Risk Factors Associated with Sporadic *Campylobacter*, *Salmonella* and Verotoxin-Producing *Escherichia coli* in Different Regions within Manitoba

by

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A Thesis submitted to the Faculty of Graduate Studies of

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ABSTRACT

Campylobacter, *Salmonella* and VTEC infect hundreds of Manitobans annually. Thus the purpose of this study is to determine the risk factors for the three infections and the geographic distribution of these factors.

The population under study was identified through Manitoba Health's surveillance system. Data was collected using a standard questionnaire and controls were case nominated. Data analysis included case-case comparisons by infection, location and infection stratified by location.

Infection rates were higher in rural youth, particularly for those infected with *Campylobacter,* which was found to be associated with 'rural' risk factors. *Salmonella* was found to be associated with urban or semi-urban lifestyle. VTEC infection was found to be related to outdoor recreational activities.

The methodology used provided a cost-effective technique of accessing almost all eligible lab-confirmed cases, however, the nomination of controls by cases was unsuccessful and an alternate method would be needed if future studies of this type are undertaken.

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CHAPTER 1: INTRODUCTION AND STUDY OBJECTIVES

1.0 Introduction:

Campylobacter, *Salmonella* and verotoxin-producing *Escherichia coli* (VTEC) infect hundreds of Manitobans annually [1]. Many previous studies have examined the risk factors associated with these pathogens in different populations all over the world, generating substantial knowledge in this domain. Although the general risk factors and geographic distribution of *Campylobacter*, *Salmonella* and VTEC are known, the specific risk factors for the individual pathogens within individual geographic areas may vary. It is therefore important to conduct local investigations to inform disease control efforts. It is also important to periodically conduct and repeat investigations of this type as risk factors within a region could change over time in response to changing environmental and demographic variables.

A study conducted in the Province of Manitoba highlighted the need for regional analysis as distinct differences in the prevalence patterns for *Campylobacter* were found between Winnipeg and the remainder of the province [2]. The prevalence in rural Manitoba was found to be higher when compared to Winnipeg, especially for those aged zero to four [2]. Similar patterns were seen for *Salmonella* and VTEC [3]. Further geographic analysis has found higher than expected prevalence of infection in particular regions that differed between pathogens. This suggested differences in risk factors or environmental sources between the different pathogens, and lead to the conclusion that a complex interplay of risk factors which varied between specific geographical regions was involved in infection [3].

Odoi et al. [4] did a similar study on the enteric pathogen *Giardia* in Ontario. They found that clusters of Giardasis were linked to livestock density, but that this was not always the case. It was determined that agricultural animal density and manure spreading was only moderately associated with spatial patterning in *Giardia.* Although both of these particular studies attempted to determine risk factors for enteric infections and to link these to environmental and geographical information, no specific information regarding unique risk factors for *Campylobacter*, *Salmonella* and VTEC by region were determined.

1.1 Purpose and Objectives:

With hundreds of Manitobans being infected by these pathogens each year, knowledge of the risk factors for each pathogen in each particular area is crucial in order to see a decrease in these rates. Thus the purpose of this study is to determine the risk factors for *Campylobacter*, *Salmonella* and VTEC and to determine the geographic distribution of these factors. The specific objectives of the study include:

1. Determining the risk factors for sporadic *Campylobacter, Salmonella* and VTEC infections in the Province of Manitoba.
2. Examining the difference between specific risk factors for sporadic *Campylobacter, Salmonella* and VTEC infection in the Province of Manitoba.
3. Examining how risk factors for sporadic *Campylobacter, Salmonella* and VTEC infection differ by location in the Province of Manitoba.

A second purpose of the study is to pilot a method utilizing existing public health practice to attain cases and to determine the ability to enroll case-nominated controls in the study. The specific objectives associated with this purpose are:

1. Determining the percentage of cases that consent to enrollment in the study.
2. Examining the percentage of study participants willing to nominate controls for the study, and the percentage of controls that consent to enrollment in the study.

1.2 Significance of the Study:

This study adds to the literature on *Campylobacter*, *Salmonella* and VTEC in the Province of Manitoba. There have been numerous studies on the risk factors for these bacterial infections but much less on the geographical distribution of the risks.

Studies have shown that particular geographic locations have increased rates of enteric infections and it is therefore thought that areas differ in the specific risk factors. Through the knowledge obtained from this study there is a potential to decrease the rates of *Campylobacter*, *Salmonella* and VTEC in Manitoba by allowing public health practitioners and the general public to know the risk factors for infection in their geographical area and thus be able to protect themselves from infection more effectively. As a Manitoba-specific study, the study has the potential to influence public health practice at a local level, and lead to changes in the public health investigations conducted by the Regional Health Authorities (RHAs).

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

A literature review was completed for *Campylobacter, Salmonella* and VTEC, providing background epidemiology including the major risk factors. The Manitoba surveillance system for reportable enteric infections and the methodology of case-case comparisons were also reviewed.

2.0 *Campylobacter*:

Campylobacter is a major cause of diarrheal illness in humans and regarded as being the most common bacterial cause of gastroenteritis worldwide [5]. *Campylobacter* infection is most commonly caused by two species *C. jejuni* and *C. coli.* These species are part of the genus *Campylobacter* which consists of slender, motile rod bacteria under the Epsilonproteobacteria class [6].

2.0.1 Reservoirs:

Campylobacter is found in the intestinal tract of warm blooded animals most commonly poultry [7]. It has also been associated with; pigs [8], rodents [9], wild birds [10] and domestic animals including dogs, cats [11], puppies and kittens [12].

2.0.2 Transmission and risk factors:

The bacteria are transmitted by a fecal-oral route from reservoirs to humans several ways, including direct contact with infected animals, contact with contaminated

water or food and from other infected individuals. *Campylobacter* can be transmitted by direct contact with infected domestic pets including cats and dogs [11,12], particularly those with diarrhea [13], domestic farm animals [14] and wild birds [15]. Infection can be spread to individuals upon consumption or direct contact with untreated water [16], as surface water can become contaminated due to runoff from animal production units, farms and meat processing plants [17]. Foodborne transmission can also occur and has been linked to undercooked meat, especially poultry [18,19], and contaminated milk [20]. Cross contamination of surfaces such as cutting boards during food preparation and storage has also been implicated in infection [21]. Vegetables and fruit can be contaminated if exposed to fecal matter during growth, harvesting or processing or as a result of cross-contamination during food preparation and storage [22]. Person-to-person transmission also occurs and is usually associated with young children and occurs within families [23].

2.0.3 Occurrence:

Campylobacter is an important cause of diarrheal illness worldwide in all age ranges [24]. The World Health Organization states that *Campylobacter* is the major cause of diarrheal illness in humans and the most common bacterial cause of gastroenteritis worldwide [25]. Globally, the bacteria is responsible for five to 14% of all reported diarrheal infections [23]. In industrialized countries it most commonly infects children younger than five and young adults [26]. In Canada, most *Campylobacter* cases occur sporadically [27] and infection rates exhibit distinct seasonal patterns with most cases occurring between June and September [28].

2.0.4 Incubation and communicability:

The incubation period for *Campylobacter* ranges from one to ten days [29] with an average of two to five [30]. Communicability occurs throughout the course of infection which is typically several days to several weeks [23].

2.0.5 Symptoms and diagnosis:

Campylobacter causes acute enteritis of variable severity [30]. Symptoms include diarrhea, abdominal pain, malaise, fever, nausea, and vomiting [23].

Diagnosis occurs through isolation of *Campylobacter* bacterium from a cultured stool specimen [24].

2.0.6 Complications:

Several complications can occur if *Campylobacter* infection becomes systemic. These include reactive arthritis, hepatitis, pancreatitis, febrile convulsions, thyroid-like syndrome and meningitis [25]. Another serious complication associated with *Campylobacter* infections is Guillaume-Barre Syndrome [31]. Although rare, occurring in only 0.1% of *Campylobacter* cases [32], this acute paralytic disease of the peripheral nervous system can be severe and result in irreversible neurological damage or death [17].

2.0.7 Treatment:

The illness is typically self-limiting [33] with symptoms clearing within approximately two to five days [24]. Simple electrolyte replacement and rehydration can be

undertaken. In invasive cases or to eliminate carrier state antimicrobial treatment can be administered and is usually erythromycin, tetracycline, or quinolones [25].

2.0.8 *Campylobacter* in Manitoba:

Campylobacter infects over 200 Manitobans annually. It occurs most often in young children aged zero to four as well as young adults aged 20 to 39 with most cases occurring during July and August [24]. Infection rates in rural Manitoba are higher overall and in almost all age categories, particularly for those aged zero to four. Infections in Manitoba vary geographically according to agricultural animal density, where higher infection rates are associated with greater animal density [2].

A study conducted on the risk factors of *Campylobacter* in Manitoba found consuming unpasteurized milk and undercooked poultry, as well as contact with contaminated surface water, domestic pets and various farm animals were related to infection [2]. Previous antibiotic use and foreign travel were also found to be associated with increased risk [24].

2.1 *Salmonella*:

Salmonellosis is one of the most common and widely distributed foodborne illnesses worldwide [34]. *Salmonella* is a species of motile rod bacteria belonging to the class Gamma Proteobacteria [35], all human pathogens are classified in the subspecies Enterica and the most common disease causing serotypes in Manitoba, representing 63%

of all *Salmonella* infections in 2000, are Typhimurium (25%), Enteritidis (22%) and Heidelberg (16%) [36].

2.1.1 Reservoirs:

A wide range of domestic and wild animals serve as reservoirs for *Salmonella.* The bacteria live in the intestines of various animals particularly poultry and swine [37], but also cattle, horses, rodents, and pets such as hedgehogs, iguanas, tortoises, dogs and cats [38,39]. Humans can also be reservoirs, although this chronic carrier state is rare [37].

2.1.2 Transmission and risk factors:

The bacteria are transmitted fecal-orally from reservoirs to humans by several routes, including direct contact with infected animals or persons, from food derived from infected animals or from cross-contamination of food products or objects [40]. *Salmonella* bacteria has been associated with various food products namely; chicken [41-44], eggs [42,43,45], especially those which are raw or undercooked [46], pork [47], cheese [43], beef [44,48], dairy [34,44], unpasteurized milk products [36], raw fruits and vegetables [40,48], sprouts [49] and unpasteurized juices [41,44]. Meat products may be contaminated during slaughter and processing and produce may become infected when contaminated water is used to irrigate crops or wash produce [44,48], or if produce is not properly cleaned when manure is used to fertilize crops [34]. Contact with contaminated surface water [36,40], and subsequently participating in recreational water activities in contaminated water bodies [50], has also been found to be associated with *Salmonella* infection. Transmission can occur from contact with animals [43] including; broiler chickens [41,42], reptiles [51,52], especially turtles

and iguanas [36,40,44], dogs and cats [44], particularly those with diarrhea [52], rodents [44], pet birds, horses and African pygmy hedgehogs [38]. Pet treats including those made from pigs ears have also been implicated in infection [53]. Cross contamination of objects, such as cutting boards, with *Salmonella* during food preparation and storage can also transmit the bacteria [54]. This can also occur due to poor hand washing [50] and poor food hygiene practices [55]. Eating at home was found to be protective [42,43,51] when compared to eating at a restaurant [50]. This may be due to restaurants being more likely to pool eggs, store pooled eggs and undercook foods. Poor general sanitation or infected food handlers may also contribute [56]. Person-to-person transmission has also been reported [39,43,51]. It is often associated with young children [46,55], particularly in children attending daycare with children who have diarrhea [51] and in elderly in institutional settings [40], particularly the diaper-using elderly [50]. International travel has also been implicated as a risk factor for *Salmonella* infection [42,57].

2.1.3 Occurrence:

Salmonellosis is the leading cause of hospitalization and death caused by known foodborne bacterial infection in the United States each year [50]. In Canada 6,000 to 12,000 cases of *Salmonella* are reported each year, although the actual number is estimated to be many times more as numerous mild or asymptomatic cases are not diagnosed [52]. In Canada the majority of cases of *Salmonella* occur sporadically [40], and most commonly in infants and children under the age of 5 [36,39,40], although peaks have been reported in those aged 20 to 29 in other countries [58]. Most cases in Canada occur

between June and September [49,52] with small peaks in January and February which may be associated with travel [59].

2.1.4 Incubation and communicability:

The incubation period for *Salmonella* ranges from six to 72 hours [36,39] with symptoms typically occurring 12 to 36 hours after infection [36]. Communicability occurs throughout the course of infection which ranges from several days to several weeks [36], typically lasting four to seven days [44,52]. A temporary carrier state may continue for months after infection, particularly in infants [36].

2.1.5 Symptoms and diagnosis:

Salmonella causes acute bacterial disease most commonly manifested in acute enterocolitis with sudden onset headache, abdominal pain, diarrhea, nausea, vomiting [36,39] and fever [42,44].

Cases are diagnosed through the isolation of *Salmonella* species from any site, regardless of symptoms [36], but most commonly through presence in stool samples [39].

2.1.6 Complications:

Severe dehydration can occur in some cases, particularly in the elderly, the very young and the immunocompromised [40]. In a small number of cases the bacteria can move into the bloodstream and other body sites which can lead to severe illness [36,52] including focal infections such as meningitis, septic arthritis and osteomyelitis [32]. These complications occur most commonly in children and those with underlying conditions

such as AIDS, malignancies, immunosuppressive therapy, hemolytic anemia, and inflammatory bowel disease [32]. A small number of cases also develop a condition called Reiter's Syndromes which is characterized by pain in the joints, irritation in the eyes and painful urination [52].

2.1.7 Treatment:

Infections are self-limiting [32,37] and antibiotic treatment is not recommended but can be necessary for the immunocompromised and the elderly or if the infection proceeds to bacteremia or other invasive disease [44].

2.1.8 *Salmonella* in Manitoba:

Salmonella infects almost 200 Manitobans annually [36], it is most commonly found in young children ages one to nine and adults aged 30 to 49 and those over the age of 60. Infection rates peak in March and August with 11% and 13% of infections respectively. The highest rates have been observed in the South Eastman and Central Regional Health Authorities (RHAs) [36].

In Manitoba, general risk factors that are commonly associated with *Salmonella* in most areas are of concern, although there are certain factors that are of increased concern locally in the province. These risk factors relate to the large amount of farming and animal production in some parts of the province which results in exposure to various domestic farm animals and animal products such as unpasteurized milk [60]. Concerns are increased with unsanitary farm practices and the presence of unhealthy animals [61]. In

Manitoba, *Salmonella* has been found in hog manure and on vegetation as well as in ground water samples after manure application [62].

The Province of Manitoba also has many recreational water bodies which are frequented by many Manitobans in the summer months. The second most common infection related to water recreation, after infection of the eyes, ears and throat, is gastroenteritis which is typically caused by *Salmonella* [63]. Recreational water bodies in Manitoba are thus frequently monitored for fecal coliform levels to ensure the health and safety of bathers [63].

2.2 VTEC:

The term VTEC refers to strains of *Escherichia coli (E.coli)* which produce verotoxins causing human illness [64,65]. Although when compared to other common enteric infections VTEC has a lower incidence, the high rate of serious illness and complications has led to a continued interest in this pathogen [66]. *E.coli* is a species of motile rod bacteria belonging to the class Gamma Proteobacteria [67]. The verotoxin-producing serotype most common in Manitoba is O157:H7 [68].

2.2.1 Reservoirs:

VTEC is widely distributed in the intestines of animals and humans, especially cattle[69], although other animals such as sheep [70], poultry [71], pigs, goats and wild animals such as deer are also reservoirs [72]. The bacteria can survive in manure and water sediments for months and house flies can carry the bacteria in farm environments [72].

2.2.2 Transmission and risk factors:

VTEC infection has several transmission patterns. It can originate from food, water and zoonotic sources, and be transmitted from person-to-person. Humans most often become infected via consumption of contaminated foods or by direct transmission from infected individuals or animals [73]. The most commonly implicated food is undercooked meat of bovine origin [74,75]. Other foods that have been implicated in VTEC infection are pork, lamb, poultry [64], unpasteurized milk [76] and milk products [77], unpasteurized juice [78] alfalfa sprouts [79] and raw fruit and vegetables [80,81]. Fruit and vegetables are often contaminated in the field by improperly composted manure, contaminated water, wildlife and poor hygienic practices by farm workers [71]. Food can also be contaminated if it comes into contact with feces from infected animals at any stage during cultivation or handling or via cross-contamination during food preparation [72]. Untreated water can also be a source of infection [82]. This waterborne transmission occurs from drinking contaminated water and swimming in contaminated water bodies, including naturally occurring bodies of water, as well as improperly chlorinated swimming pools [72,83]. Zoonotic transmission is common as domestic farm animals and pets often shed VTEC [84]. Thus, direct contact with animals, particularly cattle [85], and attending petting zoos [71] or agricultural fairs [85] increases risk of VTEC infection. Infection can also be transmitted person-to-person particularly within families and child care settings [72]. Personal care homes and other institutional settings have also been found to be common sites of this type of transmission [76,86].

2.2.3 Occurrence:

The annual incidence of infection in industrialized countries ranges from one to 30 cases per 100,000 population [72]. In 2000, 8.81 cases per 100,000 population were reported in Canada [72]. Cases were both sporadic and associated with outbreaks although the vast majority were sporadic [72]. Infection occurs most commonly in Canada during the summer with the highest percentage among young children and seniors [49].

2.2.4 Incubation and communicability:

The incubation period for VTEC ranges from 2 to 8 days with symptoms typically occurring three to four days after infection [87]. Communicability occurs throughout the course of infection which is typically up to one week [76,86]. However, duration of communicability can be prolonged in children, with one third of infected children shedding the bacteria for as long as three weeks [76].

2.2.5 Symptoms and diagnosis:

Symptoms of VTEC infection range from asymptomatic to gastroenteritis with or without bloody diarrhea. Infection is characterized by severe abdominal cramps and non-bloody diarrhea that typically progresses to bloody diarrhea [88]. Nausea and vomiting may also occur as well as fever, although this is less common [72].

Cases of VTEC are diagnosed by the isolation of VTEC or verotoxin from a stool specimen [72].

2.2.6 Complications:

VTEC infection is associated with risk of two life-threatening complications; thrombocytopenic purpura (TPP) and hemolytic uremic syndrome (HUS) [88,89]. HUS is characterized by acute renal failure, haemolytic anaemia and low platelet count [86] and occurs in up to 10% of VTEC cases [86]. Neurological complications such as seizure, stroke and coma can be associated with HUS and are most common in children under five and the elderly. Mortality rates due to HUS range from three to 17% but can be as high as 87% in the elderly [66].

TPP resembles HUS in its clinical features although it differs in that neurological signs and fever are more predominant and it is most common in those in their thirties [64].

2.2.7 Treatment:

Most infected individuals recover without treatment, although severe cases or those with complications may require intravenous fluids for rehydration, blood transfusions or kidney dialysis [72].

2.2.8 VTEC in Manitoba:

In 2007, 46 VTEC cases were reported in Manitoba [1]. Although VTEC serotype O157:H7 [72] is the most common serotype in the province, a recent Manitoba study found that 63% of infections were associated with non-O157 VTEC. It was determined that these non-O157:H7 cases were mostly sporadic and occurred in rural areas [68]. The higher rates in rural areas suggested that contact with animals, rather than simple consumption

of animal products may play a significant role in VTEC infection [68]. Thus as with *Salmonella,* concerns reside in the large amount of farming and animal production in the province which results in exposure to various domestic farm animals and animal products such as unpasteurized milk products [60].

2.3 Surveillance of *Campylobacter, Salmonella* and VTEC in Manitoba:

Surveillance is defined as the routine collection, analysis and dissemination of various data to describe the occurrence and distribution of disease, events or conditions [90]. It is conducted with the objective of determining the extent of infections and the risk of disease transmission in order to ensure that prevention and control measures can be applied and burden of illness can be minimized [90].

In Manitoba, *Campylobacter*, *Salmonella* and VTEC are considered reportable diseases in the Reporting of Diseases and Conditions Regulation under *The Public Health Act* C.C.S.M c.P120 [91]. Therefore, health professionals must report infections if they believe that the presence of the condition will not be confirmed by a positive laboratory test and laboratories must report all positive test results to the director of the Communicable Disease Control Division (CDC) at Manitoba Health and Healthy Living (MHHL) and to public health authorities in the relevant RHA [91].

When a patient presents to a physician with symptoms indicative of an enteric infection, a stool sample may be collected and sent for testing. If laboratory results are positive for *Campylobacter, Salmonella* or VTEC, MHHL and the relevant RHA will be informed. At this time a public health nurse (PHN) from the relevant RHA will contact

and interview the case. During this routine follow up they will obtain case history, provide education, and discuss etiology, epidemiology and treatment of the infection as well as preventative measures such as safe food handling and good hygiene practices. Each single incident is investigated thoroughly to determine if the case is part of an outbreak and to prevent and control the spread of illnesses. The PHN will also determine occupation of the case, assess risk factors for transmission and obtain a follow up stool specimen as required [92]. If necessary the PHN will consult with their communicable disease coordinator to determine if it is necessary to exclude a food-handler/caregiver from employment to prevent potential transmission of the disease causing organism from the employee to other individuals. The PHN would also notify a Public Health Inspector/Environmental Health Officer if a public premise is involved such as a daycare or a restaurant where special preventative measures may be required [92].

The ability of a surveillance system to detect all cases is the sensitivity [93]. This is influenced by likelihood of seeking medical attention, proportion of appropriate lab tests, the ability of the test to confirm true cases and the relaying of the information through the reporting system [93]. Due to the fact that *Campylobacter*, *Salmonella* and VTEC can cause a range of symptoms from asymptomatic carriage to severe gastroenteritis the proportion of cases with infections captured by surveillance is low. For example, for every VTEC case reported there was an estimated ten to 47 cases in the Canadian population [28], this number is even higher for *Campylobacter* and *Salmonella* as VTEC cases have a higher proportion of bloody diarrhea, which increases the likelihood of presenting to a health professional [93]. Thus the system will capture individuals with infections which are severe enough to require medical attention.

Although only a fraction of cases are captured by the system, those which are collected represent the most severe cases, providing a wealth of information on severe enteric illness in the province. The information is complete for all diagnosed cases and timely in its collection and has been frequently used for prevalence estimates and other research.

2.4 Case-Case Comparisons:

Case-control studies are frequently used for analysis of the risk factors associated with disease. The standard procedure is to compare well (control) individuals to ill (case) individuals. A less frequently used analysis are case-case studies. These latter studies are described in greater detail below. Briefly they involve comparisons between ill individuals with other ill individuals. Thus in a case-case comparison all individuals under study have been infected with an illness and various groups of cases are compared to each other [94]. For a given pathogen, cases from one geographic area may be compared to those from other areas or cases infected with one type of pathogen (e.g. a species or sub-species) may be compared to those infected with a different type of pathogen. In this way case-case comparisons provide a method of creating a group that has similar risk factors but different infectious exposure without any matching of individuals [95,96]. Thus, case-case comparisons allow researchers to see how risk factors differ between pathogens or between specific sub-species of the same pathogen. This method highlights how cases become exposed to the infecting strain apart from factors known and unknown that cause a general predisposition to infection(s) under study [95]. It can also highlight the specific

risk factors of a less prevalent species which would have normally been lost if lumped together with more predominant species [96].

Case-control studies investigating risk factors and sources of disease are often the best way to characterize overall transmission pathways for a given disease within populations and for sporadic cases (e.g. the general risk factors for acquisition of an enteric infection of any type). However, such studies can be expensive and may be beyond the capacity of many researchers or public health departments [94]. Thus an advantage with case-case studies for notifiable infectious diseases such as *Campylobacter, Salmonella* and VTEC is that surveillance systems already routinely collect information on exposures for sporadic cases and outbreak situations.

When conducting a case-control study, comparing ill with well, there can also be methodological problems such as selection and recall bias [97]. This is found to be especially true for data collected by a surveillance system. McCarthy and Giesecke [95] suggest that only one to 2.5% of individuals infected with *Salmonella* are recorded by a surveillance system. They also suggest that the process of reporting is highly selective in that only 50% of individuals have symptoms, of those only a percentage seek medical attention and have a stool sample taken and tested. This process is not random but is highly selective, it has been found to be related to socio-economic status as well as occupation and health seeking behavior causing the group to be distinctly different from the overall population [95]. The use of case-case comparisons was suggested as a resolution to this issue as all participants will have been selected by the same method thus making comparisons made more valid. There is also a decrease in selection bias as all individuals can be selected in the same manner and over the same time period [95].

There are also some disadvantages associated with case-case comparisons. Case-case comparisons have no comparison between the ill and the well and therefore do not allow a common etiology of infection to be determined [95]. This can be seen as a selection bias and decrease the range of exposures which can be studied. For example, if each of *Campylobacter*, *Salmonella*, and VTEC are equally associated with contaminated drinking water, this risk factor would not be detected in a case-case study. If however, *Campylobacter* was associated with unpasteurized milk, while *Salmonella* and VTEC were associated with contaminated water, this difference between *Campylobacter* and *Salmonella*/VTEC would be detected.

In this study it was intended to conduct both a standard case-control study as well as a case-case comparison, although the focus of the study was intended to be on the latter in order to illustrate what can be learned from this less common approach.

CHAPTER 3: METHODS

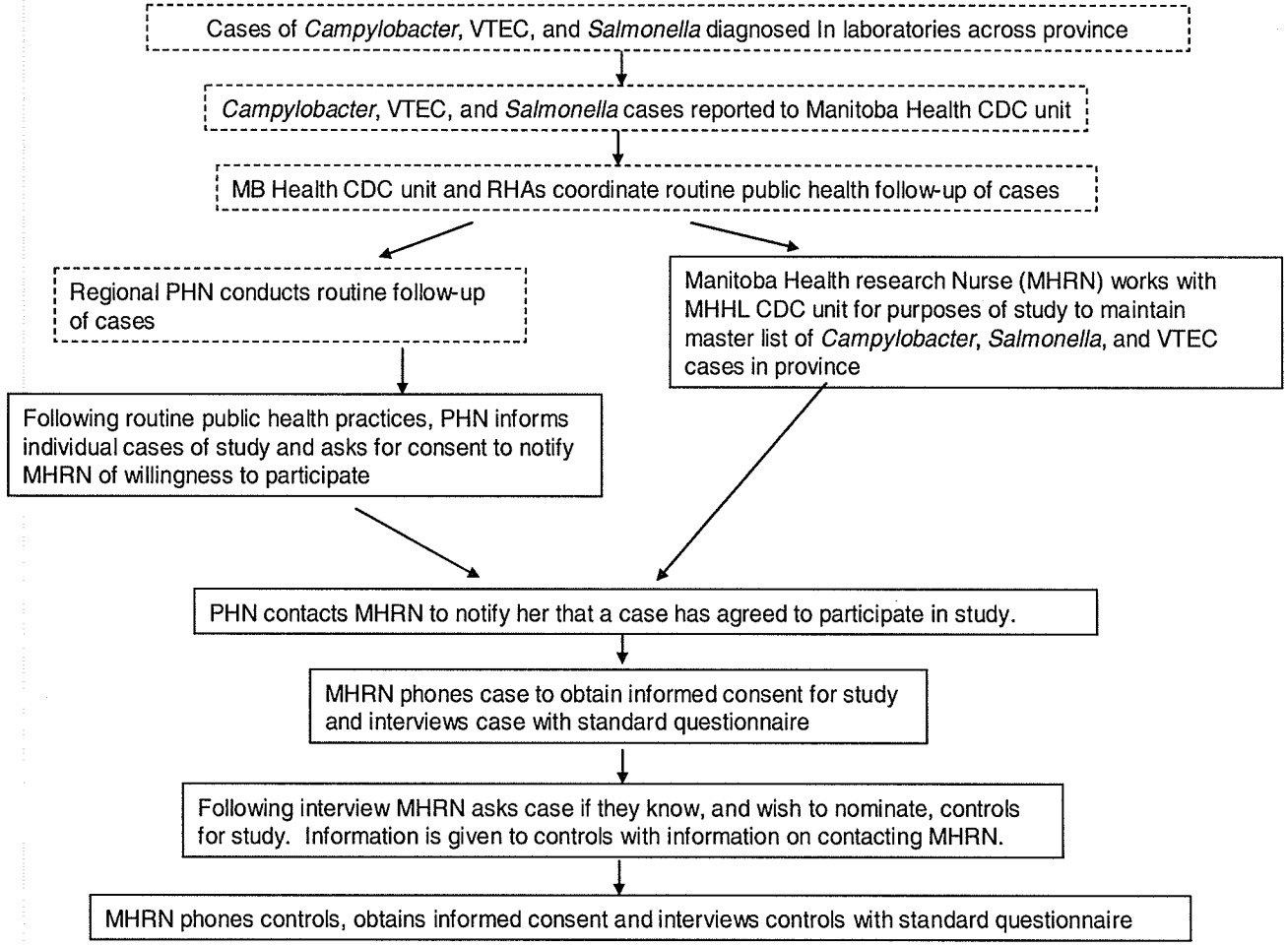
The study utilized a cross-sectional questionnaire administered to Manitobans with lab-confirmed cases of *Campylobacter*, *Salmonella* and VTEC. Infections caused by these pathogens were identified through MHHL's surveillance system. The questionnaire was designed to determine risk factors for infection overall, by pathogen and by location.

3.0 Population and Sample:

The population under study was Manitobans who were diagnosed with *Campylobacter*, *Salmonella* or VTEC during the study period (May – September of 2007), identified through MHHL's surveillance system.

Figure 1. Methodology of participant and control attainment [98]

Dashed lines indicate routine public health practices and solid lines indicate processes put in place for study purposes



The methodology of obtaining study participants is outlined in figure 1. As part of routine public health practice, when an individual presents to a health care provider with symptoms indicative of a reportable infection such as *Campylobacter, Salmonella* or VTEC a stool sample may be sent for testing to a provincial laboratory. If the presence of one of these infections is found it is reported to the CDC of MHHL and the relevant RHA. The CDC and RHA then coordinate a routine follow up of all confirmed cases, where each individual receives a call or visit from a PHN in their RHA.

The study was able to utilize this routine practice by having the PHN inform the cases about the study during routine follow up. Thus, when the PHN contacted the case for follow up they would provide information about the study including background and what participation would entail. A script was provided to the PHN in order to standardize and facilitate the process. The script included all the information mentioned above, as well as a section to document case information. Documentation consisted of whether or not they agreed to participate, and if so, the preferred time and phone number they would like to be contacted at. A list of potential questions cases may have for the PHN about the study with answers was also included (Appendix A). The PHNs were also provided with an information sheet, available to the case upon request, which provided additional information about the study as well as contact information for the research group (Appendix B).

After informing the case about the study the PHN asked for consent to notify the Manitoba Health Research Nurse (MHRN) of willingness to participate. If cases chose to participate they were asked for a telephone number as well as a preferred time to be

contacted by the MHRN. For individuals who chose to participate the PHN would fill out a provided case documentation form, with all contact information and any additional comments (Appendix C). This form was then sent to the MHRN by secure fax.

Participants were then contacted by the MHRN, who obtained informed consent by telephone using a scripted text (Appendix D). Once informed consent had been obtained the questionnaire was administered. For the purposes of the study the MHRN also collaborated with the CDC to maintain a master list of all reported cases of *Campylobacter*, *Salmonella* and VTEC.

At the time of questionnaire administration, participants were asked by the MHRN to nominate potential controls. This was done by asking study participants to nominate a friend or family member, of similar age and geographic location, who could serve as a control. This methodology of control attainment was chosen as literature states that the process by which infections are captured by surveillance systems is not random; there are specific characteristics of the individuals that lead them to be part of this group [95]. Thus friend or family controls were used to provide a control population similar to the sample population allowing for more reliable comparisons to be made.

If the participant had an individual they wished to nominate they were asked to provide them with the phone number of the MHRN in order for the potential control to contact her. In this way, control-initiated contact acts as implied consent to potentially participate in the study. Once contact was established the MHRN would brief the control on the study, carry out the telephone consent (Appendix E), and administer the same questionnaire (minus the symptoms section).

Unfortunately, this method of recruiting controls was unsuccessful, as cases were unwilling to approach friends. This resulted in recruiting of only 14 controls, all of which were household family members of cases.

3.0.1 RHA participation:

This methodology required collaboration between researchers, MHHL staff and Medical Officers of Health (MOH) as well as other staff members from several of Manitoba's RHAs. A total of eight of eleven RHAs participated in the study. Norman did not have a MOH at the time the study was initiated, and attempts to contact North Eastman RHA were unsuccessful, therefore these RHAs did not participate in the study. See Appendix F for a map of the RHAs in Manitoba.

3.0.2 Exclusion criteria:

Certain cases were excluded from the study. These included those who were institutionalized and those in hospital at time of PHN follow-up. First Nations on reserve were also excluded as their health care is federally regulated and not under provincial jurisdiction. Siblings of enrolled cases were excluded as a near simultaneous episode would likely represent same-source exposure or secondary human-human transmission. Any cases that were found to be part of an outbreak were also excluded as the purpose of the study was to determine risk factors of sporadic infection, although no outbreaks occurred during the study period.

3.1 Instrument Used:

The questionnaire used was specifically designed for the project (Appendix G). It included general questions about the participant's environment and risk factors. Participants were asked about the number of individuals in their home and their ages, their source of water and if they had eaten specific foods before becoming ill. They were also asked about contact with farm animals as well as other risk factors such as swimming, contact with other ill individuals or international travel. The questions were either yes/no or multiple choice. The questionnaire then linked possible risk factors to geographic location using the first three digits of the postal code which was obtained from MHHL and noted on each questionnaire (for controls, this information was gathered directly during the interview). The first three digits of the participant's postal code were used to indicate the RHA in which they resided. It also gave an indication of the environment the individual was living in such as a larger urban area, a small town or a rural area.

Content validity was addressed by consulting the existing literature on risk factors in order to formulate the questions used in the questionnaire. The reliability of the survey was not tested for test-retest reliability as the majority of the questionnaire pertained to events which occurred before the onset of symptoms and it was administered as soon as possible after diagnosis. The methodology utilized in the study allowed a single research nurse to administer all questionnaires and therefore inter-rater reliability was not an issue.

3.2 Data Analysis:

Questionnaire responses were entered into an access spreadsheet for all cases and controls. All yes and no responses were coded as one and zero respectively, all responses which were unknown/unsure were coded as 77, refused as 88 and not applicable as 99. Responses to multiple choice questions were coded as one, two, three, etc.

Data cleaning consisted removing all non-numerical data to ensure it was suitable for statistical analysis. The duration of illness was calculated using the date of onset of first symptom and the date of recovery (questions G13 and G14 of questionnaire respectively). A severity score was also calculated based on a system developed for calicivirus which assigns numerical values to symptoms which, when summed, give a numerical score that can be compared across cases [99]. Appendix G shows the numerical values assigned. Most symptoms received a value of one, with the exception of blood in stool and fever which were given a value of two as they are considered to be more severe symptoms. Frequency and duration of diarrhea and vomiting were given a score between one and three depending on the reported value. If a response was listed as unknown it was excluded from the analysis, this resulted in the exclusion of 80 responses. No responses of refused or not applicable were given. A copy of the original data was retained. The cleaned data was then transferred into a Stata dataset to allow for analysis using Stata statistical analysis software (STATA Corporation, Texas, US). Stata (version 9.0) was used for all statistical analysis of the data.

The data analysis included the following analyses;

- Infection analysis:

- Statistical significance was calculated using Fishers Exact test for all binary variables; this included 2x3 case-case comparison of all three infections and 2x2 case-case comparisons of *Campylobacter* versus *Salmonella* and VTEC versus *Campylobacter* and *Salmonella* pooled. *P* values were partitioned in this manner following the methodology proposed by Everitt [100].
- Everitt states that sub-tables can be used to break up the Fishers *P* value into more interpretable pieces to enable the categories responsible for a significant overall *P* value to be identified[100]. The number of sub-tables that can be made is equal to the degrees of freedom of the original table. Therefore, only two additional tables could be made resulting in the requirement to pool two of the columns in the 2x3 table[100]. The decision of which columns to combine must be made prior to investigating the data based on prior knowledge of the classification categories concerned[100]. Therefore it was chosen to compare *Campylobacter* and *Salmonella* and then VTEC to *Campylobacter* and *Salmonella* pooled as *Campylobacter* and *Salmonella* have the highest infection rates in the population in question and therefore were expected to have the greatest number of participants and consequently represent the most robust comparison.
- Relative risk for each of the explanatory variables was also calculated using *Campylobacter* as a reference category. *Campylobacter* was chosen as a reference category as it has the highest rate of infection in Manitoba and was expected to be the category with the most participants.

- Although the number of participants with VTEC infection was small leading to unstable relative risk and large confidence intervals it was not excluded from the analysis. The relative risk and 2x2 comparisons between *Campylobacter* and *Salmonella* demonstrate what the results would show without the presence of VTEC in the comparison and therefore its inclusion simply adds more breadth to the analysis.
- Statistical significance was calculated using Kruskal-Wallis test for all ordinal variables.
- Participants co-infected with more than one of the infections under study were included in both of the respective infection categories.

- Location/Infection analysis:
  - Participants were separated into two location groupings; urban/WRHA and Rural/non-WRHA. The City of Winnipeg, Manitoba's largest urban center was represented by the Winnipeg Regional Health Authority (WRHA) and all other participants represented the rural/non-WRHA grouping. These two location groupings were then stratified by infection for *Campylobacter* and *Salmonella.* This was not done for VTEC infections as the number of participants with this type of infection were too small to allow for stratification.
  - Statistical significance was calculated using Fishers Exact test for binary variables; this included a 2x2 case-case comparison of urban/WRHA *Campylobacter* participants and rural/Non-WRHA *Campylobacter*

participants and a 2x2 case-case comparison of urban/WRHA *Salmonella* participants and rural/Non-WRHA *Salmonella* participants.

- Statistical significance was calculated using Kruskal-Wallis test for all ordinal variables.

- Location analysis:
  - Participants were separated into three location groupings for analysis. The City of Winnipeg, Manitoba's largest urban center was represented by the WRHA. Western Manitoba was represented by Parkland, Brandon and Assiniboine RHAs, and South/Central Manitoba was represented by South Eastman, Interlake and Central RHAs. Further stratification into smaller locations (i.e. individual RHAs) was not possible due to the small number of cases per RHA. One case who resided in Burntwood RHA was excluded from the location analysis as they did not fit into the location groupings.
  - Statistical significance was calculated using Fishers Exact test for binary variables; this included 2x3 case-case comparisons of all three locations and 2x2 case-case comparisons of Winnipeg versus South/Central Manitoba and Western Manitoba versus Winnipeg and South/Central Manitoba pooled. *P* values were partitioned in this manner following the methodology proposed by Everitt [100].
  - Relative risk for each of the explanatory variables was also calculated using Winnipeg as a reference category. Winnipeg was chosen as a

reference category as it has the largest population in Manitoba and was expected to be the category with the most participants.

- Statistical significance was calculated using Kruskal-Wallis test for all ordinal variables.
- Participants co-infected with more than one of the infections under study were included as a single case in their respective location.
- This analysis was conducted to aid in the case-case analysis of infection.

An alpha level of 0.10 ($P<0.10$) was used for all statistical tests. This less stringent P value (i.e. as opposed to 0.05) was used since this study was considered a pilot study of enteric infections in Manitoba and a lower sensitivity level was desirable to ensure that all possible avenues for future investigation were identified.

3.3 Limitations:

There are some limitations associated with this study. First was the lack of sufficient controls. The inability to recruit an acceptable number of controls into the study limited the ability to draw conclusions on etiology of bacterial enteric infections in general and limited the study to a case-case analysis of the differences between infections caused by the three individual pathogens under study. The presence of controls would have significantly added to the richness of the study results.

The data analysis was also limited by the number of participants which were enrolled in the study. The relatively small number of cases available for analysis was more a function of the population size and infections in the province, as opposed to

participation in the study. Overall, participation in the study did reach acceptable proportions, and a different study design or extending the study over several years would have been necessary to increase overall case numbers. Due to the final sample size, multivariate analysis was not possible, thus the effect of an explanatory variable on illness outcome could be due to the association of the variable with other factors or explanatory variables that influence the occurrence of the outcome. For example, if living on a farm and exposure to farm animals are found to be significant risk factors for infection, univariate analysis can not determine if both of these variables are uniquely associated to enteric infection or rather those who live on a farm have more contact with animals and only one of these variable is driving the significant relationship to infection. The relatively small sample size also only allowed for stratification into large groupings. The analysis was limited to RHA groupings, and *Campylobacter* and *Salmonella* participants could only be stratified by urban/WRHA and rural/non-WHRA, while VTEC participants could not be stratified at all.

Another potential limitation, common to most studies on enteric infections, is found in the under-reporting of enteric infections such as *Campylobacter*, *Salmonella* and VTEC. This occurs as these disorders have a range of severity of symptoms from asymptotic to very severe. Literature states that for Salmonellosis only 50% of individuals show symptoms and only a small amount of individuals with symptoms will seek medical attention, and those who do seek medical attention have specific characteristics [95]. These may include particular personality traits, medical history, geographic locations and social factors. Thus the individuals diagnosed may not be representative of the overall population that has experienced an infection, and will be

skewed towards infections severe enough to seek medical attention [95]. The utilization of a case-case analysis removes this problem as all cases are selected in the same manner. Also, the potential inability to generalize to all infections caused by these pathogens is not a major concern as public health interest would frequently be on those cases which are severe enough to seek medical attention rather than all those infected.

Another potential limitation of the study was the exclusion of First Nations on Reserve and non-participating RHAs. This would impact the findings and interpretation of the study as it may not be representative of the province as a whole. These specific groups may have particular risk factors associated with their particular lifestyle or living conditions that may not be reflected in the participating RHAs. Manitoba is a diverse province with variation in environment, lifestyle and living conditions, therefore the exclusions of First Nations on Reserve as well all those living in Norman and North Eastman RHAs would potentially result in an under-representation or exclusion of risk factors associated with these particular groups. However, given that these individuals only represented a small proportion of the potential study participants with a similar pattern in type of infection as the overall study participants, this effect is expected to be minor.

3.4 Ethical Considerations:

Prior to beginning this study formal ethics approval was obtained from the University of Manitoba Research Ethics Board, the Health Information Privacy Committee of MHHL as well as individual ethics boards for each participating RHA (Appendix H).

Ethical issues that pertain to the study have been carefully considered. For the study, informed consent was obtained from all participants including controls. Also, no personal identifying information was collected or attached to the surveys. The surveys are being stored in a locked cabinet and will be shredded after five years. Only the study staff has access to the documents and RHAs can obtain access to the data for their region upon request.

CHAPTER 4: RESULTS

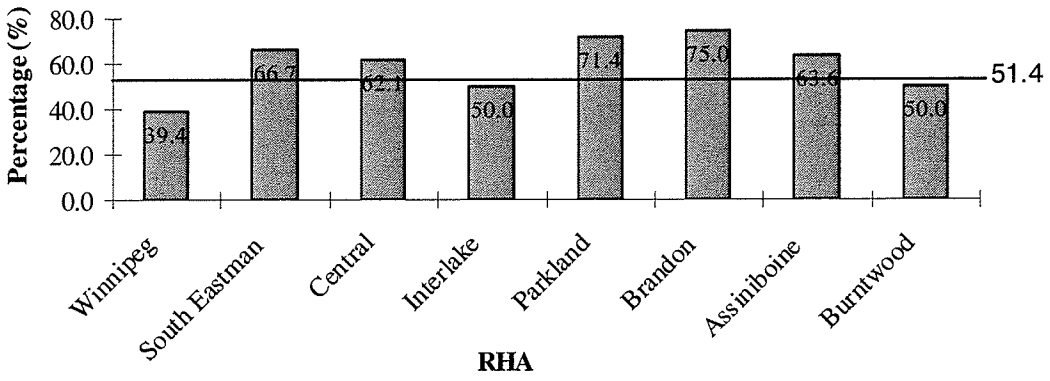
4.0 Response Rate:

A total of 220 people were diagnosed with *Campylobacter*, *Salmonella* and VTEC during the study period. Of these cases 39 (17.7%) were not eligible for the study. Reasons for ineligibility were; the PHN was unable to contact the case [28.2% (n=11)], the case was First Nations on Reserve and therefore out of provincial jurisdiction [20.5% (n=8)], consent information was not received by the MHRN [15.4% (n=6)], the case was not a Manitoba resident or lived in a non-participating RHA [20.5% (n=8)], or other reasons such as being ill with an underlying condition or the diagnosis was a second follow up diagnosis [15.4% (n=6)]. First Nations on Reserve only represented 3.6% (n=8) of all cases and only 1.4% (n=3) lived in one of the excluded RHAs. Of the excluded participants the majority [63.6% (n=7)] diagnosed with *Campylobacter* followed by *Salmonella* [36.4% (n=4)] and none were infected with VTEC.

Of the remaining 181 eligible cases 75 (41.4%) refused participation and 13 (7.2%) consented to be contacted but the MHRN was unable to contact them. A total of 93 participants consented to enrollment in the study and completed the questionnaire, representing a 51.4% response rate. No studies conducted with the same methodology could be found in the literature. Several similar studies were found, where potential cases were identified using public health surveillance data, although potential participants were then contacted through their physician rather than a nurse and were only interviewed a single time. The response rates for these studies ranged from 50 to 65%[33,46,135].

The response rate varied by RHA. The rate for rural (non-Winnipeg) cases was higher (64.4%) when compared to Winnipeg (39.4%), with the highest rate in Brandon (figure 2).

Figure 2. Response rate by RHA



4.1 Patient Demographics:

Figure 3 shows the distribution of study participants in Manitoba. The largest number of participants were residents of Winnipeg (39.8%) and Central (19.4%) RHAs, and a total of 60.2% participants (n=56) resided in rural (non-Winnipeg) regions.

Figure 3. Distribution of participants by RHA

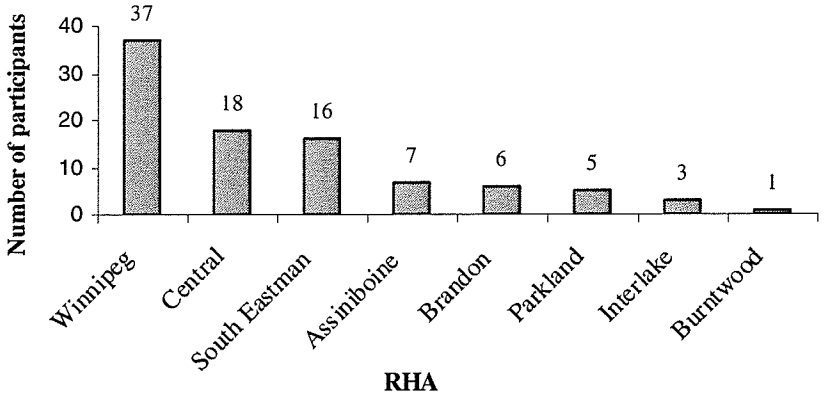


Table 1 shows the rates of infection per 100,000 population for *Campylobacter*, *Salmonella* and VTEC. Rates were based on the number of diagnosed cases during the study period (May-September) in each participating region. The population is based on records of residents registered with MHHL, residing in participating RHAs, on June 1, 2007 [101]. These rates reflect a defined time period and therefore will be lower than annual rates.

Campylobacter had the highest rate per 100,000 population followed by *Salmonella.* When broken down by RHA the rate of *Campylobacter* infection ranged from 29.9 in South Eastman to 3.9 in Interlake RHA. Central RHA had the highest rate of *Salmonella* followed by South Eastman, and Interlake RHA had the highest rate of VTEC followed by South Eastman RHA.

Table 2 shows the number of participants in each region by infection; *Campylobacter* represented the highest number of cases for most regions followed by *Salmonella.*

Table 1. Rates of infection during the study period (May –September, 2007) per 100,000 population by RHA

	Campylobacter	*Salmonella*	VTEC
Winnipeg	5.8	7.2	1.9
Central	14.6	13.6	4.9
South Eastman	29.9	12.6	6.3
Assiniboine	16.2	4.4	0.0
Brandon	10.0	6.0	4.0
Parkland	9.6	7.2	2.4
Interlake	3.9	3.9	6.5
Burntwood	10.7	2.1	2.1
Total	9.0	7.4	2.8

Note: Rates based on the number of diagnosed cases during the study period (May-September) in each participating region and the population is based on records or residents registered with MHHL, residing in participating RHAs on June 1, 2007 [101]

Table 2. Number of participants by RHA and infection and the percentage of participants with *Campylobacter, Salmonella* and VTEC in each region

	Campylobacter		*Salmonella*		VTEC		Total
RHA	**n**	**%**	**n**	**%**	**n**	**%**	**n**
Winnipeg	20	54.1%	15	40.5%	3	8.1%	37
Central	8	44.4%	7	38.9%	3	16.7%	18
South Eastman	11	68.8%	4	25.0%	2	12.5%	16
Assiniboine	6	85.7%	1	14.3%	0	0.0%	7
Brandon	4	66.7%	1	16.7%	1	16.7%	6
Parkland	3	60.0%	1	20.0%	1	20.0%	5
Interlake	1	33.3%	2	66.7%	0	0.0%	3
Burntwood	0	0.0%	0	0.0%	1	100.0%	1
TOTAL	53	55.8%	31	32.6%	11	11.6%	95

4.2 Infection Analysis:

This section highlights significant findings of the case-case analysis by infection. Risk ratios as well as Fishers Exact tests were calculated for all categorical variables including 2x3 comparisons of all three infections as well as 2x2 comparisons of *Campylobacter* versus *Salmonella* and VTEC versus *Campylobacter* and *Salmonella* pooled. *P* values were partitioned in this manner following the methodology proposed by Everitt [100]. The 2x3 tables were partitioned into two 2x2 tables to break up the Fishers *P* value into more interpretable pieces to enable the categories responsible for a significant overall *P* value to be identified[100]. The number of sub-tables that can be made is equal to the degrees of freedom of the original table. Therefore, only two additional tables could be made resulting in the requirement to pool two of the columns in the 2x3 table[100]. The decision of which columns to combine must be made prior to investigating the data based on prior knowledge of the classification categories concerned[100]. Therefore it was chosen to compare *Campylobacter* and *Salmonella* and then VTEC to *Campylobacter* and *Salmonella* pooled as *Campylobacter* and *Salmonella* have the highest infection rates in the population in question and therefore were expected to have the greatest number of participants and consequently represent the most robust comparison. Although the number of participants with VTEC infection was small leading to unstable relative risk and large confidence intervals it was not excluded from the analysis. The relative risk and 2x2 comparisons between *Campylobacter* and *Salmonella* demonstrate what the results would show without the presence of VTEC in the comparison and therefore its inclusion simply adds more breadth to the analysis.

Kruskal-Wallis tests were calculated for all ordinal variables. Appendix I shows percentages of participants reporting exposure to all explanatory variables by infection grouping as well as Fishers Exact and Kruskal-Wallis test results for 2x3 comparisons.

4.2.1 Distribution of participants:

Appendix I, table i displays detailed numerical data on the distribution of participants. There were 95 infections within the 93 study participants. Of the 93 study participants 57.0% (n=53) were infected with *Campylobacter*, 33.3% (n=31) with *Salmonella* and 11.8% (n=11) with VTEC. The additional infections reflect two participants (2.2%) being co-infected with *Salmonella* and *Campylobacter*. The following infection analysis is based on the number of infections rather than the number of study participants.

The participants ranged in age from less than one to 87 years with a mean age of 36.4 years. The age groups found to be most affected were those under the age of ten and those aged 40 to 49 with a total of 17.2% participants (n=16) falling into each of these age groups. When stratified by infection the mean age of participants was very similar for *Campylobacter* (36.6), *Salmonella* (36.2) and VTEC (36.6). In terms of the age groups found to be most affected for *Campylobacter* and VTEC it was those less than ten years of age followed by those in their thirties and forties, and for *Campylobacter* those in there fifties and sixties were also more commonly affected. For *Salmonella* most participants were in their teens or twenties followed by those in theirs thirties and forties.

All participants were questioned on number of people and children in the household. The average number of people in the household was 3.5 with a range of one to

12, the average number of children under the age of five was 0.5 with a range of zero to four and the average number of children in diapers was 0.3 with a range of zero to three. These patterns did not vary significantly by infection.

Dates of self reported onset of symptoms were determined for all study participants (figure 4). The highest number cases reported onset of symptoms occurred at the end of July and in early June with 21.5% (n=20) of participant infections occurring in the two week period from June 24 to July 7. Another peak in participant infection was seen in August with an additional 18.3% (n=17) of participant infections occurring in the two week period from August 5 to 18.

Figure 4. Epidemic curve by infection: Infections in Manitoba from May to September by date of onset of symptoms

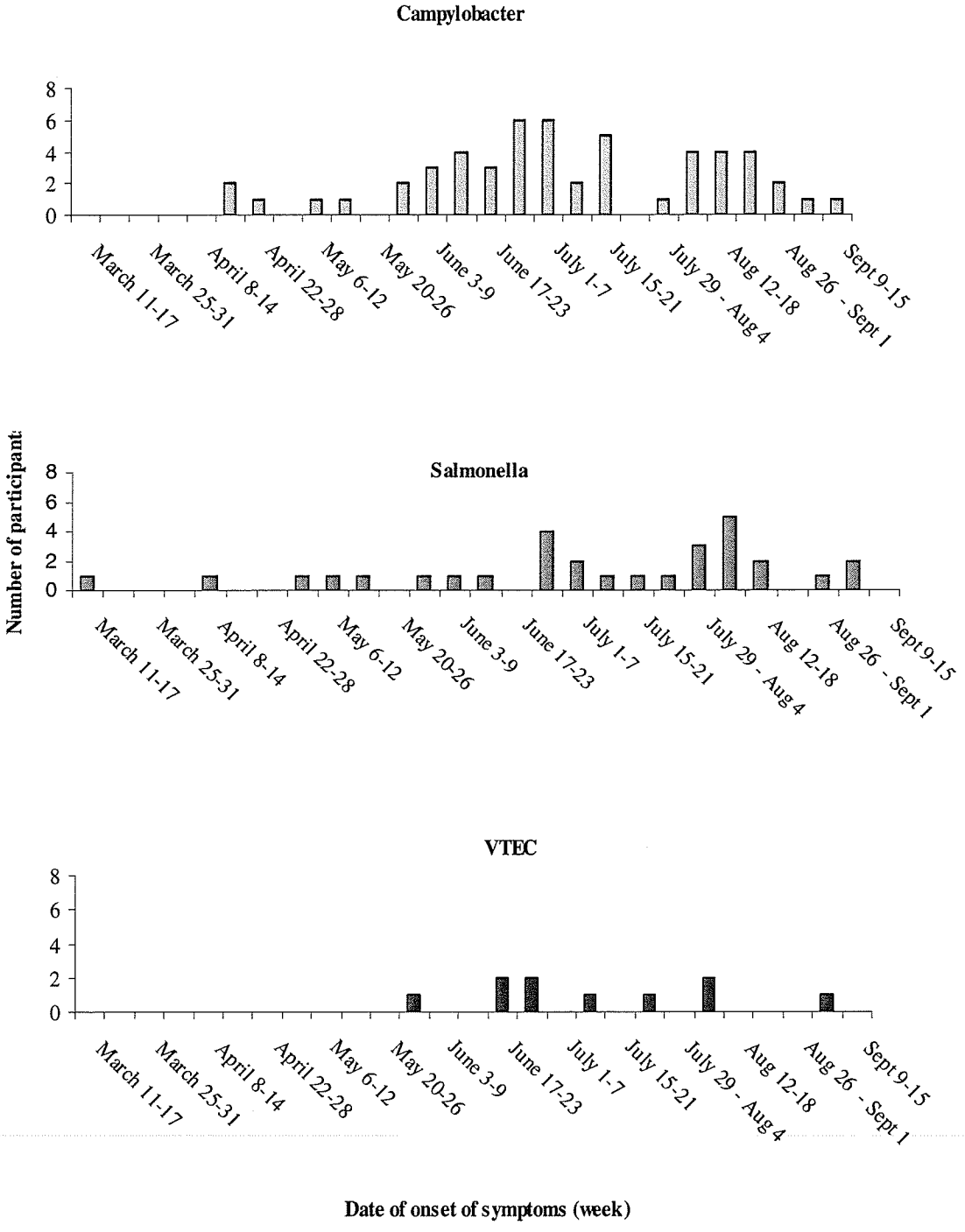


Table 3. Statistically significant univariate case-case associations between exposure to water/sewage and infection using Fishers Exact test and relative risk

					P-Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Infections**	**2x2 *Campylobacter* vs. *Salmonella***	**2x2 VTEC vs. *Campylobacter* and *Salmonella pooled***
Private well water for drinking in household							
Campylobacter	18 (34.0)	35 (66.0)	Ref	-			
Salmonella	4 (12.9)	27 (87.1)	0.42	0.16-1.06	0.094	0.041	0.726
VTEC	2 (18.2)	9 (81.8)	0.49	0.12-2.06			
Municipal water for bathing/showering or washing dishes in household							
Campylobacter	34 (64.2)	19 (35.8)	Ref	-			
Salmonella	27 (87.1)	4 (12.9)	2.55	1.00-6.48	0.055	0.025	0.502
VTEC	7 (63.6)	4 (36.4)	0.98	0.32-3.00			
Private well water for bathing/showering or washing dishes in household							
Campylobacter	20 (37.7)	33 (62.3)	Ref	-			
Salmonella	4 (12.9)	27 (87.1)	0.37	0.15-0.95	0.042	0.023	0.726
VTEC	4 (36.4)	7 (63.6)	0.95	0.31-2.91			
Water purification system in household							
Campylobacter	26 (49.1)	27 (50.9)	Ref	-			
Salmonella	7 (22.6)	24 (77.4)	0.44	0.22-0.90	0.033	0.021	0.350
VTEC	6 (54.6)	5 (45.5)	1.20	0.42-3.54			
Bathroom facilities - Indoor plumbing connected to a municipal system							
Campylobacter	31 (58.5)	22 (41.5)	Ref	-			
Salmonella	26 (83.9)	5 (16.1)	2.46	1.06-5.71	0.050	0.017	1.000
VTEC	8 (72.7)	3 (27.3)	1.71	0.50-5.84			
Used drinking water while staying overnight with friends or family at their residence							
Campylobacter	11 (20.8)	42 (79.3)	Ref	-			
Salmonella	1 (3.2)	30 (96.8)	0.20	0.03-0.33	0.067	0.048	1.000
VTEC	1 (9.09)	10 (90.9)	0.43	0.06-3.07			

4.2.2 Contact with water/sewage:

Appendix I table ii displays detailed numerical data on reported contact with water/sewage and infection, with more information on significant variables in table 3. The main source of water for participants, regardless of type of infection, was municipal water supplied by the town or city of residence.

Participants diagnosed with *Campylobacter* reported using more well water for drinking when compared to participants with *Salmonella* infection (p=0.094). Participants diagnosed with *Salmonella* utilized more municipal water and subsequently less well water for bathing/showering and washing dishes (p=0.055 and 0.042 respectively). Of the cases that reported using well water for drinking, the majority reported having the water monitored or tested and reported that tests never showed evidence of contamination.

Overall forty-one percent of participants reported having a water purification system, with the majority (84.2% [n=32]) reporting a type of filter. When infections were compared participants with *Salmonella* had significantly less of these systems in place (p=0.033).

The most common type of bathroom facility in the household, regardless of type of infection, was indoor plumbing connected to a municipal system. When infections were compared there was a significant difference in the number of participants reporting indoor plumbing connected to a municipal system, with less participants with *Campylobacter* reporting having this type of facility when compared to those with *Salmonella* (p=0.05).

Fourteen percent of participants reported drinking water while staying overnight at the residence of a friend or family. Although the specific water source was not

queried, ten of the residences visited were in rural Manitoba, one was in Winnipeg and the other in Ireland. Participants with *Campylobacter* were significantly more likely to report drinking water while staying overnight with friends or family at their residence (p=0.067).

4.2.3 Food history:

Appendix I table iii displays detailed numerical data on reported food consumption and infection. Fifty-one percent of participants ate at a fast food restaurant and 47.3% ate at another type of restaurant seven days before becoming ill. When study participants were asked what types of food they had consumed seven days prior to becoming ill, chicken was the most commonly consumed food, regardless of type of infection, followed by eggs for those with *Salmonella* and VTEC, and ground beef for those with *Campylobacter*. There were no significant differences in food history between infections.

4.2.4 Contact with animals or farms:

Appendix I table iv displays detailed numerical data on reported contact with animals or farms and infection, with more information on significant variables in table 4. In terms of contact with farm animals the most common was cattle for all infections, followed by hogs for participants with *Salmonella* and egg-laying chickens for participants with *Campylobacter*. Overall, participants had little contact with domestic farm animals with 65.6% (n=61) reporting no contact.

When infection was compared participants with *Campylobacter* had more contact with domestic farm animals when compared to those with *Salmonella* and VTEC (p=0.011).

In terms of domestic pets, contact was most commonly reported with adult dogs and adult cats for all types of infection. Very few participants reported having contact with fish, amphibians, reptiles, birds or other mammals as 71% (n=66) of participants reported that they had no contact with these animals. Participants with *Campylobacter* reported significantly less contact with pet birds (p=0.089).

4.2.5 Other occupational, recreational, or incidental exposures:

Appendix I table v displays detailed numerical data on reported occupational, recreational and incidental exposures and infection, with more information on significant variables in table 5. There was a significant difference between infections in the number of participants reporting the consumption of food prepared while on an outdoor recreational trip. Participants with *Salmonella* and particularly VTEC infection consumed more than those with *Campylobacter* (p=0.003).

In terms of recreation most participants, regardless of infection type, did not swim or participate in water related activities (65.6% [n=61]), although participants with VTEC reported swimming or participating in watersports in a lake more often (p=0.069).

Thirty-three percent (n=31) of participants attended a large gathering seven days prior to the onset of symptoms. The types of gatherings varied, and included family gatherings, concerts, barbeques, picnics and church functions. Of all participants 10.8% (n=10) had contact with someone else with symptoms and of those who had contact 40% (n=4) cared for that person.

4.2.6 Travel:

Appendix I table vi displays detailed numerical data on reported travel. Travel was common among the participants and there were no significant differences found between infections with respect to travel.

Table 4. Statistically significant univariate case-case associations between contact with animals or farms and infection using Fishers Exact test and relative risk

					P-Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Infections**	**2x2 *Campylobacter* vs. *Salmonella***	**2x2 VTEC vs. *Campylobacter* and *Salmonella* pooled**
Contact with any domestic farm animals							
Campylobacter	25 (47.2)	28 (52.8)	Ref	-			
Salmonella	7 (22.6)	24 (77.4)	0.47	0.23-0.97	0.011	0.036	0.090
VTEC	1 (9.09)	10 (90.9)	0.11	0.01-0.94			
Contact with pet birds							
Campylobacter	0 (0.0)	53 (100.0)	Ref	-			
Salmonella	1 (3.2)	30 (96.8)	2.77	2.08-3.68	0.089	0.369	0.219
VTEC	1 (9.09)	10 (90.9)	6.30	3.57-11.12			

Table 5. Statistically significant univariate case-case associations between other occupational, recreational or incidental exposures and infection using Fishers Exact test and relative risk

					P-Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Infections**	**2x2 *Campylobacter* vs. *Salmonella***	**2x2 VTEC vs. *Campylobacter* and *Salmonella* pooled**
Consumed food prepared on an outdoor recreational trip							
Campylobacter	0 (0.0)	53 (100.0)	Ref	-			
Salmonella	3 (9.7)	28 (90.3)	2.89	2.14-3.90	0.003	0.047	0.019
VTEC	3 (27.3)	8 (72.7)	7.63	4.00-14.55			
Swam or participated in watersports in a lake							
Campylobacter	1 (1.9)	52 (98.1)	Ref	-			
Salmonella	2 (6.5)	29 (93.5)	1.86	0.80-4.36	0.069	0.552	0.101
VTEC	2 (18.2)	9 (81.8)	4.52	1.69-12.31			

Table 6. Statistically significant univariate case-case associations between reported symptoms and infection using Fishers Exact test and relative risk

					P-Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Infections**	**2x2 *Campylobacte* r vs. *Salmonella***	**2x2 VTEC vs. *Campylobacter* and *Salmonella* pooled**
Diarrhea							
Campylobacter	53 (100.0)	0 (0.0)	Ref	-			
Salmonella	28 (90.3)	3 (9.7)	0.35	0.26-0.47	0.067	0.047	1.000
VTEC	11 (100.0)	0 (0.00)	-	-			
Nausea							
Campylobacter	37 (69.8)	16 (30.2)	Ref	-			
Salmonella	25 (80.6)	6 (19.4)	1.48	0.70-3.12	0.086	0.315	0.077
VTEC	5 (45.5)	6 (54.6)	0.44	0.15-1.27			
Fever							
Campylobacter	39 (73.6)	14 (26.4)	Ref	-			
Salmonella	25 (80.6)	6 (19.4)	1.30	0.62-2.72	0.001	0.598	0.000
VTEC	2 (18.2)	9 (81.8)	0.12	0.03-0.53			
Chills							
Campylobacter	36 (67.9)	17 (32.1)	Ref	-			
Salmonella	22 (71.0)	9 (29.0)	1.10	0.59-2.04	0.098	0.812	0.045
VTEC	4 (36.4)	7 (63.6)	0.34	0.11-1.05			
General muscle aches							
Campylobacter	31 (58.5)	22 (41.5)	Ref	-			
Salmonella	14 (45.2)	17 (54.8)	0.71	0.41-1.25	0.010	0.264	0.008
VTEC	1 (9.1)	10 (90.9)	0.10	0.01-0.74			
Hospitalized							
Campylobacter	6 (11.3)	47 (88.7)	Ref	-			
Salmonella	10 (32.3)	21 (67.7)	2.02	1.20-3.40	0.008	0.024	0.061
VTEC	5 (45.5)	6 (54.6)	4.02	1.49-10.84			

4.2.7 Symptoms:

Appendix I tables i and vii display detailed numerical data on reported symptoms and infection for ordinal and binary variables respectively, with more information on significant binary variables in table 6. Participants were questioned on a variety of possible symptoms. The most common symptom reported, regardless of type of infection, was diarrhea followed by fatigue for participants with *Campylobacter*, abdominal cramps for those with *Salmonella* and abdominal pain for those with VTEC.

Participants with *Salmonella* reported significantly less diarrhea (p=0.067) and those with VTEC reported significantly less nausea (p=0.086), fever (p=0.001), chills (p=0.098) and general muscle aches (p=0.010).

Participants were asked to report the first symptom they experienced. Cramps were most commonly reported by participants with *Campylobacter*, and diarrhea was the most common for those with *Salmonella* and VTEC (table 7).

Table 7. Frequency of first symptom reported by participants by infection

	Campylobacter	***Salmonella***	**VTEC**
Diarrhea	13 (24.5)	8 (25.8)	5 (45.5)
Chills	5 (9.4)	1 (3.2)	0 (0.0)
Fatigue	4 (7.5)	3 (9.7)	0 (0.0)
Cramps	16 (30.2)	4 (12.9)	3 (27.3)
Fever	6 (11.3)	1 (3.2)	0 (0.0)
Stomach/Abdominal Pain	11 (20.8)	7 (22.6)	1 (9.1)
Headache	3 (5.7)	1 (3.2)	0 (0.0)
Nausea/Vomiting	1 (1.9)	4 (12.9)	0 (0.0)
Other	4 (7.5)	5 (16.1)	2 (18.2)
Unknown	0 (0.0)	3 (9.7)	0 (0.0)

Note : Percentage may add up to over 100% as some cases reported two or more concurrent first symptoms

Participants with *Salmonella* reported more frequent vomiting, with all cases reporting two or more events in 24 hours, compared to *Campylobacter* participants, where 79.9% of cases reported this frequency (p=0.09).

The severity of infection varied across the participants. A total of 22.6% (n=21) were hospitalized and the time spent in hospital ranged from one to 14 days with an mean stay of 4.5 days for those who were hospitalized. When infections were compared significantly less *Campylobacter* participants reported being hospitalized (p=0.008).

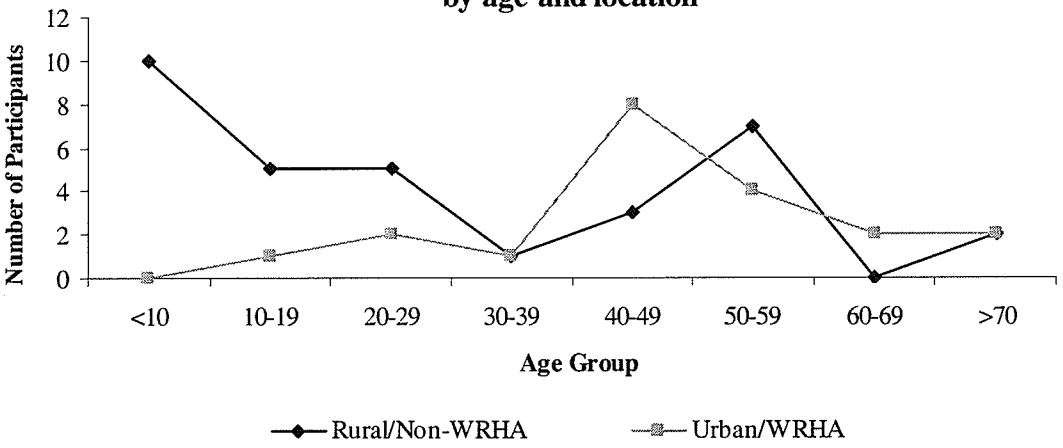
A severity score was calculated for each participant based on a system developed by Rockx et al. [99]. The scores ranged from one to 21 with a mean score of 13.2. When infections were compared participants with *Campylobacter* had the highest mean severity score of 13.79, followed by *Salmonella* (13.00). Participants with VTEC had a significantly lower mean severity score at 10.36 (p=0.004)

4.3 Analysis *Campylobacter*-Urban/WRHA and Rural/Non-WRHA:

This section highlights significant findings of the case-case analysis of participants with *Campylobacter* residing in urban and rural Manitoba. Urban participants were defined as those residing in the WRHA, which consists of the City of Winnipeg, Manitoba's largest urban center and rural participants were defined as those residing outside of this region. The analysis includes calculation of Fisher Exact tests for all categorical variables as well as Kruskal-Wallis tests for ordinal variables. (Only significant findings shown in tables 8 and 9).

4.3.1 Distribution of participants:

Figure 5. Number of participants with *Campylobacter* infection by age and location



Sixty two percent (n=33) of participants with *Campylobacter* lived outside the WRHA with the remaining 37.73% (n=20) residing within the WRHA.

The age of participants with *Campylobacter* differed significantly when urban and rural participants were compared (p=0.012), where urban participants were significantly older (mean age=48.5) than those in rural areas (mean age=29.4) (figure 5).

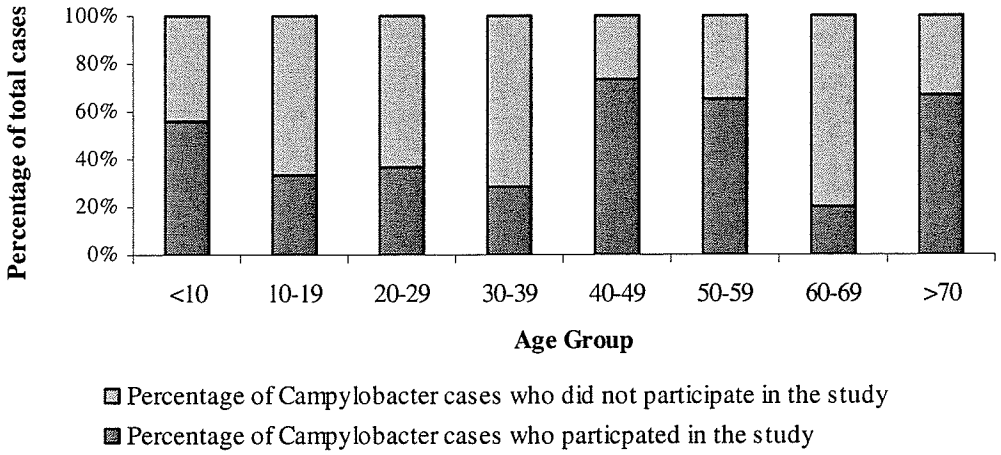
Response rates for participants with *Campylobacter* also varied by age where those 40 to 60 years of age and those over 70 years of age were more likely to participate. Those aged 10 to 39 and those in their 60s were less likely to participate (figure 6).

Urban participants had significantly less people living in their home, with an average of three persons compared to four persons in rural regions (P=0.057).

Table 8. Statistically significant univariate case-case associations between reported risk factor exposure in urban/WRHA and rural/non-WRHA participants with *Campylobacter* infection using Kruskal-Wallis test

	Urban/WRHA		Rural/non-WRHA		*P*
Explanatory Variables	**n**	**%**	**n**	**%**	
Demographics					
Age					
<10	0	0.0	10	30.3	
10-19	1	5.0	5	15.2	
20-29	2	10.0	5	15.2	
30-39	1	5.0	1	3.0	
40-49	8	40.0	3	9.1	0.006
50-59	4	20.0	7	21.2	
60-69	2	10.0	0	0.0	
≥70	2	10.0	2	6.1	
Number of persons living in household					
1-2	13	65.0	10	30.3	
3-4	3	15.0	15	45.5	0.057
≥5	4	20.0	8	24.2	

Figure 6. Number of study participants with *Campylobacter* as a percentage of total *Campylobacter* cases recorded by MHHL[1] by age grouping



4.3.2 Contact with water/sewage:

As expected urban participants used more municipal water and less well water and had more indoor plumbing connected to a municipal system and less connected to a septic field than those in outside the region.

4.3.3 Food history:

Chicken was the most commonly consumed food regardless of location. Rural participants reported consumed significantly more ground beef and unpasteurized milk (p=0.023 and 0.072 respectively).

4.3.4 Contact with animals or farms:

As expected significantly more rural participants with *Campylobacter* lived on farms or visited farms (p=0.002 and 0.001 respectively) and reported more contact with cattle and domestic farm animals in general (p=0.001 and 0.004 respectively). There was also significant differences in contact with domestic animals, where rural participants had more contact with dogs, cats, puppies and kittens overall and with adult dogs and cats specifically (p=0.087 and 0.088 respectively).

4.3.5 Other occupational, recreational, or incidental exposures:

Urban participants diagnosed with *Campylobacter* reported significantly more utilization of public pools before becoming ill when compared to rural participants (p=0.049).

4.3.6 Travel:

More rural participants with *Campylobacter* reported travel in general, and more travel specifically for business, when compared to urban participants (p=0.049 and 0.053 respectively), although the majority of this travel was within the province.

4.3.7 Symptoms:

In terms of symptoms, urban participants with *Campylobacter* reported less blood in stool when compared to the rural participants (p=0.085) and urban participants reported significantly more nausea, abdominal cramps and chills (p=0.073, 0.019 and 0.067 respectively).

Table 9. Statistically significant univariate case-case associations between risk factor exposure in urban/WRHA and rural/non-WRHA participants with *Campylobacter* infection using Fishers Exact test and relative risk

Explanatory Variables	Yes (%)	No (%)	RR	95% CI	*P*
Contact with water/sewage					
Municipal water for drinking in household					
Urban/WRHA	17(85.0)	3(15.0)	Ref	-	0.002
Rural/Non-WRHA	13(39.4)	20(60.6)	0.50	0.29-0.74	
Private well water for drinking in household					
Urban/WRHA	1(5.0)	19(95.0)	Ref	-	0.001
Rural/Non-WRHA	17(51.5)	16(48.5)	2.07	1.42-3.02	
Municipal water for bathing/showering or washing dishes in household					
Urban/WRHA	19(95.0)	1(5.0)	Ref	-	0.000
Rural/Non-WRHA	15(45.5)	18(54.5)	0.47	0.31-0.69	
Private well water for bathing/showering or washing dishes in household					
Urban/WRHA	2(10.0)	18(90.0)	Ref	-	0.001
Rural/Non-WRHA	18(54.5)	15(45.5)	1.98	1.33-2.96	
Bathroom facilities - Indoor plumbing connected to a municipal system					
Urban/WRHA	18(90.0)	2(10.0)	Ref	-	0.000
Rural/Non-WRHA	13(39.4)	20(60.6)	0.46	0.30-0.71	
Bathroom facilities - Indoor plumbing connected to a septic					
Urban/WRHA	1(5.0)	19(95.0)	Ref	-	0.002
Rural/Non-WRHA	15(45.5)	18(54.5)	1.93	1.35-2.75	
Food history					
Consumed beef (ground)					
Urban/WRHA	8(40.0)	12(60.0)	Ref	-	0.023
Rural/Non-WRHA	24(72.7)	9(27.3)	1.75	1.03-2.98	
Consumed milk (unpasteurized)					
Urban/WRHA	0(0.0)	20(100.0)	Ref	-	0.072
Rural/Non-WRHA	6(18.2)	27(81.8)	1.74	1.36-2.23	
Contact with animals or farms					
Live on a farm					
Urban/WRHA	1(5.0)	19(95.0)	Ref	-	0.002
Rural/Non-WRHA	15(45.5)	18(54.5)	1.93	1.35-2.75	
Visited a Farm					
Urban/WRHA	2(10.0)	18(90.0)	Ref	-	0.001
Rural/Non-WRHA	19(57.6)	14(42.4)	2.07	1.36-3.14	
Contact with domestic cattle					
Urban/WRHA	1(5.0)	19(95.0)	Ref	-	0.001
Rural/Non-WRHA	16(48.5)	17(51.5)	2.00	1.38-2.87	
Contact with domestic egg laying chickens					
Urban/WRHA	0(0.0)	20(100.0)	Ref	-	0.072
Rural/Non-WRHA	6(18.2)	27(81.8)	1.74	1.36-2.23	
Contact with any domestic farm animals					
Urban/WRHA	4(20.0)	16(80.0)	Ref	-	0.004
Rural/Non-WRHA	21(63.6)	12(36.4)	1.96	1.24-3.12	
Contact with adult dogs					
Urban/WRHA	8(40.0)	12(60.0)	Ref	-	0.087
Rural/Non-WRHA	22(66.7)	11(33.3)	1.53	0.95-2.47	
Contact with adult cats					
Urban/WRHA	6(30.0)	14(70.0)	Ref	-	0.088
Rural/Non-WRHA	19(57.6)	14(42.4)	1.52	0.99-2.34	
Any contact with adult dogs, puppies, adult cats or kittens					
Urban/WRHA	12(60.0)	8(40.0)	Ref	-	0.039
Rural/Non-WRHA	29(87.9)	4(12.1)	2.12	0.93-4.84	

Table 9 *continued.* Statistically significant univariate case-case associations between risk factor exposure in urban/WRHA and rural/non-WRHA participants with *Campylobacter* infection using Fishers Exact test and relative risk

Explanatory Variables	Yes (%)	No (%)	RR	95% CI	*P*
Other occupational, recreational or incidental exposures					
Swam in a public pool					
WRHA	3(15.0)	17(85.0)	Ref	-	0.049
Non-WRHA	0(0.0)	33(100.0)	-	-	
Travel					
Travel in the last month					
WRHA	7(35.0)	13(65.0)	Ref	-	0.053
Non-WRHA	21(63.6)	12(36.4)	1.56	0.99-2.48	
Symptoms					
Blood in stool					
WRHA	5(25.0)	15(75.0)	Ref	-	0.085
Non-WRHA	17(51.5)	16(48.5)	1.50	0.99-2.25	
Nausea					
WRHA	17(85.0)	3(15.0)	Ref	-	0.073
Non-WRHA	20(60.6)	13(39.4)	0.067	0.46-0.97	
Abdominal cramps					
WRHA	20(100.0)	0(0.0)	Ref	-	0.019
Non-WRHA	25(75.8)	8(24.2)	0.56	0.43-0.72	
Chills					
WRHA	17(85.0)	3(15.0)	Ref	-	0.067
Non-WRHA	19(57.6)	14(42.4)	0.64	0.44-0.94	

4.4 Analysis *Salmonella*-Urban/WRHA and Rural/Non-WRHA:

This section highlights significant findings of the case-case analysis of participants with *Salmonella* residing in urban and rural Manitoba. Urban participants were defined as those residing in the WRHA, which consists of the City of Winnipeg, Manitoba's largest urban center, and rural participants were defined as those residing outside of this region. This includes calculation of Fisher Exact tests for all binary variables as well as Kruskal-Wallis tests for ordinal variables. (Only significant findings shown in table 10).

4.4.1 Distribution of participants:

Fifty-one percent of *Salmonella* participants lived outside the WRHA with the remaining 48.4% (n=15) residing within the WRHA.

4.4.2 Contact with water/sewage:

For participants with *Salmonella*, there were no significant differences in utilization of municipal and well water for drinking, bathing/showering and washing dishes. There was a significant difference between the two locations in the use of purchased bottled water for drinking where urban participants used significantly more ($p=0.029$).

As expected, urban participants with *Salmonella* reported having more bathroom facilities where indoor plumbing was connected to a municipal system.

4.4.3 Food history:

There were no significant differences between urban and rural participants with *Salmonella* in food history, including food consumed and eating outside the home.

4.4.4 Contact with animals or farms:

As expected rural participants diagnosed with *Salmonella* reported they lived on farms or visited farms more frequently and reported more contact with cattle. In terms of domestic pets, rural participants with *Salmonella* also reported having more contact with adult cats ($p=0.003$).

4.4.5 Other occupational, recreational, or incidental exposures:

There were no significant differences between urban and rural participants with *Salmonella* with respect to occupational, recreational, or incidental exposures.

4.4.6 Travel:

There were no significant differences between urban and rural participants with *Salmonella* with respect to travel.

4.4.7 Symptoms:

Symptoms were relatively similar for cases in both locations, the only significant difference was that urban participants with *Salmonella* reported less blood in stool when compared to the rural participants ($p=0.001$).

Table 10. Statistically significant univariate case-case associations between risk factor exposure in urban/WRHA and rural/non-WRHA participants with *Salmonella* infection using Fishers Exact test and relative risk

Explanatory Variables	Yes (%)	No (%)	RR	95% CI	*P*
Contact with water/sewage					
Purchased bottled water for drinking in household					
Urban/WRHA	9(60.0)	6(40.0)	Ref	-	0.029
Rural/Non-WRHA	3(18.8)	13(81.3)	0.37	0.13-1.02	
Bathroom facilities - Indoor plumbing connected to a					
Urban/WRHA	15(100.0)	0(0.0)	Ref	-	0.043
Rural/Non-WRHA	11(68.8)	5(31.3)	0.42	0.27-0.66	
Contact with animals or farms					
Live on a farm					
Urban/WRHA	0(0.0)	15(100.0)	Ref	-	0.018
Rural/Non-WRHA	6(37.5)	10(62.5)	2.50	1.55-4.04	
Visited a farm					
Urban/WRHA	0(0.0)	15(100.0)	Ref	-	0.018
Rural/Non-WRHA	6(37.5)	10(62.5)	2.50	1.55-4.04	
Contact with domestic cattle					
Urban/WRHA	0(0.0)	15(100.0)	Ref	-	0.043
Rural/Non-WRHA	5(31.3)	11(68.8)	2.36	1.51-3.70	
Contact with any domestic farm animals					
Urban/WRHA	14(93.3)	1(6.7)	Ref	-	0.083
Rural/Non-WRHA	10(62.5)	6(37.5)	0.49	0.28-0.85	
Contact with adult dogs					
Urban/WRHA	2(13.3)	13(86.7)	Ref	-	0.003
Rural/Non-WRHA	11(68.8)	5(31.3)	3.05	1.40-6.65	
Symptoms					
Blood in stool					
Urban/WRHA	2(13.3)	13(86.7)	Ref	-	0.001
Rural/Non-WRHA	12(75.0)	4(25.0)	3.64	1.51-8.81	

4.5 Location Analysis:

The previous section described the results of a case-case analysis by infection, where the characteristics of participants with *Campylobacter, Salmonella* and VTEC were compared to each other. The following section describes a case-case analysis with the focus on location (i.e. the characteristics of cases in different parts of the province, regardless of infection type, are compared to each other). The location of all participants was determined to the RHA level. Participating RHAs were then combined to form three large geographical areas for analysis. Region one - Winnipeg (Winnipeg RHA), region two – South/Central Manitoba (Central, Interlake and South Eastman RHAs) and region three- Western Manitoba (Parkland, Brandon and Assiniboine RHAs). Risk ratios as well as Fishers Exact tests were calculated for all binary variables, including 2x3 comparisons of all three location groupings as well as 2x2 comparisons of Winnipeg versus South/Central Manitoba, and Western Manitoba versus Winnipeg and South/Central Manitoba pooled. *P* values were partitioned in this manner following the methodology proposed by Everitt [100]. Kruskal-Wallis tests were calculated for all ordinal variables. Appendix J shows percentages of participants reporting exposure to all explanatory variables by location grouping as well as Fishers Exact and Kruskal-Wallis test results for 2x3 comparisons.

Much of the data presented below simply reflects expected differences between urban and rural lifestyles, such as Winnipeg households being more likely to have municipal water supplies over private wells when compared to rural households. However this data aids in the illustration of the differences in symptoms and severity of

enteric illness in different parts of the province and assists in the interpretation of the case-specific analysis presented in previous sections.

4.5.1 Distribution of participants:

Appendix J table i displays detailed numerical data on the distribution of participants. The majority of participants resided in South/Central Manitoba and Winnipeg (40.2% [n=37] in each), with the remaining participants residing in Western Manitoba (19.6% [n=18]).

There was a significant difference in age as participants in South/Central Manitoba were younger, with a mean age of 26.2, than those in Winnipeg and Western Manitoba (mean age 44.6 and 38.6 respectively) (p=0.008). In terms of age groups most affected, for Western Manitoba it was those in their teens and twenties and those in their fifties and sixties. In South/Central Manitoba it was those under the age of 30, and in Winnipeg it was those in their thirties and forties.

A significant difference was also seen in the number of children under the age of five in the household, as participants from South/Central Manitoba had more children under the age of five (mean=0.7) than those in Winnipeg and Western Manitoba (means=0.3 and 0.5 respectively). The average number of people in the household and the average number of children in diapers did not vary significantly by location.

Dates of self reported onset of symptoms were determined for all study participants. Figure 7 shows epidemic curves for the three location groupings. Peaks in the number of participants can be seen at the end of June and throughout August.

Figure 7. Epidemic curve by location: Infections in Manitoba from May to September by date of onset of symptoms

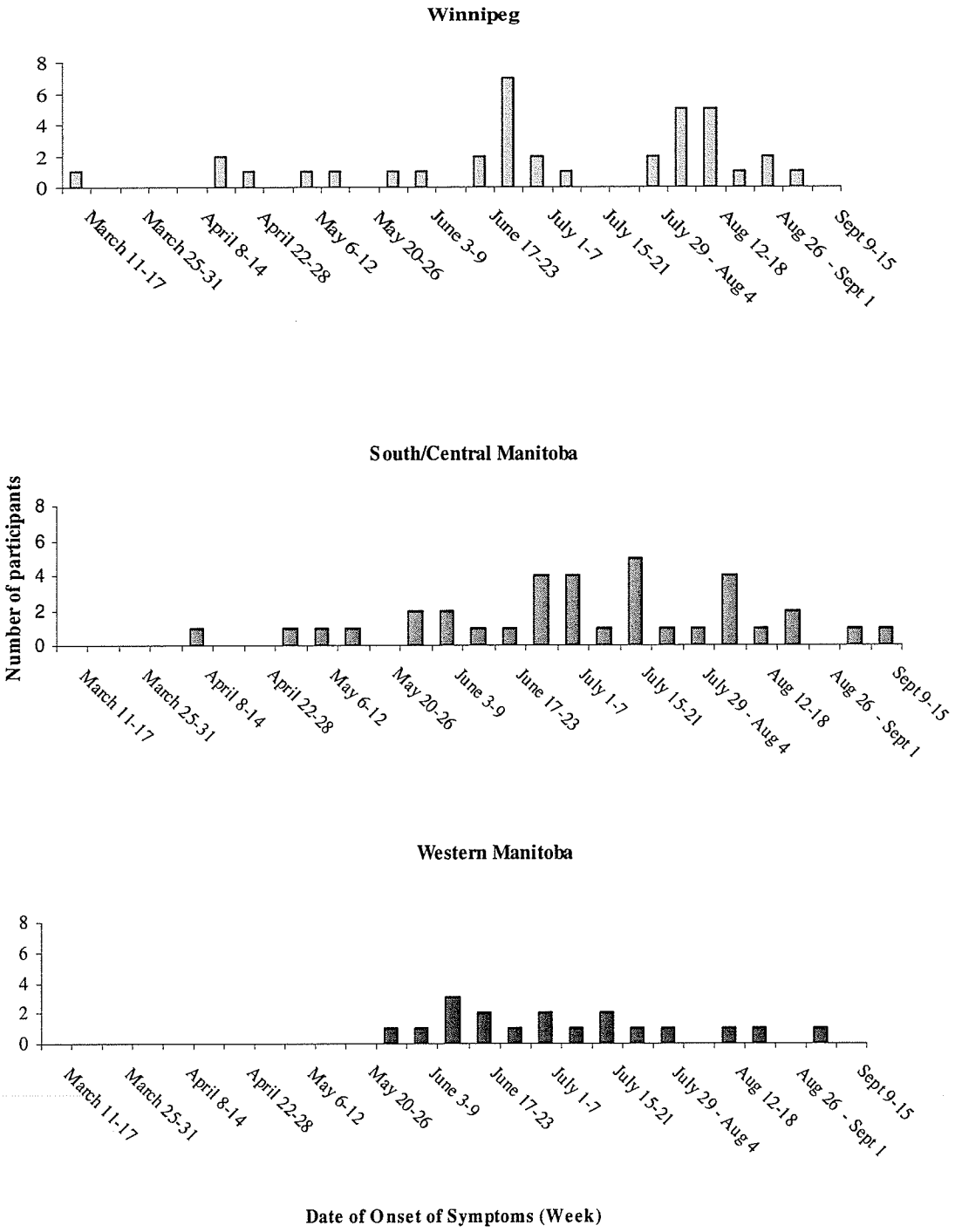


Table 11. Statistically significant univariate case-case associations between reported contact with water/sewage and location using Fishers Exact test and relative risk

					P -Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Locations**	**2x2 Winnipeg vs. South/Central Manitoba**	**2x2 Western Manitoba vs. Winnipeg and South/Central Manitoba pooled**
Municipal water for drinking in household							
Winnipeg	29 (78.4)	8(21.6)	Ref	-			
South/Central Manitoba	21(56.8)	16(43.2)	0.63	0.41-0.97	0.029	0.081	0.101
Western Manitoba	8(44.4)	10(55.6)	0.39	0.19-0.82			
Private well water for drinking in household							
Winnipeg	1(2.7)	36(97.3)	Ref	-			
South/Central Manitoba	15(40.5)	22(59.5)	2.47	1.74-3.52	0.000	0.000	0.357
Western Manitoba	6(33.3)	12(66.7)	3.43	1.93-6.10			
Municipal water for bathing/showering or washing dishes in household							
Winnipeg	36(97.3)	1(2.7)	Ref	-			
South/Central Manitoba	22(59.5)	15(40.5)	0.40	0.28-0.58	0.000	0.000	0.035
Western Manitoba	9(50.0)	9(50.0)	0.22	0.12-0.41			
Private well water for bathing/showering or washing dishes in household							
Winnipeg	2(5.4)	35(94.6)	Ref	-			
South/Central Manitoba	15(40.5)	22(59.5)	2.29	1.58-3.31	0.000	0.001	0.038
Western Manitoba	9(50.0)	9(50.0)	4.00	2.10-7.63			
Bathroom facilities - Indoor plumbing connected to a municipal system							
Winnipeg	35(94.6)	2(5.4)	Ref	-			
South/Central Manitoba	20(54.1)	17(45.9)	0.41	0.28-0.60	0.000	0.000	0.084
Western Manitoba	9(50.0)	9(50.0)	0.25	0.13-0.48			
Bathroom facilities - Indoor plumbing connected to a septic field							
Winnipeg	1(2.7)	36(97.3)	Ref	-			
South/Central Manitoba	13(35.1)	24(64.9)	2.32	1.65-3.27	0.000	0.001	0.113
Western Manitoba	7(38.9)	11(61.1)	3.74	2.09-6.68			

4.5.2 Contact with water/sewage:

Appendix J table ii displays detailed numerical information on contact with water/sewage with more information on significant variables in table 11. As expected participants living in Winnipeg used more municipal water and less well water, and had more indoor plumbing connected to a municipal system and less connected to a septic field.

4.5.3 Food history:

Appendix J table iii displays detailed numerical information on food history with more information on significant variables in table 12. Chicken was the most commonly consumed food, regardless of location, followed by eggs in Winnipeg, ground beef in South/Central Manitoba, and eggs and ground beef in Western Manitoba.

There was a significant difference between locations in the consumption of eggs and unpasteurized milk. Participants in South/Central Manitoba consumed less eggs and more unpasteurized milk ($p=0.090$ and 0.007 respectively).

Table 12. Statistically Significant univariate case-case associations between reported food history and location using Fishers Exact test and relative risk

					P -Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Locations**	**2x2 Winnipeg vs. South/Central Manitoba**	**2x2 Western Manitoba vs. Winnipeg and South/Central Manitoba pooled**
Consumed eggs							
Winnipeg	28(75.7)	9(24.3)	Ref	-			
South/Central Manitoba	19(51.4)	18(48.6)	0.61	0.39-0.94	0.090	0.052	1.000
Western Manitoba	11(61.1)	7(38.9)	0.64	0.31-1.36			
Consumed milk (unpasteurized)							
Winnipeg	0(0.0)	37(100.0)	Ref	-			
South/Central Manitoba	7(18.9)	30(81.1)	2.23	1.71-2.91	0.007	0.011	1.000
Western Manitoba	1(5.6)	17(94.4)	3.18	2.14-4.71			

4.5.4 Contact with animals or farms:

Appendix J table iv displays detailed numerical data on contact with animals or farms with more information on significant variables in table 13. A total of 23.7% percent of participants (n=22) reported living on a farm and, as expected, participants in Winnipeg reported less contact with farms and domestic farm animals than those outside this region. Of those who reported living on a farm the most common types of farms reported were mixed (ranching and crops) (36.4% [n=8]), ranching (31.8% [n=7]), hobby (9.1% [n=2]), and crop production (4.5% [n=1]). Four participants reported that farms were not active (9.1% [n=2]) or that they were unsure of the type (9.1% [n=2]). The type of farm varied significantly by location where more participants in South/Central Manitoba reported farms were primary ranching while those in Western Manitoba reported having more mixed farms (ranching and crops) (p=0.003). The types of animals produced were most commonly stated to be cattle, with 68.2% (n=15) of farms producing this animal, followed by hogs (22.7% [n=5]), turkeys (13.6% [n=3]), horses (9.1% [n=2]), chickens, goats and bison (4.5% [n=1] each).

Participants in Western Manitoba reported more contact with domestic farm animals overall (p=0.001), particularly with hogs (p=0.058), broiler chickens (p=0.097), egg-laying chickens (p=0.007) and other domestic farm animals (p=0.045).

In terms of domestic pets, contact was most commonly reported with cats in Winnipeg and Western Manitoba, and with dogs in South/Central Manitoba. Participants in Winnipeg had significantly less contact with any dogs, puppies, cats or kittens (p=0.003), while participants in South/Central Manitoba had more contact with adult

dogs (p=0.000) and kittens (0.038) specifically, and participants in South/Central Manitoba and Western Manitoba had more contact with cats (p=0.043).

4.5.5 Other occupational, recreational, or incidental exposures:

Appendix J table v displays detailed numerical information on other occupational, recreational or incidental exposures. In terms of recreation, most participants, regardless of location, did not swim or participate in water related activities. No significant differences were found in occupational, recreational or incidental exposures between the three RHA groupings.

Table 13. Statistically significant univariate case-case associations between reported contact with animals or farms and location using Fishers Exact test and relative risk

					P -Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Locations**	**2x2 Winnipeg vs. South/Central Manitoba**	**2x2 Western Manitoba vs. Winnipeg and South/Central Manitoba pooled**
Live on a farm							
Winnipeg	1(2.7)	36(97.3)	Ref	-			
South/Central Manitoba	13(35.1)	24(64.9)	2.32	1.65-3.27	0.000	0.001	0.032
Western Manitoba	8(44.4)	10(55.6)	4.09	2.26-7.41			
Visited a farm							
Winnipeg	2(5.4)	35(94.6)	Ref	-			
South/Central Manitoba	16(43.2)	21(56.8)	2.37	1.63-3.45	0.000	0.000	0.020
Western Manitoba	10(55.6)	8(44.4)	4.48	2.28-8.79			
Contact with domestic cattle							
Winnipeg	1(2.7)	36(97.3)	Ref	-			
South/Central Manitoba	13(35.1)	24(64.9)	2.32	1.65-3.27	0.000	0.001	0.032
Western Manitoba	8(44.4)	10(55.6)	4.09	2.26-7.41			
Contact with domestic hogs							
Winnipeg	1(2.7)	36(97.3)	Ref	-			
South/Central Manitoba	3(8.1)	34(91.9)	1.54	0.83-2.86	0.058	0.615	0.044
Western Manitoba	4(22.2)	14(77.8)	2.86	1.53-5.33			
Contact with broiler chickens							
Winnipeg	0(0.0)	37(100.0)	Ref	-			
South/Central Manitoba	1(2.7)	36(97.3)	2.03	1.61-2.56	0.097	1.000	0.097
Western Manitoba	2(11.1)	16(88.9)	3.31	2.20-4.99			
Contact with egg laying chickens							
Winnipeg	0(0.0)	37(100.0)	Ref	-			
South/Central Manitoba	2(5.4)	35(94.6)	2.06	1.62-2.61	0.007	0.493	0.013
Western Manitoba	4(22.2)	14(77.8)	3.64	2.33-5.69			
Contact with other domestic farm animals							
Winnipeg	0(0.0)	37(100.0)	Ref	-			
South/Central Manitoba	2(5.4)	35(94.6)	2.06	1.62-2.62	0.045	0.493	0.050
Western Manitoba	3(16.7)	15(83.3)	3.47	2.26-5.31			
Contact with any domestic farm animals							
Winnipeg	5(13.5)	32(86.5)	Ref	-			
South/Central Manitoba	16(43.2)	21(56.8)	1.92	1.28-2.90	0.001	0.009	0.013
Western Manitoba	11(61.1)	7(38.9)	3.83	1.81-8.09			

Table 13 ***continued.*** **Statistically Significant univariate case-case associations between reported contact with animals or farms and location using fishers exact test and relative risk**

					P -Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Locations**	**2x2 Winnipeg vs. South/Central Manitoba**	**2x2 Western Manitoba vs. Winnipeg and South/Central Manitoba pooled**
Contact with adult dogs							
Winnipeg	10(27.0)	27(73.0)	Ref	-			
South/Central Manitoba	28(75.7)	9(24.3)	2.95	1.62-5.35	0.000	0.000	0.793
Western Manitoba	8(44.4)	10(55.6)	1.64	0.78-3.45			
Contact with adult cats							
Winnipeg	11(29.7)	26(70.3)	Ref	-			
South/Central Manitoba	19(51.4)	18(48.6)	1.55	0.99-2.42	0.043	0.097	0.185
Western Manitoba	11(61.1)	7(38.9)	2.36	1.08-5.14			
Contact with kittens							
Winnipeg	4(10.8)	33(89.2)	Ref	-			
South/Central Manitoba	13(35.1)	24(64.9)	1.82	1.21-2.72	0.038	0.025	0.753
Western Manitoba	3(16.7)	15(83.3)	1.37	0.53-3.56			
Any contact with adult dogs, puppies, adult cats or kittens							
Winnipeg	21(56.8)	16(43.2)	Ref	-			
South/Central Manitoba	31(83.8)	6(16.2)	2.19	1.07-4.48	0.003	0.021	0.036
Western Manitoba	17(94.4)	1(5.6)	7.61	1.10-52.61			

4.5.6 Travel:

Appendix J table vi displays detailed numerical information on travel with more information on significant variables in table 14. Significantly less participants from Winnipeg reported travel (p=0.055). Of all participants forty-eight percent (n=45) reported traveling within the last month. Of those who did travel, 68.9% (n=31) of participants did so within Manitoba, 17.8% (n=8) traveled outside of Manitoba but within Canada, 13.3% (n=6) outside of Canada but within North America and 11.1% (n=5) outside of North America. (Total percentage exceeds 100 as participants reported more than one trip or trips with multiple destinations). When travel was examined further by these destination groupings (within Manitoba, within Canada, within North America and International) no significant differences were found.

There was a significant difference between regional groupings in the amount of participants reporting travel for business (p=0.032) and for reasons other than business, recreation or military such as school or doctors appointments (p=0.097), although the majority of this travel was also within the province.

Table 14. Statistically significant univariate case-case associations between reported risk factor exposure and location using Fishers Exact test and relative risk

					P -Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Locations**	**2x2 Winnipeg vs. South/Central Manitoba**	**2x2 Western Manitoba vs. Winnipeg and South/Central Manitoba pooled**
Travel in the last month							
Winnipeg	12(32.4)	25(67.6)	Ref	-			
South/Central Manitoba	22(59.5)	15(40.5)	1.73	1.08-2.76	0.055	0.035	0.600
Western Manitoba	10(55.6)	8(44.4)	1.88	0.88-4.00			
Travel in the last month for business							
Winnipeg	0(0.0)	37(100.0)	Ref	-			
South/Central Manitoba	4(10.8)	33(89.2)	2.12	1.66-2.72	0.032	0.115	0.133
Western Manitoba	3(16.7)	15(83.3)	3.47	2.26-5.31			
Travel in the last month for reasons other than business, recreation or military							
Winnipeg	0(0.0)	37(100.0)	Ref	-			
South/Central Manitoba	1(2.7)	36(97.3)	2.03	1.61-2.56	0.097	1.000	0.097
Western Manitoba	2(11.1)	16(88.9)	3.31	2.20-4.99			

Table 15. Statistically Significant univariate case-case associations between reported symptoms and location using Fishers Exact test and relative risk

					p -Values Fishers Exact Test		
Explanatory Variables	**Yes (%)**	**No (%)**	**RR**	**95% CI**	**2x3 All three Locations**	**2x2 Winnipeg vs. South/Central Manitoba**	**2x2 Western Manitoba vs. Winnipeg and South/Central Manitoba pooled**
Symptoms							
Blood in stool							
Winnipeg	9(24.3)	28(75.7)	Ref	-			
South/Central Manitoba	23(62.2)	14(37.8)	2.16	1.33-3.48	0.004	0.002	0.610
Western Manitoba	9(50.0)	9(50.0)	2.06	0.99-4.28			
Chills							
Winnipeg	28(75.7)	9(24.3)	Ref	-			
South/Central Manitoba	18(48.6)	19(51.4)	0.58	0.37-0.90	0.028	0.030	0.276
Western Manitoba	14(77.8)	4(22.2)	1.08	0.43-2.72			
Headache							
Winnipeg	21(56.8)	16(43.2)	Ref	-			
South/Central Manitoba	19(51.4)	18(48.6)	0.90	0.57-1.41	0.047	0.816	0.019
Western Manitoba	4(22.2)	14(77.8)	0.34	0.13-0.91			

4.5.7 Symptoms:

Appendix J tables i and vii display detailed numerical information on symptoms for ordinal and binary variables respectively, with more detailed information on significant binary variables in table 15. Participants were questioned on a variety of possible symptoms. The most common symptom reported, regardless of location, was diarrhea. This was followed by fatigue for participants in Western and South/Central Manitoba, and abdominal cramps for participants in Winnipeg. There were some differences between RHA groupings in terms of symptoms reported by participants. Participants in Winnipeg reported less blood in stool (p=0.004), those in South/Central Manitoba reported less chills (p=0.028) and those in Western Manitoba reported less headaches (p=0.047).

Participants reported the first symptom they experienced. For those in Winnipeg and South/Central Manitoba the most commonly reported first symptom was diarrhea, and for those in Western Manitoba it was cramps (table 16).

Table 16. Frequency of first symptom reported by participants by infection

	Winnipeg	South/Central Manitoba	Western Manitoba
Diarrhea	10 (27.0)	12 (32.4)	2 (11.1)
Chills	2 (5.4)	3 (8.1)	1 (5.6)
Fatigue	4 (10.8)	1 (2.7)	2 (11.1)
Cramps	9 (24.3)	6 (16.2)	7 (38.9)
Fever	1 (2.7)	4 (10.8)	2 (11.1)
Stomach/Abdominal Pain	9 (24.3)	7 (18.9)	2 (11.1)
Headache	0 (0.0)	3 (8.1)	1 (5.6)
Nausea/Vomiting	5 (13.5)	0 (0.0)	0 (0.0)
Other	5 (13.5)	6 (16.2)	0 (0.0)
Unknown	2 (5.4)	0 (0.0)	1 (5.6)

Note: Percentage may add up to over 100% as some cases reported two or more concurrent first symptoms

There was also a significant difference in duration of diarrhea as participants in Western Manitoba reported shorter durations with only 38% reporting 6 or more days, compared to 68% and 73% in South/Central Manitoba and Winnipeg respectively (p=0.052). Locations also reported significant differences in illness duration, where participants in Western Manitoba had the shortest duration, with a mean of 8.1 days, followed by South/Central Manitoba with a duration of 13.2 days, and participants in Winnipeg had the longest duration of 18.8 days (p=0.019).

4.6 Summary of Significant Results:

Table 17 reviews and summarizes all of the analyses undertaken and presented above, as well as which pathogens showed significant differences for a given variable. Only variables with significant results are shown.

In terms of age, rural participants were found to be younger overall and participants infected with *Campylobacter* in rural areas were significantly younger and had more people living in their household than those in urban areas.

Significant differences were seen in water sources utilized by participants where those with *Campylobacter* reported using more well water and those with *Salmonella* who reported using more municipal water. Purchased bottled water was utilized more often by urban participants with *Salmonella* when compared to those in rural areas. Participants with *Campylobacter* reported having less bathroom facilities with indoor plumbing connected to a municipal system and consuming water while staying overnight with friends or family at their residence more often.

Significant differences were found in the number of participants with *Campylobacter* reporting they consumed ground beef and unpasteurized milk before becoming ill, with rural participants reporting more consumption.

Participants with *Campylobacter* reported more contact with domestic farm animals and those with VTEC reported more contact with pet birds. Rural *Campylobacter* participants reported more contact with adult cats. Rural participants with *Campylobacter* and *Salmonella* reported more contact with adult dogs than their respective urban counterparts.

Engaging in watersports in a lake and eating food prepared on a recreational trip was reported more often by participants with VTEC. Urban participants with *Campylobacter* reported swimming in a public pool more often than those in rural areas.

Significantly more rural participants and particularly rural participants with *Campylobacter* reported travel. Although when examined by location of reported travel it was found that most occurred within the province.

In terms of symptoms, participants with *Salmonella* reported less diarrhea and more frequent vomiting, while participants with VTEC reported less nausea, chills and general muscle aches. Participants with VTEC had significantly lower reported severity scores and *Campylobacter* participants were hospitalized less often. Rural participants with *Campylobacter* and *Salmonella* reported more blood in stool and rural *Campylobacter* participants reported less nausea, abdominal cramps and chills. Participants living in Winnipeg reported less blood in stool and those in South/Central Manitoba reported less chills. Participants in Western Manitoba reported fewer headaches, shortest diarrhea duration, and shortest illness durations.

Table 17. Schematical overview of significant associations for all relevant[a] analysis

Explanatory Variables	Analysis linked to significant association				Infection implicated in association		
	Case -case	Location overall	Location - *Campylobacter*	Location - *Salmonella*	*Campylobacter*	*Salmonella*	VTEC
Demographics							
Age		X	X		✓		
Number of persons living in household			X		✓		
Contact with Water/Sewage							
Private well water utilized in household	X				✓		
Municipal water utilized in household						✓	
Purchased bottled water for drinking in household				X		✓	
Water purification system in household	X					✓	
Bathroom facilities - Indoor plumbing connected to a municipal system	X				✓	✓	
Used drinking water while staying overnight with friends or family at their residence	X				✓		
Food History							
Consumed milk (unpasteurized)		X	X		✓		
Consumed beef (ground)			X		✓		
Contact with Animals or Farms							
Contact with any domestic farm animals	X				✓		
Contact with pet birds	X						✓
Contact with adult dogs		X	X	X		✓	
Contact with adult cats		X	X		✓		
Any contact with adult dogs, puppies, adult cats or kittens		X	X		✓		
Other occupational, recreational or incidental exposures							
Consumed food prepared on an outdoor recreational trip	X						✓
Swam or participated in watersports in a lake	X						✓
Swam in a public pool			X		✓		
Travel							
Travel in the last month		X	X				
Symptoms							
Diarrhea	X					✓	
Duration of Diarrhea			X				
Nausea	X		X				✓
Fever	X						✓
Chills	X	X	X				✓
Headache			X				✓
General Muscle Aches	X						✓
Blood in Stool		X	X	X	✓	✓	
Abdominal Cramps			X		✓		
Number of Vomiting Events in 24 hours	X					✓	
Duration of Illness			X				
Severity Score	X						✓
Hospitalized	X				✓		

[a] Some significant variable from the location analysis were excluded as they refected obvious differences between urban and rural lifestyles. Boxes that appear lightly shaded also represent these findings.

CHAPTER 5: DISCUSSION

This study was conducted with two main objectives; first, to determine the risk factors for infection by *Campylobacter*, *Salmonella* and VTEC and their geographic distribution and second, to pilot a methodology utilizing existing public health practice to attain cases and to determine the ability to enroll case-nominated controls.

5.0 Risk Factors for *Campylobacter*, *Salmonella* and VTEC in Manitoba:

The results presented in the previous section and the subsequent discussion are not meant to infer definitive etiology but rather to build hypotheses and demonstrate the need for further research with respect to location specific risk factors for *Campylobacter*, *Salmonella* and VTEC in Manitoba.

5.0.1 Distribution of participants:

Participants living in rural areas were significantly younger than those living in urban areas. When stratified by infection this difference was seen for participants with *Campylobacter* but not for those with *Salmonella*. This indicates that the age difference seen for *Campylobacter* could be impacting the age difference for infections overall, given that almost 60% of study participants were infected with *Campylobacter*.

Previous literature has demonstrated age differences in the incidence patterns of *Campylobacter* in Manitoba [2] and elsewhere [102]. In Manitoba, the rates of *Campylobacter* infection are approximately seven times higher for those living in rural areas, with particularly high rates in rural children aged zero to four [2]. For study

participants with *Campylobacter,* infection was most common in those less than 1ten years of age. Of these cases, all were from rural areas. A study in New Zealand found that those under the age of 15 living in rural areas had significantly higher rates of infection. This pattern was attributed to an increased vulnerability to infection in rural children and youth due to contact with farm animals and contaminated environments [102]. The reason why these factors affect children more than adults could be due to the fact that rural adults over time may have developed a decreased sensitivity to infection. Compounding this pattern is the possibility that case finding in areas where children are disproportionately affected may be more likely in this age group, as parents or caregivers could be more likely to seek medical attention for children, as opposed to themselves.

Only three participants with *Salmonella* and 3 participants with VTEC were under the age of ten. For these *Salmonella* cases all were rural residents, and for VTEC, two were rural residents. These patterns for *Salmonella* and VTEC are consistent with the association between infections in children being in rural areas. However, given the relatively small number of *Salmonella*/VTEC cases, a larger sample size would be necessary to verify the association for these two pathogens.

Other peaks in the number of study participants were seen for those in their forties and fifties, where the majority of those in their forties were urban residents and those in their fifties were more often rural residents. This does not coincide with typical incidence patterns from the literature, as peaks in young adults, rather than those in their forties and fifties are reported [2,103].

It appears that the different age distributions in the study participants with *Campylobacter* versus the overall infected population, at least partially reflects a different

likelihood to participate in the study. Figure 6 shows the percentage of study participants as a percentage of the total *Campylobacter* cases reported to the CDC at MHHL. This figure demonstrates that the study was more likely to enroll people less than ten, those in their forties and fifties, as well as those in their seventies. Overall, this slight skewing of the data should be kept in mind when interpreting the risk factors below, as they would tend to represent the risk factors for the above age groups to a slightly greater extent.

5.0.2 Contact with water/sewage:

Campylobacter infection was found to be associated with the utilization of well water as well as a greater likelihood of having bathroom facilities connected to non-municipal water systems. These two variables would be highly correlated and it cannot be determined from the present results whether it is the private water, the bathroom facilities connected to that water, or both that is the key risk factor. *Salmonella* infection was found to be associated with the utilization of municipal water.

In Manitoba, approximately 80% and 15% of the population is serviced by public and private water systems, respectively [104]. This distribution is similar to the water supplies as reported by study participants with *Salmonella.* For participants with *Salmonella*, 87% reported being serviced by public water, while 13% reported having a private system [105]. Conversely, for study participants with *Campylobacter* only 36% reported having a public water system while 64% reported having a private system.

The consumption of contaminated drinking water has been found to be a source of *Campylobacter* and other enteric infections [17,36]. In Manitoba public water supply systems are regulated under the Manitoba Public Health act which requires systems to

meet certain design, operational, and monitoring requirements under the Drinking Water Safety Act [104]. This includes routine testing of disinfectant levels (such as chlorine) as well as levels of bacteria and other specified substances [106,107]. These tests must be conducted at specified time intervals depending on the size of the population being served by the water supply in question [107]. While this routine monitoring is regulated for public water systems, private systems, defined as those which supply water to only one private residence, are the responsibility of the system owner including construction, operation and monitoring [104]. This difference leads to increased potential of contamination in private systems if they are not operated and monitored adequately by the owner.

The Government of Manitoba recommends that private water supplies be tested at least once a year [105]. Of study participants with private water sources, all those with *Salmonella* and 72% of those with *Campylobacter* reported having their private well water tested or monitored. The increased percentage of participants with *Campylobacter* who utilize private well water combined with the decreased percentage of those reporting routine testing could demonstrate a link between exposure to *Campylobacter* and consumption of private well water.

Additionally, more participants with *Campylobacter* reported consuming water while staying overnight with friends or family at their residence. Although the water sources (private well or municipal) were not queried for this variable, the majority of these residences [72.7% (n=8)] were located in rural Manitoba. This again suggests that increased risk of infection by this pathogen may be associated with consumption from a private water supply.

Given the level of municipal water quality control in the province it is doubtful that the observed correlation between municipal water and *Salmonella* is directly related to the consumption of this water. Rather this association is likely serving as a marker for increased infection rates in urban areas. Of all *Salmonella* participants 48% were residents of Winnipeg and 44% were residents of South/Central Manitoba (compared to 38% for *Campylobacter* participants for both locations). Although South/Central Manitoba is considered a rural location, participants from this region reported living on a farm or having contact with farm animals less often when compared to Western Manitoba. Therefore, for *Salmonella*, the association with municipal water seems to be a proxy marker for an urban or semi-urban lifestyle. *Campylobacter* differs in that it seems to be related to a rural lifestyle, where well water utilization may be one of several rural-related risk factors associated with infection.

Urban participants with *Salmonella* also consumed significantly more bottled water than those in rural areas. Bottled water sold in Canada is regulated and although no known outbreaks due to consumption of bottled water have been reported in Canada [108], outbreaks of *Salmonella* caused by consumption of contaminated bottled water have been reported internationally [109]. Literature also states that bottled water has been found to be contaminated with Norovirus which has a common ecology and epidemiology to *Salmonella* [110]. This association could indicate an increased risk for *Salmonella* infection is related to the consumption of bottled water and could be a factor in urban *Salmonella* infection.

5.0.3 Food history:

Rural participants with *Campylobacter* consumed more ground beef and unpasteurized milk when compared to urban participants with *Campylobacter*. A larger proportion of rural residents live on farms and therefore have greater access to unpasteurized milk. The dairy act of Manitoba states that the distribution and sale of unpasteurized milk is prohibited, and under this act violators can be fined and imprisoned for up to six months [106]. While this law allows health inspectors and government officials to stop the sale and distribution of unpasteurized milk it does not allow any action on dairy farmers who choose to consume unpasteurized milk and milk products obtained on their own farm [106].

There are many individuals who choose to consume unpasteurized milk as they believe that the pasteurization process kills many beneficial bacteria, destroys enzymes and diminishes vitamin content [111]. The consumption of unpasteurized milk has been well documented as a risk factor for *Campylobacter* and other enteric infections ([13,20,27,36,76,112]), although this difference was only seen between the urban and rural participants with *Campylobacter*. No difference in consumption was found in the case-case comparison between the three infections. Rural rates of unpasteurized milk consumption are expected to be higher, although this would also be expected to be seen for *Salmonella* infections. The consumption of unpasteurized milk as a risk behaviour may therefore be greater for *Campylobacter* in comparison to *Salmonella*.

The link for *Campylobacter* with ground beef consumption in rural areas could be associated with the beef consumed in rural areas being more likely to be contaminated, as ground beef may be obtained from a local source and not from a centralized food

system. It could also be that more beef overall is consumed in rural areas, although data on food consumption in Canada is limited to nutrient intake and status, not individual consumptions [113]. Studies have been completed on individual food consumption for specific regions and sub-populations but no specific Manitoba data could be found [114-118]. Therefore the presence of a control population would have been useful in this situation to provide data as food consumption patterns in different locations within Manitoba may differ. If there was no difference in beef consumption between urban and rural Manitobans these findings could indicate an increased risk of *Campylobacter* contamination in rural beef over beef obtained in Winnipeg.

5.0.4 Contact with animals or farms:

Campylobacter infection was associated with increased contact with animals. Studies have shown that rates of *Campylobacter* as well as other enteric infections coincide with animal density patterns [2,4]. As this finding was significant in the case-case comparisons, it indicates that animal exposure is more of a factor for *Campylobacter* compared to *Salmonella* and VTEC. Participants were questioned on contact with particular farm animals and no significant differences between infections were found for any specific animals. As discussed previously, transmission patterns for *Campylobacter* in Manitoba appear to be related to more 'rural' risk factors. Farm animals in rural Manitoba may be more likely to be infected with *Campylobacter* than with *Salmonella* or VTEC and exposure to these infected animals could cause increased rates in the Manitoba rural population. Exposure to farm animals could also be a marker for other

factors associated with rural living such as contaminated water or unpasteurized milk consumption.

Rural *Campylobacter* and *Salmonella* participants had more contact with adult dogs. This was also seen in the location comparison, where rural participants overall had more contact with cats, dogs, puppies and kittens. No research could be found on the levels of bacterial infection in rural and urban dogs. Some studies have been conducted on parasitic infections comparing rates in urban and rural dogs [119,120]. These studies found that rural dogs had higher prevalence of infection of *Echinoccosis* [119] and a higher frequency of antibodies for *Neospora caninu* [120] although no similar studies of bacterial infections could be found. It is hypothesized that rural dogs would have higher infection rates, due to closer proximity to farm animals and contaminated environments and could spread this infection to pet owners. Thus examination of the enteric infection rates of rural and urban dogs may provide a partial explanation of the increased rates of infection in rural Manitoba.

5.0.5 Other occupational or incidental exposures:

VTEC was found to be related to recreational activities, specifically swimming in a lake and eating food prepared on an outdoor trip. In terms of swimming, exposure to contaminated surface water is a risk factor for VTEC [65,84] and other common enteric infections [17,36], thus recreational water bodies in Manitoba are routinely monitored for contamination by disease causing organisms during the summer months. Guidelines for the testing of Manitoba water bodies, using fecal coliforms as indicator bacteria, were put in place to protect recreational water from contamination by organisms with the potential

to cause illness. While fecal coliforms do not normally cause illness themselves, large numbers of them often indicate the presence of more harmful organisms and thus can serve as indicator bacteria. The recreational water quality guideline for indicator bacteria is 200 fecal coliforms per 100ml sample. If a particular water body tests over these limits, it will be retested. If the water quality issue persists, warning signs for bathers may be posted at the discretion of the regional Medical Officer of Health [63].

The most common illness contracted by bathers are infections of the eyes, ears, nose and throat followed by gastroenteritis typically caused by *Salmonella* or enteric viruses [63]. However, studies have also found infections of *Campylobacter* and VTEC to be related to recreational water bodies in Canada [121,122]. The elevated percentage of participants with VTEC with reported exposure to recreational water bodies could indicate that Manitoba water bodies are more likely to be contaminated with VTEC over *Salmonella* and *Campylobacter,* or that recreational exposure to contaminated water sources is a better transmission vehicle for VTEC. Since only indicator bacteria are tested, rather than specific pathogens, it is unknown what bacterial species may or may not be present. Testing water bodies for individual bacteria coupled with molecular typing could help determine whether this is a significant source of infection for bathers.

Participants with VTEC also reported consuming more food prepared on an outdoor recreational trip, in comparison to participants with *Salmonella* and *Campylobacter.* This association could be related to types of food eaten and how the food was prepared. For example, if the food consumed is more often ground beef, which has been associated with VTEC, over chicken, which is more commonly associated with *Campylobacter* and *Salmonella,* this could be driving the higher reported consumption of

food prepared on recreational trips by participants with VTEC. Consuming food on a recreational trip may also increase risk as it can be more difficult to clean and conduct correct hygiene practices leading to an increased possibility of contaminating food. Although cases were not questioned about the type of food eaten on an outdoor trip or about cooking hygiene practices during these outings, this information would be useful in determining the specific association. This risk factor could also be related to the recreational water activity, where those who swam in a lake did so during a recreational trip and also ate food on that trip. However, the majority of those who swam in a lake (60%) did not report eating food prepared on an outdoor recreational trip. If the data were more robust, multivariate analysis could have been conducted to determine any links between these indicators.

5.0.6 Symptoms:

The majority of symptoms did not vary by infection. VTEC was found to be less severe than the other infections (severity score calculation described in methods), and reported less nausea, chills and general muscle aches. However, VTEC cases did report being hospitalized more often. Perhaps specific symptoms, demographics or risk factors are related to increased levels of hospitalization. Multivariate analysis would be necessary to determine what relationship specific symptoms or explanatory variables are related to hospitalization. In the present study the number of participants was too low to conduct an analysis of this type.

Differences in symptoms were also observed for the various location groupings. Winnipeg participants reported less blood in stool, those in South/Central Manitoba

experienced less chills and those in Western Manitoba reported less headaches, as well as shorter diarrhea and illness durations. It has been hypothesized that rural cases are less severe, particularly in adults as they are thought to have more widespread immunity resulting from greater exposure to infections during childhood [103]. However, no differences were seen in the reported severity of infection or in the number of cases hospitalized between urban and rural areas. Participants in Western Manitoba did have significantly shorter illness duration which may be related to a decreased severity in rural areas as more participants living in this region reported exposure to 'rural' risk factors such as living on a farm.

Urban *Campylobacter* and *Salmonella* participants also reported less blood in stool when compared to their rural counterparts. Rural participants with *Campylobacter* also experienced less nausea, abdominal craps and chills. Although rural cases had more blood in stool, they reported other symptoms less. This pattern was especially noticeable for *Campylobacter*, however given the larger number of cases of *Campylobacter*, these differences may have also been seen for *Salmonella* if the number of participants was greater. Many studies have examined the differences in prevalence rates between urban and rural locations but few have examined the differences in disease manifestation between these different areas. Further study on the differences in symptoms could offer new information on how different lifestyles, environments and exposure routes can affect enteric illness outcome.

5.1 Methodology of Case and Control Attainment:

5.1.1 Response rate – Case attainment:

The methodology utilized was able to capture the majority of eligible cases. Of all 220 diagnosed infections only 7.7% (n=17) were lost, either due to the PHNs not being able to contact them [5% (n=11)] or the information not being received by the MHRN from the PHN [2.3% (n=6)], an additional 22 cases were also excluded based on the exclusion criteria. Thus, this was a cost effective method of accessing 92% (n=203) of lab confirmed cases of *Campylobacter, Salmonella* and VTEC. Utilizing this method allowed all of the final 93 participants to be contacted by a single MHRN, cutting down costs and increasing consistency of participant interviews. The participating PHNs felt that participation in the study did not negatively impact their work load, and the MHRN stated that "working with the RHAs ... was also not a problem. I didn't have to contact anyone too often and when I did, I usually got a quick and helpful response." [123]

The response rate for the study was 51%. This was higher than anticipated as cases were asked to participate after completing a questionnaire administered by the PHN similar to that used for the study. This was an issue that was raised by the PHNs in the WRHA as it was stated that, "generally problems always arise when you have to interview individuals twice; they may not agree to a second interview or the story changes" [124]. Although the method of utilizing PHNs to recruit cases in the study results in the potential issues associated with administering two questionnaires it may also have a positive effect on response rate as studies have shown that having advance notice of a telephone survey, such as sending introductory letters in advance, does increase the

response rate [125]. Thus having a public health practitioner inform cases of the study may result in increased credibility and thus increase the level of response.

Regional differences in response rate were found in the study, as rural cases were more likely to agree to participate. Refusal of telephone surveys has been found to be associated with particular characteristics. Low response rates have been associated with higher income, higher immigrant population, crowded housing, urban areas [126] and lower levels of education [127]. Studies have found that compositional effects such as differences in socioeconomic status have the greatest impact on non-response [128]. These conclusions are reflected in the Winnipeg population as Winnipeg, with the lowest study response rate (55.2%), has the highest number of immigrants at 17.2% [129], [130] and is Manitoba's largest urban center with the highest average personal income of $28,520 [131], although it also had the highest percentage of residents with post-secondary education (55.2% of population aged 25-54). However, the size of the urban center may also influence this relationship, as Brandon, a much smaller urban center in comparison to Winnipeg, had the highest response rate (75%) and second highest income with an average personal income in 2001 of $25,937[131], but a low percentage of immigrants (5.3%)[130] and the second highest percentage of residents with post secondary education (52%)[130]. The difference between urban/rural response rates is somewhat controversial as some studies have found relatively large differences in response rates between urban and rural populations [14,132] while others have not [125,133]. An additional factor that could lead to regional differences in response rates could be the different PHN teams present and in their manner of and time available for follow-up of cases.

5.1.2 Control attainment:

The method of obtaining controls through participant nomination was unsuccessful. Participants were unwilling to contact friends and non-household family members to recruit them into the study, and overall no non-household controls were enrolled in the study. In the beginning of the study, when this became evident, participants were given the option to enroll household family members as controls and this was more successful. The MHRN stated that "it [was] really difficult initially to get any non-household controls involved. Then when it was changed to household controls, there was a bit more cooperation" [123]. However, as this process was started well into the study, only 14 household controls were recruited. Another potential method of attaining controls would be to have MHHL utilize the database of residents registered with MHHL to randomly select age, location matched controls with initial contact by mail. In this particular study, this was not done as the necessary logistics for this process could not have been set up until after closure of the study period in the fall.

5.2 Summary:

Infection rates overall and particularly for *Campylobacter* appear to be higher in Manitoba's rural youth. This has been reported by previous studies and has been hypothesized to be related to an increased vulnerability to infection from contact with farm animals and contaminated environments [102].

Campylobacter infection was found to be higher in rural areas and was often connected with risk factors commonly associated with rural living. These included

increased utilization of well water, more contact with farm animals and consumption of unpasteurized milk.

Participants with *Salmonella* reported utilizing significantly more municipal water and there was no significant difference between urban and rural participants in terms of type of water utilized in the home. As public water supplies in Manitoba are unlikely to be contaminated with *Salmonella* it is hypothesized that this is acting as a marker for the fact that more of the participants with *Salmonella* lived in urban or semi-urban areas. There was also an increased consumption of bottled water for urban *Salmonella* participants which could be related to infection.

The participation in outdoor recreational activities was found to be related to VTEC infection, particularly swimming in a lake and consuming food prepared on an outdoor recreational trip. This could be due to direct contact with contaminated surface water, eating undercooked food or cross contamination during food preparation outdoors where it is often more difficult to maintain hygiene practices.

Contact with dogs was found to be related to both *Campylobacter* and *Salmonella* infection in rural areas. Although no research could be found on the levels of these pathogens in rural and urban dogs, it is hypothesized that rural dogs would have higher infection rates. This would be due to closer proximity to farm animals and contaminated environments.

The methodology used for this study provided a cost effective technique of accessing almost all eligible lab-confirmed cases, and provided valuable data covering most of Manitoba's RHAs, utilizing existing systems. Utilization of case-nominated, non-

household, controls was unsuccessful, although cases were willing to recruit household family members.

If this study were to be repeated I would retain the method of case attainment and would continue to collect household controls. Although, in addition I would request age and location matched controls be selected and contacted by MHHL utilizing existing knowledge on enteric infection rates in Manitoba to attempt to match the expected population of infected individuals prior to commencing the study.

The study highlighted some particular questions that could be explored in future studies in order to provide more information on potential causes of *Campylobacter*, *Salmonella* and VTEC in Manitoba. The knowledge of food consumption patterns for urban and rural areas in the province would be useful to allow for comparisons of typical food consumption patterns between different locations. The incidence of enteric bacterial infections in urban and rural dogs would also aid in determining if these pets could be a factor in the increased rates in rural Manitoba. The levels of VTEC and other specific bacterial pathogens in Manitoba water bodies would aid in the determination of the relationship between VTEC and recreational activities. Further studies should also be conducted on the differences in symptom manifestation between urban and rural *Campylobacter, Salmonella* and VTEC cases to increase understanding in how enteric infections affect urban and rural residents differently.

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APPENDIX A – Public Health Nurse Script

Researchers at Manitoba Health are carrying out a province-wide study on diarrhea-causing bacteria (*Salmonella*, *Campylobacter* and *E. coli* bacteria). They are trying to identify how people in different parts of the province are becoming infected with these bacteria.

To do this, a Manitoba Health research nurse would like to ask you** a series of questions that takes about 20 minutes to complete. The questions she will ask will be similar to the types of questions that I have just asked you. Manitoba Health needs to use their own questionnaire since it is very important that they collect exactly the same kind of information from people in all the different parts of the province. All of the information that you provide to the research nurse will remain confidential.

***If the person is less than 18 years of age a parent or guardian should answer the questions.*

If you are interested in taking part in this study, the Manitoba Health research nurse would like to phone you to tell you more about the study and see if you would like to complete the questionnaire. Everything can be done over the phone. If you agree to be contacted I will give her your phone number. Even if she contacts you, you can still decide to not take part in the study. At this point, you are only agreeing to be contacted by the research nurse.

Documentation*

Case has: □ Agreed / □ Refused to be contacted by the Manitoba Health Research Nurse.

**If case is followed by a WRHA Public Health Nurse, please document consent within the corresponding iPHIS notes.*

Phone number* case wishes to be contacted at: _____
Is there a preferred time of day to be contacted (e.g., day time, evening)? _____

**If case is followed by a WRHA Public Health Nurse, please ensure that the demographic module for the client in iPHIS is up to date.*

Potential Questions and Answers
Client: Will the research nurse provide feedback to me at the end of the study?
PHN: Questionnaires will be coded (anonymous) preventing individual information to be reported back directly to clients. However, a final study report will be available on the Manitoba Health website at the end of the study.

Client: I'd like to think about this and get back to you? When do you need a response about my participation?
PHN: Ideally, within 24 hours so that the research nurse can contact you in a timely manner as some of the questions rely on your recall of events, activities, foods etc.

Client: Where can I learn more about this study?
PHN: (If home interview) The attached fact sheet describes the study in more detail. Or, you can speak to the research nurse for more information before deciding whether you want to participate in the study. However, if you want to speak to the research nurse, I need your consent to be contacted by the research nurse.
PHN: (If phone interview) I can fax you a fact sheet describing the study in more detail. Or, you can speak to the research nurse for more information before deciding whether you want to participate in the study. However, if you want to speak to the research nurse, I need your consent to be contacted by the research nurse.

APPENDIX B – Case Information Sheet

Manitoba

Health
Healthy Living

Cadham Provincial Laboratory
Public Health Branch

P.O. Box 8450
750 William Avenue
Winnipeg MB R3C 3Y1
PH: (204) 945-6123
Fax: (204) 786-4770
www.gov.mb.ca/health/publichealth/cpl

Title of Study: "Risk factors associated with *Escherichia coli*, *Salmonella* and *Campylobacter* cases in Manitoba".

Principal Investigator: John Wylie, Cadham Provincial Laboratory, Manitoba Health, Winnipeg, 204-945-7473

Manitoba Health would like your help with a research study that we are conducting. The study will include both people who have been recently diagnosed with a bacterial infection and healthy people who have not recently been sick. By comparing these two groups we hope to better understand how people become infected with these bacteria.

If you take part in the study, you will be asked several questions that are designed to gather information on the most common sources of these bacteria. For example, they involve questions about drinking water, types of foods eaten or contact with animals. The questionnaire will take about 20 minutes to complete. By gathering information from a large number of people we can get a better idea of how these infections are spread. We expect about 100-150 people with diarrhea infections to participate in this study. It is important to do this within the individual regions of Manitoba as the sources of these infections can differ in different areas.

If you are interested in helping with this research, the Manitoba Health Research Nurse (Krista Klassen) will contact you and provide more information about the study and answer any questions you may have. You are also welcome to contact the study investigator (John Wylie) at 204-945-7473.

Manitoba
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APPENDIX C – Case Documentation Form

Current case list for documentation of consent to contact:

2007 – Week _____

RHA:_____

Last name	First name	Address	City	PCode	RHA	Age	Infection type
Smith	John	123 Anywhere	Winnipeg	R3C 4L5	Winnipeg	50	None

Status of above case regarding consent to be contacted by Manitoba Health Research Nurse:
- ☐ Accepted
- ☐ Refused
- ☐ Pending

Phone number case wishes to be contacted at: _____

Known cautions for above case (e.g. case is hospitalized, case is deceased):

Last name	First name	Address	City	PCode	RHA	Age	Infection type
Doe	Jane	123 Somewhere	Winnipeg	R3C 4L6	Winnipeg	25	None

Status of above case regarding consent to be contacted by Manitoba Health Research Nurse:
- ☐ Accepted
- ☐ Refused
- ☐ Pending

Phone number case wishes to be contacted at: _____

Known cautions for above case (e.g. case is hospitalized, case is deceased):

APPENDIX D – Case Consent Form

Manitoba

Health
Healthy Living

Cadham Provincial Laboratory
Public Health Branch

P.O. Box 8450
750 William Avenue
Winnipeg MB R3C 3Y1
PH: (204) 945-6123
Fax: (204) 786-4770
www.gov.mb.ca/health/publichealth/cpl

Telephone script for participant information

Title of Study: "Risk factors associated with *Escherichia coli*, *Salmonella* and *Campylobacter* cases in Manitoba".

Principal Investigator: John Wylie, Cadham Provincial Laboratory, Manitoba Health, Winnipeg, 204-945-7473

We would like your help with a research study that Manitoba Health is conducting. The study will include both people who have been recently diagnosed with a bacterial infection and healthy people who have not recently been sick. By comparing these two groups we hope to better understand how people become infected with these bacteria. First, I will read you some information on the study. As I go through this information, please ask me to explain any parts that are not clear.

We will be asking you questions that are designed to gather information on the most common sources of these bacteria. For example, they involve questions about drinking water, types of foods eaten or contact with animals. The questionnaire will take about 20 minutes to complete. By gathering information from a large number of people we can get a better idea of how these infections are spread. We expect about 100-150 people with diarrhea infections to participate in this study. It is important to do this within the individual regions of Manitoba as the sources of these infections can differ in different areas.

We will also ask if you know any friends or neighbors who may be interested in participating in the study as healthy people. I will talk to you about this at the end of the questionnaire. You do not have to participate in that part of the study if you do not wish.

Participation in all parts of the study is entirely voluntary and you can stop participating at any time. There are no direct benefits to you for helping us with this study, but we hope the information you and others provides will help prevent some people from being infected in the future.

We expect to produce a government report with the information that we collect. All of the information recorded on the questionnaire is anonymous, so no identifying information will ever appear in any report. The University of Manitoba Health Research Ethics Board may review records related to the study for quality assurance purposes. All of the questionnaires for this study will be stored at Cadham Provincial Laboratory.

You are free to ask any questions about the study and your rights as a research participant. If you have questions about the study, please ask them now or you can also contact the study investigator (John Wylie, 204-945-7473). If you have any questions about your rights as a research participant, you may contact the University of Manitoba, Bannatyne Campus Research Ethics Board office at 204-789-3389.

If you have no (other) questions, we can begin the questionnaire.

Research nurse documentation of telephone consent:

Case study code:_____

Signature of research nurse:_____

Date (yyyy/mm/dd):_____/____/____

Manitoba
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APPENDIX E – Control Consent Form

Manitoba

Health
Healthy Living

Cadham Provincial Laboratory
Public Health Branch

P.O. Box 8450
750 William Avenue
Winnipeg MB R3C 3Y1
PH: (204) 945-6123
Fax: (204) 786-4770
www.gov.mb.ca/health/publichealth/cpl

Telephone script for participant information

Title of Study: "Risk factors associated with *Escherichia coli*, *Salmonella* and *Campylobacter* cases in Manitoba".

Principal Investigator: John Wylie, Cadham Provincial Laboratory, Manitoba Health, Winnipeg, 204-945-7473

We would like your help with a research study that Manitoba Health is conducting. The study will include both people who have been recently diagnosed with a bacterial infection and healthy people who have not recently been sick. By comparing these two groups we hope to better understand how people become infected with these bacteria. First, I will read you some information on the study. As I go through this information, please ask me to explain any parts that are not clear.

We will be asking you questions that are designed to gather information on the most common sources of these bacteria. For example, they involve questions about drinking water, types of foods eaten or contact with animals. The questionnaire will take about 20 minutes to complete. By gathering information from a large number of people we can get a better idea of how these infections are spread. We expect about 100-150 people with diarrhea infections to participate in this study. It is important to do this within the individual regions of Manitoba as the sources of these infections can differ in different areas.

We will also ask if you know any friends or neighbors who may be interested in participating in the study as healthy people. I will talk to you about this at the end of the questionnaire. You do not have to participate in that part of the study if you do not wish.

Participation in all parts of the study is entirely voluntary and you can stop participating at any time. There are no direct benefits to you for helping us with this study, but we hope the information you and others provides will help prevent some people from being infected in the future.

You will be free to ask any questions about the study and your rights as a research participant. If you have questions about the study, you can ask the research nurse when you phone her or you can also contact the study investigator (John Wylie, 204-945-7473). If you have any questions about your rights as a research participant, you may contact the University of Manitoba, Bannatyne Campus Research Ethics Board office at 204-789-3389.

Research nurse documentation of telephone consent by control:

Control study code:_____

Signature of research nurse:_____

Date (yyyy/mm/dd):_____/____/____

Manitoba
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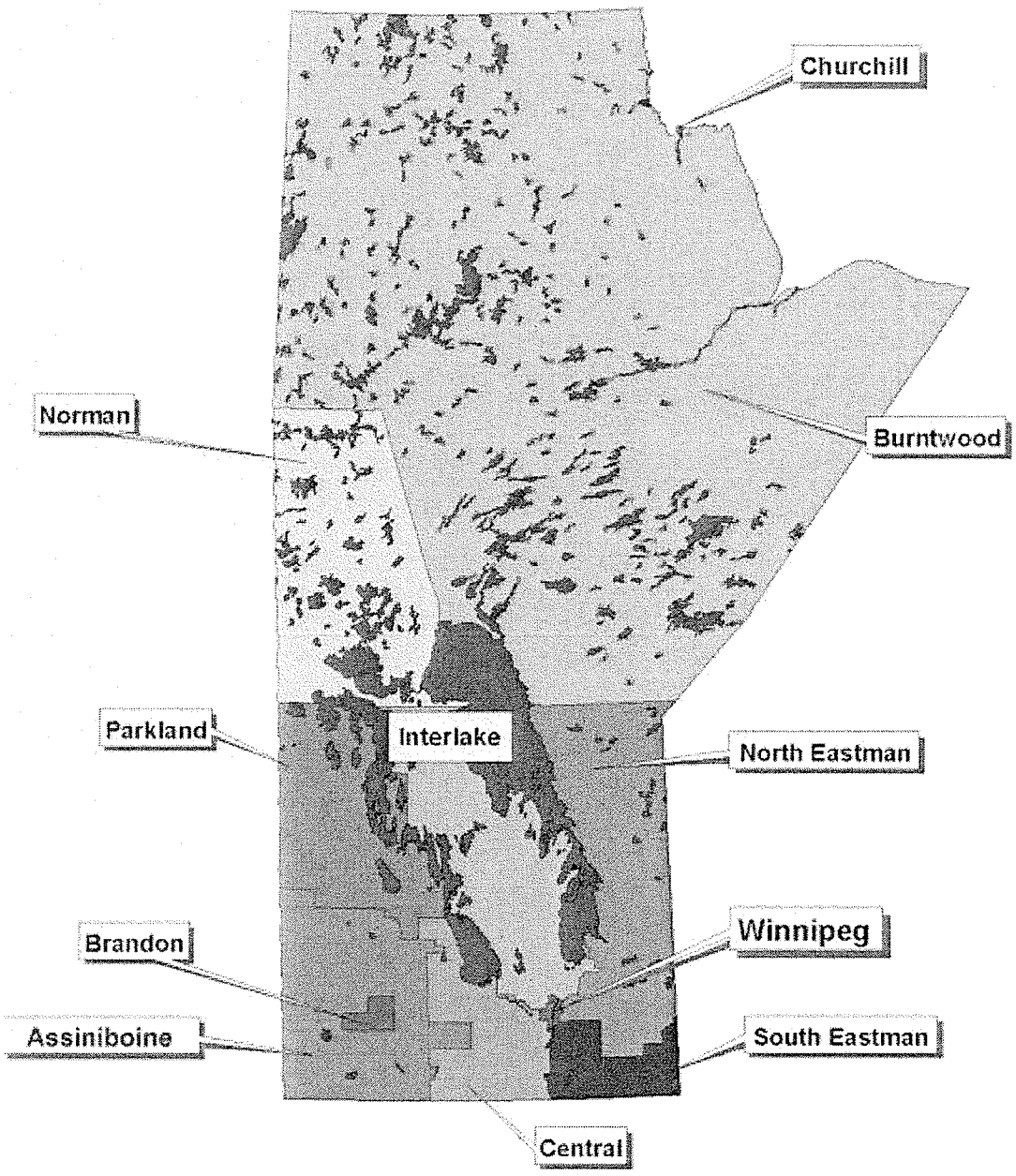
APPENDIX F – Map of Manitoba's Regional Health Authorities [134]



APPENDIX G – Questionnaire

Person is:
Case _____ Case study code number: CA_____ (assign sequential number)

Control _____ Control study code number: CO___(assign sequential number)

Interview date: _____/_____/_____ (YYYY/MM/DD)

A. Patient demographics:

Controls **Cases**

For cases - Complete A.2 and A.3. from Manitoba Health Records
A.2 Age ____
A.3 Postal code: (first 3 digits only) ___

For controls – Ask the following three questions

A.1 Confirm that the control has not been ill with a diarrheal illness in the past 30 days

☐ Yes ☐ No ➡ If No, stop interview and explain reason to control

A.2 Household control _____ Non-household control_____

A.3 Age ____ (this can be indicated as an age group if person prefers)
☐ 0-4
☐ 5-11
☐ 12-19
☐ >19
☐ Refused

A.4 Postal code: (first 3 digits only) _____

Standard interview for both cases and controls begins here:

A.4. How many persons live in your household? _____

A.5. How many persons are 5 years of age or less in your household? _____

A.6. How many diapered children are in the household?_____

B. Contact with water/sewage

B. 1 What type of water do you usually use for drinking in your household? (*read responses and choose all that apply*):

1. ☐ Municipal water supplied by town/city of residence
2. ☐ Private well water
3. ☐ Untreated lake/river water (name of lake or river)
4. ☐ Purchased bottled water
5. ☐ Other ➡ **Specify**:_____
77. ☐ Unsure/don't know
88. ☐ Refused

If "yes" to private well water above please answer the following two questions: –

B.1.1 Is the quality of this water monitored or tested (samples sent to testing laboratory)?

0. ☐ Yes ➡ **If yes**, approximate date of last test _____ (yyyy/mm/dd)
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused
99. ☐ Not applicable

B.1.2 Have water testing results from your well ever shown evidence of contamination? (*read responses*)

0. ☐ Yes, results showed presence of Total Coliform (TC) bacteria only
1. ☐ Yes, results showed presence of Total Coliform (TC) and E.coli (EC) bacteria
3. ☐ Yes, but unsure of what results were
4. ☐ No.
5. ☐ other ➡ **Specify**_____
77. ☐ Unsure/don't know
88. ☐ Refused
99. ☐ Not applicable

B.2. What type of water do you usually use for bathing or showering in your household? (*read responses and choose all that apply*)

1. ☐ Municipal water supplied by town/city of residence
2. ☐ Private well water
3. ☐ Untreated lake/river water (name of lake or river)
4. ☐ Other ➡ **Specify**:_____
77. ☐ Unsure/don't know
88. ☐ Refused

B.3. What type of water do you usually use for washing dishes in your household?
(*read responses and choose all that apply*)

1. ☐ Municipal water supplied by town/city of residence
2. ☐ Private well water
3. ☐ Untreated lake/river water (name of lake or river)
4. ☐ Purchased bottled water
5. ☐ Other ➡ **Specify**:_____
77. ☐ Unsure/don't know
88. ☐ Refused

B.4. Have you ever had a boil water advisory in your district for the drinking water supplied to your residence?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

B.4.1 If "yes" to B.4 - Is this boil water advisory currently still in effect?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused
99. ☐ Not applicable

B.5 How is water delivered to your house?
(*read responses*)

1. ☐ Piped into house
2. ☐ Delivered to house and stored in barrel in house
3. ☐ Delivered to house and stored in a cistern
4. ☐ Other ➡ **Specify**:_____
77. ☐ Refused
88. ☐ Unsure/don't know

B.6 Is there a water purification system within your house (e.g. attachment on faucet or filtration unit set on top of a water container)?

0. ☐ Yes ➡ specify: _____
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

B.7 What type of bathroom facilities are in your household?
(read responses)

1. ☐ Indoor plumbing connected to municipal system
2. ☐ Indoor plumbing connected to holding tank
3. ☐ Indoor plumbing connected to septic field
4. ☐ Outhouse
5. ☐ Indoor buckets
77. ☐ Unsure/don't know
88. ☐ Refused

B.8 In the previous 7 days have you used drinking water *(read and check all that apply)*?

1. ☐ from your own or a friend's cottage
   *If yes, specify location of cottage (e.g. which lake):*_____
2. ☐ while staying overnight with friends or family in their residence
   *If yes, specify location (e.g. town, municipality):*_____
3. ☐ while staying overnight at a hotel or motel
   *If yes, specify location (e.g. town, municipality):*_____
4. ☐ Other – If yes, specify:_____
77. ☐ Unsure/don't know
88. ☐ Refused

C. Food history

C.1. Did you (did your child) eat in any Fast Food restaurants during the seven days before your (their) illness?

0. ☐ Yes ➡ specify: _____
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

C. 2. Did you (did your child) eat in any other type of restaurant during the seven days before your (their) illness?

0. ☐ Yes ➡ specify: _____
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

C.3. In the 7 days prior to the onset of symptoms, did you (your child) consume any of the following foods (*read through list and check as many as apply*):

1. ☐ Eggs
2. ☐ Chicken
3. ☐ beef (ground)
4. ☐ pork
5. ☐ Locally acquired wild game
6. ☐ Pre-washed salad greens (purchased)
7. ☐ Alfalfa/clover/bean sprouts
8. ☐ Unpasteurized fruit juices → If yes, ask which type of juice:
   - 8a. ☐ freshly prepared in house
   - 8b. ☐ purchased
9. ☐ Milk (unpasteurized)
10. ☐ Cheese (unpasteurized)
11. ☐ Other dairy (unpasteurized)
12. ☐ None of the above
77. ☐ Unsure/don't know
88. ☐ Refused

D. Contact with animals or farms

D.1. Do you (does your child) live on a farm?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

If yes:

Type of farm

1. ☐ Mixed (ranching and crops)
2. ☐ Primarily crop production
3. ☐ Primarily ranching

77. ☐ Unsure/don't know
88. ☐ Refused
99. ☐ Not applicable

Types of animals produced

_____
_____
_____
_____

In the 7 days (applies to questions D.2 to D.13) prior to the onset of symptoms:
(would need to indicate to person that next 13 questions are all for previous 7 days – may need to remind person of this at various points)

D.2. Did you (your child) visit a farm

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.3. Did you (your child) visit a petting zoo

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.4. Did you (your child) have contact with, or visit a place that had any domestic *(read through list and check all that apply):*

1. ☐ Cattle
2. ☐ Hogs
3. ☐ broiler chickens
4. ☐ egg-laying chickens
5. ☐ sheep
6. ☐ Other domestic farm animals
7. ☐ None of the above
77. ☐ Unsure/don't know
88. ☐ Refused

D.5. Did you (your child) handle any raw animal products (e.g. in preparation to cook)?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.6. Did you (your child) handle any animal carcasses or manure (e.g. on a farm or while gardening)?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.7. Did you (your child) have any contact with, or visited a place that had, any: *(read through list and check all that apply)*

1. ☐ adult dogs
2. ☐ puppies
3. ☐ adult cats
4. ☐ kittens
5. ☐ None of the above
77. ☐ Unsure/don't know
88. ☐ Refused

D.8. Did you (your child) have any contact with any of the following types of pets *(read through list and check all that apply)*

1. ☐ Fish
2. ☐ Amphibians
3. ☐ Reptiles
4. ☐ Birds
5. ☐ other mammals (e.g. pygmy hedgehogs, mice, guinea pigs)
   If yes, specify_____
6. ☐ None of the above.
77. ☐ Unsure/don't know
88. ☐ Refused

D.9. Did you (your child) have any contact with/eat any pet treats (e.g., pig ears, etc)?

0. ☐ Yes ➡ specify:_____
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.10 Did you (your child) clean or fill a bird feeder?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.11. Did you (your child) clean or remove bird droppings from an area

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.12. Did you (your child) tend or touch any sick or dead wild birds.

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

D.13 Did you (your child) tend or touch any other types of sick or dead wild animals (i.e. other than birds)?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E. Other occupational, recreational, or incidental exposures

E.1. In the 7 days prior to your symptoms, did you (your child) have any contact with any children who attend a daycare centre?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E.2. In the 7 days prior to your symptoms, did you (your child) change any diapers?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E.3. In the 7 days prior to your symptoms, did you (your child) have any contact with children who use diapers?

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E.4 Do you (does your child) work in the meat processing industry?
(examples of meat processing includes slaughterhouses, butchers, and meat sections at grocery stores – farming is not included here as it was queried in question D.1)

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E.5 Have you (your child) consumed any food prepared while on an outdoor recreational trip (e.g. camping, canoeing)

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E.6 In previous 7 days did you (your child) *(read through list and check all that are applicable)*:

1. ☐ Swim in public pool – if yes, name of pool and location:_____
2. ☐ Use a public hot tub – if yes, location:_____
3. ☐ Swim or watersports in river – if yes, name of river:_____
4. ☐ Swim or watersports in lake – if yes, name of lake:_____
5. ☐ Swim in private pool
6. : ☐ Use a private hot tub
7. ☐ Use a private wading pool
8. ☐ Swim/watersports or contact with any other type of water (e.g. flood water, drainage ditch, ocean).
9. ☐ None of the above.
77. ☐ Unsure/don't know
88. ☐ Refused

E.7 Did you (your child) attend any large gatherings in the 7 days before their illness (wedding, receptions, showers, parties, festivals, fairs, conferences, picnics, etc.)?

0. ☐ Yes Specify:_____
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E.8 In previous 7 days did you (your child) have any contact with anyone else who had symptoms of vomiting or diarrhea?

0. ☐ Yes If yes, then state number of hours of contact:_____
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

E.9 **If yes to above -** In previous 7 days did you (your child) care for any other person with above symptoms

0. ☐ Yes
1. ☐ No
77. ☐ Unsure/don't know
88. ☐ Refused

If yes, length of care period and number of persons cared for?

_____

F. Travel history

F.1 Did you (your child) travel in the last month? Yes or no.

0. ☐ Yes
1. ☐ No – *If "No", go to section G: symptoms*
77. ☐ Unsure/don't know
88. ☐ Refused

If yes to above, then:

F.2 Name of city/towns visited (or countries if travel involved many location changes)

_____
_____
_____
_____

99. ☐ Not applicable

F.3 Length of time on travel

_____

99. ☐ Not applicable

F.4 Type of travel

1. ☐ Business
2. ☐ Military
3. ☐ Recreational
4. ☐ Other – Specify:_____

77. ☐ Unsure/don't know
88. ☐ Refused
99. ☐ Not applicable

G. Symptom history – For cases read through symptom list and indicate all that apply. Skip symptoms section if control is being interviewed.

	Symptom	Response	Standard score for given symptom[2]	
1	Diarrhea	Yes No *If No, go to 2.*		
	1a. Date of first diarrhea	YYYY/MM/DD		
	1b. Diarrhea duration: (number of days)		1-4 days 5 days >=6 days	**1** **2** **3**
	1c. Diarrhea/loose stools per 24 hours[1]		1-3 4-5 >=6	**1** **2** **3**
2	Blood in stool	Yes No		**2**
3	Vomiting	Yes No *If No, go to 4.*		
	3a. Vomiting events per 24 hours[1]		1 time 2-4 times >=5 times	**1** **2** **3**
	3b. Vomiting duration: *number of days*		1 2 >=3	**1** **2** **3**
4	Nausea	Yes No		**1**
5	Abdominal cramps	Yes No		**1**
6	Abdominal pain	Yes No		**1**
7	Fever	Yes No		**2**
8	Chills	Yes No		**1**
9	Headache	Yes No		**1**
10	General muscle aches	Yes No		**1**
11	Fatigue	Yes No		**1**
12	What was first symptom?			
13	Date of onset of first symptoms	YYYY/MM/DD		
14	Date of recovery (define as absence of all or most symptoms	YYYY/MM/DD		
15	Hospitalized	Yes No	# days hospitalized	

1 maximum number of events that occurred during a 24 hour period
2 Severity score based on a score developed for *calicivirus* (from Rockx *et al.* 2002. Natural history of human calicivirus infection: a prospective cohort study. Clin. Infect. Dis. 35:246)

APPENDIX H – Ethics Approvals

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. J. Wylie
Sponsor: Manitoba Health

Protocol Reference Number: H2007:045
Date of REB Meeting: February 26, 2007
Date of Approval: March 26, 2007
Date of Expiry: February 26, 2008

Protocol Title: "Risk Factors Associated with Escherichia coli, Salmonella, and Campylobacter cases in Manitoba"

The following is/are approved for use:

- **Revised Protocol Version dated March 24, 2007**
- **Research Participant Information and Consent Form (Control) Version dated March 24, 2007**
- **Research Participant Information and Consent Form (Case) Version dated March 24, 2007**
- **Control Information Sheet Version dated March 24, 2007**
- **Questionnaire dated January 30, 2007**

The above was approved by Dr. John Arnett, Ph.D., C. Psych., Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated March 24, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

This approval is valid for one year from the date of the REB meeting at which the study was reviewed. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought from the relevant institution, if required.

Sincerely yours,

John Arnett, Ph.D., C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone:** (204) 789-3255 / Fax: (204) 789-3414

UNIVERSITY of MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. J. Wylie
Sponsor: Manitoba Health

Protocol Reference Number: H2007:045
Date of Approval: May 3, 2007

Protocol Title: "Risk Factors Associated with Escherichia coli, Salmonella, and Campylobacter cases In Manitoba"

The following is/are approved for use:

- **Public Health Nurse Script dated 2007_05_01**
- **Case Information sheet dated March 24, 2007**

The above was approved by Dr. John Arnett, Ph.D., C. Psych, Chair, Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated March 24, 2007 received May 2, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations*.

A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought from the relevant institution, if required.

Sincerely yours,

John Arnett, Ph.D. C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to the REB Secretary **Telephone:** (204) 789-3255 / Fax: (204) 789-3414

UNIVERSITY OF MANITOBA

BANNATYNE CAMPUS
Research Ethics Boards

P126-770 Bannatyne Avenue
Winnipeg, Manitoba
Canada R3E 0W3
Tel: (204) 789-3255
Fax: (204) 789-3414

APPROVAL FORM

Principal Investigator: Dr. J. Wylie

Protocol Reference Number: H2007:164
Date of Approval: August 13, 2007
Date of Expiry: August 13, 2008

Protocol Title: "Risk Factors Associated with Escherichia coli, Salmonella, and Campylobacter Cases in Manitoba" linked to H2007:045

The following is/are approved for use:

- **Protocol dated May 24, 2007**
- **Control Consent Form dated March 24, 2007**
- **Telephone Script for Participant Information dated March 24, 2007**
- **PHN Script dated May 1, 2007**
- **Case and Control Information form dated January 30, 2007**

The above underwent expedited review and was **approved as submitted** on August 13, 2007 by Dr. John Arnett, Ph.D., C. Psych., Health Research Ethics Board, Bannatyne Campus, University of Manitoba on behalf of the committee per your letter dated July 19, 2007. The Research Ethics Board is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement, and the applicable laws and regulations of Manitoba. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the *Food and Drug Regulations.*

This approval is valid for one year only. A study status report must be submitted annually and must accompany your request for re-approval. Any significant changes of the protocol and informed consent form should be reported to the Chair for consideration in advance of implementation of such changes. The REB must be notified regarding discontinuation or study closure.

This approval is for the ethics of human use only. For the logistics of performing the study, approval must be sought form the relevant institution, if required.

Sincerely yours,

John Arnett, Ph.D., C. Psych.
Chair, Health Research Ethics Board
Bannatyne Campus

Please quote the above protocol reference number on all correspondence.
Inquiries should be directed to REB Secretary
Telephone: (204) 789-3883 / Fax: (204) 789-3414

Manitoba

Health
Health Information Privacy Committee
4045 - 300 Carlton Street
Winnipeg MB R3B 3M9
Phone: (204) 786-7204
FAX: (204) 944-1911

John Wylie, PhD
Cadham Provincial Laboratory
750 William Avenue
Winnipeg, MB
R3C 3Y1

May 1, 2007

File No. 2006/2007 - 38

Dear Dr Wylie:

Re: Risk factors associated with *Escherichia coli, Salmonella* and *Campylobacter* cases in Manitoba

Thank you for your response to the Committee's concerns. The Health Information Privacy Committee has considered and ***conditionally approved*** your request for data for this project pending:

- Proof of approval from the individual RHAs involved. The Committee noted that the RHAs have withheld approval until receiving approval from HIPC. However, final HIPC approval will remain contingent upon approval from the RHAs.

Please note that as this project is conditionally approved it will not have to go back to the full Committee for approval once satisfactory responses to the above have been documented. Conditional approval is valid for one year only from the date of this letter. If the requested information is not provided within this time period a full proposal should be resubmitted.

If you have any questions or concerns, please do not hesitate to contact Patricia Caetano, Committee Coordinator at 786-7204.

Yours truly,

Dr R. Walker
Chair

Please quote the file number on all correspondence

cc: L. Barre

Email confirmation of ethics approval for Brandon and Assiniboine RHAs

From: Robertson, Theresa [mailto:RobertsonT@brandonrha.mb.ca]
Sent: Monday, June 18, 2007 4:12 PM
To: Weiss, Elise (HEALTH)
Subject: RE: CPL Enteric Study

Hi Elise -- The Ethics Committee approved the above study at their meeting on June 15th, 2007.

Thanks for the reminder - I almost forgot to notify you of this information.

-Theresa

Email confirmation of ethics approval for Central RHA

From: Buchan, Shelley (HEALTH)
Sent: Wednesday, May 09, 2007 9:05 AM
To: Wylie, John (HEALTH)
Subject: RE: enteric study update

Hello John,
Last week I met with the Ethics Review Chairperson and we completed our internal process and forms. Your study has approval by the RHA – Central to proceed. We look forward to your inservice on May. 28th and wish you the best.

Please let me know if you need anything more.

Shelley

Dr. Shelley Buchan
Medical Officer of Health
RHA-Central

Email confirmation of ethics approval for South Eastman RHA

Please be advised that the South Eastman Health/Sante Sud-Est. Ethics Committee, at their May 17, 2007 meeting, gave approval for the RHA's participation in the said study.

Trust this is satisfactory.

David I. Driedger
Vice President
Acute Care & Corporate Services
Tel/Tél (204) 424-6023
Fax/Télécopieur (204) 424-5868
ddriedger@sehealth.mb.ca

"Partnering with Community to Optimize Health."

« Être partenaire de la communauté afin d'en optimiser la santé.»

FICE RÉGIONAL DE LA SANTÉ
ES PARCS
ARKLAND
REGIONAL HEALTH AUTHORITY

Parkland Regional Health Authority
Office Régional de la Santé des Parcs
Room 112 – 27 2nd Avenue SW, Dauphin, MB R7N 3E5 Tel: 204-622-6222 Fax: 204-622-6232 Toll Free: 1-800-259-7541

May 17, 2007

Dr. J. Wylie
Cadham Provincial Laboratory
750 William Avenue
Winnipeg, Manitoba
R3C 3Y1

Dear Dr. Wylie:

Re: Research Proposal – Risk Factors associated with *Escherichia coli*, *Salmonella*, and *Campylobacter* cases in Manitoba

The Parkland Regional Health Authority is pleased to support this study and looks forward to working with you.

The Public Health program has received the materials you have sent and has identified no issues with the process. We look forward to working with you on this important piece of work.

Sincerely,

Maggie Campbell
Interim VP Community Health Services

Cc. Sherri Buhler, Director – Public Health Programs

"Individuals, families and communities achieving the best possible health and wellness."

Winnipeg Regional Health Authority
Office régional de la santé de Winnipeg

1800 - 155 Carlton St.
Winnipeg, Manitoba
R3C 4Y1 CANADA
TEL: 204/926.7000
FAX: 204/926.7007
www.wrha.mb.ca

155, rue Carlton, suite 1800
Winnipeg Manitoba
R3C 4Y1 CANADA
TÉL: 204/926.7000
TÉLÉC: 204/926.7007
www.wrha.mb.ca

March 2, 2007

Dr. John Wylie
Cadham Provincial Public Health Laboratory
750 William Avenue
Winnipeg MB R3E 3J7

Dear Dr. Wylie,

Re: Proposal "Risk Factors Associated with Escherichia Coli, Salmonella, and Campylobacter Cases in Manitoba"
WRHA Reference No: 2007-025

We are pleased to inform you that your research access request for the above-named study has been approved by the Winnipeg Regional Health Authority (WRHA) Research Review Committee pending confirmation that the following conditions are met or agreed to:

- Approved pending REB approval and clarification of the following:
  - The algorithm seems to be slightly out of keeping with the way we think the recruitment will be done. It seems to indicate that the PHN informs cases about the study and the lets MHRN know and that he/she has agreed to participate in the study. We think that box should read he/she has agreed to be contacted to hear more about the study.
  - How will you be approaching minor children?
  - If HREB does agree that written consent is not required, we think that there ought to be documentation on the study form of the verbal consent.
- You, your co-investigators, and your research assistants comply with the relevant privacy legislation as indicated below.
  - [X] *The Personal Health Information Act*
  - [ ] *The Freedom of Information and Protection of Privacy Act*
  - [ ] *The Personal Health Information Act* and *The Freedom of Information and Protection of Privacy Act*
- You complete and return the attached Confidentiality Agreement(s) to Cathy Pope, WRHA, 1800 – 155 Carlton Street, Winnipeg, MB R3C 4Y1;
- You submit to our attention any significant changes in your proposal prior to implementation or any significant changes during the course of the study;
- You submit a summary of the final results of the study to the WRHA and provide us with a copy of any publications arising from the study;
- It is an expected courtesy that WRHA will be given a minimum of five working days advance notice of publication or presentation of results with policy implications, in order to be prepared for public response;
- You agree to be accountable for appropriate storage and elimination of material.

Thank you for selecting the Winnipeg Regional Health Authority as the site to conduct your research. Please let us know should you encounter any site-related difficulties during the course of your study.

We extend best wishes for successful completion of your study.

Sincerely,

Dr. Mike Moffatt
Executive Director, Division of Research & Applied Learning
Chair, Research Review Committee
Winnipeg Regional Health Authority

cc. Dr. B. Postl
Ms. L. Esposito
Chair, HREB

Encl: *PHIA Agreement*

Burntwood
Regional Health Authority

May 1, 2007

Dr. John Wylie
Cadham Provincial Laboratory
Manitoba Health
PO Box 8450
750 William Avenue
Winnipeg, MB
R3C 3Y1

Dear Dr. John Wylie:

Please find attached the signed agreement between Burntwood Regional Health Authority and John Wylie, Manitoba Health for Access to Personal Health Information for Research Purposes. Thank you and we look forward to the results of the study that you are leading.

Sincerely,

Marie O'Neill
CEO

MO/gp

Copy: Dr. Randy Gesell, Medical Officer of Health
Gloria King, V.P. Health Services / Chief Nursing Officer
Dr. J. Botha, V.P. Medical Services
File

APPENDIX I – Percentage of and Fishers Exact/Kruskal-Wallis *p*-values for participants reporting exposure to explanatory variables: Case-case analysis by infection

Table i. Univariate case-case associations between reported risk factor exposure and infection using Kruskal-Wallis test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Demographics							
Age							
<10	10	18.9	3	9.7	3	27.3	
10-19	6	11.32	7	22.6	1	9.1	
20-29	7	13.2	4	12.9	1	9.1	
30-39	2	3.8	5	16.1	1	9.1	
40-49	11	20.8	3	9.7	2	18.2	0.380
50-59	11	20.8	4	12.9	0	0.0	
60-69	2	3.8	1	3.2	1	9.1	
≥70	4	7.5	4	12.9	2	18.2	
Number of persons living in household							
1-2	23	43.4	10	32.3	4	36.4	
3-4	18	34.0	13	41.9	5	45.5	0.692
≥5	12	22.6	8	25.8	2	18.2	
Number of persons 5 years of age or less living in							
0	37	69.8	23	74.2	6	54.5	
1-2	13	24.5	7	22.6	5	45.5	0.552
>3	3	5.7	1	3.2	0	0.0	
Number of diapered children living in household							
0	42	79.2	27	87.1	8	72.7	
1	7	13.2	4	12.9	2	18.2	0.456
≥2	4	7.5	0	0.0	1	9.1	
Symptoms							
Diarrhea - Duration (days)							
1-4	11	20.8	6	21.4	4	36.4	
5	6	11.3	2	7.1	3	27.3	0.210
≥6	36	67.9	20	71.4	4	36.4	
Diarrhea - Number of events per 24 hours							
1-3	1	1.9	3	10.7	0	0.0	
4-5	10	18.9	7	25.0	3	27.3	0.995
≥6	41	77.4	18	64.3	8	72.7	
Unknown	1	1.9	0	0.0	0	0.0	
Vomiting - Duration (days)							
1	9	47.4	4	57.1	0	0.0	
2	7	36.8	1	14.3	1	100.0	0.137
≥3	3	15.8	2	28.6	0	0.0	
Vomiting - Number of events per 24 hours							
1	4	21.1	0	0.0	0	0.0	
2-4	11	57.9	5	71.4	0	0.0	0.094
≥5	4	21.1	2	28.6	1	100.0	
Severity score							
<10	3	5.7	5	16.1	6	54.5	
10-14	28	52.8	12	38.7	4	36.4	0.004
15-19	19	35.8	13	41.9	1	9.1	
≥20	3	5.7	1	3.2	0	0.0	
Duration of illness (days)							
<10	27	50.9	13	41.9	5	45.5	
10-14	12	22.6	7	22.6	3	27.3	0.487
15-19	9	17.0	3	9.7	0	0.0	
≥20	5	9.4	8	25.8	3	27.3	

Table ii. Univariate case-case associations between reported contact with water/sewage and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Type of water used in household for drinking							
Municipal							
Yes	30	56.6	22	71.0	7	63.6	0.446
No	23	43.4	9	29.0	4	36.4	
Private well							
Yes	18	34.0	4	12.9	2	18.2	0.094
No	35	66.0	27	87.1	9	81.8	
Purchased bottled							
Yes	22	41.5	12	38.7	3	27.3	0.720
No	31	58.5	19	61.3	8	72.7	
Other							
Yes	6	11.3	2	6.5	1	9.1	0.879
No	47	88.7	29	93.5	10	90.9	
Quality of well water (if used) monitored or tested							
Yes	13	72.2	4	100.0	2	100.0	1.000
No	2	11.1	0	0.0	0	0.0	
Unsure	3	16.7	0	0.0	0	0.0	
If tested: the results from well showed evidence of contamination							
Yes - Total Coliform (TC) bacteria only	0	0.0	1	25.0	0	0.0	
No	10	76.9	3	75.0	2	100.0	0.375
Unsure	3	23.1	0	0.0	0	0.0	
Type of water used in household for bathing/showering							
Municipal							
Yes	34	64.2	27	87.1	7	63.6	0.055
No	19	35.8	4	12.9	4	36.4	
Private well							
Yes	20	37.7	4	12.9	4	36.4	0.042
No	33	62.3	27	87.1	7	63.6	
Type of water used in household for washing dishes							
Municipal							
Yes	34	64.2	27	87.1	7	63.6	0.055
No	19	35.8	4	12.9	4	36.4	
Private well							
Yes	20	37.7	4	12.9	4	36.4	0.042
No	33	62.3	27	87.1	7	63.6	
Had a boil water advisory in your district for the drinking water supplied to your residence							
Yes	5	9.4	1	3.2	0	0.0	
No	48	90.6	29	93.5	10	90.9	0.459
Unsure	0	0.0	1	3.2	1	9.1	
If so, is the advisory still in effect							
Yes	1	20.0	0	0.0	0	0.0	1.000
No	3	60.0	1	100.0	0	0.0	
Unsure	1	20.0	0	0.0	0	0.0	
Water delivery system							
Piped into home							
Yes	52	98.1	31	100.0	11	100.0	1.000
No	1	1.9	0	0.0	0	0.0	
Delivered to home and stored in a cistern							
Yes	2	3.8	0	0.0	0	0.0	0.632
No	51	96.2	31	100.0	11	100.0	
Delivered to home by other method							
Yes	1	1.9	0	0.0	0	0.0	1.000
No	52	98.1	31	100.0	11	100.0	
Water purification system in household							
Yes	26	49.1	7	22.6	6	54.5	0.033
No	27	50.9	24	77.4	5	45.5	

Table ii *continued*. Univariate case-case associations between reported contact with water/sewage and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Indoor plumbing connected to a municipal system							
Yes	31	58.5	26	83.9	8	72.7	0.050
No	22	41.5	5	16.1	3	27.3	
Indoor plumbing connected to a holding tank							
Yes	5	9.4	1	3.2	2	18.2	0.250
No	48	90.6	30	96.8	9	81.8	
Indoor plumbing connected to a septic field							
Yes	16	30.2	4	12.9	2	18.2	0.202
No	37	69.8	27	87.1	9	81.8	
Used drinking water							
From own or a friends cottage							
Yes	0	0.0	0	0.0	1	9.1	0.116
No	53	100.0	31	100.0	10	90.9	
While staying overnight with friends or family at their residence							
Yes	11	20.8	1	3.2	1	9.1	0.067
No	42	79.2	30	96.8	10	90.9	
While staying overnight at a hotel or motel							
Yes	5	9.4	6	19.4	0	0.0	0.231
No	48	90.6	25	80.6	11	100.0	

Table iii. Univariate case-case associations between reported food history and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Ate at a fast food restaurant 7 days before becoming ill							
Yes	23	43.4	19	61.3	5	45.5	
No	25	47.2	12	38.7	5	45.5	0.488
Unsure	5	9.4	0	0.0	1	9.1	
Ate at a any other restaurant 7 days before becoming ill							
Yes	24	45.3	16	51.6	5	45.5	
No	27	50.9	13	41.9	6	54.5	0.792
Unsure	2	3.8	2	6.5	0	0.0	
Consumed the following foods (7 days prior to illness)							
Eggs							
Yes	29	54.7	23	74.2	8	72.7	0.187
No	24	45.3	8	25.8	3	27.3	
Chicken							
Yes	42	79.2	25	80.6	9	81.8	1.000
No	11	20.8	6	19.4	2	18.2	
Beef (ground)							
Yes	32	60.4	20	64.5	7	63.6	0.953
No	21	39.6	11	35.5	4	36.4	
Pork							
Yes	21	39.6	10	32.3	6	54.5	0.467
No	32	60.4	21	67.7	5	45.5	
Locally acquired wild game							
Yes	1	1.9	3	9.7	1	9.1	0.184
No	52	98.1	28	90.3	10	90.9	
Pre-washed salad greens							
Yes	18	34.0	6	19.4	4	36.4	0.304
No	35	66.0	25	80.6	7	63.6	
Alfalfa/clover/been sprouts							
Yes	4	7.5	3	9.7	0	0.0	0.744
No	49	92.5	28	90.3	11	100.0	
Fruit juice (unpasteurized)							
Yes	0	0.0	1	3.2	0	0.0	0.442
No	53	100.0	30	96.8	11	100.0	
Milk (unpasteurized)							
Yes	6	11.3	2	6.5	0	0.0	0.654
No	47	88.7	29	93.5	11	100.0	
Other dairy (unpasteurized)							
Yes	1	1.9	0	0.0	0	0.0	1.000
No	52	98.1	31	100.0	11	100.0	
None of the above							
Yes	1	1.9	1	3.2	0	0.0	1.000
No	52	98.1	30	96.8	11	100.0	

Table iv. Univariate case-case associations between reported contact with animals or farms and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Live on a farm							
Yes	16	30.2	6	19.4	1	9.1	0.269
No	37	69.8	25	80.6	10	90.9	
Type of farm							
Mixed (ranching and crops)	6	37.5	2	33.3	0	0.0	
Primarily crop production	1	6.3	0	0.0	0	0.0	
Primarily Ranching	6	37.5	2	33.3	0	0.0	0.672
Other (hobby farm)	2	12.5	2	33.3	1	100.0	
Unknown	1	6.3	0	0.0	0	0.0	
Visited a farm							
Yes	21	39.6	6	19.4	2	18.2	0.112
No	32	60.4	25	80.6	9	81.8	
Visited a petting zoo							
Yes	1	1.9	0	0.0	0	0.0	
No	51	96.2	31	100.0	11	100.0	1.000
Unknown	1	1.9	0	0.0	0	0.0	
Contact with							
Domestic cattle							
Yes	17	32.1	5	16.1	1	9.1	0.136
No	36	67.9	26	83.9	10	90.9	
Domestic hogs							
Yes	5	9.4	3	9.7	0	0.0	0.76
No	48	90.6	28	90.3	11	100.0	
Broiler chickens							
Yes	3	5.7	0	0.0	0	0.0	0.513
No	50	94.3	31	100.0	11	100.0	
Egg laying chickens							
Yes	6	11.3	0	0.0	0	0.0	0.116
No	47	88.7	31	100.0	11	100.0	
Domestic sheep							
Yes	1	1.9	0	0.0	0	0.0	1.000
No	52	98.1	31	100.0	11	100.0	
Other domestic farm animals							
Yes	5	9.4	0	0.0	0	0.0	0.233
No	48	90.6	31	100.0	11	100.0	
None of the above							
Yes	28	52.8	24	77.4	10	90.9	0.011
No	25	47.2	7	22.6	1	9.1	
Handled raw animal products							
Yes	17	32.1	10	32.3	7	63.6	
No	33	62.3	15	48.4	3	27.3	0.116
Unknown	3	5.7	6	19.4	1	9.1	
Handled animal carcasses or manure							
Yes	6	11.3	3	9.7	1	9.1	
No	43	81.1	24	77.4	9	81.8	1.000
Unknown	4	7.5	10	32.3	1	9.1	

Table iv *continued*. Univariate case-case associations between reported contact with animals or farms and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Contact with							
Adult dogs							
Yes	30	56.6	13	41.9	5	45.5	0.403
No	23	43.4	18	58.1	6	54.5	
Puppies							
Yes	9	17.0	5	16.1	0	0.0	0.452
No	44	83.0	26	83.9	11	100.0	
Adult cats							
Yes	25	47.2	13	41.9	4	36.4	0.799
No	28	52.8	18	58.1	7	63.6	
Kittens							
Yes	12	22.6	6	19.4	2	18.2	0.936
No	41	77.4	25	80.6	9	81.8	
None of the above							
Yes	12	22.6	8	25.8	3	27.3	0.835
No	41	77.4	23	74.2	8	72.7	
Contact with							
Fish							
Yes	5	9.4	6	19.4	2	18.2	0.395
No	48	90.6	25	80.6	9	81.8	
Amphibians							
Yes	3	5.7	0	0.0	0	0.0	0.513
No	50	94.3	31	100.0	11	100.0	
Reptiles							
Yes	3	5.7	5	16.1	0	0.0	0.170
No	50	94.3	26	83.9	11	100.0	
Pet birds							
Yes	0	0.0	1	3.2	1	9.1	0.089
No	53	100.0	30	96.8	10	90.9	
Other mammals							
Yes	1	1.9	2	6.5	0	0.0	0.691
No	52	98.1	29	93.5	11	100.0	
None of the above							
Yes	40	75.5	20	64.5	8	72.7	0.543
No	13	24.5	11	35.5	3	27.3	
Contact with pet treats							
Yes	10	18.9	6	19.4	4	36.4	
No	42	79.2	23	43.4	7	63.6	0.448
Unknown	1	1.9	2	6.5	0	0.0	
Cleaned or filled a bird feeder							
Yes	5	9.4	3	9.7	0	0.0	0.760
No	48	90.6	28	90.3	11	100.0	
Removed bird droppings from an area							
Yes	2	3.8	2	6.5	1	9.1	
No	46	86.8	25	80.6	7	63.6	0.368
Unknown	5	9.4	4	12.9	3	27.3	
Tended to or touched any sick or dead wild birds							
Yes	2	3.8	0	0.0	0	0.0	
No	49	92.5	30	96.8	11	100.0	0.634
Unknown	2	3.8	1	3.2	0	0.0	
Tended to or touched any sick or dead wild animals							
Yes	1	1.9	1	3.2	0	0.0	
No	51	96.2	29	93.5	11	100.0	1.000
Unknown	1	1.9	1	3.2	0	0.0	

Table v. Univariate case-case associations between reported occupational, recreational or incidental exposures and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Contact with children who attend daycare (7 days prior to illness)							
Yes	7	13.2	1	3.2	2	18.2	
No	45	84.9	28	90.3	9	81.8	0.223
Unknown	1	1.9	2	6.5	0	0.0	
Changed diapers (7 days prior to illness)							
Yes	4	7.5	1	3.2	2	18.2	
No	48	90.6	30	96.8	9	81.8	0.250
Unknown	1	1.9	0	0.0	0	0.0	
Contact with children who use diapers (7 days prior to illness)							
Yes	18	34.0	7	22.6	4	36.4	
No	34	64.2	23	74.2	6	19.4	0.463
Unknown	1	1.9	1	3.2	1	9.1	
Work in the meat processing industry							
Yes	3	5.7	1	3.2	0	0.0	1.000
No	50	94.3	30	96.8	11	100.0	
Consumed food prepared on an outdoor recreational trip							
Yes	0	0.0	3	9.7	3	27.3	0.003
No	53	100.0	28	90.3	8	72.7	
In the previous 7 days							
Swam in a public pool							
Yes	3	5.7	5	16.1	2	18.2	0.172
No	50	94.3	26	83.9	9	81.8	
Swam or participated in watersports in a river							
Yes	2	3.8	0	0.0	1	9.1	0.214
No	51	96.2	31	100.0	10	90.9	
Swam or participated in watersports in a lake							
Yes	1	1.9	2	6.5	2	18.2	0.069
No	52	98.1	29	93.5	9	81.8	
Swam in a private pool							
Yes	3	5.7	1	3.2	2	18.2	0.239
No	50	94.3	30	96.8	9	81.8	
Used a private hot tub							
Yes	1	1.9	2	6.5	0	0.0	0.691
No	52	98.1	29	93.5	11	100.0	
Swam/watersports or contact with any other type of water							
Yes	2	3.8	3	9.7	0	0.0	0.396
No	51	96.2	28	90.3	11	100.0	
None of the above							
Yes	38	71.7	19	61.3	6	54.5	0.382
No	15	28.3	12	38.7	5	45.5	
Attended a large gathering							
Yes	20	37.7	8	25.8	3	27.3	
No	33	62.3	21	67.7	8	72.7	0.662
Unknown	0	0.0	2	6.5	0	0.0	
Contact with someone who had symptoms of vomiting and diarrhea							
Yes	7	13.2	3	9.7	0	0.0	
No	43	81.1	27	87.1	11	100.0	0.574
Unknown	3	5.7	1	3.2	0	0.0	
Did you care for someone who had symptoms of vomiting and diarrhea							
Yes	3	30.0	1	25.0	0	0.0	1.000
No	7	60.0	3	50.0	0	0.0	

Table vi. Univariate case-case associations between reported travel and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Travel in the last month							
Yes	28	52.8	13	41.9	5	45.5	0.639
No	25	47.2	18	58.1	6	54.5	
Reason for travel							
Business							
Yes	5	9.4	2	6.5	0	0.0	0.864
No	48	90.6	29	93.5	11	100.0	
Recreation							
Yes	22	41.5	12	38.7	5	45.5	0.913
No	31	58.5	19	61.3	6	54.5	
Other							
Yes	2	3.8	1	3.2	0	0.0	1.000
No	51	96.2	30	96.8	11	100.0	

Table vii. Univariate case-case associations between reported symptoms and infection using Fishers Exact test

	Campylobacter		*Salmonella*		VTEC		*P*
Explanatory Variables	n	%	n	%	n	%	
Diarrhea							
Yes	53	100.0	28	90.3	11	100.0	0.067
No	0	0.0	3	9.7	0	0.0	
Blood in stool							
Yes	22	41.5	14	45.2	7	63.6	0.446
No	31	58.5	17	54.8	4	36.4	
Vomiting							
Yes	19	35.8	7	22.6	1	9.1	0.165
No	34	64.2	24	77.4	10	90.9	
Nausea							
Yes	37	69.8	25	80.6	5	45.5	0.086
No	16	30.2	6	19.4	6	54.5	
Abdominal cramps							
Yes	45	84.9	26	83.9	9	81.8	1.000
No	8	15.1	5	16.1	2	18.2	
Abdominal pain							
Yes	41	77.4	24	77.4	10	90.9	0.674
No	12	22.6	7	22.6	1	9.1	
Fever							
Yes	39	73.6	25	80.6	2	18.2	0.001
No	14	26.4	6	19.4	9	81.8	
Chills							
Yes	36	67.9	22	71.0	4	36.4	0.098
No	17	32.1	9	29.0	7	63.6	
Headache							
Yes	28	52.8	15	48.4	1	9.1	0.024
No	25	47.2	16	51.6	10	90.9	
General muscle aches							
Yes	31	58.5	14	45.2	1	9.1	0.001
No	22	41.5	17	54.8	10	90.9	
Fatigue							
Yes	47	88.7	25	80.6	8	72.7	0.279
No	6	11.3	6	19.4	3	27.3	
Hospitalized							
Yes	6	11.3	10	32.3	5	45.5	0.008
No	47	88.7	21	67.7	6	54.5	

APPENDIX J – Percentage of and Fishers Exact/Kruskal-Wallis *p*-values for participants reporting exposure to explanatory variables: Case-case analysis by location

Table viii. Univariate case-case associations between reported risk factor exposure and infection using Kruskal-Wallis

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Age							
<10	1	2.7	12	32.4	3	16.7	
10-19	3	8.1	8	21.6	3	16.7	
20-29	4	10.8	5	13.5	2	11.1	
30-39	5	13.5	1	2.7	1	5.6	0.014
40-49	11	29.7	3	8.1	2	11.1	
50-59	6	16.2	4	10.8	5	27.8	
60-69	3	8.1	0	0.0	0	0	
≥70	4	10.8	4	10.8	2	11.1	
Number of persons living in household							
1-2	17	45.9	10	27.0	8	44.4	
3-4	11	29.7	17	45.9	7	38.9	0.320
≥5	9	24.3	10	27.0	3	16.7	
Number of persons 5 years of age or less living in household							
0	30	81.1	21	56.8	13	72.2	
1-2	6	16.2	14	37.8	4	22.2	0.083
>3	1	2.7	2	5.4	1	5.6	
Number of diapered children living in household							
0	33	89.2	27	73.0	14	77.8	
1	3	8.1	8	21.6	2	11.1	0.214
≥2	1	2.7	2	5.4	2	11.1	
Diarrhea - Duration (days)							
1-4	6	16.2	7	20.6	8	44.4	
5	4	10.8	2	5.9	3	16.7	0.052
≥6	27	73.0	25	73.5	7	38.9	
Diarrhea - Number of events per 24 hours							
1-3	3	8.1	0	0.0	1	5.6	
4-5	7	18.9	6	17.6	6	33.3	0.735
≥6	27	73.0	27	79.4	11	61.1	
Unknown	0	0.0	1	2.9	0	0.0	
Vomiting - Duration (days)							
1	1	7.7	3	30.0	0	0.0	
2	9	69.2	6	60	1	25.0	0.514
≥3	3	23.1	1	10.0	3	75.0	
Vomiting - Number of events per 24 hours							
1	6	46.2	3	30.0	1	25.0	
2-4	5	38.5	6	60.0	1	25.0	0.485
≥5	2	15.4	1	10.0	2	50.0	
Severity Score							
<10	4	10.8	8	21.6	2	11.1	
10-14	16	43.2	13	35.1	12	66.7	0.332
15-19	14	37.8	15	40.5	4	22.2	
≥20	3	8.1	1	2.7	0	0.0	
Duration of illness (days)							
<10	13	35.1	19	51.4	13	72.2	
10-14	7	18.9	11	29.7	2	11.1	0.019
15-19	7	18.9	2	5.4	2	11.1	
≥20	10	27.0	5	13.5	1	5.6	

Table ix. Univariate case-case associations between reported contact with water/sewage and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Type of water used in household for drinking							
Municipal							
Yes	29	78.4	21	56.8	8	44.4	0.029
No	8	21.6	16	43.2	10	55.6	
Private well							
Yes	1	2.7	15	40.5	6	33.3	0.000
No	36	97.3	22	59.5	12	66.7	
Purchased bottled							
Yes	18	48.6	10	27.0	8	44.4	0.145
No	19	51.4	27	73.0	10	55.6	
Other							
Yes	5	13.5	2	5.4	2	11.1	0.532
No	32	86.5	35	94.6	16	88.9	
Quality of well water (if used) monitored or tested							
Yes	1	100.0	12	80.0	4	66.7	
No	0	0.0	1	6.7	1	16.7	0.554
Unsure	0	0.0	2	13.3	1	16.7	
If tested: the results from well showed evidence of contamination							
Yes - Total Coliform (TC) bacteria only	0	0.0	1	8.3	0	0.0	
No	0	0.0	9	75.0	4	100.0	1.000
Unsure	1	100.0	2	16.7	0	0.0	
Type of water used in household for bathing/showering							
Municipal							
Yes	36	97.3	22	59.5	9	50.0	0.000
No	1	2.7	15	40.5	9	50.0	
Private well							
Yes	2	5.4	15	40.5	9	50.0	0.000
No	35	94.6	22	59.5	9	50.0	
Type of water used in household for washing dishes							
Municipal							
Yes	36	97.3	22	59.5	9	50.0	0.000
No	1	2.7	15	40.5	9	50.0	
Private well							
Yes	2	5.4	15	40.5	9	50.0	0.000
No	35	94.6	22	59.5	9	50.0	
Had a boil water advisory in your district for the drinking water supplied to your residence							
Yes	3	8.1	1	2.7	2	11.1	
No	34	91.9	34	91.9	16	88.9	0.561
Unsure	0	0.0	2	5.4	0	0.0	
If so, is the advisory still in effect							
Yes	1	33.3	0	0.0	0	0.0	
No	1	33.3	1	100.0	2	100.0	1.000
Unsure	1	33.3	0	0.0	0	0.0	

Table ix *continued*. Univariate case-case associations between reported contact with water/sewage and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Water delivery system							
Piped into home							
Yes	37	100.0	37	100.0	17	94.4	0.196
No	0	0.0	0	0.0	1	5.6	
Delivered to home and stored in a cistern							
Yes	1	2.7	0	0.0	1	5.6	0.673
No	36	97.3	37	100.0	17	94.4	
Delivered to home by other method							
Yes	0	0.0	0	0.0	1	5.6	0.196
No	37	100.0	37	100.0	17	94.4	
Water purification system in household							
Yes	12	32.4	16	43.2	9	50.0	0.404
No	25	67.6	21	56.8	9	50.0	
Type of bathroom facility in the household							
Indoor plumbing connected to a municipal system							
Yes	35	94.6	20	54.1	9	50.0	0.000
No	2	5.4	17	45.9	9	50.0	
Indoor plumbing connected to a holding tank							
Yes	1	2.7	4	10.8	2	11.1	0.397
No	36	97.3	33	89.2	16	88.9	
Indoor plumbing connected to a septic field							
Yes	1	2.7	13	35.1	7	38.9	0.000
No	36	97.3	24	64.9	11	61.1	
Used drinking water							
From own or a friends cottage							
Yes	0	0.0	0	0.0	1	5.6	0.196
No	37	100.0	37	100.0	17	94.4	
While staying overnight with friends or family at their residence							
Yes	2	5.4	8	21.6	3	16.7	0.137
No	35	94.6	29	78.4	15	83.3	
While staying overnight at a hotel or motel							
Yes	4	10.8	5	13.5	1	5.6	0.907
No	33	89.2	32	86.5	17	94.4	

Table x. Univariate case-case associations between reported food history and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Ate at a fast food restaurant 7 days before becoming ill							
Yes	22	59.5	16	43.2	9	50.0	
No	12	32.4	18	48.6	9	50.0	0.361
Unknown	3	8.1	3	8.1	0	0.0	
Ate at a any other restaurant 7 days before becoming ill							
Yes	19	51.4	15	40.5	10	55.6	
No	16	43.2	20	54.1	8	44.4	0.582
Unknown	2	5.4	2	5.4	0	0.0	
Consumed the following foods (7 days prior to illness)							
Eggs							
Yes	28	75.7	19	51.4	11	61.1	0.090
No	9	24.3	18	48.6	7	38.9	
Chicken							
Yes	33	89.2	27	73.0	14	77.8	0.220
No	4	10.8	10	27.0	4	22.2	
Beef (ground)							
Yes	21	56.8	25	67.6	11	61.1	0.696
No	16	43.2	12	32.4	7	38.9	
Pork							
Yes	15	40.5	12	32.4	9	50.0	0.449
No	22	59.5	25	67.6	9	50.0	
Locally acquired wild game							
Yes	2	5.4	2	5.4	1	5.6	1.000
No	35	94.6	35	94.6	17	94.4	
Pre-washed salad greens							
Yes	10	27.0	12	32.4	5	27.8	0.955
No	27	73.0	25	67.6	13	72.2	
Alfalfa/clover/been sprouts							
Yes	5	13.5	1	2.7	1	5.6	0.271
No	32	86.5	36	97.3	17	94.4	
Fruit juice (unpasteurized)							
Yes	0	0.0	1	2.7	0	0.0	1.000
No	37	100.0	36	97.3	18	100.0	
Milk (unpasteurized)							
Yes	0	0.0	7	18.9	1	5.6	0.007
No	37	100.0	30	81.1	17	94.4	
Other dairy (unpasteurized)							
Yes	0	0.0	0	0.0	1	5.6	0.196
No	37	100.0	37	100.0	17	94.4	
None of the above							
Yes	1	2.7	0	0.0	0	0.0	1.000
No	36	97.3	37	100.0	18	100.0	

Table xi. Univariate case-case associations between reported contact with animals or farms and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Live on a farm							
Yes	1	2.7	13	35.1	8	44.4	0.000
No	36	97.3	24	64.9	10	55.6	
Type of farm							
Mixed (ranching and crops)	0	0.0	2	15.4	6	75.0	
Primarily crop production	0	0.0	0	0.0	1	12.5	
Primarily Ranching	0	0.0	7	53.8	0	0.0	0.003
Other (hobby farm)	0	0.0	4	30.8	1	12.5	
Unknown	1	100.0	0	0.0	0	0.0	
Visited a farm							
Yes	2	5.4	16	43.2	10	55.6	0.000
No	35	94.6	21	56.8	8	44.4	
Visited a petting zoo							
Yes	0	0.0	0	0.0	1	5.6	
No	37	100.0	37	100.0	16	88.9	0.187
Unknown	0	0.0	0	0.0	1	5.6	
Contact with							
Domestic cattle							
Yes	1	2.7	13	35.1	8	44.4	0.000
No	36	97.3	24	64.9	10	55.6	
Domestic hogs							
Yes	1	2.7	3	8.1	4	22.2	0.058
No	36	97.3	34	91.9	14	77.8	
Broiler chickens							
Yes	0	0.0	1	2.7	2	11.1	0.097
No	37	100.0	36	97.3	16	88.9	
Egg laying chickens							
Yes	0	0.0	2	5.4	4	22.2	0.007
No	37	100.0	35	94.6	14	77.8	
Domestic sheep							
Yes	0	0.0	0	0.0	1	5.6	0.196
No	37	100.0	37	100.0	17	94.4	
Other domestic farm animals							
Yes	0	0.0	2	5.4	3	16.7	0.045
No	37	100.0	35	94.6	15	83.3	
None of the above							
Yes	32	86.5	21	56.8	7	38.9	0.001
No	5	13.5	16	43.2	11	61.1	
Handled raw animal products							
Yes	14	37.8	9	24.3	8	44.4	
No	20	54.1	24	64.9	7	38.9	0.196
Unknown	3	8.1	4	10.8	3	16.7	
Handled animal carcasses or manure							
Yes	2	5.4	3	8.1	4	22.2	
No	35	94.6	26	70.3	13	72.2	0.153
Unknown	0	0.0	8	21.6	1	5.6	

Table xi *continued.* Univariate case-case associations between reported contact with animals or farms and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Contact with							
Adult dogs							
Yes	10	27.0	28	75.7	8	44.4	0.000
No	27	73.0	9	24.3	10	55.6	
Puppies							
Yes	5	13.5	4	10.8	4	22.2	0.543
No	32	86.5	33	89.2	14	77.8	
Adult cats							
Yes	11	29.7	19	51.4	11	61.1	0.043
No	26	70.3	18	48.6	7	38.9	
Kittens							
Yes	4	10.8	13	35.1	3	16.7	0.038
No	33	89.2	24	64.9	15	83.3	
None of the above							
Yes	16	43.2	6	16.2	1	5.6	0.003
No	21	56.8	31	83.8	17	94.4	
Contact with							
Fish							
Yes	5	13.5	4	10.8	4	22.2	0.543
No	32	86.5	33	89.2	14	77.8	
Amphibians							
Yes	0	0.0	2	5.4	1	5.6	0.411
No	37	100.0	35	94.6	17	94.4	
Reptiles							
Yes	4	10.8	2	5.4	2	11.1	0.702
No	33	89.2	35	94.6	16	88.9	
Pet birds							
Yes	1	2.7	1	2.7	0	0.0	1.000
No	36	97.3	36	97.3	18	100.0	
Other mammals							
Yes	0	0.0	2	5.4	1	5.6	0.411
No	37	100.0	35	94.6	17	94.4	
None of the above							
Yes	26	70.3	27	73.0	12	66.7	0.955
No	11	29.7	10	27.0	6	33.3	
Contact with pet treats							
Yes	5	13.5	9	24.3	5	27.8	
No	32	86.5	25	67.6	13	72.2	0.294
Unknown	0	0.0	3	8.1	0	0.0	
Cleaned or filled a bird feeder							
Yes	2	5.4	3	8.1	3	16.7	0.399
No	35	94.6	34	91.9	15	83.3	
Removed bird droppings from an area							
Yes	3	8.1	2	5.4	0	0.0	
No	34	91.9	27	73.0	14	77.8	0.843
Unknown	0	0.0	8	21.6	4	22.2	
Tended to or touched any sick or dead wild birds							
Yes	1	2.7	1	2.7	0	0.0	
No	35	94.6	36	97.3	16	88.9	1.000
Unknown	1	2.7	0	0.0	2	11.1	
Tended to or touched any sick or dead wild animals							
Yes	1	2.7	0	0.0	0	0.0	
No	35	94.6	37	100.0	17	94.4	0.589
Unknown	1	2.7	0	0.0	1	5.6	

Table xii. Univariate case-case associations between reported occupational, recreational or incidental exposures and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Contact with children who attend daycare (7 days prior to illness)							
Yes	5	13.5	3	8.1	2	11.1	
No	30	81.1	33	89.2	16	88.9	0.763
Unknown	2	5.4	1	2.7	0	0.0	
Changed diapers (7 days prior to illness)							
Yes	3	8.1	2	5.4	2	11.1	
No	34	91.9	34	92.0	16	88.9	0.877
Unknown	0	0.0	1	2.7	0	0.0	
Contact with children who use diapers (7 days prior to illness)							
Yes	10	27.0	15	40.5	4	22.2	
No	26	70.3	20	54.1	14	77.8	0.264
Unknown	1	2.7	2	5.4	0	0.0	
Work in the meat processing industry							
Yes	1	2.7	1	2.7	1	5.6	1.000
No	36	97.3	36	97.3	17	94.4	
Consumed food prepared on an outdoor recreational trip							
Yes	3	8.1	2	5.4	1	5.6	1.000
No	34	91.9	35	94.6	17	94.4	
In the previous 7 days							
Swam in a public pool							
Yes	6	16.2	3	8.1	1	5.6	0.504
No	31	83.8	34	91.9	17	94.4	
Swam or participated in watersports in a river							
Yes	0	0.0	3	8.1	0	0.0	0.220
No	37	100.0	34	91.9	18	100.0	
Swam or participated in watersports in a lake							
Yes	1	2.7	3	8.1	1	5.6	0.838
No	36	97.3	34	91.9	17	94.4	
Swam in a private pool							
Yes	1	2.7	4	10.8	1	5.6	0.559
No	36	97.3	33	89.2	17	94.4	
Used a private hot tub							
Yes	3	8.1	0	0.0	0	0.0	0.220
No	34	91.9	37	100.0	18	100.0	
Swam/watersports or contact with any other type of water							
Yes	2	5.4	2	5.4	1	5.6	1.000
No	35	94.6	35	94.6	17	94.4	
None of the above							
Yes	22	59.5	23	62.2	15	83.3	0.194
No	15	40.5	14	37.8	3	16.7	
Attended a large gathering							
Yes	15	40.4	9	24.3	6	33.3	
No	20	54.1	28	75.7	12	66.7	0.272
Unknown	2	5.4	0	0.0	0	0.0	
Contact with someone who had symptoms of vomiting and diarrhea							
Yes	5	13.5	4	10.8	1	5.6	
No	32	86.5	32	86.5	14	77.8	0.909
Unknown	0	0.0	1	2.7	3	16.7	
Did you care for someone who had symptoms of vomiting and diarrhea							
Yes	1	20.0	2	40.0	1	25.0	1.000
No	4	80.0	3	60.0	3	75.0	

Table xiii Univariate case-case associations between reported travel and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Travel in the last month							
Yes	12	32.4	22	59.5	10	55.6	0.055
No	25	67.6	15	40.5	8	44.4	
Reason for travel							
Business							
Yes	0	0.0	4	10.8	3	16.7	0.032
No	37	100.0	33	89.2	15	83.3	
Recreation							
Yes	12	32.4	18	48.6	7	38.9	0.371
No	25	67.6	19	51.4	11	61.1	
Other							
Yes	0	0.0	1	2.7	2	11.1	0.097
No	37	100.0	36	97.3	16	88.9	

Table xiv. Univariate case-case associations between reported symptoms and location using Fishers Exact test

	Winnipeg		South/Central Manitoba		Western Manitoba		*P*
Explanatory Variables	n	%	n	%	n	%	
Diarrhea							
Yes	37	100.0	34	91.9	18	100.0	0.220
No	0	0.0	3	8.1	0	0.0	
Blood in stool							
Yes	9	24.3	23	62.2	9	50.0	0.004
No	28	75.7	14	37.8	9	50.0	
Vomiting							
Yes	13	35.1	10	27.0	4	22.2	0.635
No	24	64.9	27	73.0	14	77.8	
Nausea							
Yes	30	81.1	23	62.2	11	61.1	0.155
No	7	18.9	14	37.8	7	38.9	
Abdominal cramps							
Yes	34	91.9	29	78.4	14	77.8	0.197
No	3	8.1	8	21.6	4	22.2	
Abdominal pain							
Yes	32	86.5	29	78.4	11	61.1	0.110
No	5	13.5	8	21.6	7	38.9	
Fever							
Yes	27	73.0	24	64.9	14	77.8	0.635
No	10	27.0	13	35.1	4	22.2	
Chills							
Yes	28	75.7	18	48.6	14	77.8	0.028
No	9	24.3	19	51.4	4	22.2	
Headache							
Yes	21	56.8	19	51.4	4	22.2	0.047
No	16	43.2	18	48.6	14	77.8	
General muscle aches							
Yes	18	48.6	17	45.9	11	61.1	0.593
No	19	51.4	20	54.1	7	38.9	
Fatigue							
Yes	31	83.8	32	86.5	15	83.3	1.000
No	6	16.2	5	13.5	3	16.7	
Hospitalized							
Yes	9	24.3	6	16.2	5	27.8	0.606
No	28	75.7	31	83.8	13	72.2	