that the majority of acute gastrointestinal illnesses (AGIs) can be attributed to human-environment interactions; only that it is worth quantifying any that are.

At a time when humans are drastically modifying the biophysical landscape of the Earth to support our exponentially growing population, it is critical to investigate how pathogens are responding to these changes. The next logical step is to combine this information with what is already known about climate change to identify and work towards mitigating any potential increases in disease risk. This study aims to link health outcomes to reliable regional climatic information, which has been identified as an integral component in developing a climate change/health research agenda in BC (Ostry, Ogborn, Bassil, Takaro, & Allen, 2010). Any insight about health effects of

climate change justifies the implementation of pre-emptive policies and informed adaptive strategies (McMichael, Woodruff, & Hales, 2006).

This study utilizes spatial information to investigate links between AGI and the environment at various scales. A lack of knowledge about scale-dependency of pathogen survival, at the watershed scale in particular, has been identified by Blaustein et al. (2013). Watersheds were selected as the unit of analysis to investigate associations between AGI and agricultural land. Watersheds are biologically meaningful boundaries when investigating waterborne AGI, an idea supported by the increased numbers of managers subscribing to watershed management approaches to safe drinking water and away from the treatment-only based approaches (Davies & Mazumder, 2003). In simple terms, gravity acts upon the water that enables downstream exposures to upstream pathogen sources. When taken in its totality, however, the watershed unit represents more than just a drainage basin; it describes a complex socio-ecological system evolved over millennia connected by this single chemical compound upon which all life depends (Parkes et al., 2010).

Another variable that may be associated with AGI is aquifer type. The permeability of unconsolidated material and the degree and connectivity of fractures in the bedrock, among other variables, determine how much interaction a pathogen on the ground surface will have with the aquifer below (Schmoll, 2006). This relationship, combined with climatic conditions, will ultimately influence the amount of pathogens capable of reaching the groundwater (Schmoll, 2006). Furthermore, the size, shape and density of the species of pathogen also influence how they will interact with the aquifer (Schmoll, 2006).

This study also seeks to quantify the relationship between AGI and select independent climatic variables such as temperature and precipitation; however, it also utilizes ecosystem-level data to investigate the *net* association of AGI with multiple interrelated climatic variables. In this way, the subtle and complex interactions among AGI and the climatic variables are left intact but so too are links between AGI and biota whose ranges and numbers may be directly or indirectly associated with climate. Biogeoclimatic (BGC) zones are used for this purpose. These are geographic areas with

"similar patterns of energy flow, vegetation and soils as a result of a broadly homogenous macroclimate (Meidinger & Pojar, 1991)". A map and list of the zones are included in the Appendix A (Figure A1 and Table A1). They were delineated using field observations of plant communities and soils which, when considered simultaneously, integrate all components of the ecosystem: climate, biota, topography, parent material and time (Meidinger & Pojar, 1991). An assortment of quantifiable climate variables has been calculated for each zone, and satellite data have been used to corroborate that BGC zonation accurately describes unique macroclimatic regions (Delong, Griesbauer, Mackenzie, & Foord, 2009). BGC zones are suitable for long-term risk analysis because they are essentially historical averages and thus are considered to be perfectly predictable (although not perfectly), like geology or elevation. In contrast, unless annual averages are calculated, time-varying site specific variables such as precipitation and temperature are suitable for prediction but not long-term risk analysis because future values are unknown (Crainiceanu, Stedinger, Ruppert, & Behr, 2003). BGC zones are recommended to be used as the basic unit when investigating climate change at a variety of scales, from the province to the watershed (Delong et al., 2009). The future distributions of the BGC zones have been projected until the 2080s and are included in Appendix A (Figures A2 and A3).

Although disentangling the individual effects of each environmental variable on AGI is useful (even if many climatic variables are human constructs), variables do not exist in isolation in this open system called Earth. Therefore, isolating and analyzing variables as though they operate in a closed system has its limitations. While each study has its limitations, and this study has many, one of them is not viewing the environment as though it is a closed system that can be reduced to its individual parts, each with a regression coefficient and p-value. Rather, it utilizes a lens through which the environment is perceived to be comprised of systems nested within systems, all open and interrelated, where patterns emerge at some spatiotemporal scales but not at others, and where humans are very much a part of the ecosystem.

## 1.1. Study Area: British Columbia

British Columbia (BC) is situated in the temperate region of the Northern Hemisphere and complex topography causes large variations in climate over short distances (Pacific Climate Impacts Consortium, 2013). This makes it an excellent site to study the effects of climate on AGI, since adjacent cities may have similar socioeconomic status or share the same governments and yet be exposed to very different climates. The till left behind by past glaciations has also resulted in a variety of aquifer types that can change over very short distances. The mountainous terrain in many parts of the province creates watersheds that tend to be separated by steep slopes covered in a relatively thin layer of soil. As the mountains erode over time, much of the sediment reaches the valley bottoms, enters streams and eventually becomes deposited anywhere the velocity of the water slows enough for the particles to settle out. The result is rich alluvial soils that form on the inside bank where a stream bends, on floodplains and where a stream enters a lake or a slower moving stream. Often in valley bottoms, these areas tend to have microclimates that are much less harsh than the surrounding area. It is not surprising, then, that this is also where agricultural activities tend to occur and where settlements become established, many of which have since grown into large cities with complex socio-ecological systems that continue to have deep ties to the watersheds within which they are embedded.

On the provincial scale, BC consistently ranks among the highest in Canada with respect to active boil water advisories (Boettger & Young, 2012) and between the years 1974 and 2001 had the highest number of waterborne disease outbreaks (Dods, Copes, Lazanik, & Gillespie, 2007). Annual precipitation is projected to increase by 10% by the 2080s (relative to the 1961-1990 baseline) in all seasons but summer, for which it is projected to decrease by 10%, and is expected to occur in more intense events (Pacific Climate Impacts Consortium, 2013). Drought conditions are projected to increase in some regions and temperatures are projected to increase throughout the entire province by between 1.5°C and 4.1°C by the 2080s (Pacific Climate Impacts Consortium, 2013).

## 1.2. Purpose and Objectives

Hypothesis: Climate, aquifer type and agriculture influences incidences of campylobacterioisis, cryptosporidiosis, giardiasis, salmonellosis, and/or Verotoxin-producing *Escherichia coli* (VTEC) in BC.

The primary aim of this thesis was to investigate these relationships at the population level using biologically meaningful boundaries to define populations; climate data for each AGI case are derived from a BGC zone, the agricultural index is calculated at the watershed level, and the groundwater susceptibility is derived from underlying aquifer information. The second aim was to examine these findings in the context of a changing climate and make inferences about future AGI risk in BC.

The specific objectives were:

- To calculate and compare AGI rates among five pathogens (campylobacterioisis, VTEC, salmonellosis, giardiasis and cryptosporidiosis) for every BGC zone, aquifer type, and agricultural land class.
- To create a model describing associations between population AGI risk and climate using variables calculated at the BGC zone level. Climatic variables included are: Annual temperature, frost-free days, frost-free period, degree days below $0^{o}$C, above $5^{o}$C, below $18^{o}$C and above $18^{o}$C, annual precipitation, annual snowfall, heat-moisture index, reference evaporation and climatic moisture deficit.
- To discuss results in the context of other studies to identify possible trends and interactions across scales.
- To project future provincial AGI burden using regression coefficients and to project future spatial distributions of risk using BGC zone projections.

# Chapter 2. Background

The following chapter summarizes much of what has been described in the literature related to environmental associations with AGI. While much information is available, there remains a great deal to be learned about how the many variables interact and are related to one another in an open socio-ecological system.

## 2.1. Acute gastrointestinal illness

In Canada, the most common food and waterborne pathogens are the bacteria *campylobacter*, non-typhoidal *salmonella* (herein referred to as *salmonella*), and VTEC and protozoan parasites *cryptosporidium* and *giardia* (Thomas et al., 2013), all of which are zoonotic (Heymann, 2004 as cited in Uhlmann et al., 2009). In Canada, AGI caused by these bacteria is predominantly via the consumption of contaminated food and for these parasites, via water. While estimating percentages of AGI attributable to food or water is difficult, national estimates attributed to food are 68% for *campylobacter*, 80% for VTEC, 80% for *salmonella*, 9% for *cryptosporidium* and 7% for *giardia,* with water accounting for much of the remainder (Thomas et al., 2013). In drinking water, the bacteria are effectively treated using chemical disinfectants but the protozoa are difficult to eliminate by treatment due to the cyst stages in their lifecycles (American Water Works Association, 1999). Protozoa require higher doses of chemical disinfectant and so the preferred method is to physically remove them with filtration systems (American Water Works Association, 1999).

Infectious doses, incubation times and symptoms vary among the pathogens but, generally speaking, symptoms include diarrhoea and sometimes vomiting, abdominal pain, dehydration and fatigue (American Water Works Association, 1999). Symptoms generally last up to seven days for the bacteria and from two weeks to months for the parasites (Leggat & Goldsmid, 2004). Populations that are at greatest risk of

experiencing serious health complications related to AGI are young children, the elderly, pregnant women and immunocompromised individuals (Patz, Vavrus, Uejio, & McLellan, 2008).

The pathologies of the five pathogens in this study are similar in that they generally target the intestinal tract, however, differences do exist. The susceptibility of the host as well as the relative virulence of the strain are both important factors. *Campylobacter sp*. can infect a host with as few as 800 organisms where they adhere to the intestines and release toxins that induce diarrhoea (Wallis, 1994). The type of toxins released, mostly enterotoxin and cytotoxins, vary among strains and determine the severity of the enteritis (Wallis, 1994). The usual incubation period is 1-7 days and the average duration of illness is 1-7 days (Leggat & Goldsmid, 2004).

Unlike the other pathogens or even other *E. coli* strains, VTEC strains produce Shiga toxins. The most common VTEC strain in North America is *E. coli* 0157. VTEC has the lowest infectious dose of the bacterial pathogens in this study of between 10-100 organisms (Hellberg & Chu, 2015). VTEC colonizes the large intestines where it releases Shiga toxins that may be absorbed into the blood stream and distributed to other organs (Nguyen & Sperandio, 2012). The usual incubation period is 12-48 hours and average duration of illness is 24-48 hours (Leggat & Goldsmid, 2004). Infection often includes abdominal cramps and bloody diarrhoea and possibly haemolytic uremic syndrome, a potentially life-threatening complication (Nguyen & Sperandio, 2012).

The *salmonella* species in this study do not include *S. Typhi* and *S. Paratyphi*, since they cannot be acquired domestically in British Columbia. *Salmonella* attach to the mucosa of the small and large intestines where they produce toxins. Invading the epithelial cells induces an inflammatory reaction, which causes diarrhoea and potentially leads to destruction of the mucosa (Murray, Rosenthal, & Pfaller, 2013). The usual incubation period is 6-48 hours and average duration of illness is 3-4 days (Leggat & Goldsmid, 2004).

*Giardia* infection is initiated when at least 10-25 cysts are ingested (Murray et al., 2013). Gastric acid stimulates the organisms to release trophozoites, which multiply and attach to the intestine (Murray et al., 2013). Approximately half of infections are without

symptoms, with the remainder suffering from a range of symptoms from mild diarrhoea to severe malabsorption syndrome (Murray et al., 2013). Spread of disease outside of the intestinal tract is rare (Murray et al., 2013). The usual incubation period is 12-15 days and average duration of illness is weeks to months (Leggat & Goldsmid, 2004).

Cryptosporidiosis is the least common of the five AGIs in this study. Like giardia, *cryptosporidium* targets the intestinal tract where the organism attaches to the cell surface and reproduces oocysts. These oocysts either infect new cells or are excreted into the environment (Murray et al., 2013). In healthy individuals, infection may be mild and limited to watery diarrhoea without blood, however in immunocropmomised people, they may lose large amounts of fluid in over 50 stools per day (Murray et al., 2013). The usual incubation period is 5-10 days and average duration of illness is 2 weeks to months (Leggat & Goldsmid, 2004) and sometimes even years (Murray et al., 2013).

## 2.2. Environment and AGI

Climate and weather are not interchangeable terms. Weather refers to the *state* of the atmosphere in a particular region at a particular time (minutes to days) and is described using climatic variables such as temperature and precipitation whereas climate is commonly described as the average weather of a particular region over a much longer time, typically decades.

Each pathogen has a large and unique set of potential pathways to a host (Semenza et al., 2012), and once outside the host, pathogens can have very different rates of survival (which may influence capacity for both proliferation and dispersal) depending on the climate (American Water Works Association, 1999). For example, *salmonella* thrives in warmer environments (McMichael et al., 2006) while *giardia* can survive in cooler ones (American Water Works Association, 1999), and drought generally increases the infection risk of persistent pathogens like *cryptosporidium* while decreasing it for rapidly inactivating pathogens like *campylobacter* (Schijven et al., 2013).

Some environmental factors that have been found to influence pathogen survival and growth in water include water type (surface versus ground), temperature, pH, dissolved oxygen, presence of organic material, nutrient availability, antibiosis, algal toxins, ultraviolet light and heavy metals, the specifics of which vary among pathogens (American Water Works Association, 1999). Outside of the water, ambient air temperature has been observed to have a significant effect on the survival and growth of some pathogens, which is particularly relevant for foodborne transmission pathways (D'Souza, Becker, Hall, & Moodie, 2004; Fleury, Charron, Holt, Allen, & Maarouf, 2006).

The ways in which the environment interacts with and contaminates water are more intuitive than the ways in which it interacts with food. Common foods that may become contaminated with these bacteria include poultry, raw milk, eggs, meat, produce and unpasteurized juice (Altekruse, Cohen, Swerdlow, & others, 1997). In many cases, hygiene and food preparation methods are responsible for foodborne pathogen transmission and the effects of the environment are probably minimal. However, it is not always so clear. For example, produce can become contaminated through their stomata, the microscopic pores in leaf tissue that control gas exchange, via pathogen-laden irrigation water (Liu, Hofstra, & Franz, 2013; Painter et al., 2013) and the bacteria on contaminated raw chicken interacts with the climate of a warm trunk of a vehicle on the way home from the grocery store. Therefore, in what might initially appear to be an entirely foodborne transmission pathway, links to waterborne transmission pathways and to the climate could plausibly exist. Many similar scenarios could exist that for an individual person may pose a very small risk but on the population scale has the potential to impact many people.

A wide variety of associations between elements of the environment and reported cases of AGI and waterborne disease outbreaks has been observed. These associations involve heavy precipitation events (Curriero, Patz, Rose, & Lele, 2001), temperature (D'Souza et al., 2004; Fleury et al., 2006), streamflow (Lake, Bentham, Kovats, & Nichols, 2005), drought (Charron et al., 2005), turbidity (Schwartz, Levin, & Goldstein, 2000) and wildlife (Charron et al., 2005). Other studies have found associations between reported AGI or outbreaks and human-related factors such as livestock rearing (Hrudey, Payment, Huck, Gillham, & Hrudey, 2003), rural living

(Galanis et al., 2014), sewage disposal (Arnone & Walling, 2007), swimming (Sanborn & Takaro, 2013), drinking water system types (Uhlmann et al., 2009) and temporary drinking water system outages (Barrett, 2014). Serologic evidence indicates that rural populations and those living on or near farms are more exposed to VTEC (Haack et al., 2003).

Studies investigating the presence of these organisms in the environment have found associations with a range of wildlife (Langholz & Jay-Russell, 2013; Nichols, 2005; Smith, Cacciò, Cook, Nichols, & Tait, 2007; Waldenstrom et al., 2002), watersheds (Davies & Mazumder, 2003), urban development (Rosen, Croft, Atwill, Wade, & Stehman, 2000) and impervious surfaces (Arnone & Walling, 2007). Harmel et al., (2010) observed significantly higher *E. coli* concentrations from runoff originating from grazed fields than from cultivated sites, although the association was not entirely attributable to the presence of cattle. *Salmonella* and VTEC can persist in soil for up to several months following application of contaminated manure, and extreme weather (heavy precipitation or drought) has been observed to increase the internalization of *salmonella* into the leaves of leafy vegetables. Fresh produce is thought to be responsible for a large portion of foodborne AGI, in large part because it is irrigated with contaminated water and then consumed raw and in large quantities (Liu et al., 2013).

All zoonotic enteric diseases are seasonal, with regional variations being partly attributed to complex environment-pathogen-host interactions, the details of which are not well understood (Lal, Hales, French, & Baker, 2012). Evidence suggests that host population dynamics, local environmental influences and large-scale longer-term climatic variability may interact and affect future enteric disease risk (Lal et al., 2012). In humans, population dynamics include seasonal variations in behaviour such as diet choices and recreational activities (Nylen et al., 2002).

The population health effects of pathogen exposure below the level that would cause infection are not entirely clear. For example, dogs are known hosts for some zoonotic pathogens and yet Robertson et al. (2002) observed dogs to be negatively associated with human AGI. Similarly, one study found intensive farming activities to be negatively correlated with AGI (Febriani et al., 2010). Hunter and Thompson (2005)

speculate that these protective-like associations may be due in part to a strengthened immune system caused by repeated low dose exposure to these pathogens. Potential for this inverse relationship is discussed more in section 5.2.7.

## 2.3. VBNC state, biofilms and potential for environmental reservoirs outside of hosts

The parasites *cryptosporidium* and *giardia* have cyst stages in their lifecycles that allow them to go dormant for long periods of time when environmental conditions are unfavourable (American Water Works Association, 1999). Less understood, is that the three bacterial pathogens in this study also go through a similar stage where they are considered to be viable but nonculturable (VBNC) (Oliver, 2009). For *E. coli*, this temperature threshold has been observed when temperatures reach 5$^{o}$C or lower (Blaustein et al., 2013). In this stage they are alive but cannot divide or infect a host; however, following resuscitation, they become culturable again and able to carry out a typical life cycle. Organisms in the VBNC state may not be detected when using typical methods for testing drinking water and yet can make up portions of biofilms, which can potentially act as persistent environmental reservoirs (Wingender & Flemming, 2011). Approximately 95% of the total bacteria in a drinking water system are attached to surfaces as biofilms with only 5% suspended in the water (Wingender & Flemming, 2011). However, even though no pathogens may be measured in the water at a particular time, biofilms supporting pathogens may still grow, periodically becoming dislodged and potentially contaminating the water for short periods of time (Wingender & Flemming, 2011). This raises the question about whether pathogenic biofilms are contaminating refillable drinking water bottles (that are increasingly being used), which often go un-cleaned for many weeks despite being used daily. (Tatchou-Nyamsi-König et al., 2008) found that 1-2% of *campylobacter jejuni* cells adhered to polyethylene terephthalate, a widely used polymer for water bottles, which is the first step towards establishing a biofilm. Another study examined the bacterial counts of drinking water sampled from refillable water bottles at a public school (Oliphant, Ryan, & Chu, 2002). They found that the Canadian Drinking Water Quality (CDWQ) guidelines criterion for total coliform was exceeded in 13.3% of samples, while the criteria for fecal coliform and

total heterotrophic were exceeded in 8.9% and 64.4% samples, respectively. The five sources of water used to refill the bottles in the school were all tested at below the CDWQ criteria. Though contamination is thought to have originated from hands and mouths coming into contact throughout the day (Oliphant et al., 2002), much like in drinking water systems, the role that climate plays in mediating pathogen growth in these bottles remains unknown. Temperatures of a water bottle probably fluctuate much more and reach higher extremes than those of a drinking water system, since they are typically transported throughout the day and exposed to many different microclimates.

The chemical composition of groundwater and pH has also been shown to influence growth and survival of these pathogens in unique ways. With *campylobacter jejuni*, in particular, it is thought that the synergistic effects of multiple compounds can play a greater role than any single compound on its own (Cook & Bolster, 2007). Increased nutrient supply can also increase biofilm growth (Wingender & Flemming, 2011). In the environment, pathogenic biofilms are more common on some materials than others. A study in BC investigated *campylobacter* incidence in biofilms growing in the streams of agricultural watersheds (Maal-Bared, Bartlett, Bowie, & Hall, 2012). *Campylobacter* were found in biofilms of 27% of sediment samples, 22% of slate rock, 13% of wood and 9% of water samples. The ability of pathogens to exist as part of a biofilm varies. The bacterial pathogens have been observed making their own biofilms as well as joining existing ones, within which they are thought to live for up to two or three weeks (Wingender & Flemming, 2011). The parasites, however, may only join existing biofilms but can attach as oocysts, which can act as reservoirs for future contamination of drinking water long after any outbreak or water advisory has passed (Wingender & Flemming, 2011).

## 2.4. Climate change influences on AGI

Observational evidence about the health implications of climate change has been accumulating. Direct effects include, for example, heat exposure, extreme weather events and natural disasters (and associated outbreaks of infectious disease, malnutrition and mental stress). Indirect effects include under-nutrition, the spread of infectious diseases and mental stress from forced relocation (Kjellstrom & McMichael,

2013). Of the three basic ways in which climate change can impact health, “effects mediated through natural systems” (Woodward et al., 2014) has been identified as one.

With respect to climate change and AGI, a review concluded that while individual weather events have been linked to AGI, general climate change phenomena have not (Semenza et al., 2012). There has been particular focus in the literature regarding the increased average temperatures, increased precipitation intensity, and increased drought frequency and length that are projected to occur in many areas of the globe (Charron et al., 2005). Less understood is how climate change will alter host wildlife species’ ranges and distributions or how it will alter livestock rearing practices, such as the use of indoor housing that can facilitate spread of disease (Hellberg & Chu, 2015). On an individual level, climate change might also increase an animal’s stress level, making it more susceptible to disease (Hellberg & Chu, 2015).

## 2.5. Knowledge gaps and challenges

Although associations have been observed between AGI and the aforementioned environmental variables, the causal pathways of these relationships remain largely unknown due, in part, to the following: AGI may be influenced by a combination of environmental factors (some with positive and others with negative associations) and not necessarily by single environmental variables alone (Sterk, Schijven, de Nijs, & de Roda Husman, 2013); pathogen abundance and exposure can be mediated through large-scale and complex processes (Plowright, Sokolow, Gorman, Daszak, & Foley, 2008); the majority of parasitic AGIs (~90%) (Hunter & Thompson, 2005) and foodborne AGIs (Fleury et al., 2006) are thought to be sporadic, which are considerably more difficult to identify sources for than with outbreaks (Hunter & Thompson, 2005); there is rarely certainty that sporadic AGI cases originate from the same sources as outbreaks (Hunter & Thompson, 2005), making extrapolating from one type of study to the next problematic; the health databases upon which these studies are typically based are biased and reflect a very small percentage of the actual disease burden due to under-reporting, which has been estimated to be at 1 in 347 (0.3%) of all infectious gastrointestinal illness cases reported in BC (MacDougall et al., 2008); and randomized

control trials are rarely used in studying the effects of the environment on AGI (Semenza et al., 2012).

# Chapter 3. Methodology

The methodology of this study involved collecting secondary data and manipulating it using a geographical information system (GIS) so that statistical analysis could be used to identify associations between the different environmental variables and AGI. While some data required little or no modification, other data required extensive processing in order to be in a useable format. During every step of the data processing, utmost care was taken to maximize data integrity. At times when the loss of information was unavoidable, multiple options were explored before choosing one that would minimize the loss of the most important information.

## 3.1. Overview

This ecological study utilizes spatial information to investigate associations between reported AGI cases and various environmental variables. ArcGIS versions 10.0, 10.1 and 10.2 were used to geocode AGI cases and to manipulate datasets for analysis. R 3.1.2, R Studio 0.98.1091, IBM SPSS Statistics 21.0.0 and Excel 14.1.0 were used for calculating statistics, organizing data and generating charts.

The BC Albers projection and the North American Datum (NAD) 83 geographic coordinate system were used for all spatial analysis. The BC Albers projection was chosen because it is an equal-area projection and therefore minimizes distortion of area, which is essential for generating accurate population density datasets.

## 3.2. Data preparation

### 3.2.1. Cases and address geocoding

All laboratory-confirmed cases of the five pathogens of interest are reportable by law in BC through the integrated Public Health Information System (Uhlmann et al., 2009). Access to case data required ethics approval. All cases with addresses were included, except salmonellosis with travel related serotypes (*S. typhi* and *S. paratyphi*). Home address, date reported, age, sex, AGI and health authority were known for each case. Every address was first geocoded at the cadastre level. If that failed then it was done at the street level, and if that failed then the postal code centroid was used (Table 3.1). Addresses that contained post office (PO) boxes were not geocoded in Greater Vancouver or Greater Victoria but were geocoded for smaller cities and rural BC because they were found to occur in a much larger proportion of cases, suggesting that many people use PO Boxes as their primary mailing address in rural BC. Although there was much less accuracy and precision in the geocoding for cases with a PO Box or postal code, because these cases were mostly in remote areas of the province, including them in the study was consider vital so as to not underestimate rates of disease in rural communities. Cases were de-identified once each was assigned a BGC zone, an aquifer type and a watershed ALR percentage.

**Table 3.1 - Complete geocoding breakdown for AGI cases**

| Geocoding method (in order of preference) | Cases | Percent | Comments |
|---|---|---|---|
| Cadastre | 28458 | 73.0 | Most accurate. Assigned case to a property. Larger cities tended to have complete cadastre information, while rural areas had less complete information. |
| Street | 7918 | 20.3 | Reasonably accurate. Estimated location along a street based on address number. Many rural addresses were geocoded this way. |
| PO Box (by Street) | 242 | 0.6 | Least Accurate. Same as street but rather than a house address, the address of a PO Box was geocoded. |
| Postal Code | 2361 | 6.1 | Least accurate. Assigned case to centroid of 6-digit postal code polygon. Most of these were rural and included many addresses with rural routes and lot numbers that could not otherwise be geocoded. |

### 3.2.2. Population

In this study, the census years 2006 and 2011 were used to calculate the populations. Census information for 2006 and 2011 at the dissemination block and the census subdivision levels were obtained from Statistics Canada (Statistics Canada, 2011). Estimating populations within ecological boundaries such as BGC zones and watersheds required some manipulation of the data. The year 2001 was not used because the data are not available at the fine dissemination block level, but instead at a coarser resolution. Incorporating data at two different resolutions would have presented opportunities for the modifiable areal unit problem (refer to section 5.1.2).

To calculate populations of the various zones, the dissemination block population data were clipped using the population ecumene layer with a 500-metre buffer. An ecumene is a description of where the population lives and is much more precise than political boundaries that can have large unoccupied areas within them. The BC Population Ecumene was created by Anthony Smith, Human Early Learning Partnership and was released in 2011 (A. Smith, 2011). Using the field calculator, the next step was to calculate a population density for each clipped dissemination block by dividing the population by the area of the polygon. The shapefile was then converted into a raster with 25-metre pixel resolution using the population density value. To calculate the population for any given overlaying variable, the 'zonal statistics as table' tool was used to sum the pixel values within each polygon. Finally, using the field calculator this sum was divided by 1600 to convert the density back into a population count. The value of 1600 was used because that is the number of 25 m by 25 m squares in a single square kilometre.

### 3.2.3. Biogeoclimatic zones, subzones and associated climatic data

Version 9 of the Biogeoclimatic Ecosystem Classification system was obtained from the Research Branch of the British Columbia Forest Service (British Columbia Forest Service, 2014). Version 5 of the corresponding BEC annual seasonal climatic data (1990-2012) was also obtained. Subzone-level climatic data were collapsed into

their respective larger zones by calculating a mean for each subzone. Table 3.2 describes the climatic variables included in the analysis.

**Table 3.2 - Climatic variables included in BGC zone and AGI correlation tests**

| Climatic variable | Description | Unit |
|---|---|---|
| Annual temp. | Mean annual temperature | $^{o}$C |
| Temp. of the warmest month | Mean warmest month temperature | $^{o}$C |
| Temp. of the coldest month | Mean coldest month temperature | $^{o}$C |
| Continentality | Temperature difference between 'mean warmest month temperature' and 'mean coldest month temperature' | $^{o}$C |
| Degree-days below 0$^{o}$C | Chilling degree-days | None |
| Degree-days above 5$^{o}$C | Growing degree-days | None |
| Degree-days below 18$^{o}$C | Heating degree-days | None |
| Degree-days above 18$^{o}$C | Cooling degree-days | None |
| Frost-free days | Number of days without frost | None |
| Frost-free period | Length of period without frost (in days) | None |
| Annual precipitation | Mean annual precipitation | mm |
| Growing season precipitation | Mean precipitation of the growing season | mm |
| Annual heat-moisture index | ('Mean annual temperature'+10) / ('Mean annual precipitation'/1000) | $^{o}$C mm-1 |
| Summer heat-moisture index | 'Mean warmest month temperature' / ('Mean annual summer (May to Sept.) precipitation'/1000) | $^{o}$C mm-1 |
| Annual snowfall | Annual precipitation as snow | mm |
| Extreme min. temp | Extreme minimum temperature over 30 years | $^{o}$C |
| Extreme max. temp | Extreme maximum temperature over 30 years | $^{o}$C |
| Reference Evaporation | Hargreaves reference evaporation.<br>This is a measure of potential evapotranspiration, which is defined as the amount of water that would evaporate from the soil (evaporation) and from the plants (transpiration) if there were unlimited water available in the soil. | mm |
| Climatic moisture deficit | Hargreaves climatic moisture deficit.<br>The amount by which potential evapotranspiration exceeds actual evapotranspiration. | mm |

### 3.2.4. Watersheds and agriculture

Watershed data from the BC Watershed Atlas 50K were obtained from DataBC at the third-order and greater level. Agricultural Land Reserve (ALR) data were obtained from the Provincial Agricultural Land Commission. Using the 'zonal statistics as table' tool in ArcGIS, total percent ALR land within each watershed was calculated and then re-classed into five levels: 0%, 0.1-24.9%, 25-49.9%, 50-74.9% and 75-100%, as well as into two levels: No ALR (0%) and ALR (>0%).

### 3.2.5. Aquifer type

The aquifer dataset was published by the BC Ministry of the Environment and acquired through DataBC (BC Ministry of the Environment, 2014). This dataset does not include all aquifers, nor necessarily the entirety of each aquifer it does include. Aquifers are generally only mapped in regions where populations exist. Overlaying aquifers were consolidated into two groups, bedrock and unconsolidated material (Figure 3.1), using geoprocessing techniques. In the event the two types were overlaying one another, the unconsolidated layer (invariably the shallower aquifer) was selected. The aquifer dataset was then split into urban and rural components by using the 2011 population density raster created previously. A cut-off of 400 people per square kilometre was selected, based on the value used by (Galanis et al., 2014) to investigate campylobacteriosis rates in BC. Urban populations were excluded from this portion of the study because of the complexity evident in many major drinking water systems in large cities, such as Vancouver and Victoria where surface water is piped in from many kilometers away. By excluding urban populations, the intention was to focus the study on populations with smaller, private and less complex drinking water systems, such as a backyard well. The intention was that the home addresses in these populations are more likely to accurately identify the aquifer from which they may be extracting drinking water.

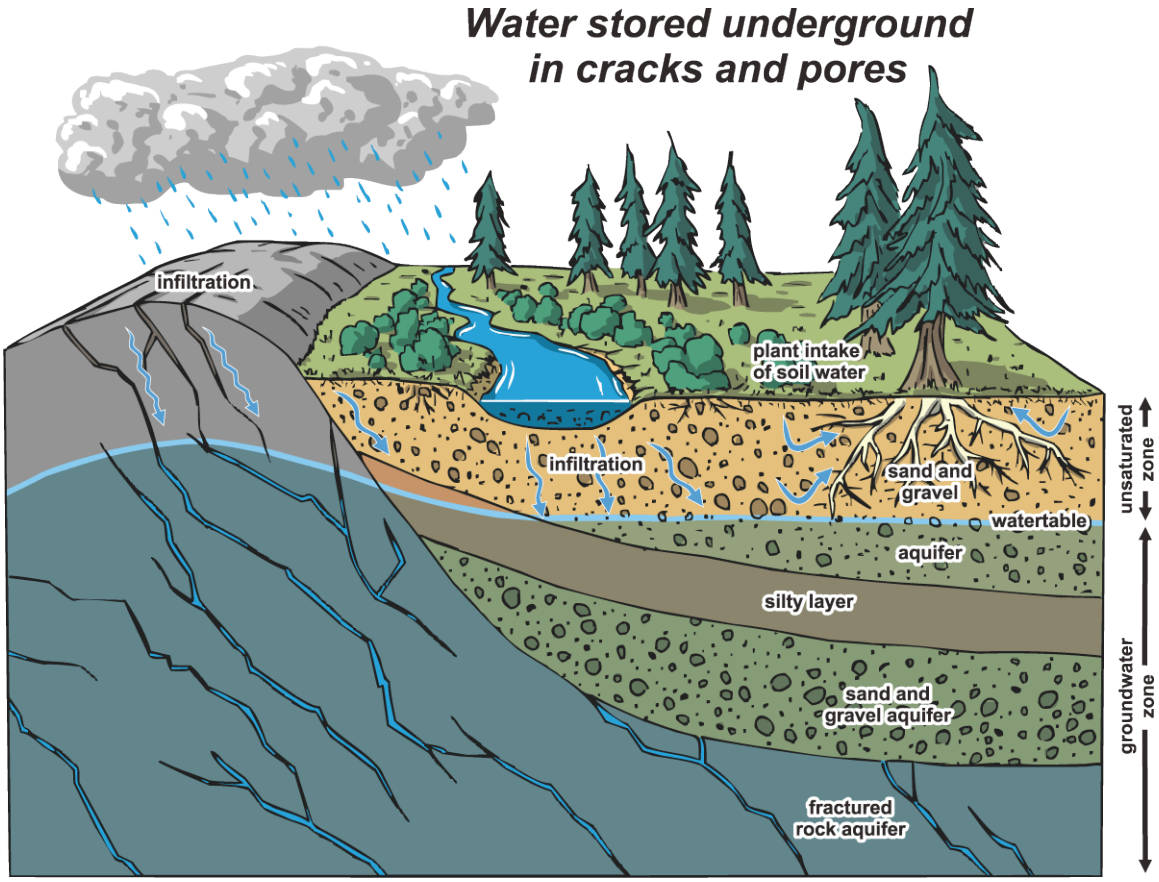


**Figure 3.1 – An Illustration highlighting some differences between bedrock aquifers and unconsolidated aquifers (Geological Survey of Canada, 2014)**

### 3.2.6. Case counts per zone

Each case is represented in ArcGIS as a single point with a unique geographical coordinate. To assign each case to a single BGC zone, agriculture class, and aquifer type, cases were selected by using the 'entirely within' option of the 'select layer by location' tool (for example, all cases located 'entirely within' the Bunchgrass BGC zone). This step was repeated for every biogeoclimatic zone and each of the five agricultural classes.

## 3.3. Statistical analysis

### 3.3.1. One-sample test of proportions

The one-sample test of proportions was used in R and R Studio to calculate the 95% confidence intervals for each AGI in every BGC zone, ALR class and aquifer type. Excel was used to graphically display the data.

### 3.3.2. Pearson's correlation

Bivariate Pearson's correlation tests were performed on all AGIs and climatic variables in the dataset using SPSS.

### 3.3.3. Univariate regression model

Negative binomial regression was used to model AGI - climate associations. Correlations among variables were identified using Pearson's correlation values. Variables were selected based upon biological plausibility, support in the literature, simplicity (to facilitate knowledge translation), and ability to be compatible with existing climate change models.

### 3.3.4. Climate change projections

BGC zone projections were obtained with ClimateWNA (Western North America) version 5.10 (Wang, Hamann, Spittlehouse, & Murdock, 2012). They were only available in raster format and so were converted to polygons. This resulted in a decrease in precision compared to the Version 9 polygons of the BEC classification that were used to investigate AGI - climate associations. In their projections, Wang, Campbell, O'Neill, & Aitken (2012) "considered the variation in projected future climates for GHG emission scenarios (A2, B1, and A1B), general circulation models (GCMs), and model runs for each GCM simultaneously, plotting projected changes in mean annual temperature and precipitation from 133 such combinations". They then selected twenty climate change scenarios to be used in the BGC zone projections (10 recommended for BC climate change analysis and 10 randomly selected), with each pixel eventually being assigned

the BGC zone that the projections predicted most often. For further details on the specific models and methods, refer to Wang, Campbell, O'Neill, & Aitken (2012). Algorithms used in the ClimateWNA software package are evaluated for accuracy using observation stations throughout WNA and were found to greatly improve accuracy of temperature variables during downscaling over medium-resolution climate surfaces (Wang, Hamann, et al., 2012).

This study utilizes BGC zone projections to project future AGI incidence using a very simple method. First, every AGI case is assigned to a BGC zone, as they are currently defined (Figure A1 in Appendix A), to calculate an AGI rate. While retaining the same AGI rate for every BGC zone, the rates are then allowed to take on new spatial dimensions based on the changes in BGC zone extents projected into the future (Figures A2 and A3 in Appendix A).

### 3.3.5. Data summary

Table 3.3 summarizes what datasets were used in this study and for what purpose. Table 3.4 summarizes when, why and how many cases were retained or excluded throughout the various stages of analysis. This information relates to the limitations of the study that are outlined in Section 5.1. Refer to Table 3.1 for breakdown of geocoding methods used on the 38,979 cases.

**Table 3.3 - Summary of data layers and how they were used**

| Data | Analysis |
|---|---|
| AGI cases | AGI data province-wide from 1993-2013 were accessed through the Integrated Public Health Information System. |
| Population | Population data were obtained at the dissemination block level from Statistics Canada (Statistics Canada, 2011). They were used to calculate AGI rates. |
| Population ecumene | By describing exactly where the population resides in BC, the ecumene layer by Anthony Smith (2011) was combined with the population data to generate population density raster layers at a fine spatial resolution (25m). The population density raster was used to create the urban/rural layer, which used 400 persons per $km^2$ as a cutoff. |
| BGC zones (current) | Version 9 of the Biogeoclimatic Ecosystem Classification system was obtained from the Research Branch of the British Columbia Forest Service (British Columbia Forest Service, 2014). This was used for assigning AGI rates to BGC zones. To investigate AGI-climate associations, climatic data for BGC zones were obtained through ClimateBC (Centre for Forest Conservation Genetics, 2014). |
| BGC zones (projected) | BGC zone projections for 2010s, 2050s and 2080s (Wang, Hamann, et al., 2012) were used to project future distributions of AGI rates. |
| Aquifer | Aquifer data for the province (BC Ministry of the Environment, 2014) were obtained through DataBC. These data were used to investigate AGI-aquifer associations. |
| Watershed | Watershed data from the BC Watershed Atlas 50K were obtained from DataBC at the third-order and greater level. These data were used as the units of analysis for investigating associations between AGI and agricultural land. |
| ALR | ALR data were obtained from the Provincial Agricultural Land Commission (Agricultural Land Commission, 2015). These data were used to assign a percentage of agricultural land to each watershed. |

**Table 3.4 – Data capture summary for all AGI cases throughout the entire analysis phase**

| Section | Cases | Brief explanation |
|---|---|---|
| Pre-analysis | 72564 | Total cases (1993-2013) |
| | -31131 | Removed cases without addresses |
| | -2454 | Removed cases before year 2000 |
| | =38979 | |
| **Each of the following analysis began with 38979 cases** | | |
| BGC Zones | -65 | Removed cases from BGC zones with populations too small for analysis |
| | +3352 | Interpolated values for years 2006-2010 for two BGC zones (Coastal Western Hemlock and Coastal Douglas Fir) because Vancouver Coastal Health Authority had many missing addresses for that time. |
| | =42266 | |
| ALR | -11729 | Removed all data from years 2006-2010 due to VCHA missing data |
| | =27250 | |
| ALR Under 10 years | -11729 | Removed all data from years 2006-2010 due to VCHA missing data |
| | -22741 | Removed all cases of people 10 years of age and over |
| | =4509 | |
| Rural/Urban | -11725 | Removed all data from years 2006-2010 due to VCHA missing data |
| | =27254 | |
| Aquifer Type | -11725 | Removed all data from years 2006-2010 due to VCHA missing data |
| | -6980 | Removed cases with unknown aquifer types |
| | -17102 | Removed cases in urban populations |
| | =3172 | |

Four cases were lost at various stages because they landed exactly on a polygon line during spatial analysis and thus were assigned to neither polygon.

# Chapter 4. Results

The results in this study are often presented first in tabular format, with a rate per 100,000 population and an upper and lower 95% confidence interval, and then again as a bar chart with whiskers that describe the confidence intervals. Because they are relatively easily understood, it is recommended that the bar charts be used primarily for interpreting these results, with the tabular data being used as a supplement when increased precision is desired.

## 4.1. Cases

There were more males than females in the dataset (53.0% and 46.9%, respectively) and the age groups 0-9 and 20-29 had the highest numbers of cases (16.1% and 17%, respectively) (Table 4.1). July, August and September had the highest numbers of cases, and November through to March had the lowest (Table 4.1b).

**Table 4.1 - Sex and age frequency table of successfully geocoded AGI cases from 2000-2013**

| | | Frequency | Percent | Cumulative Percent | Per 100,000 Pop* |
|---|---|---|---|---|---|
| **Sex** | **Female** | 18300 | 46.9 | 46.9 | 871.6 |
| | **Male** | 20652 | 53.0 | 99.9 | 1025.4 |
| | **Unknown** | 27 | 0.1 | 100.0 | - |
| **Age** | **0-9** | 6284 | 16.1 | 16.1 | 1487.1 |
| | **10-19** | 3725 | 9.6 | 25.7 | 702.1 |
| | **20-29** | 6627 | 17.0 | 42.7 | 1296.4 |
| | **30-39** | 5376 | 13.8 | 56.5 | 986.0 |
| | **40-49** | 5411 | 13.9 | 70.4 | 796.9 |
| | **50-59** | 5047 | 12.9 | 83.3 | 828.0 |
| | **60-69** | 3415 | 8.8 | 92.1 | 886.2 |
| | **70-79** | 2019 | 5.2 | 97.2 | 764.6 |
| | **80+** | 1075 | 2.8 | 100.0 | 647.7 |

* Calculated using 2006 census (Statistics Canada, 2011). Rates are underestimated since only cases that were successfully geocoded were included

**Table 4.1b – Frequency table of successfully geocoded AGI cases from 2000-2013 (continued)**

| | | Frequency | Percent | Cumulative Percent | Per 100,000 Pop* |
|---|---|---|---|---|---|
| **Month** | **1** | 2854 | 7.3 | 7.3 | - |
| | **2** | 2722 | 7.0 | 14.3 | - |
| | **3** | 2694 | 6.9 | 21.2 | - |
| | **4** | 2871 | 7.4 | 28.6 | - |
| | **5** | 3242 | 8.3 | 36.9 | - |
| | **6** | 3685 | 9.5 | 46.4 | - |
| | **7** | 3986 | 10.2 | 56.6 | - |
| | **8** | 4303 | 11.0 | 67.6 | - |
| | **9** | 4048 | 10.4 | 78.0 | - |
| | **10** | 3197 | 8.2 | 86.2 | - |
| | **11** | 2788 | 7.2 | 93.4 | - |
| | **12** | 2589 | 6.6 | 100.0 | - |
| **HA** | **FHA** | 16592 | 42.6 | 42.6 | 1171.2 |
| | **IHA** | 5747 | 14.7 | 57.3 | 858.6 |
| | **NHA** | 1204 | 3.1 | 60.4 | 438.6 |
| | **VCHA** | 8839 | 22.7 | 83.1 | 869.4 |
| | **VIHA** | 6597 | 16.9 | 100.0 | 946.3 |
| **AGI** | **Campylobacteriosis** | 19721 | 50.6 | 50.6 | 479.4 |
| | **VTEC** | 1709 | 4.4 | 55.0 | 41.5 |
| | **Salmonellosis** | 9022 | 23.1 | 78.1 | 219.3 |
| | **Giardiasis** | 7328 | 18.8 | 96.9 | 178.1 |
| | **Cryptosporidiosis** | 1199 | 3.1 | 100.0 | 29.1 |

Health Authority (HA), Fraser Health Authority (FHA), Interior Health Authority (IHA), Northern Health Authority (NHA), Vancouver Coastal Health Authority (VCHA) and Vancouver Island Health Authority (VIHA)
* Calculated using 2006 census (Statistics Canada, 2011). Rates are underestimated since only cases that were successfully geocoded were included

Data analysis revealed that disproportionate numbers of cases with missing addresses occurred before the year 2000, as well as between the years 2006-2010 for the Vancouver Coastal Health Authority (VCHA) (Figures 4.1 – 4.3). Rates for Figure 4.2 were calculated using health authority population estimates provided by BC Stats (BC Stats, 2015). During that time, but to a lesser extent, the Northern Health Authority also missed more addresses, and for the single year of 2007, Vancouver Island Health

Authority missed over 40%. For a complete breakdown of the geocoding process, refer to Table 3.4.

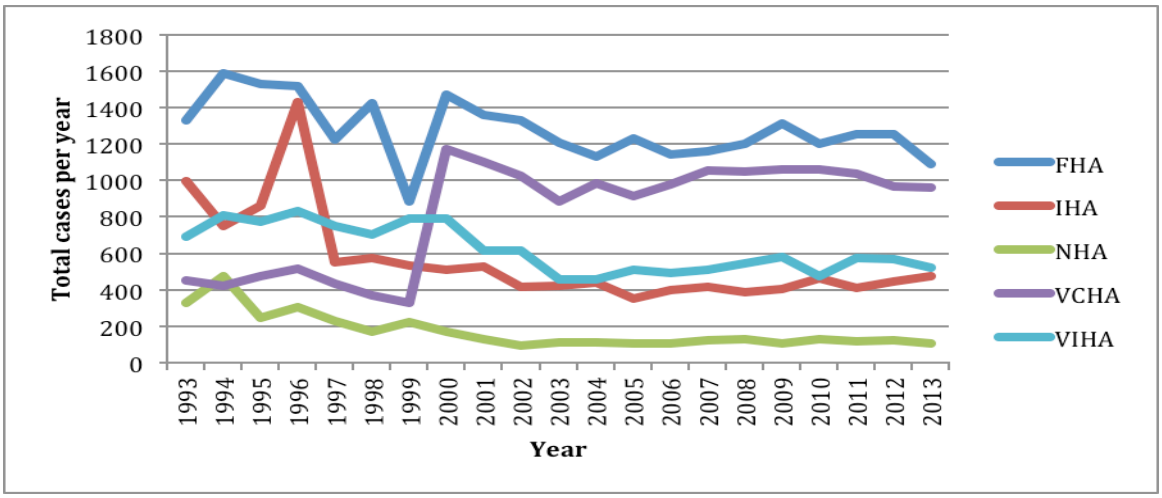


Figure 4.1 - Total AGI cases per year per health authority from 1993-2013 (including those with missing addresses)

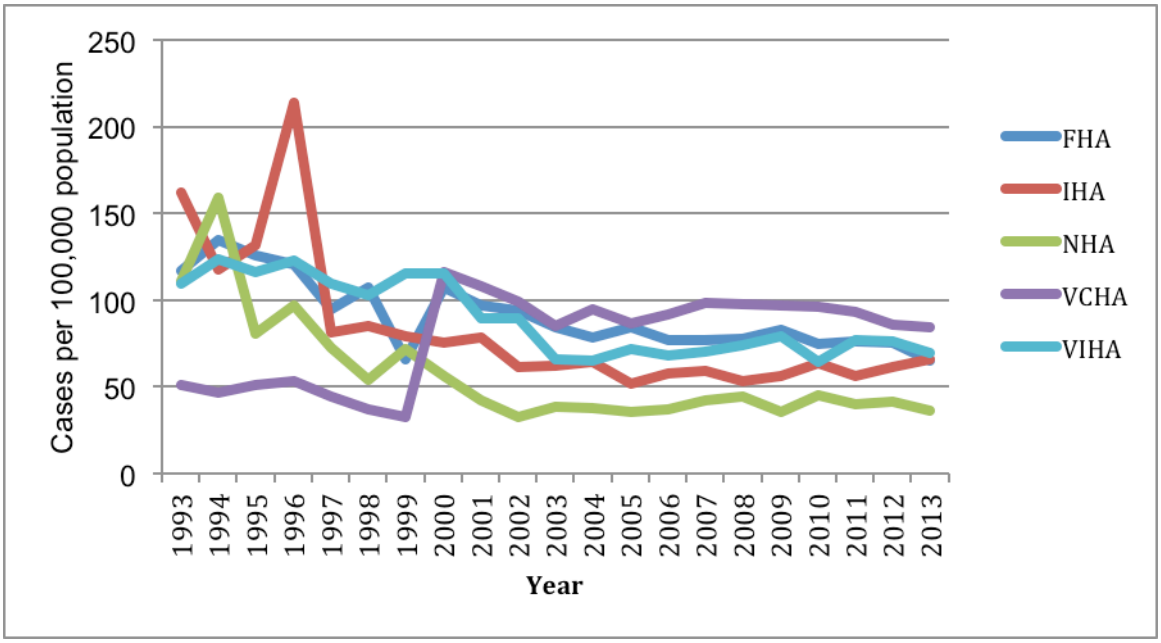


Figure 4.2 - Total AGI cases per 100,000 population per year per health authority from 1993-2013 (including those with missing addresses)

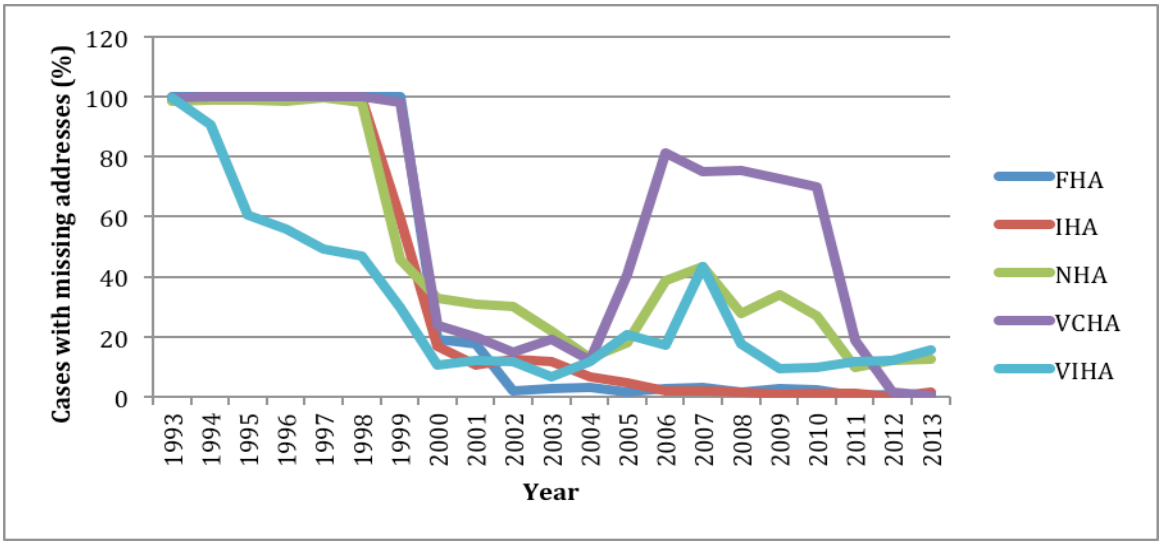


Figure 4.3 - Percent AGI cases with missing addresses per year per health authority from 1993-2013

## 4.2. Proportion estimates

### 4.2.1. BGC Zones

Due to the high numbers of missing addresses in the VCHA from 2006 to 2010 (70-80% of addresses missing during that time), missing values were imputed using the mean of the remaining years. A sensitivity analysis was performed on the estimates by repeating the AGI - climate analysis using values two standard deviations (SD) above and below the imputed values. The results tended to get stronger on the upper 2 SD and weaker on the lower 2 SD (Refer to Appendix B). It was decided that the imputed values were not likely to influence the results of the analysis significantly and so the entire 14 years of data (2000-2013) were used in the BGC zone analysis. Originally, there were a total of 38,914 cases in the 9 BGC zones (65 were removed because they resided in BGC zones with populations too small to include in the study). With imputed values, there were a total of 42,266 cases. The results of the one-sample test of proportions are presented in Tables 4.2 and 4.3 and Figure 4.4. 95% confidence intervals (CIs) are described as lower CI (CI-) and upper CI (CI+) and rates for each AGI are per 100,000 population. Refer to Table A1 in Appendix A for individual BGC zone definitions.

**Table 4.2 – AGI Rates per 100,000 among nine BGC zones (bacteria)**

| | Campylobacteriosis | | | VTEC | | | Salmonellosis | | |
|---|---|---|---|---|---|---|---|---|---|
| **BGC Zone** | Rate | CI - | CI + | Rate | CI - | CI + | Rate | CI - | CI + |
| **BG** | 28.42 | 25.86 | 31.22 | 4.04 | 3.13 | 5.21 | 14.37 | 12.58 | 16.41 |
| **BWBS** | 5.35 | 4.00 | 7.13 | 0.44 | 0.14 | 1.20 | 5.89 | 4.47 | 7.75 |
| **CDF** | 42.14 | 41.00 | 43.31 | 2.98 | 2.69 | 3.31 | 16.07 | 15.37 | 16.81 |
| **CWH** | 39.50 | 38.84 | 40.18 | 2.99 | 2.82 | 3.19 | 17.60 | 17.15 | 18.05 |
| **ICH** | 31.84 | 29.04 | 34.91 | 2.74 | 1.99 | 3.78 | 11.19 | 9.56 | 13.08 |
| **IDF** | 28.80 | 26.96 | 30.77 | 3.89 | 3.24 | 4.67 | 14.58 | 13.28 | 16.00 |
| **MS** | 32.97 | 24.65 | 43.97 | 2.02 | 0.52 | 6.43 | 15.48 | 10.05 | 23.63 |
| **PP** | 27.53 | 25.79 | 29.39 | 4.39 | 3.72 | 5.18 | 13.19 | 12.00 | 14.50 |
| **SBS** | 13.86 | 12.37 | 15.54 | 2.01 | 1.48 | 2.72 | 10.99 | 9.67 | 12.49 |

All results are statistically significant at p = 0.01
Difference between any two BGC zones is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

**Table 4.3 - AGI Rates per 100,000 among nine BGC zones (parasites)**

| | Giardiasis | | | Cryptosporidiosis | | |
|---|---|---|---|---|---|---|
| **BGC Zone** | Rate | CI - | CI + | Rate | CI - | CI + |
| **BG** | 8.60 | 7.23 | 10.21 | 3.01 | 2.24 | 4.05 |
| **BWBS** | 5.56 | 4.19 | 7.38 | 1.42 | 0.79 | 2.50 |
| **CDF** | 10.89 | 10.32 | 11.50 | 1.92 | 1.69 | 2.19 |
| **CWH** | 15.77 | 15.36 | 16.20 | 2.58 | 2.41 | 2.75 |
| **ICH** | 14.68 | 12.81 | 16.82 | 2.26 | 1.58 | 3.22 |
| **IDF** | 12.36 | 11.17 | 13.67 | 3.25 | 2.66 | 3.97 |
| **MS** | 16.15 | 10.58 | 24.43 | 1.35 | 0.23 | 5.43 |
| **PP** | 9.41 | 8.41 | 10.52 | 1.08 | 0.77 | 1.52 |
| **SBS** | 8.89 | 7.71 | 10.26 | 0.82 | 0.50 | 1.33 |

All results are statistically significant at p = 0.01
Difference between any two BGC zones is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

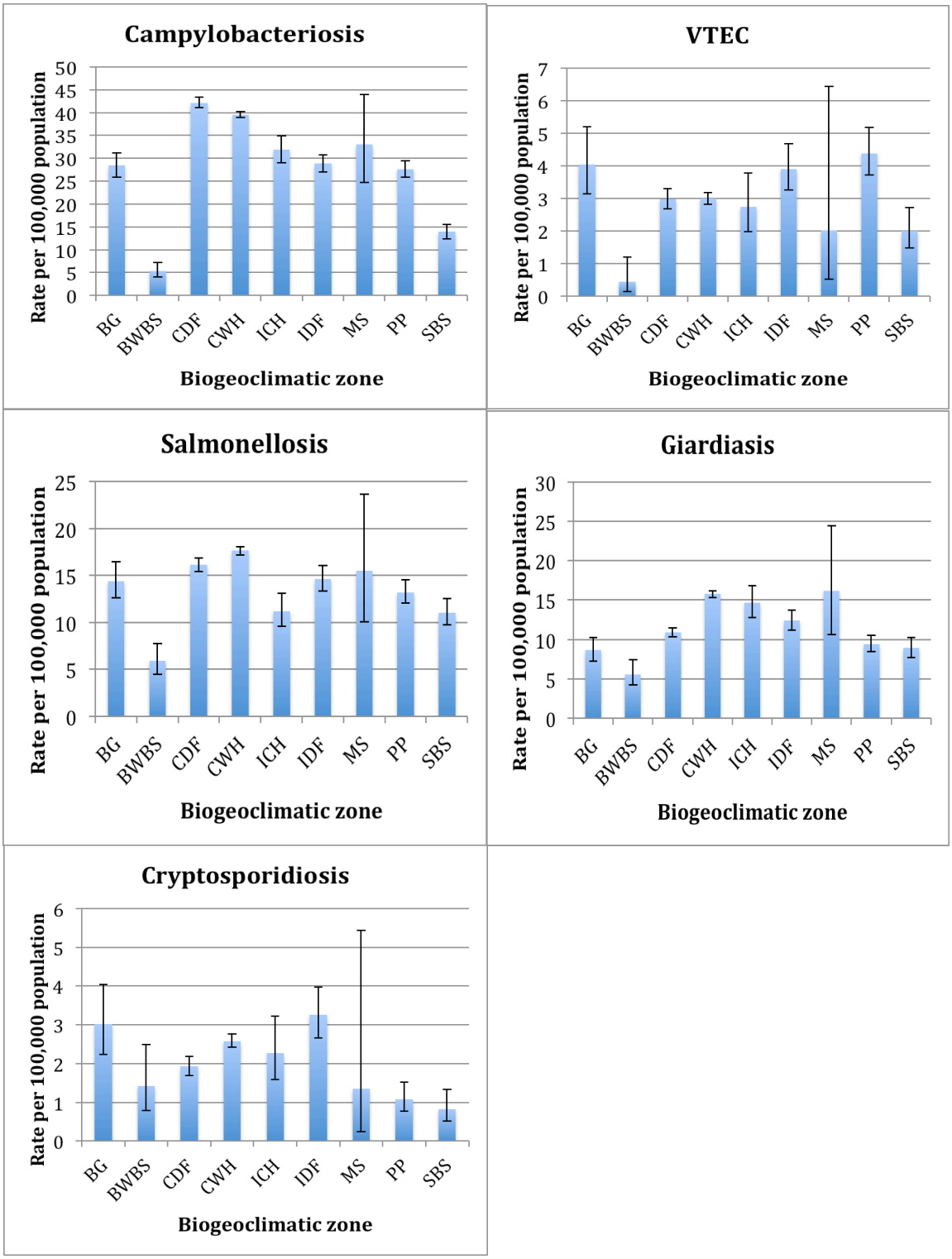


Figure 4.4 – AGI rates per 100,000 among nine BGC zones (all pathogens)

### 4.2.2. Agricultural Land Reserve

A total of 27,250 cases were included in this analysis from 2000-2005 and 2011-2013. Campylobacteriosis, salmonellosis and giardiasis had significantly higher rates of disease in the ALR watersheds than the non-ALR watersheds, while VTEC had an opposite relationship (Tables 4.4-4.5 and Figures 4.5-4.6). Of those under 10 years of age, there was a significantly higher rate of salmonellosis in non-ALR watersheds (Figures 4.7-4.8). A map of percent ALR per watershed is included in Appendix C (Figure C1), while Figure C2 presents the results of the AGI-ALR association when ALR is divided into five classes.

**Table 4.4 - AGI rates per 100,000 among watersheds with and without ALR (bacteria)**

| | **Campylobacteriosis** | | | **VTEC** | | | **Salmonellosis** | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rate | CI- | CI+ | Rate | CI- | CI+ | Rate | CI- | CI+ |
| **No ALR** | 40.84 | 39.54 | 42.18 | 2.58 | 2.27 | 2.94 | 17.46 | 16.61 | 18.35 |
| **ALR** | 35.84 | 35.15 | 36.54 | 3.24 | 3.04 | 3.46 | 15.68 | 15.23 | 16.15 |

All results are statistically significant at p = 0.01
Difference between ALR and No ALR is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

**Table 4.5 - AGI rates per 100,000 among watersheds with and without ALR (parasites)**

| | **Giardia** | CI- | CI+ | **Crypto** | CI- | CI+ |
|---|---|---|---|---|---|---|
| **No ALR** | 16.65 | 15.83 | 17.52 | 2.44 | 2.13 | 2.79 |
| **ALR** | 12.81 | 12.40 | 13.23 | 2.33 | 2.16 | 2.52 |

All results are statistically significant at p = 0.01
Difference between ALR and No ALR is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

### *AGI rate among ALR and non-ALR watersheds*

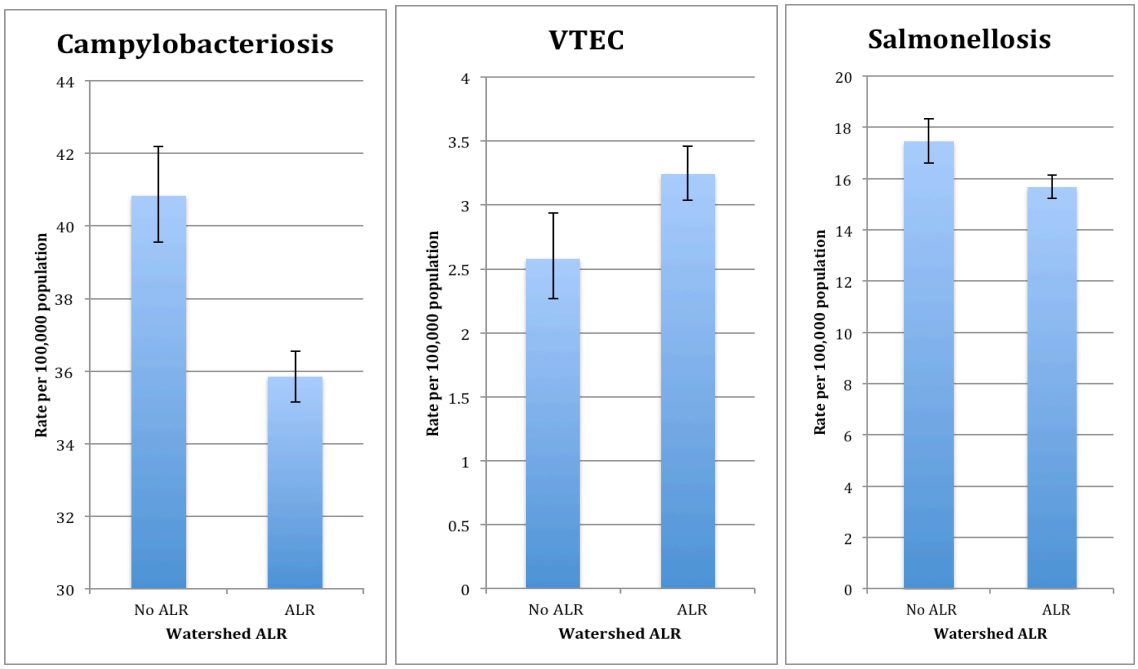


Figure 4.5 - AGI rates per 100,000 among watersheds with and without ALR (bacteria)

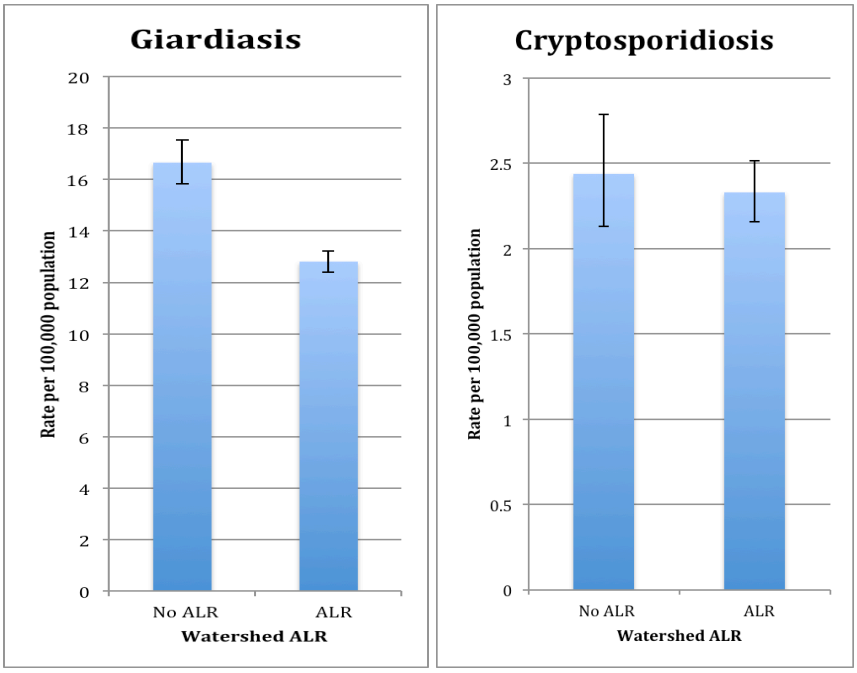


Figure 4.6 – AGI rates per 100,000 among watersheds with and without ALR (parasites)

### *ALR and children under 10 years of age*

A total of 4,509 cases were used in this analysis from 2000-2005 and 2011-2013.

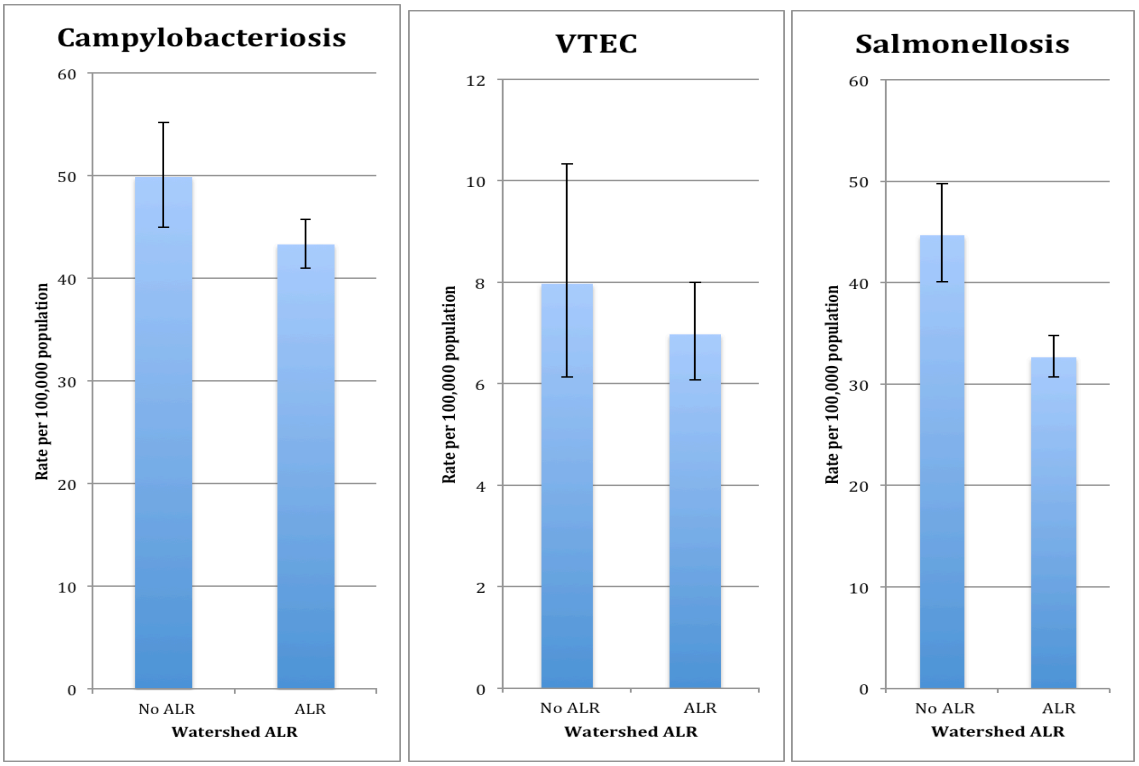


Figure 4.7 - AGI rates (under 10) among watersheds with and without ALR (bacteria)

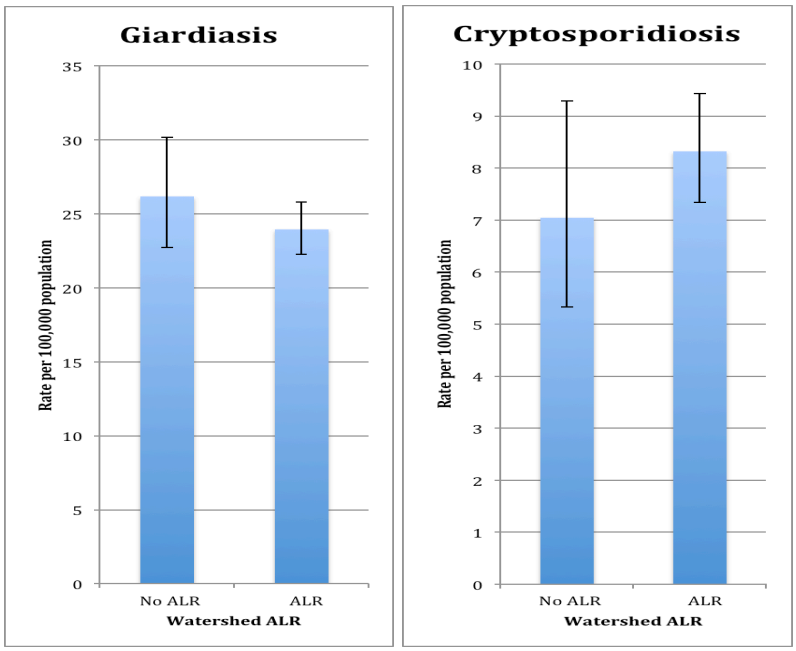


Figure 4.8 - AGI rate (under 10) among watersheds with and without ALR (parasites)

### 4.2.3. Population density (rural<400 persons/km$^2$)

A total of 27,254 cases were used in this analysis from 2000-2005 and 2011-2013. Campylobacteriosis, salmonellosis and giardiasis had higher rates of disease associated with urban population density (Tables 4.6-4.7 and Figure 4.9). A map of the urban and rural population distribution can be found in Appendix D, Figure D1.

**Table 4.6 - AGI rates per 100,000 among urban and rural populations (bacteria)**

| | Campylobacteriosis | | | VTEC | | | Salmonellosis | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rate | CI- | CI+ | Rate | CI- | CI+ | Rate | CI- | CI+ |
| **Rural** | 32.57 | 31.25 | 33.95 | 3.22 | 2.82 | 3.68 | 12.84 | 12.02 | 13.72 |
| **Urban** | 38.09 | 37.40 | 38.79 | 3.05 | 2.86 | 3.26 | 16.86 | 16.40 | 17.33 |

All results are statistically significant at p = 0.01
Difference between Rural and Urban is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

**Table 4.7 - AGI rates per 100,000 among urban and rural populations (parasites)**

| | Giardiasis | | | Cryptosporidiosis | | |
|---|---|---|---|---|---|---|
| | Rate | CI- | CI+ | Rate | CI- | CI+ |
| **Rural** | 12.47 | 11.66 | 13.33 | 2.24 | 1.90 | 2.62 |
| **Urban** | 14.03 | 13.62 | 14.46 | 2.39 | 2.22 | 2.57 |

All results are statistically significant at p = 0.01
Difference between Rural and Urban is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

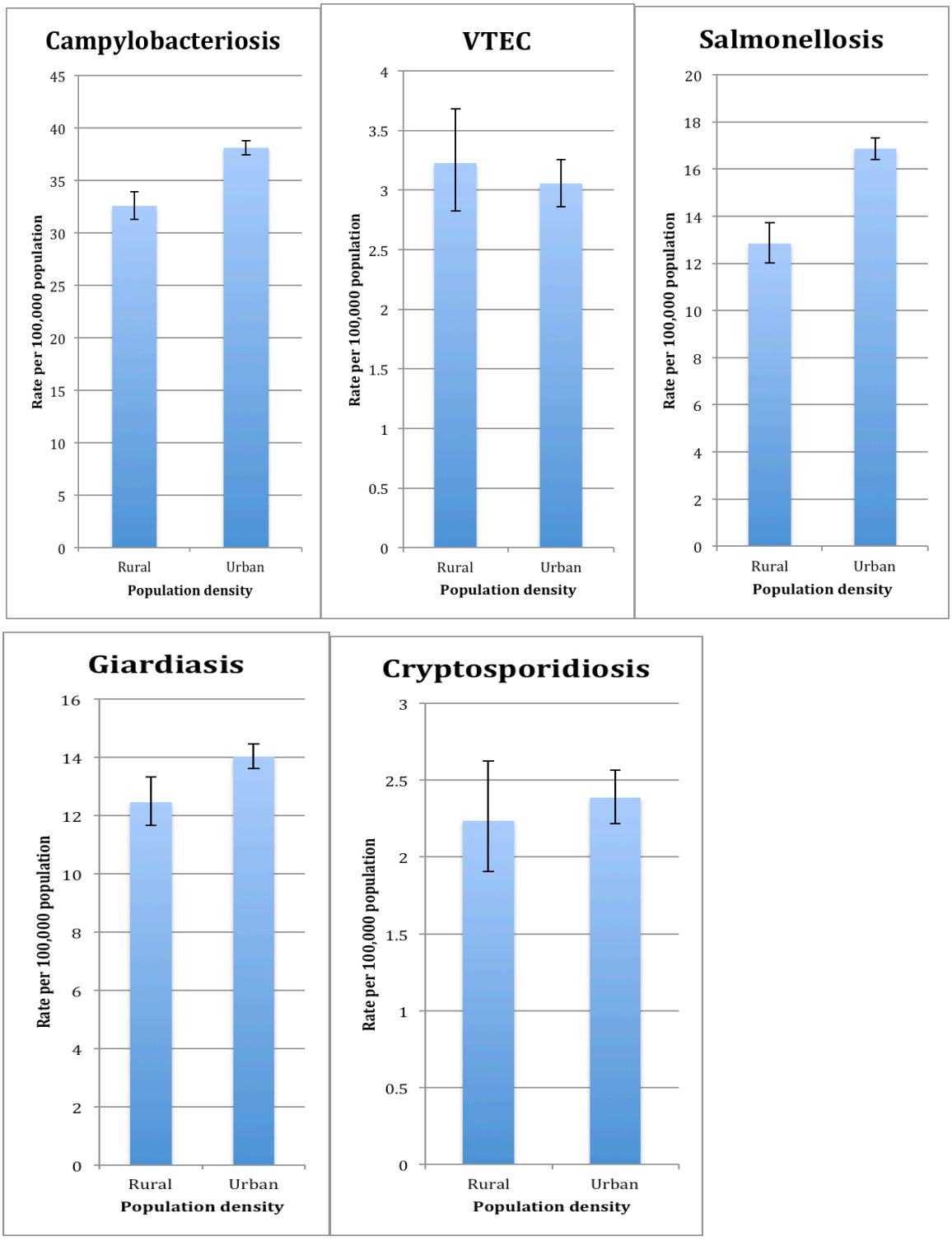


Figure 4.9 - AGI rates per 100,000 among urban and rural populations

### 4.2.4. Aquifer type in the rural population (<400 persons/km$^2$)

A total of 3,172 cases were used in this analysis. The spatial distribution of aquifers in BC is attached in Appendix E (Figure E1). Aside from BGC zones, this variable was the only other variable that imputed the missing addresses from 2006-2010. The sensitivity analysis (Appendix B - Table B3) shows that the proportions did change when compared to the results with those years omitted. Therefore, it was decided to omit the years 2006-2010 for all of the other variables except BGC zone. When analysed individually, campylobacterioisis and salmonellosis had significantly higher rates associated with unconsolidated aquifers (Tables 4.8-4.9 and Figure 4.13). When combined into bacteria and parasites, the two groups both had significantly higher rates associated with unconsolidated aquifers (Table 4.10 and Figure 4.13b).

**Table 4.8 - Rural AGI rates per 100,000 associated with two aquifer types (bacteria)**

| | Campylobacteriosis | | | VTEC | | | Salmonellosis | | |
|---|---|---|---|---|---|---|---|---|---|
| | Rate | CI- | CI+ | Rate | CI- | CI+ | Rate | CI- | CI+ |
| **Bedrock** | 33.97 | 31.28 | 36.89 | 2.65 | 1.96 | 3.58 | 11.48 | 9.95 | 13.24 |
| **Unconsolidated** | 40.15 | 37.84 | 42.59 | 4.14 | 3.44 | 4.99 | 15.32 | 13.91 | 16.86 |

All results are statistically significant at p = 0.01
Difference between Bedrock and Unconsolidated is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

**Table 4.9 - Rural AGI rates per 100,000 associated with two aquifer types (parasites)**

| | Giardiasis | | | Cryptosporidiosis | | |
|---|---|---|---|---|---|---|
| | Rate | CI- | CI+ | Rate | CI- | CI+ |
| **Bedrock** | 11.07 | 9.57 | 12.80 | 1.94 | 1.36 | 2.76 |
| **Unconsolidated** | 14.13 | 12.78 | 15.61 | 3.17 | 2.56 | 3.93 |

All results are statistically significant at p = 0.01
Difference between Bedrock and Unconsolidated is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

**Table 4.10 - Rural AGI rates per 100,000 associated with two aquifer types (bacteria and parasites combined and analyzed separately)**

| | Bacteria | | | Parasites | | |
|---|---|---|---|---|---|---|
| | Rate | CI- | CI+ | Rate | CI- | CI+ |
| **Bedrock** | 48.10 | 44.89 | 51.55 | 13.01 | 11.38 | 14.88 |
| **Unconsolidated** | 59.61 | 56.78 | 62.57 | 17.30 | 15.80 | 18.93 |

All results are statistically significant at p = 0.01

Difference between Bedrock and Unconsolidated is significant (p=0.05) when range between upper and lower confidence intervals (CI+ and CI-, respectively) do not overlap one another

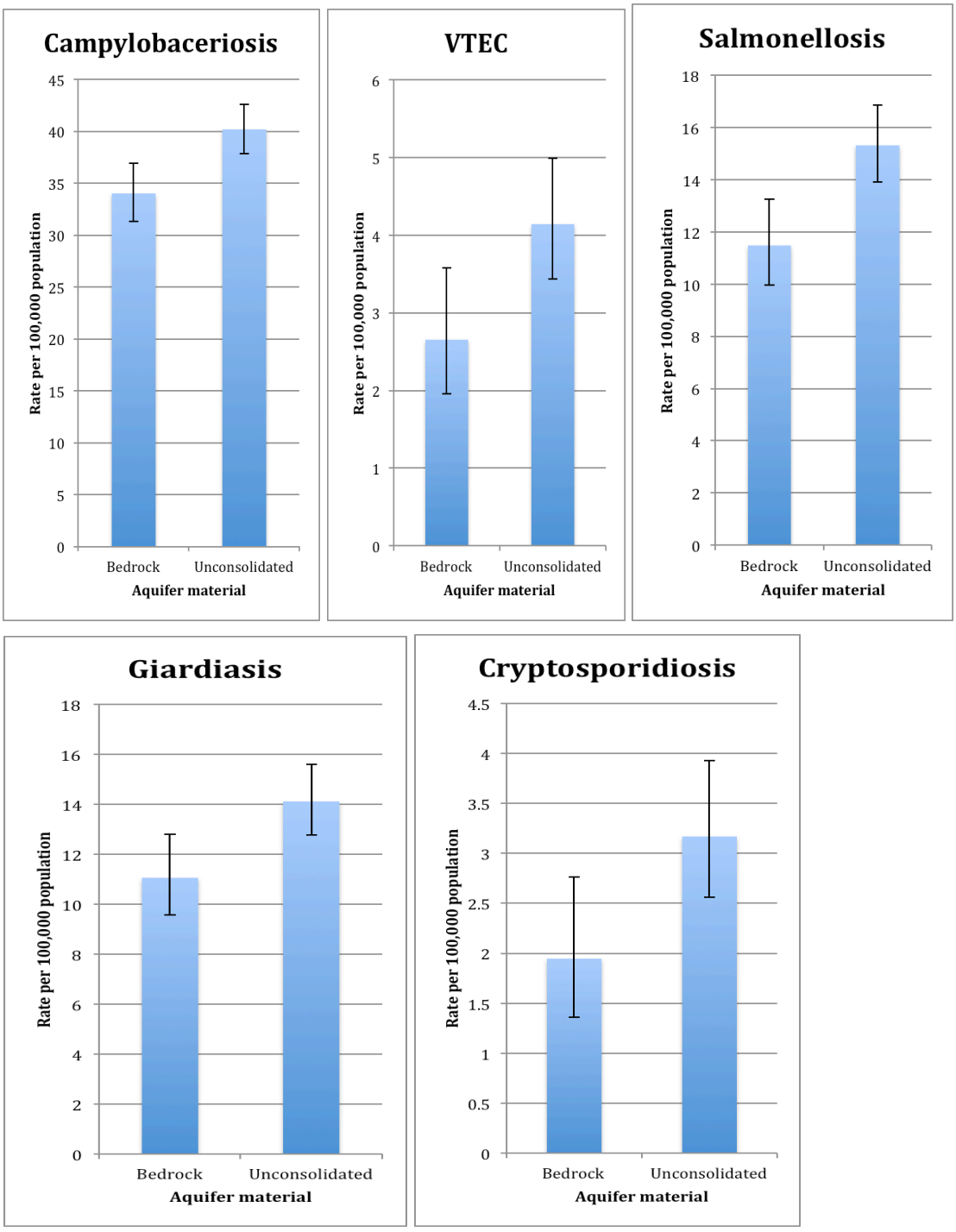


Figure 4.13 - Rural AGI rates per 100,000 associated with two aquifer types

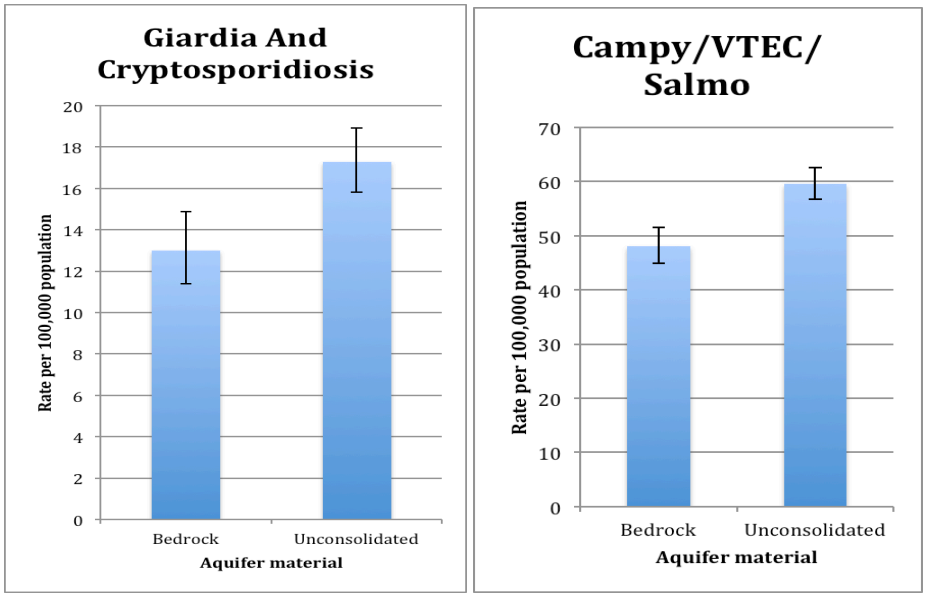


Figure 4.13b - Rural AGI rates grouped by bacteria and parasites associated with two aquifer types

## 4.3. Bivariate Pearson's Correlation tests

A total of 42,266 cases were included in this analysis from 2000-2013, of which 3,352 were imputed (7.9%). Campylobacteriosis was positively correlated with salmonellosis and giardiasis, while giardiasis was also correlated with salmonellosis (Table 4.12).

**Table 4.12 - Bivariate Pearson's correlation results among five AGIs only (rates calculated per BGC zone)**

| | | Campy | VTEC | Salmo | Giard | Crypt |
|---|---|---|---|---|---|---|
| **Campylobacteriosis** | R | 1 | 0.576 | **0.898**** | **0.730*** | 0.433 |
| | sig. | | 0.104 | 0.001 | 0.026 | 0.244 |
| **VTEC** | R | 0.576 | 1 | 0.627 | 0.197 | 0.479 |
| | sig. | 0.104 | | 0.071 | 0.612 | 0.192 |
| **Salmonellosis** | R | **0.898**** | 0.627 | 1 | **0.669*** | 0.415 |
| | sig. | 0.001 | 0.071 | | 0.049 | 0.266 |
| **Giardiasis** | R | **0.730*** | 0.197 | **0.669*** | 1 | 0.272 |
| | sig. | 0.026 | 0.612 | 0.049 | | 0.479 |
| **Cryptosporidiosis** | R | 0.433 | 0.479 | 0.415 | 0.272 | 1 |
| | sig. | 0.244 | 0.192 | 0.266 | 0.479 | |

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed)
Rates are calculated at the BGC zone scale (nine in total) and compared to one another. For example, a high correlation between two AGIs means that they both have high rates and low rates in the same BGC zones.

The parasites were not correlated with any of the variables, whereas the bacteria were all coordinated with many temperature-related variables. Generally, salmonellosis and campylobacterioisis shared similar trends, while VTEC was notably different. When combined, all of the bacteria together were also correlated with many of the temperature-related variables. All correlation results with climatic variables are included in Tables 4.13 and 4.13b, with figures 4.10 to 4.12 displaying some of the stronger correlations as scatter plots (Refer to Table 3.2 for descriptions of climatic terms).

**Table 4.13 - Bivariate Pearson's correlation results among five AGIs and climatic variables**

| | | Campy | VTEC | Salmo | Giard | Crypt | Bacteria | Parasites |
|---|---|---|---|---|---|---|---|---|
| **Annual temp.** | R | **0.717*** | **0.749*** | **0.671*** | 0.093 | 0.335 | **0.743*** | 0.159 |
| | sig. | 0.030 | 0.020 | 0.048 | 0.811 | 0.378 | 0.022 | 0.683 |
| **Temp. of the warmest month** | R | 0.429 | **0.870**** | 0.424 | -0.158 | 0.362 | 0.483 | -0.066 |
| | sig. | 0.249 | 0.002 | 0.256 | 0.685 | 0.338 | 0.188 | 0.866 |
| **Temp. of the coldest month** | R | **0.846**** | 0.581 | **0.774*** | 0.306 | 0.288 | **0.849**** | 0.344 |
| | sig. | 0.004 | 0.101 | 0.014 | 0.423 | 0.452 | 0.004 | 0.365 |
| **Continentality** | R | **-0.830**** | -0.224 | **-0.740*** | -0.488 | -0.153 | **-0.802**** | -0.482 |
| | sig. | 0.006 | 0.562 | 0.023 | 0.182 | 0.695 | 0.009 | 0.189 |
| **Annual precip.** | R | 0.401 | -0.214 | 0.382 | 0.500 | 0.127 | 0.367 | 0.487 |
| | sig. | 0.285 | 0.581 | 0.310 | 0.170 | 0.744 | 0.331 | 0.183 |
| **Growing season precip.** | R | -0.119 | -0.587 | -0.144 | 0.281 | -0.033 | -0.167 | 0.251 |
| | sig. | 0.760 | 0.096 | 0.712 | 0.464 | 0.933 | 0.667 | 0.515 |
| **Annual heat-moisture index** | R | -0.041 | **0.706*** | 0.059 | -0.407 | 0.197 | 0.039 | -0.331 |
| | sig. | 0.918 | 0.033 | 0.880 | 0.277 | 0.612 | 0.921 | 0.384 |
| **Summer heat-moisture index** | R | 0.251 | **0.806**** | 0.289 | -0.293 | 0.161 | 0.315 | -0.234 |
| | sig. | 0.514 | 0.009 | 0.451 | 0.444 | 0.678 | 0.409 | 0.545 |
| **Degree-days below 0°C** | R | **-0.838**** | **-0.773*** | **-0.800**** | -0.301 | -0.361 | **-0.864**** | -0.355 |
| | sig. | 0.005 | 0.015 | 0.010 | 0.432 | 0.341 | 0.003 | 0.349 |

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Refer to Table 3.2 for descriptions of climatic terms

**Table 4.13b - Bivariate Pearson's correlation results among five AGIs and climatic variables (continued)**

| | | Campy | VTEC | Salmo | Giard | Crypt | Bacteria | Parasites |
|---|---|---|---|---|---|---|---|---|
| **Degree-days above 5 °C** | R | 0.543 | **0.766*** | 0.500 | -0.127 | 0.304 | 0.577 | -0.050 |
| | sig. | 0.131 | 0.016 | 0.170 | 0.745 | 0.426 | 0.104 | 0.899 |
| **Degree-days below 18 °C** | R | **-0.680*** | **-0.747*** | -0.613 | -0.027 | -0.298 | **-0.703*** | -0.090 |
| | sig. | 0.044 | 0.021 | 0.079 | 0.945 | 0.435 | 0.035 | 0.818 |
| **Degree-days above 18 °C** | R | 0.276 | **0.813**** | 0.283 | -0.282 | 0.176 | 0.333 | -0.220 |
| | sig. | 0.472 | 0.008 | 0.460 | 0.463 | 0.651 | 0.381 | 0.569 |
| **Frost-free days** | R | **0.674*** | 0.467 | 0.589 | 0.090 | 0.252 | **0.671*** | 0.137 |
| | sig. | 0.046 | 0.205 | 0.095 | 0.818 | 0.514 | 0.048 | 0.724 |
| **Frost-free period** | R | 0.654 | 0.474 | 0.557 | 0.044 | 0.238 | 0.649 | 0.093 |
| | sig. | 0.056 | 0.197 | 0.119 | 0.910 | 0.537 | 0.058 | 0.813 |
| **Annual snowfall** | R | -0.149 | -0.609 | -0.130 | 0.499 | -0.193 | -0.188 | 0.416 |
| | sig. | 0.702 | 0.082 | 0.739 | 0.171 | 0.619 | 0.628 | 0.265 |
| **Extreme min. temp** | R | **0.747*** | 0.487 | 0.638 | 0.158 | 0.230 | **0.737*** | 0.195 |
| | sig. | 0.021 | 0.184 | 0.064 | 0.685 | 0.551 | 0.023 | 0.615 |
| **Extreme max. temp** | R | 0.520 | **0.942**** | 0.577 | 0.030 | 0.477 | 0.591 | 0.131 |
| | sig. | 0.151 | 0 | 0.104 | 0.940 | 0.194 | 0.094 | 0.736 |
| **Reference Evap.** | R | 0.557 | **0.963**** | 0.597 | 0.025 | 0.437 | 0.624 | 0.119 |
| | sig. | 0.120 | 0.000 | 0.089 | 0.948 | 0.240 | 0.072 | 0.761 |
| **Climatic moisture deficit** | R | 0.229 | **0.851**** | 0.305 | -0.223 | 0.275 | 0.306 | -0.145 |
| | sig. | 0.554 | 0.004 | 0.425 | 0.564 | 0.473 | 0.424 | 0.710 |

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Refer to Table 3.2 for descriptions of climatic terms

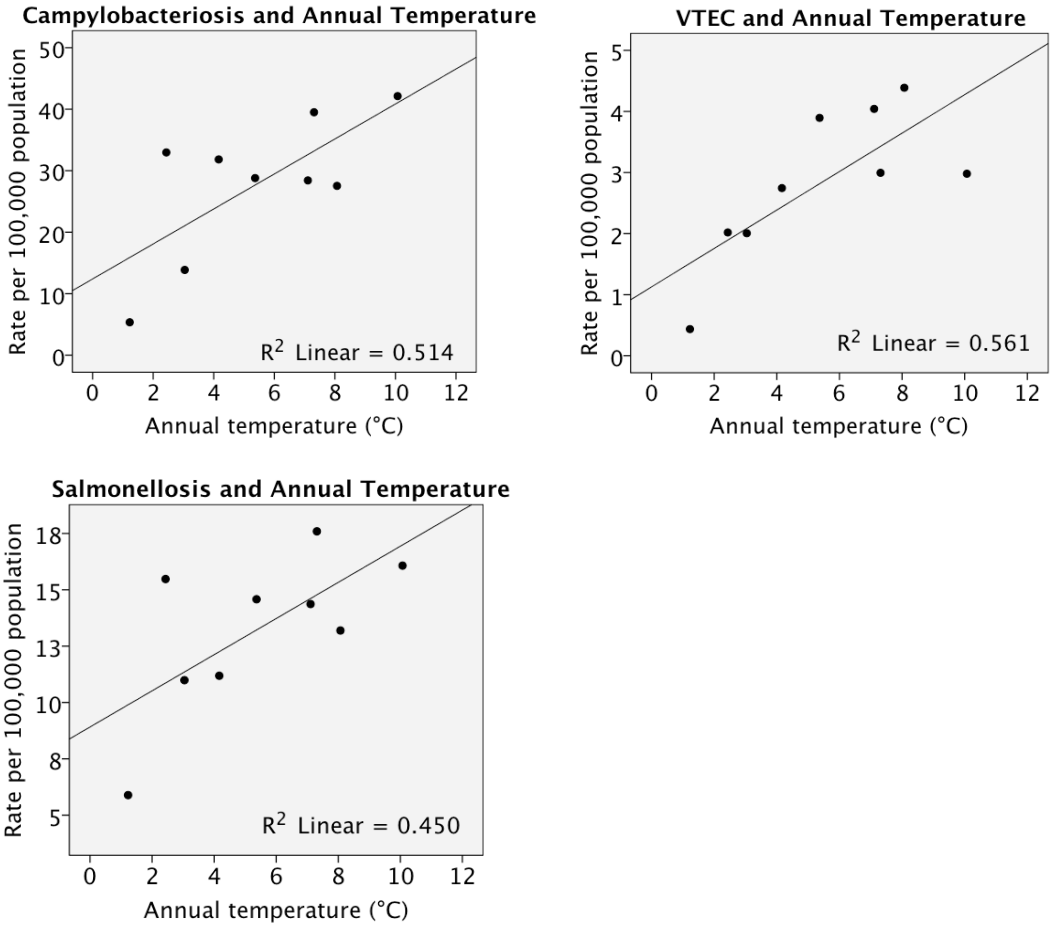


Figure 4.10 - Associations between bacterial AGIs and annual temperature at the biogeoclimatic zone level

All three associations from Figure 4.10 are significant when p=0.05. For definitions of climatic variables, refer to Table 3.2.

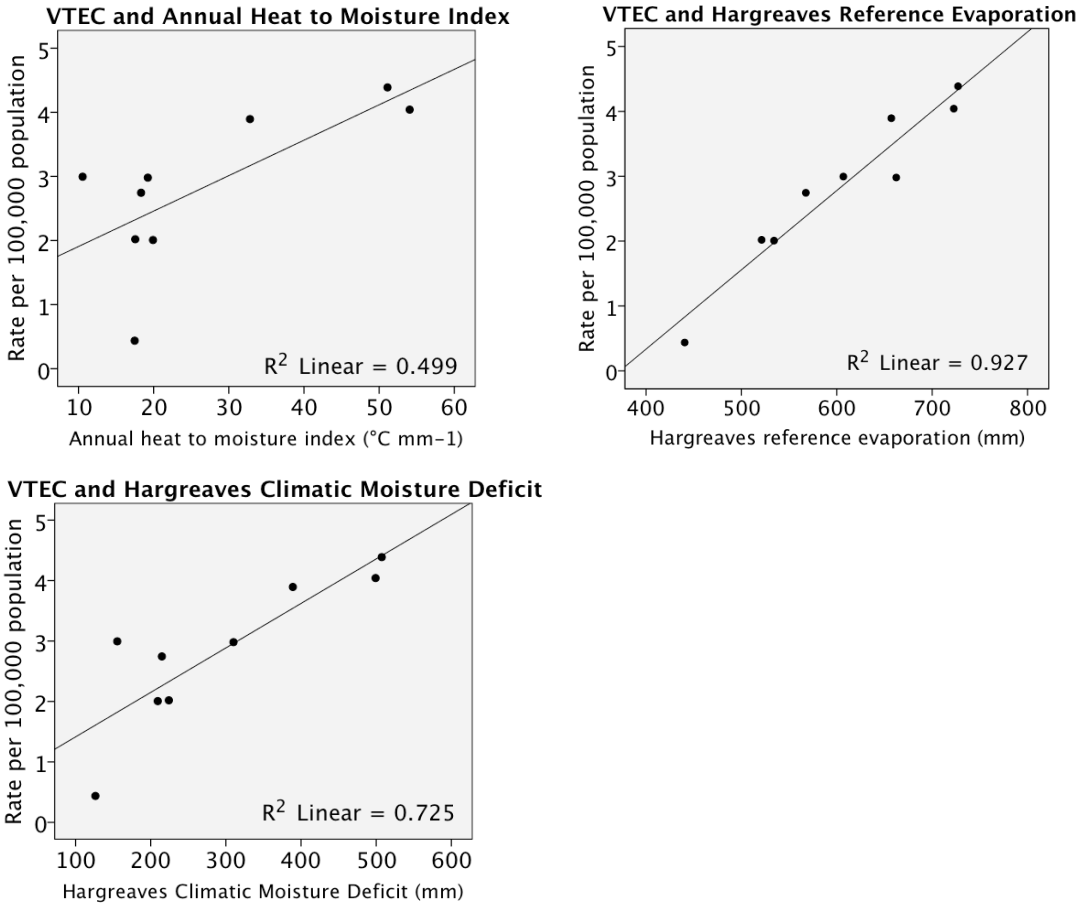


Figure 4.11 - Associations between VTEC and moisture-related variables at the biogeoclimatic zone level

All three associations from Figure 4.11 are significant when p=0.05. For definitions of climatic variables, refer to Table 3.2.

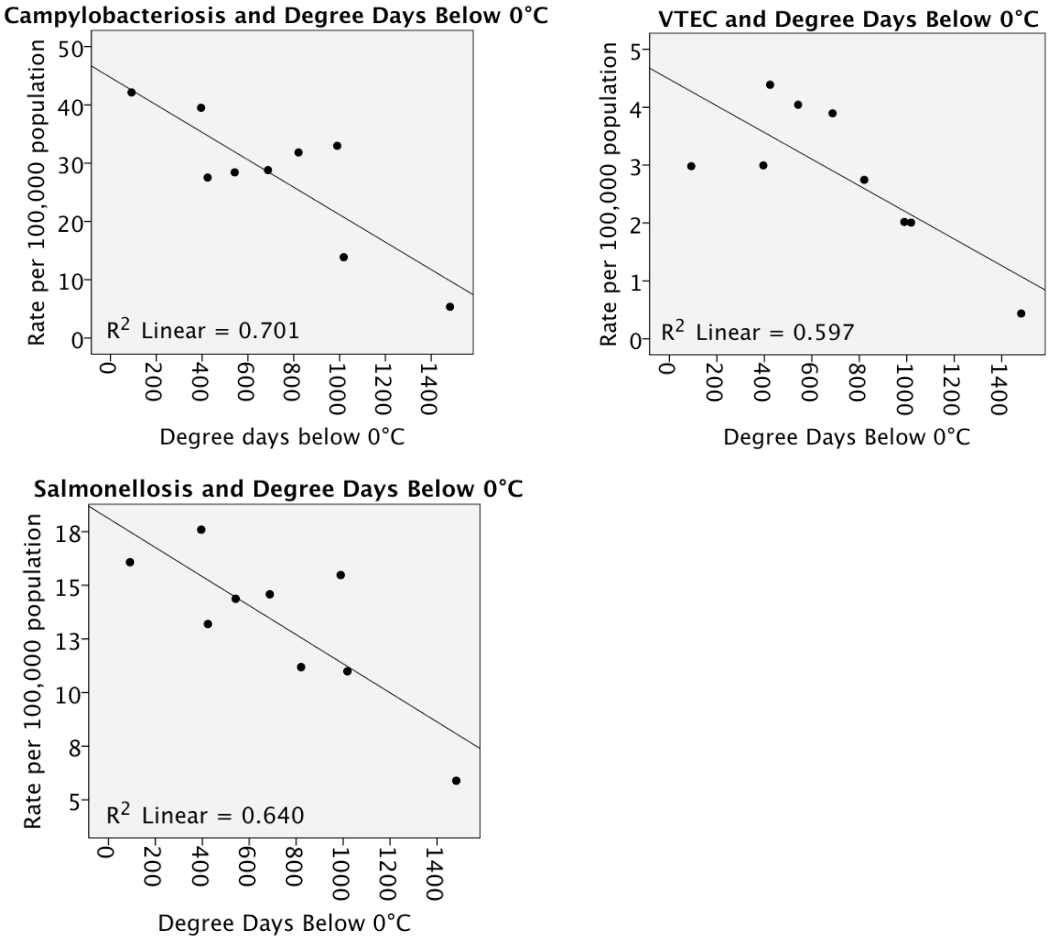


Figure 4.12 - Associations between bacterial AGIs and degree-days below 0°C at the biogeoclimatic zone level

All three associations from Figure 4.12 are significant when p=0.05. For definitions of climatic variables, refer to Table 3.2.

## 4.4. Regression models

A total of 42,266 cases were included in this analysis. Due to the high degree of collinearity among many of the climatic variables, only one variable was selected for the model (mean annual temperature) (Table 4.14). The regression output is included in Appendix F.

**Table 4.14 - Negative binomial univariate regression results for mean annual temperature ($^oC$)**

| | Coefficient ($e^x$) | Std. Error | z value | p |
|---|---|---|---|---|
| **Campylobacteriosis** | 1.12068 | 0.04902 | 2.324 | 0.0201 * |
| **VTEC** | 1.13780 | 0.04450 | 2.902 | 0.00371 ** |
| **Salmonellosis** | 1.07641 | 0.02221 | 3.315 | 0.000916 *** |
| **Bacteria (all 3)** | 1.11189 | 0.03862 | 2.746 | 0.00603 ** |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05

## 4.5. Population attributable risk (PAR) and provincial AGI projections for 2080s

Population attributable risk (PAR) of the bacteria was calculated using the coefficients from the regression model and results are presented in Table 4.15. The following equation was used:

$$PAR = p\,(RR - 1) / \{p(RR-1) + 1\} \qquad \text{(Equation 1)}$$

where p=proportion exposed (in this case it is 1 because everyone is exposed), and RR=relative risk.

Cases associated with climate change are projected for the 2080s (Table 4.15). Intermediary steps for this calculation, as well as estimates for current annual bacterial AGI rates that account for under-reporting and under-diagnosis, are shown in Table 4.16.

**Table 4.15 - Population attributable risk of 1°C increase in mean annual temperature for bacterial AGIs and projections for 2080s**

| | | Projections by 2080s associated with climate change (Increase in mean annual temperature of 1.5°C - 4.1°C)* | |
|---|---|---|---|
| | PAR | Percent | Additional cases province-wide per year ** |
| **Campylobacteriosis** | 0.10768 | 16.2% - 44.1% | 10554- 28847 |
| **VTEC** | 0.12111 | 18.2% - 49.7% | 741- 2025 |
| **Salmonellosis** | 0.07099 | 10.6% - 29.1% | 3011- 8229 |
| **Bacteria (all 3)** | 0.10063 | 15.1% - 41.3% | 14305- 39101 |

These projections only account for observed association with mean annual temperature on BGC zone scale and do not necessarily account for climate-related associations that may be occurring on other spatiotemporal scales. The projections assume that temperature will increase evenly across BC, which it likely will not.

* Pacific Climate Impacts Consortium (2013)

** The calculation used mean AGI from 2000-2013 and mean BC population from 2000-2013 to estimate future burden of disease in 2041 based on population projection at that time. The 2041 BC population projection of 6,118,000 (BC Stats, 2015) was used to represent the 2080s. Due to under-reporting and under-diagnosis of these AGIs, the following multipliers were used on the 2000-2013 mean annual AGI counts: Campylobacteriosis (27.2), VTEC (20.1) and salmonellosis (26.1) (Thomas et al., 2013).

**Table 4.16 – Projected annual bacterial AGI cases in 2080s with and without climate change**

| | Mean annual cases (2000-2013) | | Projected annual cases (2041) (no climate change) | Additional annual projected cases (2080s) under climate change | |
|---|---|---|---|---|---|
| | | | | Mean annual temperature change | |
| **AGI** | **Reported** | **Estimated with multipliers** | | **+1.5°C** | **+4.1°C** |
| Campy. | 1692 | 46020 | 65340 | 10554 | 28847 |
| VTEC | 143 | 2873 | 4079 | 741 | 2025 |
| Salmo. | 763 | 19912 | 28272 | 3011 | 8229 |

Refer to Table 4.15 notes for summary of steps involved in the calculation

For every 1°C of annual temperature warming that occurs, the PAR describes the percentage of additional AGI cases that can be expected to occur as a result. The inverse is also true, for every 1°C increase in temperature that is avoided this is the percentage of AGI cases that can be prevented. The values were: campylobacteriosis (10.8%), VTEC (12.1%), Salmonellosis (7.1%) and for all three bacteria combined (10.1%). In BC, an increase of 2.7°C is projected by 2080s using an ensemble of Representative Concentration Pathway (RCP) scenarios, with a range of between 1.5°C to 4.1°C (Pacific Climate Impacts Consortium, 2013), which translates to a 15.2% to

41.4% increase in the number of bacterial AGI cases. The PAR of these pathogens was combined with population projections and multipliers (to account for under-diagnosis and under-reporting) to estimate future additional AGI cases annually for the 2080s (Table 4.15). These were: Campylobacteriosis (10554-28847), VTEC (741-2025), salmonellosis (3011-8229), and all three combined: 14305-39101.

No associations between annual precipitation and AGI were observed at the BGC zone scale but because precipitation patterns are projected to change in BC to due climate change (Pacific Climate Impacts Consortium, 2013), a precipitation-AGI association to climate change could exist at finer temporal and spatial scales than those used in this study. It is important to keep in mind that any projections for future climate scenarios are limited not only by the complexity of the ecological systems that can have surprising and unexpected feedback loops and thresholds, but also by our inability to predict future technological advances and other social processes that will influence the amount of GHGs emitted in the future.

## 4.6. Biogeoclimatic zone climate envelope model AGI projections

The following projections (Figures 4.13 – 4.27) display the rate per 100,000 population as calculated by 42,266 cases. By the 2080s there is a noticeable province-wide increase in the spatial distribution of most AGIs. In many cases, BGC zones migrate to higher latitudes and altitudes, sometimes resulting in large expansions of area and in other BGC zones resulting in drastic reductions.

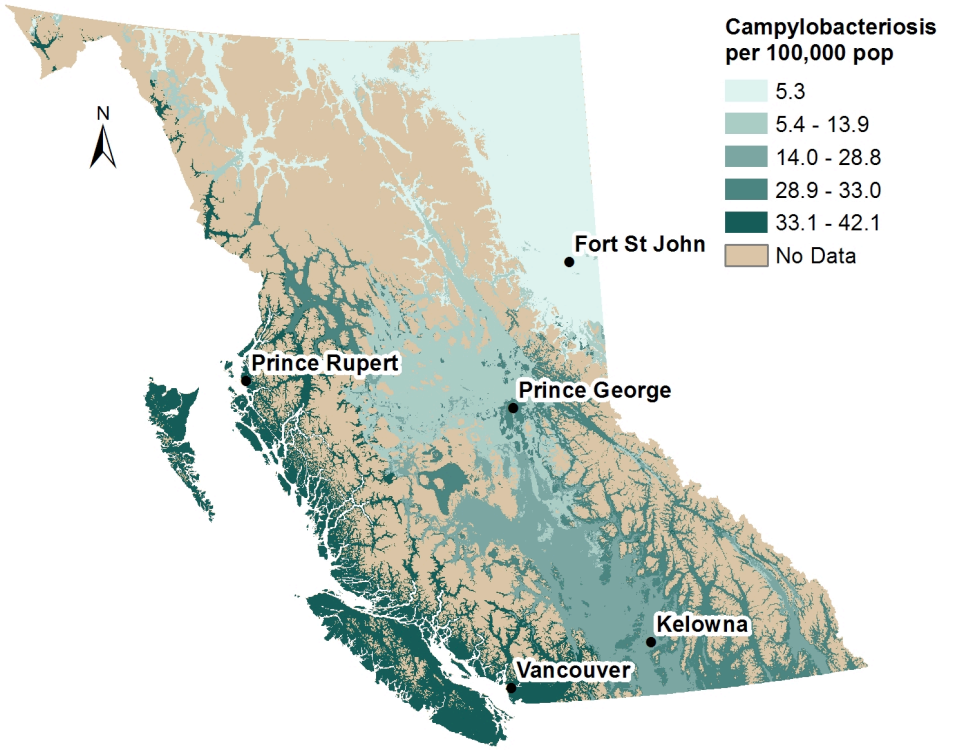


Figure 4.13 - Campylobacter incidence by BGC zone (2010s)

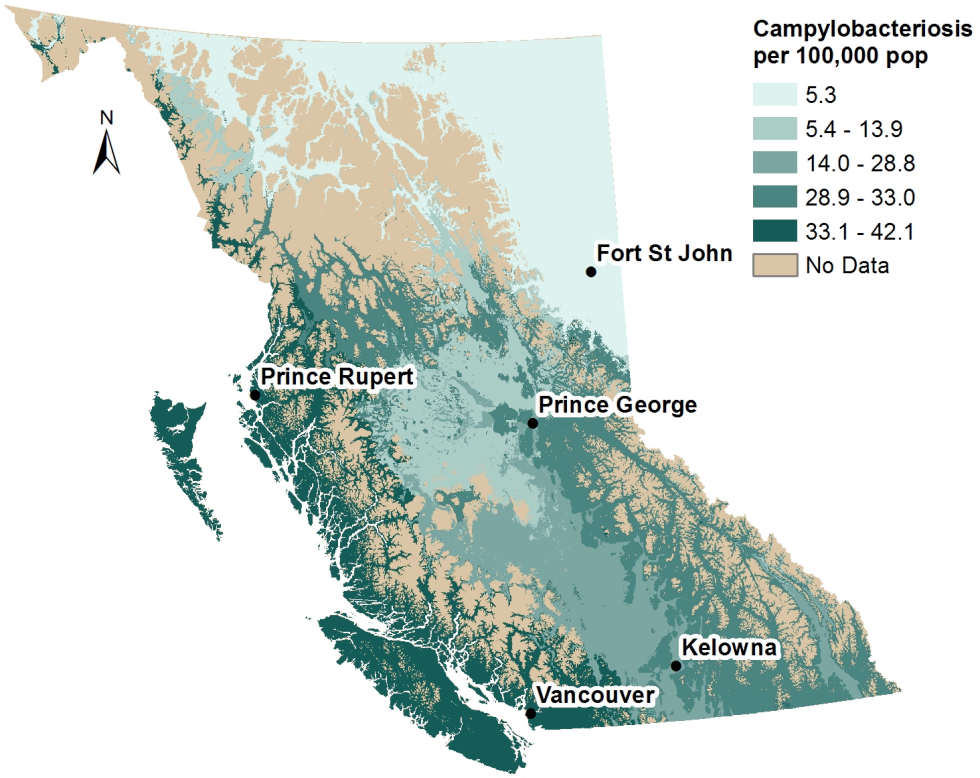


Figure 4.14 - Campylobacter incidence by BGC zone (2050s)

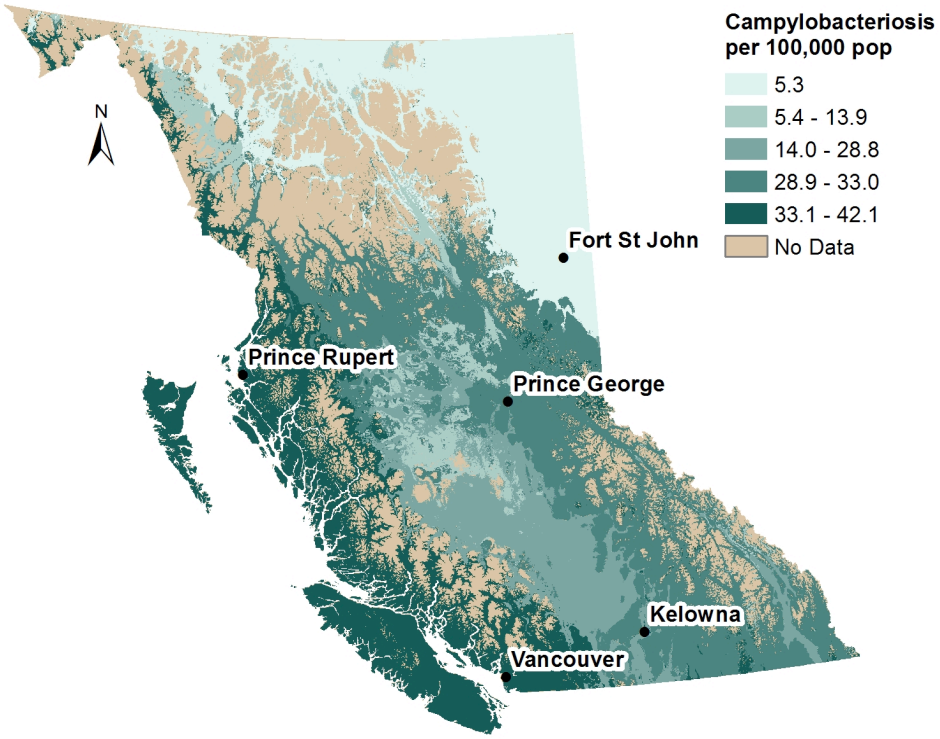


Figure 4.15 Campylobacter incidence by BGC zone (2080s)

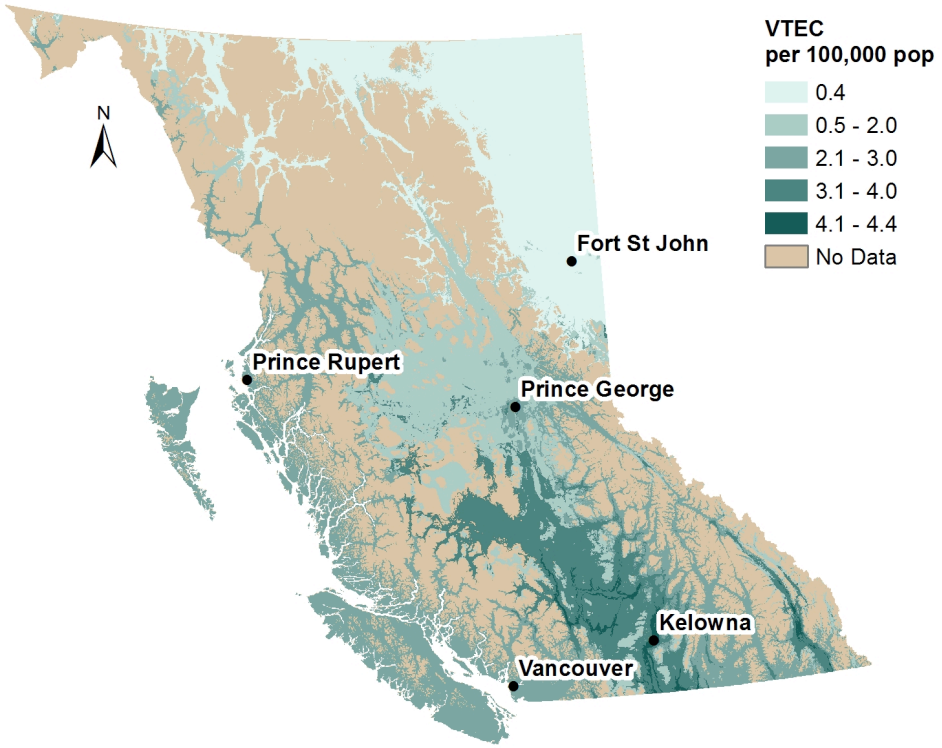


Figure 4.16 - VTEC incidence by BGC zone (2010s)

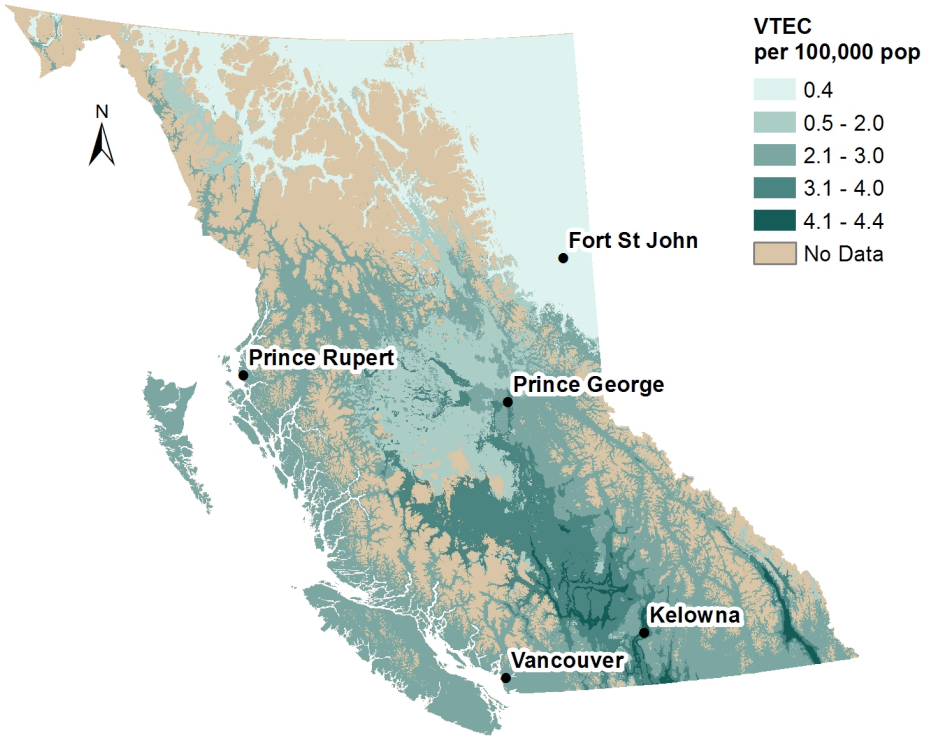


Figure 4.17 - VTEC incidence by BGC zone (2050s)

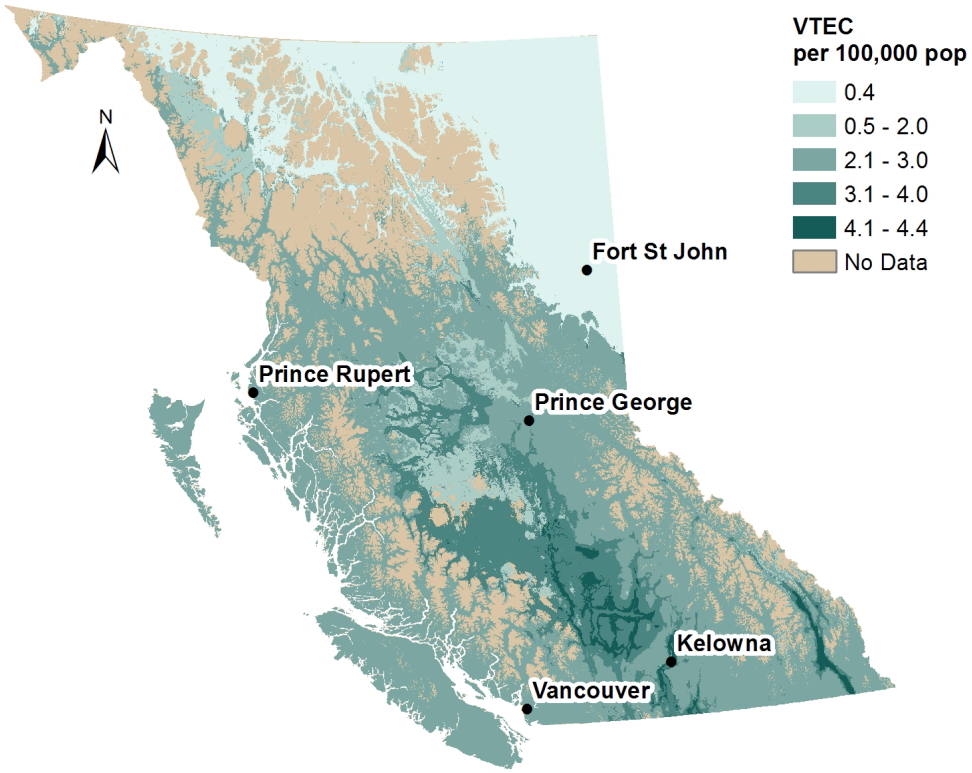


Figure 4.18- VTEC incidence by BGC zone (2080s)

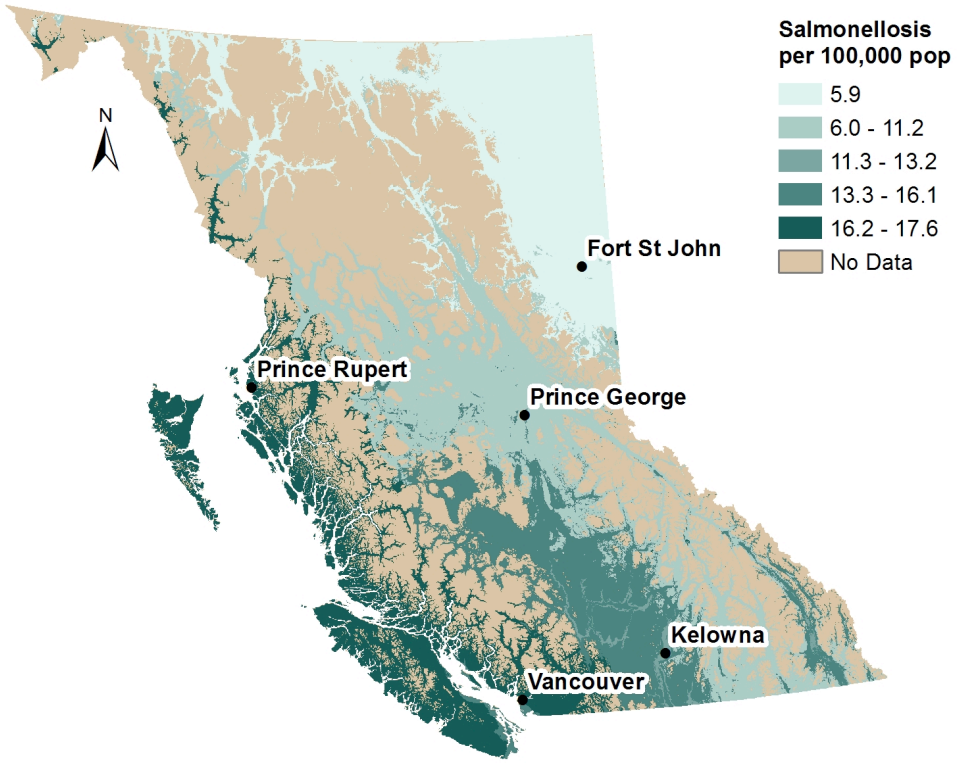


Figure 4.19 - Salmonellosis incidence by BGC zone (2010s)

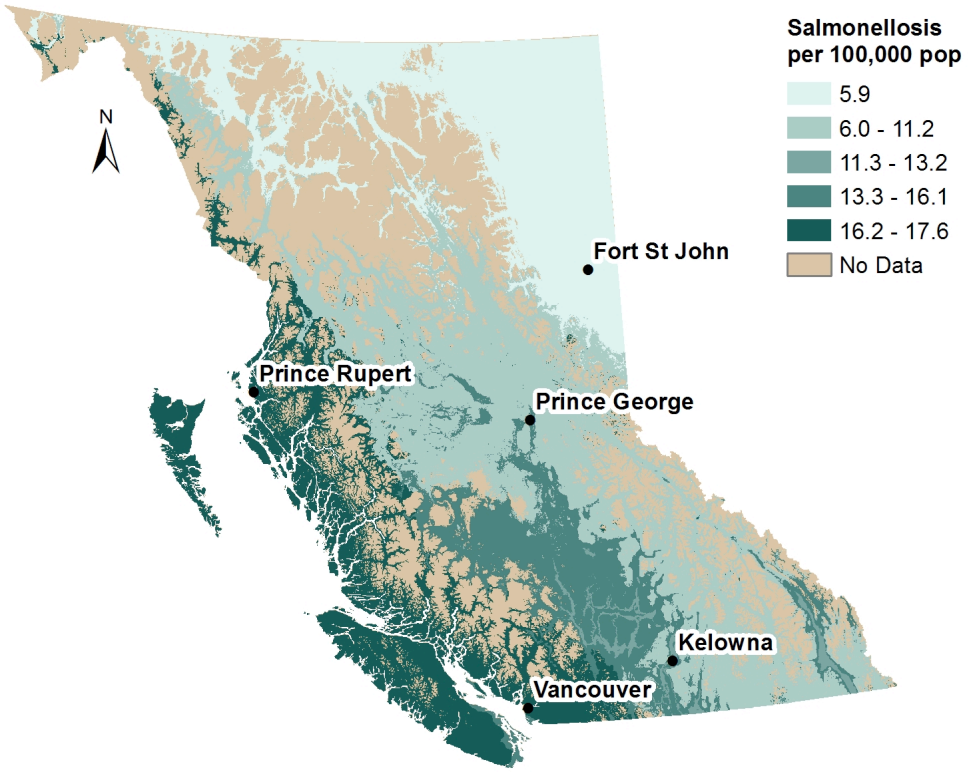


Figure 4.20 - Salmonellosis incidence by BGC zone (2050s)

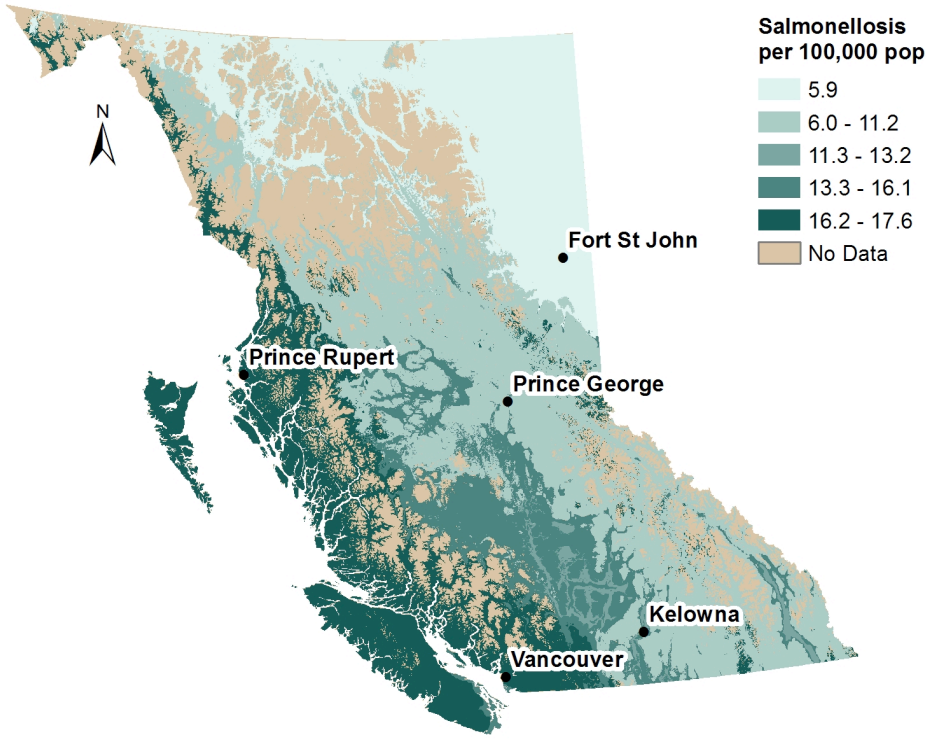


Figure 4.21 - Salmonellosis incidence by BGC zone (2080s)

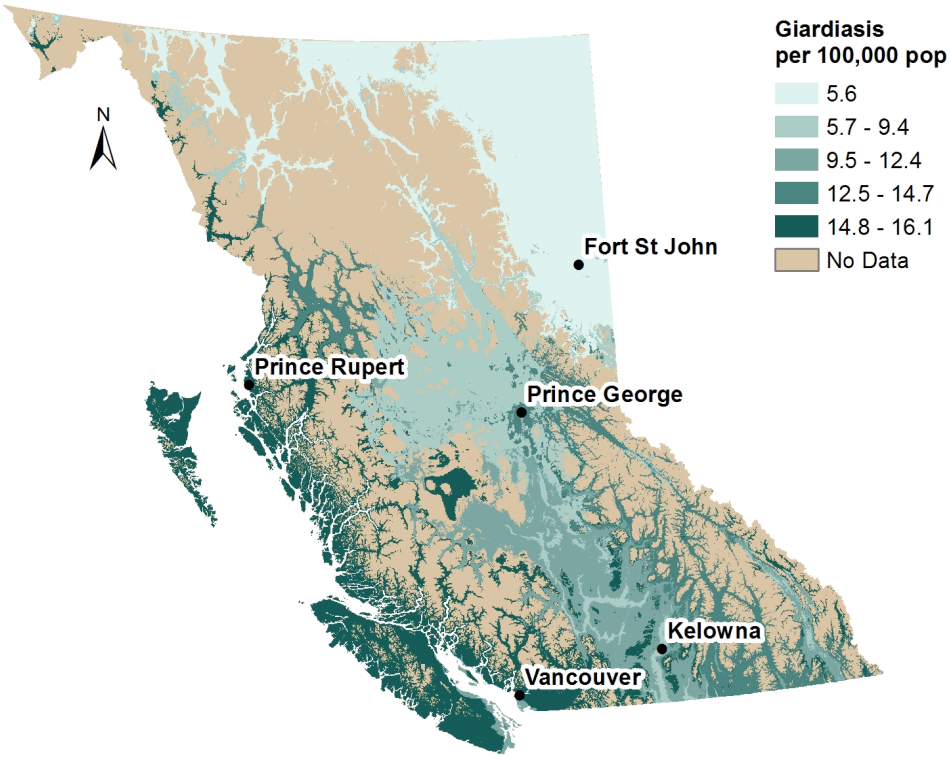


Figure 4.22 - Giardiasis incidence by BGC zone (2010s)

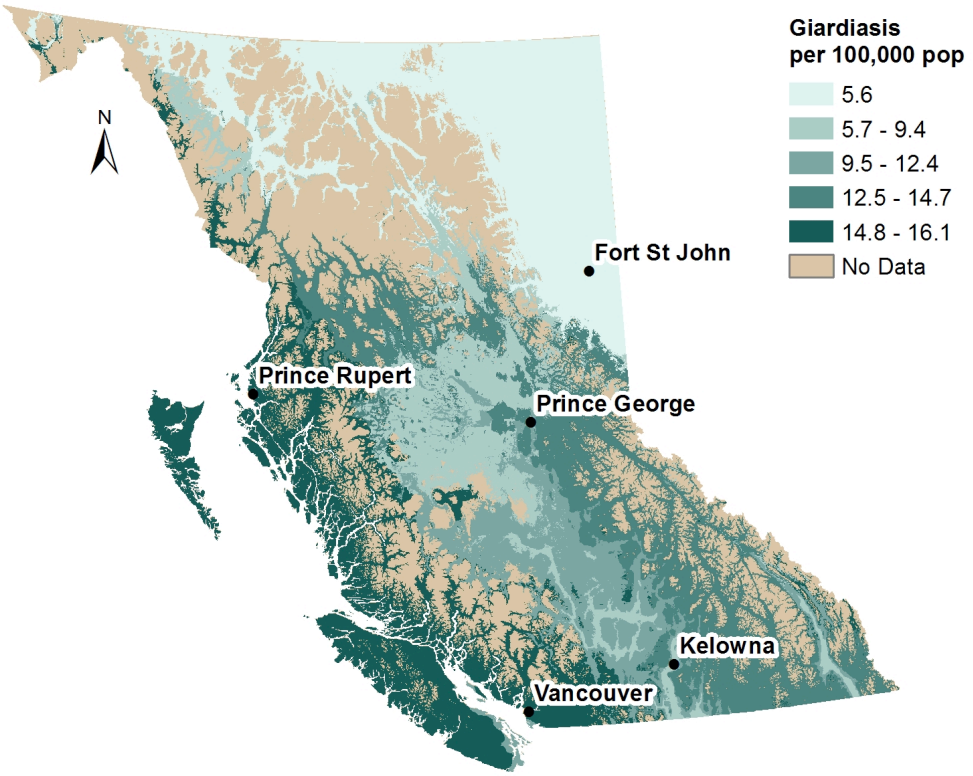


Figure 4.23 - Giardiasis incidence by BGC zone (2050s)

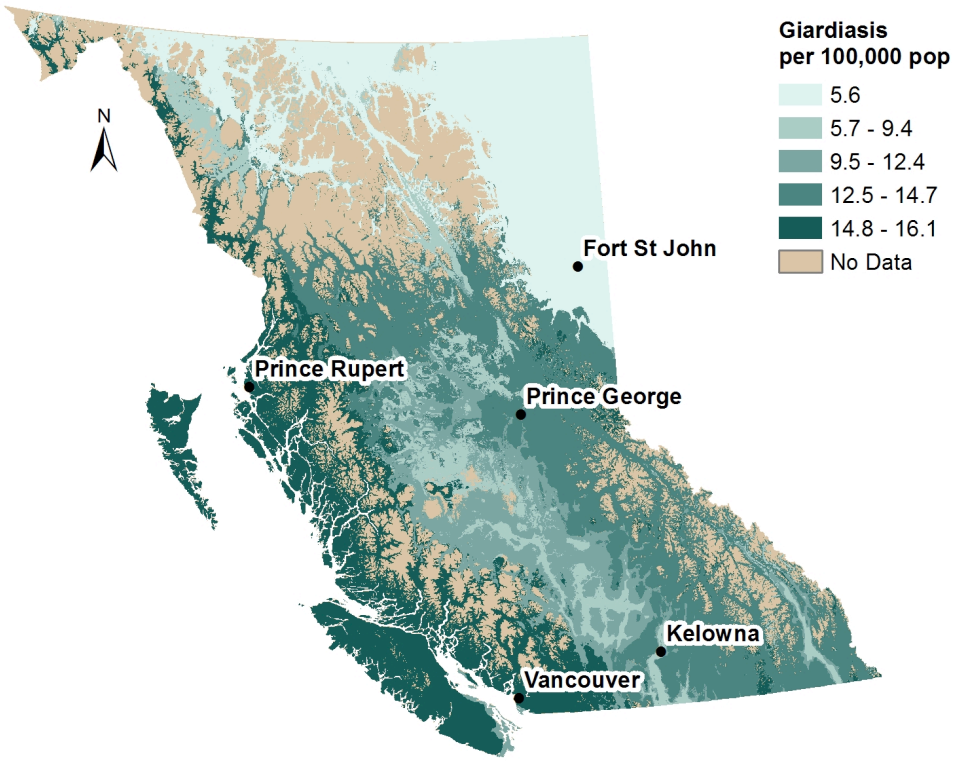


Figure 4.24 - Giardiasis incidence by BGC zone (2080s)

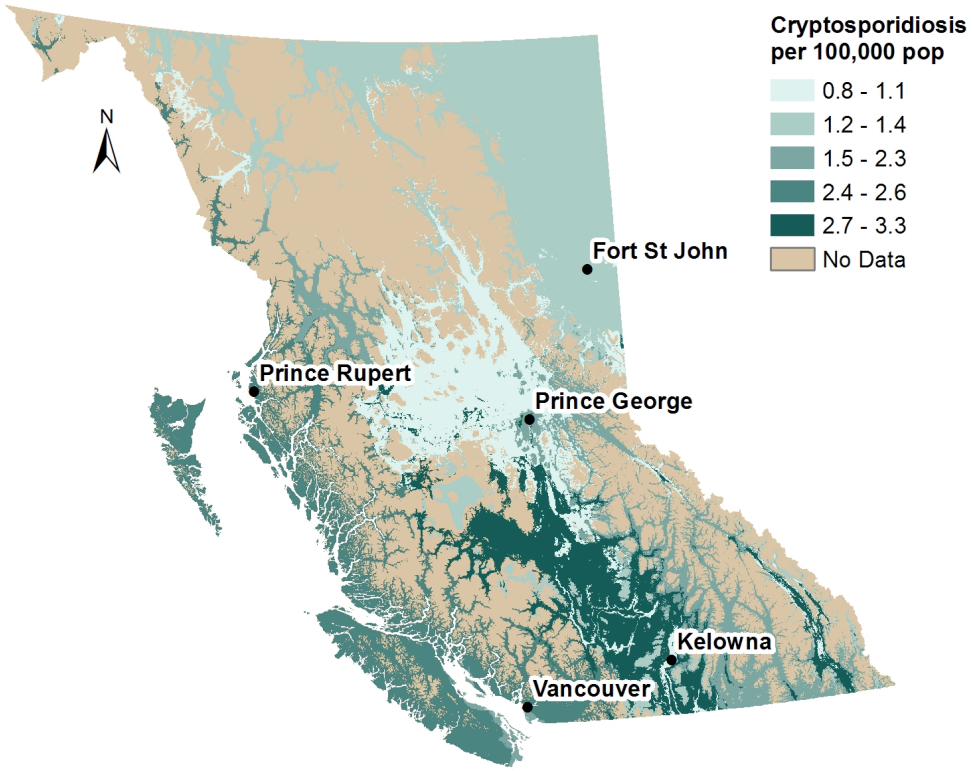


Figure 4.25 - Cryptosporidiosis incidence by BGC zone (2010s)

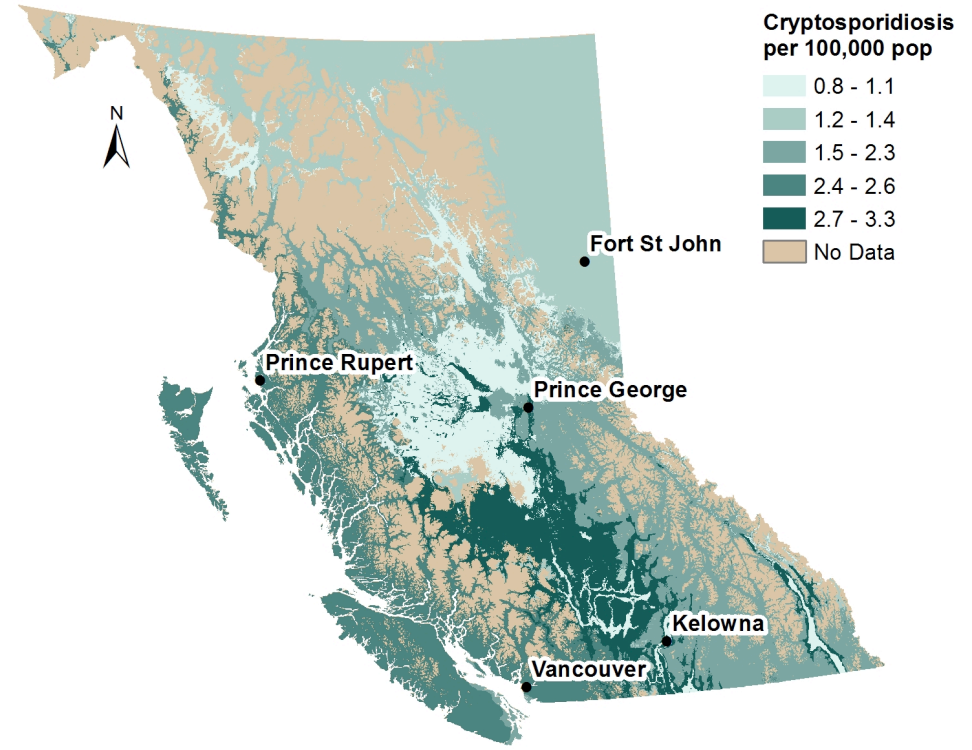


Figure 4.26 - Cryptosporidiosis incidence by BGC zone (2050s)

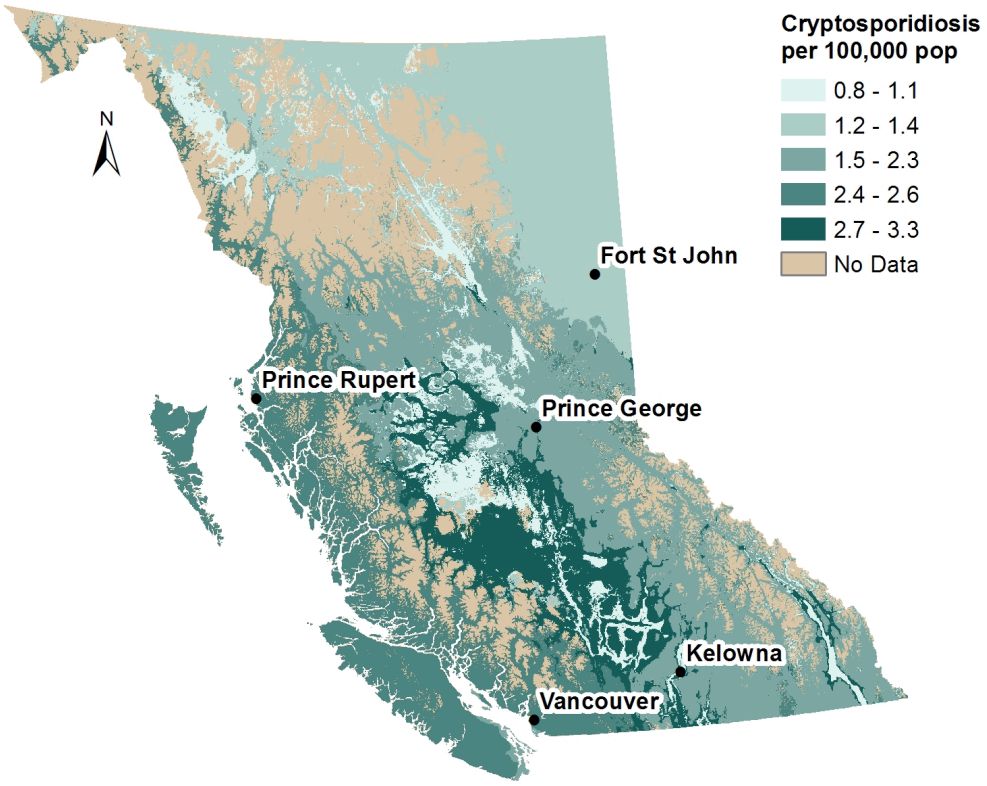


Figure 4.27 - Cryptosporidiosis incidence by BGC zone (2080s)

# Chapter 5. Discussion

On its own, this study offers more opportunities to generate new hypothesis than it does provide answers, which is consistent with the view generally held among epidemiologists regarding the utility of ecological studies such as this one (Sedgwick, 2011). However, it does present a unique perspective on environment/pathogen interactions occurring at relatively large spatiotemporal scales to which other studies, perhaps more robust individual-level studies, at different scales can be compared. Plowright et al. (2008) describe *triangulation* as the process of drawing multiple lines of evidence to reach a "verdict of causation" about pathways in disease ecology, since controlling for all variables in ecological processes is not possible. They suggest that the traditional reductionist approaches to causal inference are alone inadequate for investigating ecological drivers of disease emergence that are often mediated though large-scale and complex processes.

## 5.1. Limitations

### 5.1.1. Datasets

#### *AGI Dataset*

**Reported AGIs as surrogate measure for pathogen presence and risk**

Throughout this discussion, reported AGI is used as a means for making inferences about pathogen presence in the environment. This creates some limitations, particularly with regard to how reported AGI may be interpreted, since risk of disease

may have to do with more than simply pathogen abundance. An advantage of using data this way, aside from its accessibility, is that it integrates pathogen abundance in the environment with human exposure into a single variable, which may have value to health professionals and others that are interested in identifying at-risk populations throughout the province. However, care should be taken when interpreting this variable since a higher AGI rate in a particular zone (BGC, aquifer type, or ALR class) may indicate there is any combination of the following: higher numbers of pathogens, increased opportunity for exposure (cultural practices), higher pathogen survival/dispersal, or a vulnerable population. Some of these variables could actually be negatively correlated as long as one variable dominates and drives the perceived association. Such details cannot be discerned in this study and present opportunities for further analysis.

**Outbreaks and travel cases**

On a case-by-case basis, cases associated with foodborne outbreaks are relatively rare compared to sporadic cases (Fleury et al., 2006). To an even greater degree this is true for the parasitic AGIs, 90% of which are estimated to be sporadic (Hunter & Thompson, 2005). This study included all cases regardless of if they were sporadic or as part of outbreaks. Ideally cases that are associated with outbreaks would have been removed so as to not add a bias; however, the large number of cases and lack of complete outbreak data for the entire province presented too great a challenge. Even if known outbreaks were removed, it could artificially inflate rates for areas where undocumented outbreaks occurred (Fleury et al., 2006). Bias would be minimized if outbreaks were randomly distributed across the BGC zones, ALR classes and aquifer types, although this information is not available. Travel-related cases were not identified with the exception of salmonella cases with travel related serotypes (*S. typhi* and *S. paratyphi*), which were removed.

**Aggregating all bacteria and all parasites**

In addition to being analyzed individually, pathogens were aggregated into two groups, bacteria and parasites. The main benefit of this was an increase in statistical power, but the main drawback was that unique species-specific interactions were averaged out and potentially lost or masked by other interactions. The correlation

coefficients suggest that campylobacter and salmonellosis interact with climate at the BGC zone level in very similar ways. In some ways VTEC appeared similar (notably with annual temperature and degree-days); however, VTEC was unique in that it had very strong independent associations with reference evaporation, soil-moisture deficit and annual heat-moisture index. VTEC also stands out among the bacteria in the ALR study by exhibiting a significant positive association with ALR land while the others had significant negative associations. These results suggest that it may be appropriate to aggregate campylobacteriosis and salmonellosis at the scales used in this study; however, depending on what exactly is being investigated, VTEC may be more appropriately analyzed on its own. With the parasites, cryptosporidium had such low case counts with relatively large confidence intervals, that similarities and differences with giardia could not be as clearly observed. However, because they are both parasites with oocyst stages and are predominantly waterborne, it does seem logical to combine the two to increase statistical power.

**Effects of interventions**

The *Drinking Water Protection Act* was introduced in BC in 2003 (Government of British Columbia, 2013). This legislation outlines clear guidelines and restrictions pertaining to water systems and how they must be constructed and maintained. This was but one large-scale initiative that occurred within the timeframe of this study; however, many smaller regional initiatives have also occurred over the years. The effects of these interventions are (hopefully) influencing the trends in the study over time, though teasing out the effects of specific interventions is beyond the scope of the study. Therefore, a major assumption has been made; that any interventions have been rolled-out across the province at an even pace. It is very likely that socio-political-economic factors varying throughout the province would be breaking this assumption.

**Missing addresses, rural under-representation and under-reporting**

On the whole, these AGIs are notoriously underreported with from between 20 to 49 cases existing in the community for every one that goes reported, depending on the pathogen and location (Thomas et al., 2013). In BC, the number of all infectious gastrointestinal illnesses going reported has been estimated to be 1 in 347, or 0.3%

(MacDougall et al., 2008). Rural addresses were consistently more difficult to geocode and thus less likely to be included into the final dataset. Of those that did make it, they were much more likely to be geocoded using postal codes and so can be expected to be less accurate as well. Another factor that may introduce bias in reporting rates between urban and rural populations involves proximity to medical facilities. The increased travel time required for rural populations has been observed to dissuade people from seeking medical assistance (Regan & Wong, 2009).

Bias could also be introduced from differences in reporting among the five health authorities (VCHA in particular). For the years 2006 to 2010, missing data were added using imputation methods. If sensitivity analysis indicated that imputation changed the results significantly, those years were removed altogether to avoid bias. A more robust imputation method could have improved the outcome and quality of the study by adding more power.

Other less obvious differences probably exist throughout the HAs that were not accounted for; however, their effect was minimized in using BGC zones, watersheds and aquifer types because they cut across many HAs, a process that could distribute the bias across multiple zones. Exceptions are those BGC zones that fall entirely within one HA, such as the BWBS in the northern interior of the province.

### *Ecumene layer*

The ecumene layer is from 2011, and a 500 m buffer was applied, which probably results in slight overestimates of the area in which people live, particularly for the earlier years of the study. It was decided that in this case overestimation was better than underestimation, since the latter can result in losing entire dissemination blocks during the ecumene ‘clip’ procedure, which would significantly influence population estimates. The population density conversion following the clip corrected for much of the overestimation error; however, the result will be such that as one approaches the ecumene boundary, confidence in the population estimate decreases compared to deeper in the polygon. This could result in overestimation of those populations in the least populated BGC zones (causing an underestimation in AGI incidence), since they

appear to overlay with the ecumene layers more often along the ecumene's boundaries than do the more populated zones.

### *Census data*

Dissemination block population data and census subdivision age data were clipped by the ecumene and transformed a number of times before being re-aggregated to describe polygons of other spatial dimensions. Error could have occurred across any of these steps; however, overall error was estimated to be relatively small. As was mentioned previously, this error is probably disproportionately occurring along the boundaries of the ecumene polygons. A notable limitation of the census data is that they were only available for census years 2001, 2006 and 2011 but the resolution of 2001 was much more coarse. Therefore, to avoid the possible bias of the modifiable areal unit problem (Section 5.1.2), 2006 statistics were used for all years before 2009, and 2011 data were used for all years thereafter. Obviously the population is not stagnant for years at a time; however, population trend directions and rates were also not consistent over time, making interpolating the missing values vulnerable to error as well.

### *ALR dataset*

Confidence in the ALR dataset was low in this study regarding its ability to accurately describe the distribution of active agricultural projects. Only one year of ALR data (2013) was used for this entire study period. Furthermore, it does not distinguish between the many varieties of agricultural activities, such as animal farms versus annual or perennial crops. Even amongst farm animals themselves, variation in ability to contaminate adjacent water has been observed, likely due to a combination of unique pathogen-host relationships as well as human behaviour associated with raising each species (such as how and when manure is spread to the fields) (Soller, Schoen, Bartrand, Ravenscroft, & Ashbolt, 2010). It is possible that the 0% agriculture delineation in this study is more accurate because it is definitely not ALR land. However, there are agricultural activities occurring outside of ALR, for instance many Canadian cities have permitted raising small numbers of chickens in private yards within city limits. In one study, all of the municipalities studied in two Canadian provinces (including BC) were found to be supporting urban agriculture, although some focused more narrowly on specific practices while others remained more generalized (Huang & Drescher, 2015).

For the aforementioned reasons, more confidence was put in the binary classification of ALR (as opposed to five classes), yet it is worth mentioning that the rate estimates for each were fairly consistent with one another.

### 5.1.2. Interpretation

This study has many limitations that must be considered when interpreting its results. One of its main limitations pertains to the spatial and temporal scales within which any associations have been observed. It must not be assumed that just because an association exists on one scale that it exists on another or, inversely, because it does not exist at one scale that it also does not exist on another. Even when associations do exist on multiple scales, their directions may be different, along with any ecological processes underlying them.

#### *Ecological fallacy and the modifiable areal unit problem*

This is an ecological study whereby the climate data for each AGI case are derived from a BGC zone, the agricultural index is calculated at the watershed level, and the groundwater susceptibility is derived from underlying aquifer information. These are the boundaries used to calculate and compare AGI incidence across space. Ecological fallacy is a risk in a spatial study of this nature, whereby relationships observed at the group level are assumed to occur at the individual level (Sedgwick, 2011). However, the degree to which a pattern observed and analyzed at the group level is representative at the individual level is a function of the effects of grouping (Piantadosi, Byar, & Green, 1988). Related to the concept of the ecological fallacy is the 'modifiable areal unit problem' (MAUP). MAUP describes the bias that occurs whenever point estimates of spatial information are aggregated into regions; when boundaries are redrawn, trends may change.

Risk for these two types of biases was minimized by grouping cases at the BGC zone, aquifer type and watershed levels; groupings that are ecologically meaningful for the environment/AGI associations being investigated, or at least there is evidence to suggest they could be (see Introduction). Interpretations should be made at the same scale that the study uses (Piantadosi et al., 1988), in this case the BGC zone, the

watershed and aquifer scales, with the exception of the regression models that were calculated at the provincial scale.

### *Socio-economic factors*

This study did not control for any effects of socio-economic status (SES), which have been found to be associated with AGI in BC (Galanis et al., 2014). SES data were not available as part of the original AGI dataset and therefore would have had to be interpolated from large census subdivisions. This would have made the study guilty of the ecological fallacy exactly as described by Sedgwick (2011) because there would be no assurance that the SES statistic of a zone was in any way reflective of the SES of the relatively small number of people in the AGI dataset. Social deprivation has been observed to be negatively correlated with campylobacteriosis in urban environments but not in rural communities (Spencer et al., 2012). One logical explanation might be that more frozen poultry products (which carry lower risk than fresh ones) are being consumed more by those of higher deprivation, as well as there being fewer places to purchase fresh food in the rural communities, making access to the riskier fresh products more difficult, regardless of social depravity (Spencer et al., 2012). Furthermore, people with less social deprivation are more likely to obtain food from a larger variety of sources, which increases the likelihood of exposure through any one source (Spencer et al., 2012).

To maintain sufficient power to identify subtle environmental effects on rarely reported AGIs, large numbers of cases were needed, spread throughout a few numbers of categories, each with a high population. Controlling for age would have been ideal; however, the population age data were a) coarse and b), creating more categories would have resulted in reduced power. Controlling for age was more viable when using binary environmental exposures. Age was used to stratify the binary ALR variable in this study, although only incidence for 10 years and under was calculated.

### *BGC zones*

In reality, boundaries separating BGC zones are not abrupt but are gradual transitions between ecosystems. Satellite measurements have observed some error occurring within 1 km horizontal and 100 m vertical of their borders, within which the

BGC climate average does not as accurately predict the climate (Delong et al., 2009). Adding further error is the considerable variation in climate at the local level. One of the main forces controlling the climate is incoming solar radiation, which in a mountainous province such as BC can be incredibly variable over short distances where mountain peaks block the sunlight for certain regions and extend it for others (Aguilar, Herrero, & Polo, 2010). However, because most people probably do not spend all of their time in a single microclimate directly adjacent to their house but instead are interacting with many different microclimates every day (themselves and indirectly via the food and water they consume), this error is hopefully not that significant. Another opportunity for error is the single statistic describing an entire BGC zone that was derived from a mean of all subzones within it. Some of the subzones have very few people living in them, such as those at higher altitudes in exposed mountainous regions. Thus if most of the population resides in the warmer subzones, for example, then the mean temperature value for the entire BGC zone will underestimate the temperature of where most people reside.

This study used annual climatic data aggregated to the BGC zone level derived from the years 1990 to 2012. Alternatively, zones could be broken up into subzones or smaller, and climatic measurements could instead be measured at daily, weekly or monthly intervals. It is not clear if environment/AGI associations on one scale also occur on others. Furthermore, maintaining a constant temporal scale but changing the spatial scale could also plausibly change the results.

Thus temporal trends and variance around the mean may differ significantly among climatic variables for zones that, when observed at the annual level, appear to be the same. Some attempts are made throughout this discussion to connect trends observed at different scales in other studies to those observed here to help provide a more complete view of the ecological processes implicated with AGI. Combining studies across spatiotemporal scales is fraught with challenges but is necessary for making AGI projections with climate change. This is especially true considering that the GCMs used to project future climates are most accurate at their coarsest resolutions, with confidence decreasing as they are downscaled to smaller and smaller regions and time periods (Fowler, Blenkinsop, & Tebaldi, 2007). The opposite is true with respect to confidence in AGI/environment associations, whereby the most confidence is likely placed in

associations observed at smaller and more tightly controlled individual-level studies than compared to those observed at larger scales where causal pathways cannot be conclusively proven because they are ecological studies (such as this one).

It is tempting to use environment/AGI associations observed in BC to gain insight into what could be occurring in other regions of the world. Caution again must be taken when extrapolating complex and unique socioecological relationships observed at one location to another. At the very least, though, if environment/AGI relationships are apparent in a highly developed nation such as Canada, then it would be reasonable to hypothesize that environment/AGI relationships (but perhaps totally different ones) can also exist in lesser-developed nations where inadequate sanitation and limited access to education and healthcare services compounds risk of AGI.

### *Annual averages and controlling for seasonality*

Seasonality was not included in this study, although can be observed in Table 4.1b, but was controlled for by using annual climatic averages. This means that climatic variables with high correlation to AGI rates may be doing so in some seasons more than in others, so long as the net result is a correlation. Some of the climatic variables in this study are not annual but are seasonal measurements, such as 'growing season precipitation'. Special care must be taken when interpreting these associations that are not made with data of the same temporal scale. These variables were included in the analysis in case they influence overall risk throughout the year. An example of this would be 'mean temperature of the coldest month', which could potentially be negatively associated with the denaturing of pathogens at low temperatures, thus influencing the numbers of organisms that would effectively 'over winter' (outside of a host) and influence the risk the following year. Future analysis would benefit from being done at the seasonal level to provide a clearer picture of any such relationship. All of the BGC zone climatic variables used in this study are available at the seasonal level (four seasons).

## 5.2. Associations

### 5.2.1. Correlations among AGIs

There were a number of correlations observed among the five AGIs at the BGC zone level. It was anticipated that correlations might exist among the bacterial pathogens themselves (predominantly foodborne) and within the parasites (predominantly waterborne). Surprisingly, however, associations were observed between giardiasis and campylobacteriosis ($R^2$ 0.73, $p<0.05$) and giardiasis and salmonellosis ($R^2$ 0.669, $p<0.05$). Among the bacteria, only campylobacteriosis and salmonellosis ($R^2$ 0.898, $p<0.01$) were correlated. Further analysis would be required to see what might explain these correlations, or lack thereof.

### 5.2.2. BGC zones

#### *BGC zones versus subzones – Precision, accuracy and issues of scale*

AGI/climate trends were much stronger at the BGC zone level than at the subzone level, for which they all but disappeared. Therefore, it was decided that the BGC zones be used in the final analysis. While this resulted in fewer data points (nine zones in total), there was more confidence in the accuracy of the points. The main drawback of this decision was that the increased climatic specificity that the BGC subzone data offered was forfeited. The following reasons were identified as possible explanations as to why the AGI/climate associations were so strong at the BGC zones level, yet almost non-existent at the subzone level:

i) Influence of outbreaks – Because outbreaks were included in the dataset it is likely that with fewer zones, and larger case counts per zone, that the unwanted effects of outbreaks are diluted as compared to when smaller subzones are used. Any outbreak will have a greater effect on the data when those cases make up a greater proportion of the total cases within a particular zone.

ii) Accuracy of exposure – Because the study is investigating links between the environment and AGI, and we know that people are being exposed to pathogens in food,

drinking water, pets, wildlife, farms and many other sources, the chances of the source of exposure being within the much larger BGC zone is higher than in a subzone. For example, it is more likely that one person spends more time in the BGC zone of their home address (and interacting with food, water and animals also from within that zone) than from exclusively within their subzone. It is a case of accuracy and precision. By analyzing at the subzone level, there is greater precision but less accuracy in terms of exposure. Especially with foodborne illnesses that may originate from as far away as the farm, processing plant or restaurant, it is more likely that a source of exposure is within the larger zone of the home address than in that subzone.

iii) Accuracy of population estimates – Estimating the population of each BGC zone required converting census population data from dissemination blocks and then being clipped with a population ecumene. With the larger zones, there is more confidence in the estimate of the population denominator used for calculating proportions.

### *Zone-zone incidence comparisons*

There was a significant difference in AGI incidence across the BGC zones depending on which zone was used as a reference. It is reasonable to assume that nearer BGC zones are more similar in climate than further ones, and it would appear that most of the AGI rates gradually change from one to another, as opposed to abrupt changes among adjacent zones. Abrupt changes might be expected if there were no links between macroclimate and AGI, if climatic effects were being outweighed by some other independent factor, or if BGC zones were not biologically meaningful boundaries for these pathogens. Gradual transitions in AGI incidence between BGC zones were more evident with the bacterial pathogens than the parasites.

BGC zones not only integrate a multitude of climatic variables at the macroclimatic scale, they also describe areas with similar patterns of energy flow, vegetation and soils (Meidinger & Pojar, 1991) and by association, some animals. So when variables have positive associations and others have negative associations with AGI, a BGC zone is able to quantify the *net* association of these variables (their interactions) with AGI. The ability for BGC zones to integrate all of these elements

provides a unique opportunity to identify climate regions of BC that have higher or lower risks of AGI, without necessarily understanding all of the intricacies of the specific environment/pathogen relationships.

The findings of this study suggest that environment-AGI associations can be observed at the ecosystem level, at least with the bacteria, as well as that BGC zones are one appropriate scale for which to be doing such studies.

### 5.2.3. AGI and climate

#### *Shape of the curve*

The majority of significant linear AGI/climate relationships in this study were actually better described as non-linear (quadratic) relationships. This suggests that there may be thresholds in climate/pathogen interactions at the scale of this study, which would support previously observed trends in other studies (Fleury et al., 2006; Schijven et al., 2013). However, having only nine BGC zones (only nine points on a scatter plot), the confidence in the shape of the quadratic line is not very good. Furthermore, quadratic models are more difficult to interpret than linear ones, which may reduce the usefulness of the model. For these reasons, it was decided to model the AGI/climate associations as linear.

#### *Multivariate versus univariate regression and issues of collinearity*

Significant associations were observed between the bacterial pathogens and many of the temperature-related variables, with higher rates consistently associated with warmer temperatures (Table 4.13). However, many climatic variables were observed to be collinear with one another, especially among the temperature-related variables. Because of this collinearity, all of the variables could not be included in the regression model because it breaks the assumption that they must be independent. It was hoped that one temperature and one precipitation variable could be added, since they tended not to be correlated; however, none of the precipitation variables had a significant effect on the models. For these reasons, univariate regression analysis was chosen. Out of the various temperature-related variables to select from, annual temperature was chosen for three reasons: it is easily understood by many people (knowledge translation), it was

significant at a 0.05 significance level for all three bacterial pathogens, and perhaps most importantly, because it is used in climate change models.

Throughout this discussion the results of this study will be compared with existing literature. However, due to the high degree of collinearity among many of the climatic variables, it is impossible to know which ones (or combinations thereof) may be driving any associations observed in this study. Even so, individual variables with significant correlations with AGI were analyzed as though they were describing some of the variability, both for the purpose of discussion and to identify any support in the literature.

### *Temperature variables*

Temperature was correlated with all three bacterial pathogens in this study, depending upon the actual variable being measured (Table 4.13). Mean annual temperature was positively associated with all three, mean temperature of the warmest month was positively associated with VTEC, mean temperature of the coldest month was negatively associated with campylobacteriosis and salmonellosis, extreme minimum temperature was negatively associated with campylobacteriosis, extreme maximum temperature was positivity associated with VTEC, and frost-free period was positively associated with campylobacteriosis. When the three bacterial pathogens were analyzed together as one, they were positively associated with mean annual temperature, mean temperature of the coldest month, extreme minimum temperature and frost-free days. Overall, campylobacteriosis and salmonellosis were nearly identical in their associations with climate, although salmonellosis trends were somewhat weaker in every case. VTEC, while sharing some of the same trends as the others, stood out as being the only one correlated to extreme maximum temperature and mean temperature of the warmest month as well as the only one not correlated with mean temperature of the coldest month. These findings suggest that salmonellosis and campylobacteriosis cases might be analyzed together when investigating temperature-related links at the ecosystem level to increase power, but that VTEC might be more appropriately analyzed separately. Further analysis is required to identify species-specific environment-pathogen interactions that may be occurring with VTEC.

### *Degree-days*

Degree-days integrate time and heat into one unit. Degree-days are highly correlated with the different bacterial AGIs in this study but not the parasites (Table 4.13). They are often used in the study of insect development whereby each species requires a certain amount of heat over time to develop into a particular life cycle (Graczyk, Knight, Gilman, & Cranfield, 2001). It does not matter so much when the heat occurs for insect development, but only that a sufficient amount eventually does. Interpreting degree-days is fairly straightforward, although somewhat counter-intuitive since multiple degree-days can be accumulated in a single 24-hour period. If the temperatures were, on average, -1$^{o}$C for a full 24-hour period, that would be equal to one degree-day below 0$^{o}$C. If it were -5$^{o}$C for 24 hours, then that would be equal to 5 degree-days below 0$^{o}$C. Add them up over an entire year, and that is the scale of this study. Degree-days may help explain some of the variability in AGI either directly, or indirectly via other species that together make the ecological community within which the pathogens exist. Some insects influenced by degree-days may also be acting as mechanical vectors for pathogens. The life expectancy of the common housefly (*Musca domestica*), for example, is accurately estimated using degree-days and has been found to carry all five of the pathogens in this study (Graczyk et al., 2001). Fecal material, pathogens and oocysts can become lodged in their hairy legs in addition to passing through their intestinal tracts and eventually be deposited on or near food, water (Graczyk et al., 2001) or fresh produce (Wasala, Talley, DeSilva, Fletcher, & Wayadande, 2013). It would even be likely that by influencing insects, degree-days are also associated with larger animals.

An ideal degree-days measurement for studying AGI would be at the exact temperature where a bacterial pathogen gets stressed into VBNC state (both low and high temperature extremes). This point for VTEC has been observed when temperatures fall below 5$^{o}$C (Blaustein et al., 2013). Degree-days above 5$^{o}$C were strongly correlated with only VTEC ($R^2$ 0.587, p=0.016). All of the bacteria were negatively correlated with degree-days below 0$^{o}$C, campylobacteriosis and VTEC were negatively associated with degree-days below 18$^{o}$C, and VTEC alone had a positive association with degree-days above 18$^{o}$C. When grouped together, the three bacteria were negatively associated with degree-days below 0$^{o}$C and degree-days below 18$^{o}$C. More research is required to

identify if degree-days specifically are associated with these AGIs, or if degree-days are acting as a surrogate measure for some other temperature-related measurement. In any case, it appears as though degree-days below 0$^{o}$C is a temperature below which AGI risk for all three pathogens decreases and that generally speaking warmer temperatures are associated with increased AGI, particularly with VTEC.

### *Precipitation and precipitation-related variables*

Precipitation variables were not significantly correlated with any of the AGIs, even though many studies have observed increased incidence with extreme (upper percentile) precipitation events (Carlton et al., 2014; Curriero et al., 2001; Rizak & Hrudey, 2008). This suggests that annual precipitation at the BGC scale is not strongly correlated with frequency of extreme precipitation events and that annual BGC zone precipitation averages do not effectively describe any effect that precipitation may be having with AGI risk. However, some annual moisture-related variables were strongly correlated with VTEC, suggesting that precipitation is associated in some way with AGI at the BGC scale via interactions with other variables. In the case of VTEC, a positive association with annual heat-moisture index ($R^2$ 499, p=0.03), annual moisture deficit ($R^2$ 0.725, p=0.004), reference evaporation ($R^2$ 0.927, p<0.001) and annual temperature ($R^2$ 0.749, p=0.02) suggests that VTEC is associated with very hot and very dry annual conditions. Because there is no negative association with annual precipitation, as would be expected if VTEC was associated with dry conditions alone, it does support the idea that precipitation is involved at a different scale. The fact that the other two bacteria had no correlation with heat-moisture index, annual moisture deficit or reference evaporation suggests that there may be unique species-specific environment/pathogen interactions occurring with VTEC that is different from those of the other two primarily food-borne AGIs. Examining how these annual integrated precipitation-temperature variables are interacting with VTEC warrants further investigation.

## 5.2.4. ALR

When interpreting these results it is important to keep in mind that they are unadjusted and on a provincial scale. Types of agriculture vary throughout the province and so does the climate, which is likely mediating any risk associated with agriculture.

The ability to identify specific risks (i.e. ruminants) is lost in a study of this scale, which is why it is recommended to repeat the analysis with more precise agriculture data. Just because the rates are, for the most part, lower in ALR watersheds, it does not mean that humans are not getting sick from pathogens originating from farms, only that the incidence of AGI in ALR-related watersheds is lower than that of non-ALR ones. There may be 500 people living in a watershed, with 30 of them living at nearby farms and acquiring AGI at very high rates as a result, but still on the watershed level the rates are low; low compared to non-agricultural watersheds that may be dense urban watersheds that have been shown to at times have equal or higher pathogen contamination than agricultural watersheds. Soller et al. (2010) observed beaches within 2 km of farming activities with significantly lower pathogen contamination than those in urban settings, presumably sourced not from animals but from humans (bathing, untreated sewage, malfunctioning septic systems, etc.). Additionally, the combined sewer system capacity can be exceeded by heavy rainfall and cause sewer overflow into adjacent surface waters, a process that is exacerbated by increases in impervious surface (Delpla, Jung, Baures, Clement, & Thomas, 2009).

Because this study does not include multiple covariates simultaneously, any AGI risk associated with ALR or non-ALR watersheds could be explained by a variety of other factors. For example, popularity of recreational swimming and other water-related activities may not be as common in ALR watersheds if local residents are aware livestock are contaminating the streams. Even if an ALR watershed were more contaminated from zoonotic sources, if swimming is uncommon, an adjacent non-ALR watershed where water is less contaminated but many more people are swimming could result in higher AGI rates there. Therefore, it is not clear whether or not anthroponotic transmission of AGIs in the non-ALR watershed poses a greater public health risk than zoonotic transmission in the ALR watershed, nor is it clear how the relative risk of each could change depending upon local recreational water-use patterns. A watershed scale is appropriate for investigating such nuances, and would be facilitated through the development of enhanced surveillance that utilizes molecular genotyping technology to differentiate between zoonotic and anthroponotic transmission (Pintar et al., 2010).

As a binary variable, having any amount of ALR land located within a watershed is associated with a lower AGI incidence for campylobacteriosis, salmonellosis and giardia and a higher incidence for VTEC (Tables 4.4-4.5). When combined, bacteria and parasites were each negatively correlated with ALR. The ALR variable was also split into five groups: 0%, less than 25%, 25 to 50%, 50 to 75% and greater than 75% ALR land (Figures C1 and C2 in Appendix C). Campylobacteriosis, salmonellosis and giardiasis followed the same general trend; however, in all three there was a significant decrease in incidence when comparing 0% ALR to 0-25% ALR. For campylobacteriosis and salmonellosis, the 25-50% incidence then increased significantly back to near 0% levels. This erratic behaviour could suggest that this dataset has some problems associated with it, which might be expected if there is too much variation in farming practices and land-uses within the ALR polygon. Or it suggests that there could be non-linear associations between percent ALR land and these AGIs at the watershed scale. With children under 10 years of age, salmonella was the only AGI with a significant association with ALR, which was lower in the ALR watersheds (Figures 4.7-4.8).

Further information could be gained from this dataset by also calculating the rates of people living inside ALR land with those living outside ALR land, irrespective of watersheds. Using available satellite imagery, it is also possible to increase the resolution and identify specific farms and farming practices throughout the province. Possible scale-dependencies of the agriculture-AGI relationship could be investigated in comparing a variety of studies across scales using the same dataset.

### 5.2.5. Population density (rural vs. urban)

The AGI dataset was split into two zones, rural and urban, with the cut-off at 400 persons per square kilometre. The primary reason this variable was added was so that the aquifer data could be stratified into high and low density populations, which was a crude attempt at separating populations that are more and less likely to be using private water systems. The assumption is that people living in cities are more likely to be on a municipal system than to have a private well, and that people on a private well are more likely to have an association with the aquifer beneath their house than people living in the city. A study identified private wells as having a high risk of AGIs as compared to

municipal systems (Uhlmann et al., 2009). Since there are many urban surface water systems in BC (Uhlmann et al., 2009), which would presumably not be as influenced by the aquifer as groundwater systems, there was too much variability in the urban aquifer data to include in the analysis.

Aside from drinking water sources though, other differences in urban and rural lifestyles have been found to be associated with AGI. Agricultural land is an obvious one that will be discussed. Controlling for the effect of agriculture, Spencer et al. (2012) observed an increased risk of campylobacteriosis in urban communities compared to rural ones, except in children under four years, who had higher risk in rural environments. A lack of access to fresh food and increased reliance on frozen food was hypothesized to explain some of the reduced risk observed in rural settings (Spencer et al., 2012). Increased social connectedness of rural communities has also been associated with slower diarrheal disease spread as compared to urban ones (Zelner et al., 2012). In that study they describe two different mechanisms operating concurrently: decreased contact with outside individuals, and greater density of social ties that facilitate the spread of behavioral practices that reduce risk. The degree that an Ecuadorian study would be relevant to what is happening in BC is debatable, particularly the latter point; however, the former point could be relevant.

### 5.2.6. Aquifer type

Unlike with average soil types, BGC zones are not accurate at predicting the underlying geology where groundwater resides. This is evident when the BGC map is overlain with the aquifer geology map. Southern Vancouver Island and mainland Vancouver both share the same BGC zone but the aquifers on the island are mostly in fractured bedrock while those of Vancouver are of unconsolidated material (for aquifer map refer to Figure E1 in Appendix E). The positive association with AGI and unconsolidated material in the rural population was consistent among all of the pathogens in this study when combined into two groups: bacteria and parasites (Table 4.10). When analyzed individually, only cryptosporidiosis and salmonellosis were associated (Table 4.8-4.9). These relationships suggest that this proportion of variation

could be related in some way to groundwater; however, it is possible that aquifer type is a surrogate measure for some other variable implicated with AGI.

These findings are consistent with research that indicates unconsolidated aquifers are generally more vulnerable to pathogen contamination than bedrock aquifers (Borchardt, Bertz, Spencer, & Battigelli, 2003), even though both have been attributed to groundwater contamination (Borchardt et al., 2003; Hrudey et al., 2003; Rizak & Hrudey, 2008). Unconsolidated aquifers generally have a stronger hydraulic connection to surface water, although the direction and strength of the flow can vary throughout the year. For example, during the spring freshet water can move from streams into the groundwater, a reversal of what commonly occurs during the summer months. While it is beyond the scope of this thesis, further analysis is required to differentiate between high and low risk aquifers to determine if some highly fractured bedrock aquifers might actually be higher risk than some unconsolidated ones. Because there is no drinking water system data in the study, there is no way to know what proportion of these systems are managed and which are private systems. Further analysis is required to identify what specific types of water systems in rural BC are vulnerable to contamination, or if they are all equally so. The results of this study suggest that a rural definition of <400 people / $km^2$ is a crude but effective way to estimate where people are more likely to be on a well, either private or public. A better method would be to utilize the existing province-wide Ground Water Wells and Aquifer Database (WELLS) provided by the BC Ministry of the Environment (iMapBC, 2014).

### 5.2.7. Integrating all of the environmental variables

Independently, the environmental variables in this study have revealed some statistically significant correlations with AGI. However, since none of these variables is operating in isolation of one another, it is important to consider how they might be interacting to influence rates and how the associations among many of them are difficult to discern from one another. With an ecosystem perspective, humans are both influencing and being influenced by our environment; it is becoming more evident that where human health stops and the health of the environment begins is perhaps a line that only exists in our imagination. The innate interconnectedness of ecosystems,

health/wellbeing and social systems is a perspective that is gaining traction as a means of not only understanding our reality, but also as a tool for galvanizing multi-disciplinary collaboration around problems that many parties are concerned about but that are incapable of solving on their own. Parkes et al. (2010) has identified the watershed as an ideal setting within which such initiatives may be executed so as to maximize synergistic benefits for ecosystems, human health and society.

### *BGC zones and agriculture*

It is very likely that climate and agricultural activities are correlated since crop/livestock/environment relationships have evolved over time. If both agricultural associations and climatic associations with AGI exist, then the accuracy to which any AGI/climate relationships observed in this study could be projected into the future depends on this collinearity continuing to exist. As long as farmers continue to take advantage of changing climate conditions and expand farms into new territories, they will likely continue to bring any associated AGI risk along with them on the migration. This study would benefit by stratifying the BGC zones by agricultural classes, which would help in separating their associations with AGI from one another. It would also allow the opportunity to identify instances of specific BGC zones being more susceptible to agriculture-related AGI risk than others.

### *ALR, aquifer type and population density*

There is good reason to believe that population density is describing much the same thing as the ALR variable in this study since agriculture is intuitively inversely related to population density. Therefore, it may have been helpful to stratify the ALR classes into urban and rural to try and distinguish their effects from one another. As a result, any AGI associations observed with either of these two variables may be associated to one or both, or something else entirely which these variables are surrogate measures for. AGI rates are very similar between the two variables, with lower incidence being independently associated with both ALR and rural populations. An exception is VTEC, the only AGI that had a positive association with ALR, which had no association with population density.

In one study, survival rates of *campylobacter* and *E. coli* were compared to one another in groundwater (Cook & Bolster, 2007). It was found that *campylobacter* die off was 2.5 to 13 times faster than for that of *E. coli* as well as that *E. coli* survival was the greatest when there were high levels of dissolved nitrogen present. While AGI cases for both of these pathogens were found to be positively associated with unconsolidated aquifers in this study, only *E. coli* was positively correlated with ALR land. It could be the case that *E. coli* not only is more resistant to desiccation than *campylobacter* and therefore has the potential to travel further distances from the source (thus increasing the likelihood of finding a host), but also that the increased levels of nitrogen associated with farming are promoting *E. coli* survival and growth (Cook & Bolster, 2007). Potentially exacerbating this process further is the positive association between temperature (which VTEC has a positive association with) and nitrification in the soil (Delpla et al., 2009). In a lake environment, Pachepsky et al. (2014) compared inactivation rates of *E. coli* and *salmonella*, with the latter becoming inactivated twice as fast. When taken together, these studies suggest that *campylobacter* is the quickest to become deactivated in water, followed by *salmonella* and then by *E. coli*. The results of our study suggest that VTEC may be the slowest to deactivate on the watershed scale compared to the other two bacterial pathogens; however, without knowing more information about pathogen density on ALR lands, there is no way of knowing if VTEC is more resilient or if there is just more of it present.

By combining the climate - AGI associations in this study with ALR, some interesting trends emerge. VTEC, as has already been mentioned, has unique associations at the BGC zone level that were not observed with the other two bacterial pathogens. These relationships were with mean temperature of the warmest month, both summer and annual heat moisture index, degree-days above 5$^{o}$C and above 18$^{o}$C, extreme maximum temperature, reference evaporation and climatic moisture deficit. Taken together, these suggest a positive association between VTEC and very dry and hot annual macroclimates; however, the lack of a negative association with annual precipitation suggests that precipitation may be involved, just not at the scale being investigated here. Combined with the observation that it was the only one of the pathogens that was positively associated with ALR land, it really stands out among the five pathogens in this study. From this, it could be hypothesized that agriculture

increases VTEC pathogen supply beyond levels that occur outside of agricultural regions (cattle are thought to be the main reservoirs of VTEC in the environment (Langholz & Jay-Russell, 2013; Quilliam et al., 2012)), and that hot and dry climates are linked to increased growth, proliferation or distribution of the pathogens. The first rains following dry periods have been shown to result in the most heavily contaminated runoff, since moisture deficit of dry periods cause pathogens to accumulate on the soil surface instead of slowly percolating into the soil (Lake et al., 2005). When a precipitation event finally occurs, it takes everything with it into waterways. This is compounded by the fact that when soil is moisture deficient, it does not absorb water very easily, which will exacerbate runoff during a rain event. So even though annual precipitation is not associated with VTEC in this study, precipitation events could be playing an important role. Another potential pathway for dispersal is in dust, which these dry conditions would likely be associated with. VTEC, *salmonella* and *campylobacter* have all been observed travelling as dust and aerosols via wind over short distances; however, *campylobacter* is so sensitive to UV and wind that it likely cannot survive the voyage as well as the others (Hellberg & Chu, 2015). Especially considering how cattle are raised in open fields with few trees, during dry periods the hot sun will bake feces that may become airborne as dust. So it may be possible that dust is acting as a means of distributing the pathogens farther and to a larger groups of people that would otherwise be exposed, but also giving a ‘double dose’ to those that live nearby that are exposed via other sources like wildlife, livestock or contaminated water. In theory, then, even if a person had safe drinking water and practiced excellent hygiene, it would still be possible for bacteria to come into contact with food, drinks and body parts via dust or flies. Another trait of VTEC that might help explain why it is associated with ALR land in this study at the watershed level is that it has a very low estimated infectious dose (10 to 100 cells) (Hellberg & Chu, 2015), which in theory would mean that it could disperse over larger distances and still maintain an effective dose compared to other pathogens that could still be viable after travelling so far, but are simply not available in adequate numbers to infect a host. The possible association between VTEC and dust does raise the question as to whether or not the verotoxins produced by VTEC are potentially making people sick after the pathogen itself has died and lost ability to infect a host. Future studies might investigate the dust-VTEC association further as well as the possibility that verotoxins persist as a risk in the environment after the VTEC organism dies.

Another way of interpreting the negative associations observed between AGI and ALR land in this study is that ALR may very well be positively associated with AGI but since it is being compared to urban areas, it may just be that the urban environment is *more* associated with AGI. That is a major limitation of making relative comparisons. Turgeon et al. (2011) observed comparable levels of contamination in urban streams as those that are in agricultural streams, and attributed urban sources to storm water runoff, domestic waste, pets, urban wildlife and possible sewage overflows. Exposures to contaminated water in the city might involve direct exposure through swimming as well as indirect exposures through pets or wildlife. In regions of BC such as Kelowna, which have an abundance of lakes coupled with large populations and warm temperatures, beaches can become contaminated from an increase in swimmer density (Turgeon et al., 2011). One study found an average increase of 3% to 8% risk of AGI following swimming (Sanborn & Takaro, 2013). Children under five, in particular, were of high risk due to their behaviors that include swallowing more water, playing in shallow and warmer areas of the beach that are conducive to bacterial growth and staying in for longer periods. Another important consideration when interpreting the ALR and urban density variable is wildlife hosts. The North American beaver (*Castor canadensis*) is a notorious host for *giardia* and a major reason why even in the most pristine watersheds people still filter their water before consumption. In this study, beaver-related cases are likely predominantly in the rural group where habitat exists to support beaver populations; however, since many people from the city do travel to rural areas for recreational purposes, this may not be true. In this study, giardiasis as well as cryptosporidiosis and salmonellosis incidence was greater in the urban settings. Urban wildlife such as pigeons, pets and rodents could be linked with the relatively high incidence in urban populations. So again, it may be that agricultural practices are positively associated with these AGIs but that the urban environment is more positively associated with them, at least at the watershed scale. Further analysis is required to investigate these possibilities.

### *Eukaryotic predators and indirect effects of temperature on deactivation of pathogens*

It has been shown that temperature influences deactivation rates of *E. coli*, the degree to which varies depending on the type of water source involved (agricultural,

pristine, lakes, rivers or groundwater) (Blaustein et al., 2013). These differences may be associated with complex predatory-prey relationships at the micro-scale since the nutrient input of added *E. coli* into water sources (such as by livestock) presents a food source for eukaryotic predators that is greater than that supplied by wild or indigenous prey (Blaustein et al., 2013). Unique ecologies of the various microbial communities adds to the complexity of the temperature/pathogen deactivation relationship since protozoan predators have a temperature optimum of 15-20$^{o}$C, while the bacterial predators are more active as incubation temperatures increase. The often non-linear relationship observed between temperature and deactivation of *E. coli*, which generally slows over time, might be attributed to prey-switching that occurs when *E. coli* numbers become too low to sustain a predator species (Blaustein et al., 2013). Mauro et al. (2013) made the observation that an overall decrease in microbes in water was associated with an overall increase in VTEC. They also observed that VTEC interacted with the microcosm in fundamentally different ways than non-VTEC strains, generally appearing much more resistant to grazers. They hypothesize that this increased resistance to grazing might be resulting in increased VTEC persistence in aquatic environments relative to non-VTEC strains. This is one more characteristic of VTEC that could be helping to explain why it was the only AGI in this study positively associated with ALR at the watershed scale.

### *Hygiene hypothesis*

Watersheds can be quite large and ALR land is not necessarily being used for agriculture; therefore, it is likely there would be many watersheds in this study where people are categorized as residing in a watershed with ALR land, but may in fact live many kilometres away from any active agricultural activity. Galanis et al. (2014) found that persons living less than two kilometres from ALR in one region of BC to have a higher incidence of campylobacter (compared to other enteric diseases) than those afar. This was consistent with another study that observed during baseflow conditions, the area within two kilometres influences most of the microbiological quality of a sampling point (Turgeon et al., 2011). The study by Galanis et al. (2014) was on a much smaller region of BC and to assume that those relationships are representative of the entire province would be to ignore the possibility that environment - AGI relationships may

change in response to changes in climate and agricultural practices that vary throughout BC.

As one moves further from the ALR, what happens when the point is reached where there are still pathogens associated with ALR present but the quantity is too low to infect a host? It is possible that constant low-dose exposures could cause an immune system response, thereby offering some protection to individuals when exposed at higher doses. This effect is often called the “hygiene hypothesis”, for which urban living and agriculture have often been used as proxy measures for investigating (decreased and increased pathogen exposure, respectively) (Bloomfield, Stanwell-Smith, Crevel, & Pickup, 2006). One study measuring VTEC antibodies in blood observed that rural residents were most likely to have antibodies, followed by those living in a small city, with the largest city having the least people with antibodies (Haack et al., 2003). (Spencer et al., 2012) observed significant relationships in both directions between proximity to poultry farms and campylobacter infection. They suggested the variation was a result in the vastly different size of farms in the study populations. This might suggest there is a threshold, below which the poultry farms do not have a positive influence any longer with AGI and maybe a negative one. Belongia et al. (2003) compared diarrhoea rates among children living on farms to those who do not. They found that children on farms had a 46% lower incidence of diarrhoea (19 episodes/1000 child-years versus 35 episodes/1000 child-years with non-overlapping 95% CIs) and suggest repeated antigenic stimulation from a farm environment as being a factor.

One possible reason why there is conflicting results in the literature regarding the direction of the relationship between farming activities and AGI is because livestock, farming practices (i.e. manure spreading), climate, stream proximity and drinking water supply systems all vary across space and time. So depending on the scale of study, where it is located, what variables are included (or not), who the subjects are, and what pathogen is being investigated, variation could probably be expected. But if the hygiene hypothesis does explain some of this variation, Figure 5.2 presents one hypothetical way in which it may be doing so.

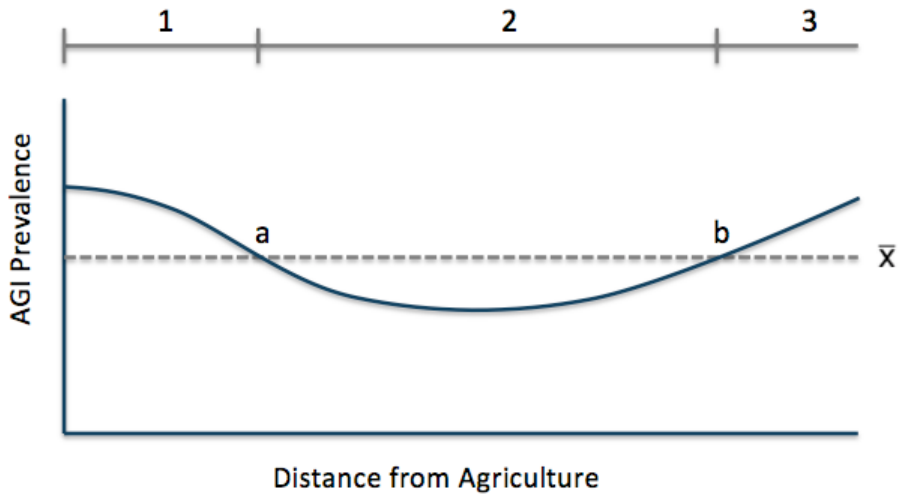


Figure 5.1 Hypothesized association between adaptive immunity and agricultural land

Zone 1 is the high-risk zone with sufficient pathogen exposure to cause infection in a relatively high proportion of those exposed, even in individuals that were previously immune. (Havelaar et al., 2009) observed that large doses can overcome campylobacteriosis immunity among rural residents. Zone 2 is the area whereby exposure is occurring but it results in an immune response more often than it causes a full-blown infection, making it relatively 'protective' on the population scale. Zone 3 represents the area where there is no exposure to pathogens from the original source, and no protective effect of the persistent low-level exposure. In this zone people are exposed to pathogens less frequently, on average, but when they are exposed it is more likely to result in a full-blown illness due to a relatively weak immune system. The exact distances of points 'a' and 'b' would widely vary throughout the province depending upon agricultural activities, physical geography, climate and season, among other reasons. The shape of the curve could also vary among pathogens.

In the case of campylobacteriosis, point 'a' could be on average around 2 km from the source (Galanis et al., 2014). In our study, distance 'b' might loosely represent the watershed boundary, except on larger watersheds with limited ALR where this point would likely reside within the watershed boundary. If 'b' is the watershed boundary, and if on the watershed population scale there are on average more people getting the low-dose 'protective' exposure (zone 2) than the high dose close-proximity exposure (zone 1), then it would re-create the negative correlations observed among

campylobacteriosis, salmonellosis and giardiasis with ALR in this study. In this case zone 3 would be describing an adjacent no-ALR watershed whose incidence is higher than in the ALR watershed, on average, uninfluenced by the distance from the original source. For an AGI like VTEC that was positively correlated with ALR land, point 'a' would move much closer to point 'b' until the combined incidence of zones 1 and 2 were greater than that of zone 3. This might occur if pathogen density originating from the source is high. There are many assumptions being made in this hypothesis; however, it does provide an attempt to integrate two studies of different scales that observed opposite associations with campylobacteriosis and ALR in BC. It is entirely possible, however, that too much variation exists within the ALR layer and that the associations upon which this model has been built are spurious. Further analysis using higher quality agricultural data at multiple scales would help to explore this idea further.

Differences in rates of AGI among children under 10 years of age can be seen in Figures 4.7 and 4.8, when compared to those of the entire general population (Figures 4.5 and 4.6). Of the four AGIs with significant differences in rates between ALR watersheds and non-ALR watersheds in the general population, only one is apparent in children under 10, salmonellosis. These differences might be attributed to variations in statistical power, since there are only 4,509 cases in the under 10 analysis compared to the 27,250 in the general population analysis. Assuming this does not explain all of the differences, it does raise the possibility as to whether children might not have yet developed the adaptive immunity for campylobacterioisis and giardiasis that may exist in the older general population (suggested by the 'protective' association with agricultural land). Something else that is different with the children in this study is that they did not display the positive associate between VTEC and agricultural land that was apparent in the general population, which could suggest that children are more resilient to VTEC or they are simply exposed less.

### 5.2.8. Climate change projections

There is much uncertainty in the AGI estimates due to climate change in this study; however, there is less uncertainty in the direction of the estimated trends.

Because regression models were calculated at the provincial scale, caution should be taken when trying to interpret what these findings mean for particular regions.

### *BGC zone rate*

Since the reference period of 1961-1990, 20% of the land in BC has already shifted to climates that more closely resemble those of other BGC zones (Wang, Hamann, et al., 2012). The AGI rates that were calculated for each BGC zone were done so without knowing what associations might exist. The results suggest that the bacterial pathogens are generally associated with BGC zones via temperature; however for VTEC, also with evaporation and climatic moisture-deficit. With the parasites, no such link was observed even though their rates did vary across the different zones to some extent. Because of this inability to associate the parasites to any climatic variables at the BGC zone level, there is less confidence in spatial projections for parasites than with the bacteria. However, it is possible that parasite-BGC zone links exist that were not investigated here.

### *Precipitation*

There were no independent associations observed between annual precipitation and AGI in this study, which suggests that any effect precipitation may be having on AGI is independent of annual precipitation averages. Since there have been observations linking AGI with extreme weather events (Carlton et al., 2014; Curriero et al., 2001; Rizak & Hrudey, 2008), and increases in high precipitation events are projected to increase throughout BC (Pacific Climate Impacts Consortium, 2013), it is likely that precipitation-related AGI will increase in coming decades even if projections (Pacific Climate Impacts Consortium, 2013) suggest that annual provincial precipitation will stay roughly the same. Further research is required to investigate if the extended droughts projected for some regions of BC (Pacific Climate Impacts Consortium, 2013) will also have an influence on AGI. Special attention might be given to VTEC, which appears to be correlated with drought conditions at the BGC zone scale in this study (high reference evaporation, high moisture deficit and high heat-moisture index).

### *Temperature*

These results suggest that increased temperatures projected for the upcoming decades due to climate change may be associated with a near-linear increase in relative risk to AGI for the three bacterial pathogens, although no such trend was observed for the parasites. In BC, an increase of 2.7°C is projected by 2080s using an ensemble of RCP scenarios, with a range of between 1.5°C to 4.1°C (Pacific Climate Impacts Consortium, 2013). These projections are mid-point values derived from 30 GCMs, each run on a high (A2) and low (B1) greenhouse gas emission scenario. The ClimateWNA software package was not used here because it is designed for downscaling climate data to specific locations whereas this portion of the study required projections on the provincial scale.

On a global scale, relative to 1986-2005 levels by 2016-2035 there will likely be a global surface temperature increase of 0.3-0.7°C. Relative to 1850-1900 levels, by the end of the 21$^{st}$ century the global temperature increase is likely to exceed 1.5°C for all RCP scenarios and likely to exceed 2°C for the higher RCP scenarios (RCP6 and RCP8.5) (Stocker et al., 2013). Although the regression coefficients presented in this study are probably very conservative as a global estimate, they do suggest there could be substantial global increases in AGI in coming decades.

### *Difficulties in extrapolating to other regions*

In addition to the direct affects that temperature has on growth and reproduction of microbes, the climate-AGI associations observed (and not observed) in this study potentially involve indirect effects of climatic relationships with hundreds if not thousands of other species; communities of organisms that evolved together to survive and exploit ecological niches unique to BC's climate and terrain. The little that is known about how these five pathogens may join or form biofilms in the environment or how heterotrophic predators can prey upon them does highlight potentially important components of their disease ecologies. These beneficial and non-beneficial interactions are occurring simultaneously and mostly in ways that we do not yet understand, all the while being influenced by climate and climate change. Even though these five pathogens are ubiquitous throughout the world, the organisms they are interacting with may not be.

Therefore, while global differences in rates of these AGIs could largely be attributed to socio-economic factors and possibly directly by climate, at this point in time the possibility that some variation might also be due to variations in microbial communities throughout the globe (and their unique associations with climate and local physical landscapes) cannot be ruled out. It is for these reasons and many others why extrapolating this study to regions beyond BC is problematic, as well as why conducting similar research around the globe is warranted.

### 5.2.9. Policy implications/recommendations

Because this is an ecological study, on its own it is not a very useful tool for making policy recommendations. However, when considered along with existing research, it does suggest that there will be an increase in both bacterial and parasitic AGI province-wide in the coming years due to climate change (the latter due to increased extreme precipitation events that increase risk independent of annual rainfall). The BGC zones with high rates identify areas of risk that were otherwise unrecognizable when rates were described in HAs. These regions might be investigated more closely to identify any gaps in existing policy or opportunities for implementing new policy that could reduce AGI incidence.

ALR land is positively associated with VTEC on a watershed scale, suggesting that additional policy surrounding cattle management could potentially impact future AGI incidence, particularly in BGC zones with high VTEC risk. A number of accessible methods exist that could make good contributions towards lowering nonpoint source pathogen contamination of cattle farms. Generally speaking, any practice that promotes infiltration of potentially contaminated water into the soil will reduce farm runoff (Swinton, Lupi, Robertson, & Hamilton, 2007), a practice that also has benefits for farmers by conserving nutrients, soil and recharging groundwater supplies. Such practices might include maintaining vegetative ground cover, which could be made possible by rotating cattle throughout a pasture so as to allow sufficient time for vegetation to recover between grazing periods. Providing cattle with sources of water that are separated from nearby waterways will also allow for ecological restoration of riparian areas. This serves the dual purpose of both separating cattle from waterways as well as creating a filtration

system (forested ecosystem) that cleans contaminated groundwater as it makes its way from a nearby field into adjacent waterways. So called 'buffer zones' have been observed to filter out many pollutants from adjacent agricultural operations, including pathogens. In one study, *E. coli* content of runoff was reduced by 48-57% in the first year alone by planting buffer zones (Duchemin & Hogue, 2009). By taking an ecosystems approach such as this, nonpoint source contamination can be reduced through riparian restoration initiatives that can be tailored to fit individual farming operations, local cultural practices and native ecosystems.

The study highlights the risks of extracting drinking water from unconsolidated aquifers. The public might benefit from tighter safety regulations for wells in those areas. However, a specific study investigating the efficacy of the *Drinking Water Protection Act* on risk associated with wells in unconsolidated aquifers ought to be completed first, in case the risk associated with unconsolidated aquifers has since dropped.

Inherent limitations of using administrative datasets have to do with the fact that data are collected before a research question is developed, which often leads to data that although useful, are far from ideal. For instance, this study lacked socio-economic information of AGI subjects in addition to information on pre-existing health conditions, whether or not a case was travel-related, food consumption patterns and hygiene. Although probably not feasible on a province-wide scale, collecting primary data designed specifically to answer the questions presented in this thesis is likely the only way to reliably separate the effects of the different environmental variables on AGI. The data could be collected in a way that would allow for robust longitudinal studies with control groups. Aside from collecting primary AGI data, the following suggestions would also improve the study: acquiring accurate drinking water data (water quality and drinking water system information as well as exposure data), utilizing aquifer vulnerability data in connection with well databases, improving accuracy and precision of agricultural data, incorporating flow dynamics during watershed analysis, inclusion of recreational water-use patterns and proximity to medical facilities.

A major strength of this study is that it includes the entire province when most other studies tend to focus on much smaller regions; this was particularly important for

studying the impacts of annual climatic conditions with AGI and making projections that would be relevant for the entire province. This strength, however, is also its weakness. It is very resource-intensive to obtain accurate and precise data on a province-wide scale for many of the variables of interest, which resulted in less-than-ideal datasets being used in many cases. Future studies seeking to separate the affects of these variables on AGI to identify specific causal pathways probably ought to be completed on much smaller spatial scales where higher quality data are more accessible. With that said, enough is already known about the existence of nonpoint sources (ie. cattle and human waste), that such studies are not necessary to rationalize the implementation of already-proven methods to reduce both pathogen numbers and exposure. In fact, it is arguably a better use of limited resources to begin implementing techniques that are already proven effective to reduce AGI risk, and of course to measure their impacts on human AGI over time, than it is to spend them trying to conclusively identify specific causal pathways that in reality do not exist in isolation.

# Chapter 6. Conclusion

This study has taken a novel approach towards investigating AGI - environment associations by comparing incidence across ecosystems, watersheds and aquifer types in BC. It also highlights the care that ought to be taken when combining multiple pathogens to investigate environmental associations. Grouping by bacteria and parasites generally appears appropriate; however, VTEC might be more appropriately analyzed separately. While incidence for all AGIs varied among the BGC zones, climatic associations for these trends were only evident in the bacterial AGIs campylobacteriosis, VTEC and salmonellosis. Of them, annual temperature-related variables were the most consistent, with a clear trend of increased incidence with increasing temperatures. A PAR of 0.10063 was calculated for the three bacteria combined, suggesting that for every degree Celsius increase in annual temperature, BC could experience a 10% increase in these AGIs. Incorporating population growth projections, climate change projections and multipliers to account for under-diagnosis and under-reporting, between 14,305 to 39,101 additional cases of bacterial AGIs could result annually by 2080s in BC. Climatic associations were observed with VTEC that were not evident in any of the other AGIs, notably positive associations with reference evaporation and moisture-deficit. VTEC stood out among the bacterial AGIs further as being the only one with a positive association with agricultural land at the watershed scale. The other AGIs were either not correlated or had a negative correlation with agricultural land, highlighting the need for future studies pertaining to the scale-sensitivity of the pathogen-agriculture relationships. Such studies would also be necessary to investigate what role, if any, that adaptive immunity plays in mediating AGI incidence across the urban/rural or agricultural divides. The positive associations that all AGIs were observed to have with unconsolidated aquifers suggests that on average, wells drilled in unconsolidated material are at a greater risk to pathogenic infection for both bacterial and parasitic AGIs in BC.

Opportunities for future research were provided throughout the report in their respective sections. Generally, much research is still required to investigate AGI/environment associations in BC and beyond, which this study has shown are apparent at large spatial scales using biologically meaningful boundaries across which to compare incidence rates. The argument underlying the methods of this research is that while health data are commonly collected and disseminated using political jurisdictions, such as health authorities, when investigating human-environment associations it may be more appropriate to re-aggregate the data using biophysical boundaries that hold meaning for the organisms or ecological processes thought to be involved. This is not to say that political boundaries do not have an important role in environmental health research, so much as it is to say that in failing to explore what other boundaries might exist that could more accurately describe regions with similar ecological processes, would be to ignore the innate complexity of the world. Future research will benefit from disentangling the associations of the various environmental terms that were independently associated with AGI here and discussing those associations in the context of both climate change and a growing population.

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# Appendix A

## Biogeoclimatic zones: Current distribution and future projections

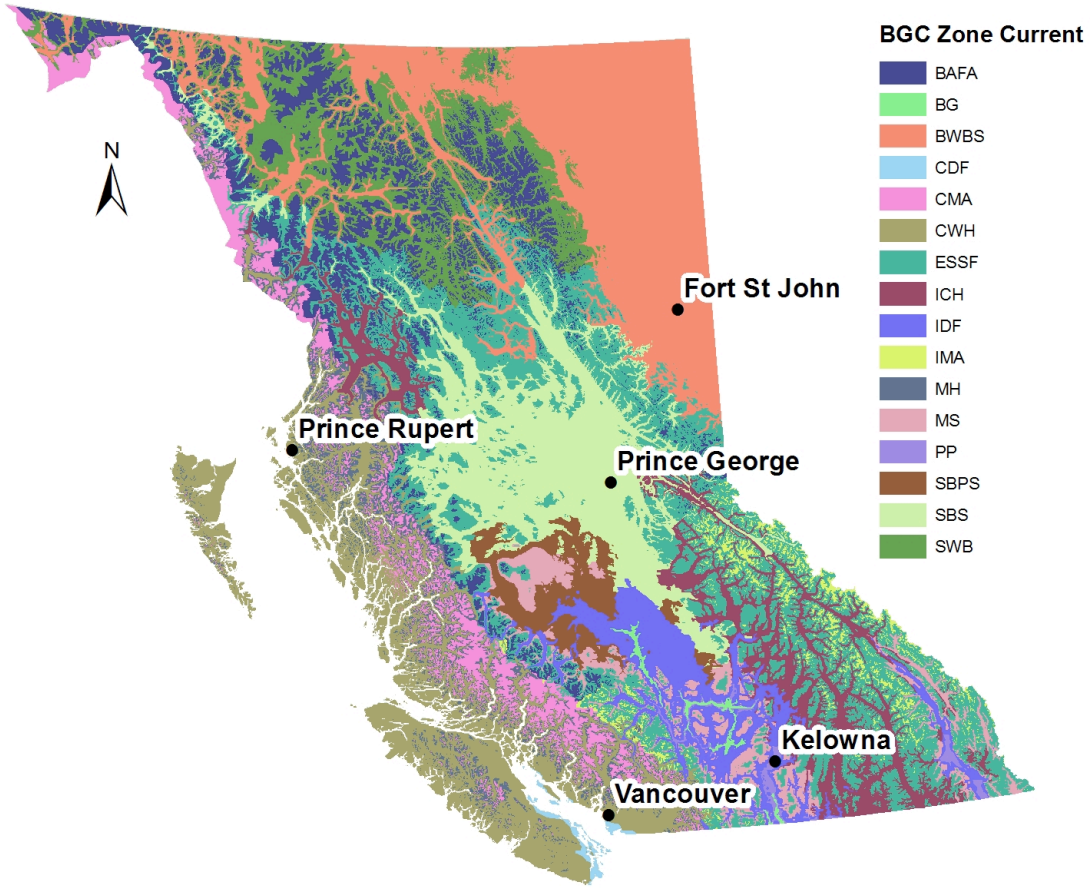


Figure A1- Current distribution of biogeoclimatic zones (Wang, Hamann, et al., 2012). See Table A1 for explanation of legend.

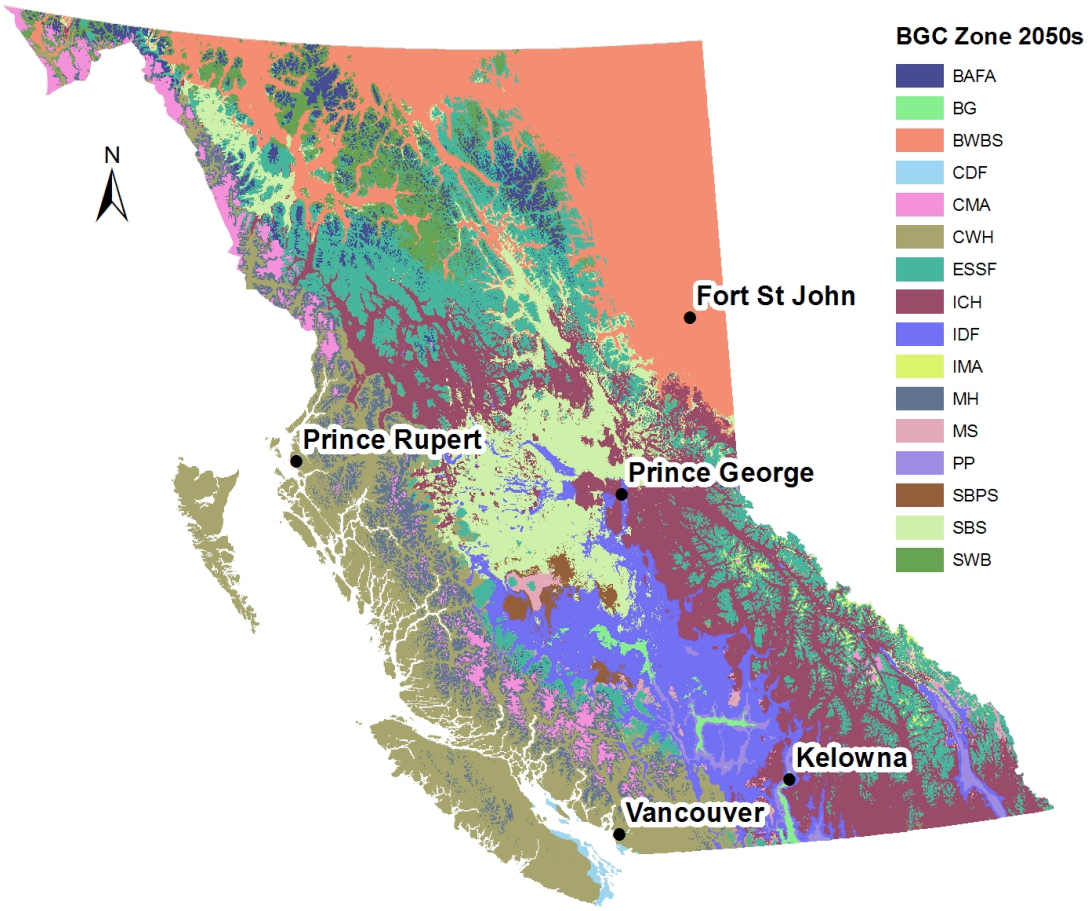


Figure A2- Projection distribution of biogeoclimatic zones (2050s) (Wang, Hamann, et al., 2012). See Table A1 for explanation of legend.

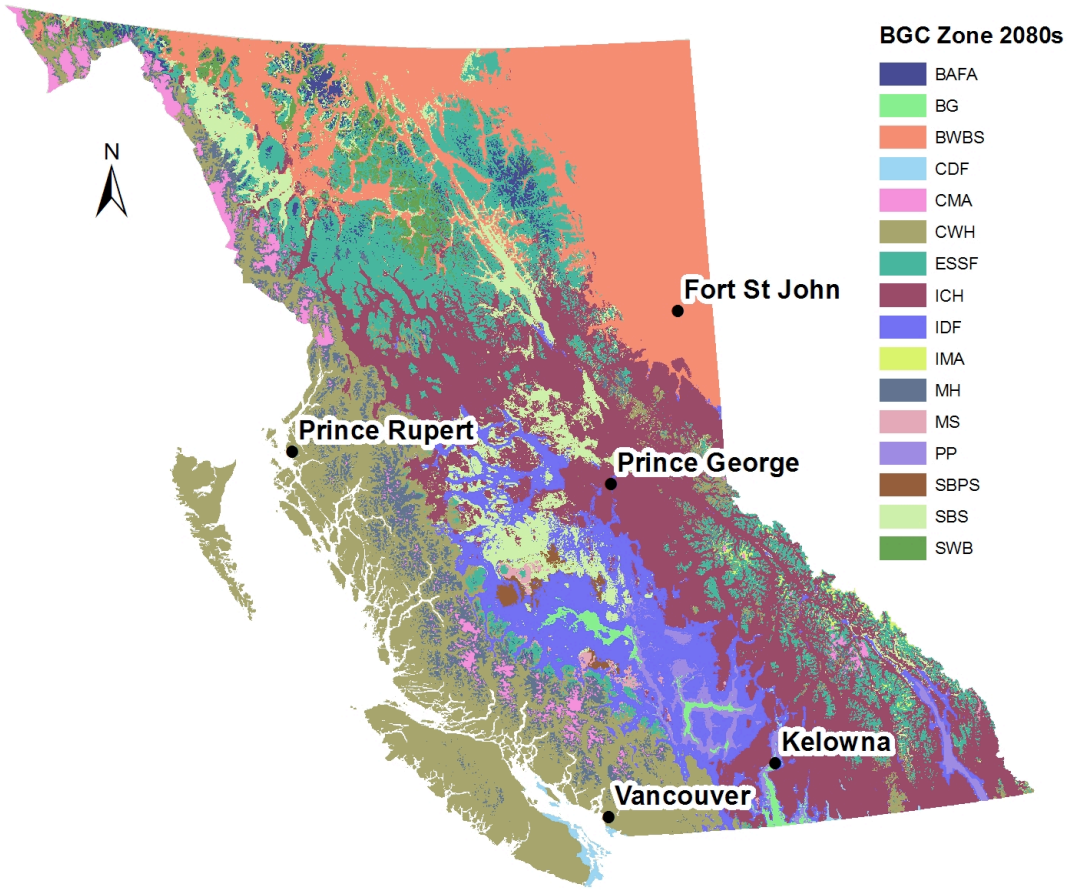


Figure A3- Projected distribution of biogeoclimatic zones (2080s) (Wang, Hamann, et al., 2012). See Table A1 for explanation of legend.

**Table A1 – BGC zone abbreviations**

| Abbreviation | BGC Zone |
|---|---|
| **BAFA, CMA, IMA** | Alpine Tundra |
| **BWBS** | Boreal White and Black Spruce |
| **BG** | Bunchgrass |
| **CDF** | Coastal Douglas-fir |
| **CWH** | Coastal Western Hemlock |
| **ESSF** | Engelmann Spruce—Subalpine Fir |
| **ICH** | Interior Cedar—Hemlock |
| **IDF** | Interior Douglas-fir |
| **MS** | Montane Spruce |
| **MH** | Mountain Hemlock |
| **PP** | Ponderosa Pine |
| **SWB** | Spruce—Willow—Birch |
| **SBPS** | Sub-Boreal Pine—Spruce |
| **SBS** | Sub-Boreal Spruce |

# Appendix B

# Sensitivity analysis for imputing missing values

**Table B1 – Pearson's correlation results using values for VCHA (2006-2010) that are 2 standard deviations lower than the average of 2000-2005 and 2011-2013**

| | | Campy | VTEC | Salmo | Giard | Crypt |
|---|---|---|---|---|---|---|
| **Annual temp.** | R | 0.621 | 0.583 | 0.578 | -0.003 | 0.128 |
| | sig. | 0.074 | 0.100 | 0.103 | 0.994 | 0.742 |
| **Temp. of the warmest month** | R | 0.413 | **0.800**** | 0.421 | -0.200 | 0.288 |
| | sig. | 0.269 | 0.010 | 0.259 | 0.606 | 0.453 |
| **Temp. of the coldest month** | R | **0.720*** | 0.376 | 0.642 | 0.190 | 0.025 |
| | sig. | 0.029 | 0.319 | 0.062 | 0.624 | 0.950 |
| **Continentality** | R | **-0.678*** | -0.003 | -0.572 | -0.365 | 0.141 |
| | sig. | 0.045 | 0.994 | 0.107 | 0.335 | 0.717 |
| **Annual precip.** | R | 0.315 | -0.385 | 0.122 | 0.409 | -0.150 |
| | sig. | 0.409 | 0.306 | 0.754 | 0.275 | 0.701 |
| **Growing season precip.** | R | -0.144 | -0.645 | -0.347 | 0.252 | -0.164 |
| | sig. | 0.711 | 0.061 | 0.361 | 0.514 | 0.673 |
| **Annual heat-moisture index** | R | 0.051 | **0.796*** | 0.233 | -0.358 | 0.346 |
| | sig. | 0.897 | 0.010 | 0.546 | 0.344 | 0.362 |
| **Summer heat-moisture index** | R | 0.269 | **0.808**** | 0.397 | -0.295 | 0.200 |
| | sig. | 0.484 | 0.008 | 0.290 | 0.440 | 0.607 |
| **Degree-days below 0** | R | **-0.763*** | -0.604 | **-0.720*** | -0.209 | -0.147 |
| | sig. | 0.017 | 0.085 | 0.029 | 0.590 | 0.705 |
| **Degree-days above 5** | R | 0.459 | 0.637 | 0.441 | -0.206 | 0.153 |
| | sig. | 0.214 | 0.065 | 0.235 | 0.595 | 0.694 |
| **Degree-days below 18** | R | -0.581 | -0.596 | -0.553 | 0.063 | -0.122 |
| | sig. | 0.101 | 0.091 | 0.123 | 0.873 | 0.755 |

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

**Table B1b - Pearson's correlation results using values for VCHA (2006-2010) that are 2 standard deviations lower than the average of 2000-2005 and 2011-2013 (continued)**

| | | Campy | VTEC | Salmo | Giard | Crypt |
|---|---|---|---|---|---|---|
| **Degree-days above 18** | R | 0.288 | **0.797*** | 0.348 | -0.294 | 0.181 |
| | sig. | 0.452 | 0.010 | 0.358 | 0.442 | 0.642 |
| **Frost-free days** | R | 0.517 | 0.253 | 0.401 | -0.038 | -0.031 |
| | sig. | 0.154 | 0.512 | 0.285 | 0.922 | 0.936 |
| **Frost-free period** | R | 0.495 | 0.266 | 0.379 | -0.082 | -0.034 |
| | sig. | 0.175 | 0.490 | 0.314 | 0.834 | 0.931 |
| **Annual snowfall** | R | -0.079 | -0.576 | -0.193 | 0.532 | -0.196 |
| | sig. | 0.839 | 0.105 | 0.619 | 0.141 | 0.614 |
| **Extreme min. temp.** | R | 0.590 | 0.276 | 0.482 | 0.033 | -0.040 |
| | sig. | 0.095 | 0.473 | 0.188 | 0.933 | 0.920 |
| **Extreme max. temp.** | R | 0.543 | **0.878**** | 0.588 | -0.002 | 0.402 |
| | sig. | 0.130 | 0.002 | 0.096 | 0.996 | 0.283 |
| **Reference Evap.** | R | 0.572 | **0.905**** | 0.648 | -0.003 | 0.383 |
| | sig. | 0.107 | 0.001 | 0.059 | 0.993 | 0.309 |
| **Climatic moisture deficit** | R | 0.291 | **0.890**** | 0.464 | -0.200 | 0.364 |
| | sig. | 0.448 | 0.001 | 0.208 | 0.605 | 0.336 |

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed)

**Table B2 – Pearson's correlation results using values for VCHA (2006-2010) that are 2 standard deviations higher than the average of 2000-2005 and 2011-2013**

| | | Campy | VTEC | Salmo | Giard | Crypt |
|---|---|---|---|---|---|---|
| **Annual temp.** | R | **0.760*** | **0.868**** | **0.700*** | 0.181 | 0.487 |
| | sig. | 0.017 | 0.002 | 0.036 | 0.641 | 0.183 |
| **Temp. of the warmest month** | R | 0.424 | **0.890**** | 0.403 | -0.116 | 0.394 |
| | sig. | 0.256 | 0.001 | 0.282 | 0.766 | 0.294 |
| **Temp. of the coldest month** | R | **0.906**** | **0.746*** | **0.825**** | 0.408 | 0.495 |
| | sig. | 0.001 | 0.021 | 0.006 | 0.275 | 0.176 |
| **Continentality** | R | **-0.911**** | -0.425 | **-0.819**** | -0.595 | -0.399 |
| | sig. | 0.001 | 0.254 | 0.007 | 0.091 | 0.287 |
| **Annual precip.** | R | 0.449 | -0.036 | 0.545 | 0.576 | 0.361 |
| | sig. | 0.225 | 0.926 | 0.129 | 0.104 | 0.339 |
| **Growing season precip.** | R | -0.096 | -0.499 | 0.007 | 0.303 | 0.086 |
| | sig. | 0.806 | 0.171 | 0.985 | 0.429 | 0.825 |
| **Annual heat-moisture index** | R | -0.107 | 0.581 | -0.067 | -0.445 | 0.045 |
| | sig. | 0.785 | 0.101 | 0.864 | 0.230 | 0.909 |
| **Summer heat-moisture index** | R | 0.229 | **0.760*** | 0.196 | -0.286 | 0.112 |
| | sig. | 0.554 | 0.018 | 0.612 | 0.456 | 0.774 |
| **Degree-days below 0** | R | **-0.861**** | **-0.893**** | **-0.814**** | -0.381 | -0.515 |
| | sig. | 0.003 | 0.001 | 0.008 | 0.312 | 0.156 |
| **Degree-days above 5** | R | 0.584 | **0.849**** | 0.515 | -0.050 | 0.410 |
| | sig. | 0.098 | 0.004 | 0.156 | 0.897 | 0.274 |
| **Degree-days below 18** | R | **-0.728*** | **-0.852**** | -0.622 | -0.110 | -0.427 |
| | sig. | 0.026 | 0.004 | 0.073 | 0.778 | 0.252 |

**Table B2b - Pearson's correlation results using values for VCHA (2006-2010) that are 2 standard deviations higher than the average of 2000-2005 and 2011-2013 (continued)**

| | | Campy | VTEC | Salmo | Giard | Crypt |
|---|---|---|---|---|---|---|
| **Degree-days above 18** | R | 0.256 | **0.784*** | 0.222 | -0.265 | 0.155 |
| | sig. | 0.506 | 0.012 | 0.565 | 0.492 | 0.691 |
| **Frost-free days** | R | **0.765*** | 0.648 | **0.689*** | 0.208 | 0.479 |
| | sig. | 0.016 | 0.059 | 0.040 | 0.592 | 0.192 |
| **Frost-free period** | R | **0.747*** | 0.650 | 0.652 | 0.161 | 0.457 |
| | sig. | 0.021 | 0.058 | 0.057 | 0.679 | 0.216 |
| **Annual snowfall** | R | -0.195 | -0.606 | -0.079 | 0.459 | -0.172 |
| | sig. | 0.615 | 0.083 | 0.840 | 0.213 | 0.658 |
| **Extreme min. temp.** | R | **0.834**** | 0.663 | **0.714*** | 0.271 | 0.448 |
| | sig. | 0.005 | 0.051 | 0.031 | 0.480 | 0.227 |
| **Extreme max. temp.** | R | 0.482 | **0.953**** | 0.539 | 0.059 | 0.499 |
| | sig. | 0.189 | 0.000 | 0.134 | 0.881 | 0.172 |
| **Reference Evap.** | R | 0.523 | **0.965**** | 0.530 | 0.052 | 0.442 |
| | sig. | 0.148 | 0.000 | 0.142 | 0.895 | 0.233 |
| **Climatic moisture deficit** | R | 0.174 | **0.767*** | 0.176 | -0.240 | 0.171 |
| | sig. | 0.655 | 0.016 | 0.650 | 0.533 | 0.661 |

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed)

**Table B3 – AGI rates among aquifer types using values for VCHA (2006-2010) that are 2 standard deviations lower than the average of 2000-2005 and 2011-2013**

| | | Rate | CI- | CI+ |
|---|---|---|---|---|
| **Campy** | **Bedrock** | 35.33 | 34.06 | 36.65 |
| | **Unconsolidated** | 35.86 | 35.23 | 36.50 |
| **VTEC** | **Bedrock** | 2.93 | 2.57 | 3.33 |
| | **Unconsolidated** | 3.12 | 2.94 | 3.32 |
| **Salmo** | **Bedrock** | 13.70 | 12.92 | 14.54 |
| | **Unconsolidated** | 17.19 | 16.76 | 17.63 |
| **Giard** | **Bedrock** | 11.47 | 10.76 | 12.24 |
| | **Unconsolidated** | 13.14 | 12.77 | 13.53 |
| **Crypt** | **Bedrock** | 1.81 | 1.54 | 2.14 |
| | **Unconsolidated** | 2.08 | 1.93 | 2.24 |

# Appendix C

## AGI rates across multiple levels of ALR per watershed

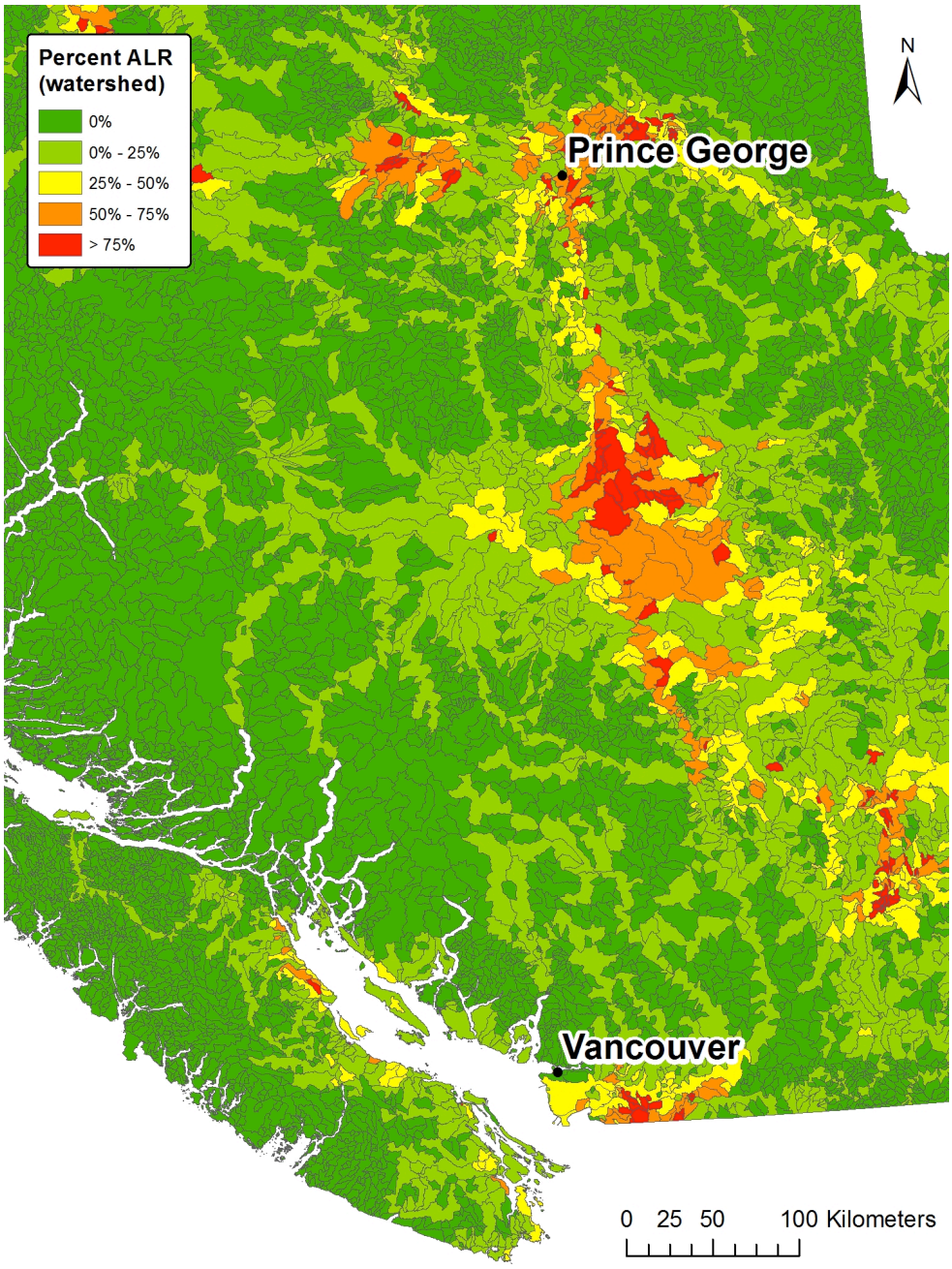


Figure C1 – ALR percentage per watershed (Agricultural Land Commission, 2015; BC Government Ecosystems Branch, 2005)

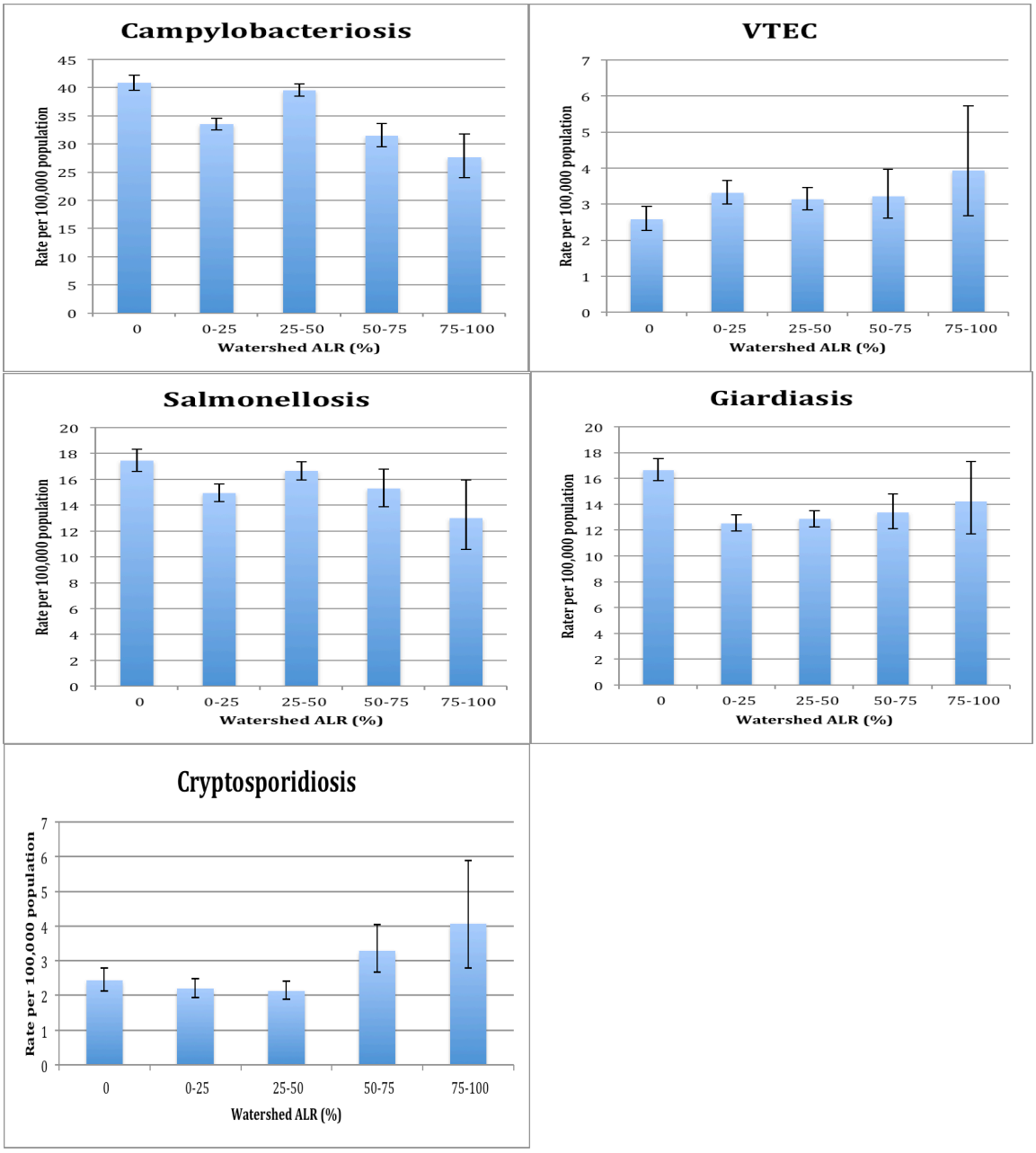


Figure C2 - AGI rates across multiple levels of ALR per watershed (years 2000-2005 and 2011-2013)

# Appendix D

## Population density (urban/rural)

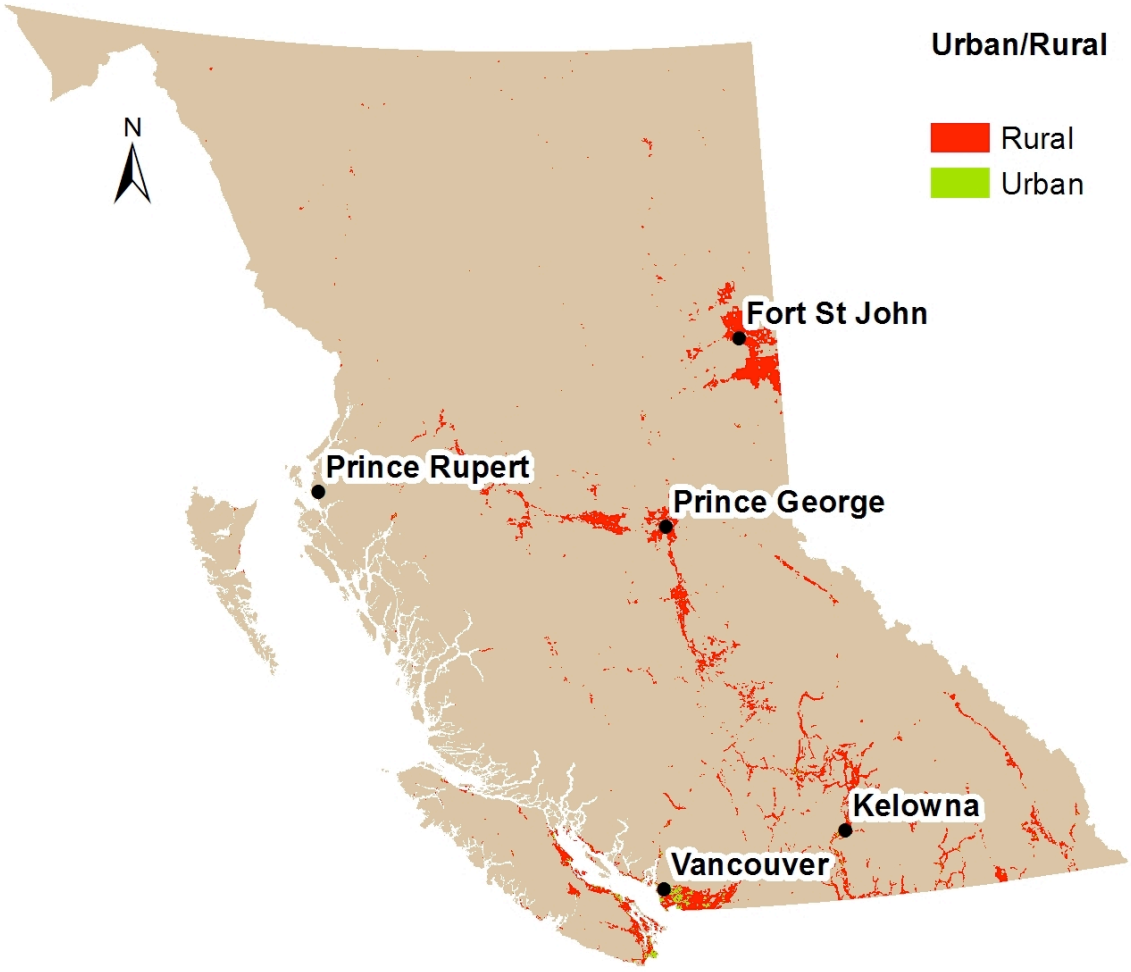


Figure D1 – 2011 Population density as defined by urban or rural (greater or less than 400 persons per km$^2$, respectively)

This layer was generated by combining census data at the dissemination block level (Statistics Canada, 2011) with a population ecumene layer created by Anthony Smith (2011).

# Appendix E

## Aquifer type distribution

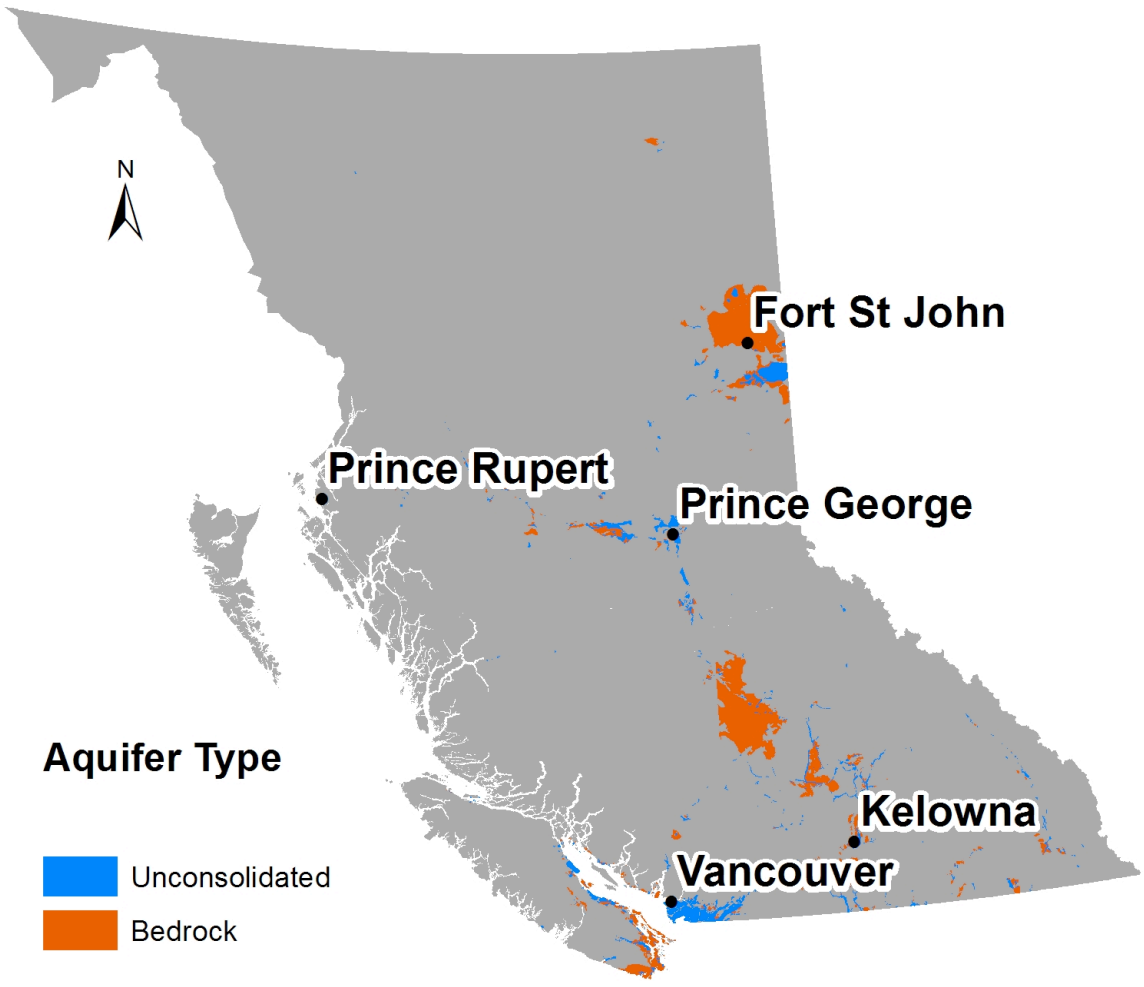


Figure E1 – Distribution of two types of aquifers in British Columbia; unconsolidated (silt, sand and gravel) and bedrock (iMapBC, 2014)

# Appendix F

# Negative binomial regression models and results for three bacterial AGIs and annual temperature

**Basic model**
glm.nb(formula = AGI_count ~ Annual_Temp + offset(log(Population))

**Campylobacteriosis**
Deviance Residuals:
```
   Min      1Q  Median      3Q     Max
-2.2440  -0.6388  -0.1805   0.5169   1.4835

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)     -8.83708    0.29443 -30.014   <2e-16 ***
data$Temp_Annual  0.11394    0.04902   2.324   0.0201 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(6.0568) family taken to be 1)
Null deviance: 14.3901  on 8  degrees of freedom
Residual deviance:  9.6346  on 7  degrees of freedom
AIC: 131.3
Number of Fisher Scoring iterations: 1
Theta:  6.06
Std. Err.:  2.98
2 x log-likelihood:  -125.301
```

**VTEC**
```
Deviance Residuals:
   Min      1Q  Median      3Q     Max
-2.2826  -0.3633   0.2001   0.5253   1.2978

Coefficients:
             Estimate Std. Error z value Pr(>|z|)
(Intercept)     -11.2020     0.2866 -39.089  < 2e-16 ***
data$Temp_Annual   0.1291     0.0445   2.902  0.00371 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(10.0133) family taken to be 1)
Null deviance: 16.8304  on 8  degrees of freedom
Residual deviance:  9.9883  on 7  degrees of freedom
AIC: 88.895
Number of Fisher Scoring iterations: 1
```

Theta:  10.01
Std. Err.:  6.38
2 x log-likelihood:  -82.895

**Salmonellosis**

Deviance Residuals:
```
    Min       1Q   Median       3Q      Max
-2.10223  -0.68503  -0.03897   0.92662   1.60766
```

Coefficients:
```
                Estimate Std. Error z value Pr(>|z|)
(Intercept)     -9.35636    0.14057 -66.559  < 2e-16 ***
data$Temp_Annual  0.07363    0.02221   3.315 0.000916 ***
---
```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(37.0659) family taken to be 1)
Null deviance: 20.889  on 8  degrees of freedom
Residual deviance: 10.789  on 7  degrees of freedom
AIC: 106.21
Number of Fisher Scoring iterations: 1
Theta:  37.1
Std. Err.:  23.3
2 x log-likelihood:  -100.208

**Campylobacteriosis, VTEC and salmonellosis (combined)**

Deviance Residuals:
```
   Min      1Q  Median      3Q     Max
-2.2681  -0.5919  -0.2160   0.5189   1.5958
```

Coefficients:
```
                Estimate Std. Error z value Pr(>|z|)
(Intercept)     -8.35126    0.23773 -35.130  < 2e-16 ***
data$Temp_Annual  0.10606    0.03862   2.746  0.00603 **
---
```
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

(Dispersion parameter for Negative Binomial(10.1222) family taken to be 1)
Null deviance: 16.184  on 8  degrees of freedom
Residual deviance:  9.581  on 7  degrees of freedom
AIC: 135.53
Number of Fisher Scoring iterations: 1
Theta:  10.12
Std. Err.:  5.05
2xlog-likelihood:-129.535