The Risk Factors for West Nile Virus Infection and West Nile Virus Disease

By Myrna Dyck, BN, BSc

A Thesis submitted to the Faculty of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science

Department of Community Health Sciences

University of Manitoba

Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION

The Risk Factors for West Nile Virus Infection and West Nile Virus Disease

BY

Myrna Dyck

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

OF

MASTER OF SCIENCE

Table of Contents

List of Tables

List of Figures

Abstract

Introduction: West Nile virus (WNV) was first documented in the West Nile district of Uganda, in 1937. In Africa this arbovirus remains endemic, with low mortality rates; while in other areas of the world, epidemics have had significant impact. Individuals in southwestern Manitoba experienced an outbreak of WNV disease during the summer of 2003. These studies determined the specific risk factors for the development of WNV infection and WNV disease, once infected, for the outbreak in southwestern Manitoba during the summer of 2003.

Methods: Using a case control study design, cases and controls (some from the Public Health Agency of Canada – Manitoba Health WNV Seroprevalence study) were compared to analyze risk factors. The MB Health – PHAC Seroprevalence study included a 20 minute telephone questionnaire and requisitioned blood work that was completed during the Spring of 2004. The questionnaire examined demographic, health, personal protective behaviours, and exposure variables. Univariate, bivariate and multivariate analyses were completed. Unmatched logistic regression was used to control for confounding variables.

Results: Living in or near a town with high levels of infected mosquitoes, having asthma, Lupus or rheumatoid arthritis, farming and walking or jogging outside increased one's odds of developing a WNV infection. The single risk factor that showed a significant association with WNV disease, after controlling for age and gender, was the recreational activity of jogging or walking outdoors (modified OR: 3.9, CI 1.1 – 13.6).

Discussion: Having specific diseases or activities that required time spent outside was associated with an increase risk in WNV infection. Living in or near a town with high levels of infected mosquitoes also increased one's odds of developing a WNV infection. Jogging or walking outdoors increased one's odds of developing WNV disease after being infected by WNV, by almost four times. Further research is needed to confirm these findings and to determine how these activities increase the chances of developing WNV infection and disease.

Acknowledgements

I wish to acknowledge my supervisor, Dr. Lawrence Elliott, who throughout this process remained a mentor, guiding, encouraging and challenging me to complete this project and thesis. My other committee members, Drs. Neil Simonsen and Pat Martens and members of the Community Health Sciences Biostatistics Unit, Dr. Tom Hassard and Ms. Mary Cheang have been consistently supportive as well. I also wish to thank Dr. Susan Roberecki and the WNV group at Manitoba Health for their assistance throughout this project. Without access to their data, these case control studies could not have been completed.

I wish to thank the Social Sciences and Humanities Research Council for funding my studies; however, most notably, without the social, mental and emotional support of my husband and both my immediate and extended families, this degree would never have been possible.

1. Introduction

1.1 Background

West Nile virus disease has been documented since the early 20th century. The first documented case was in the district of West Nile in Uganda in 1937 [1, 2]. Cases have since been diagnosed in Africa, the Middle East, Europe, and parts of North America [3, 4]. The virus has developed into different strains and has shown a variety of disease phenotypes. West Nile virus has known reservoirs in almost all bird populations [5-7], and may have reservoirs in other small reptilian or mammalian animals [8-11]. Arthropod populations (usually mosquitoes) [12-15] are required to transmit the disease within the avian population and develop a viremia amplification loop, where the disease level in the birds as well as the mosquito populations dramatically increases. The *Aedes vexans* and *Culex pipiens* mosquitoes are usually known to assist with this viral load development [16, 17]. Different mosquito species that feed on both birds and mammals (*Culex tarsalis* in Manitoba) spread West Nile virus from the bird population to humans, horses and other mammals.

Individuals in southwestern Manitoba experienced an outbreak of West Nile disease during the summer of 2003. Previously, Manitoba had not had any positive cases of West Nile virus in humans. The Assiniboine and Brandon health districts had the highest number of definitive and probable West Nile cases in Manitoba. Patients such as these, exposed to the virus and infected with the disease, experienced fevers, headaches, malaise, vomiting, confusion and, if the disease was severe, meningoencephalitis [18, 19]. Just prior to the

outbreak of human disease, the mosquito populations in the area began to test positive for the virus. Preceding the human outbreak, dead crows, magpies and other birds in the corvid bird group also began to test positive for the virus and some horses developed encephalitis as a result of contracting West Nile disease. As West Nile is a serious, newly emerging threat in North America, this recent outbreak in Manitoba deserves careful study and analysis. This project included two case-control studies of the 2003 summer outbreak in Southwestern Manitoba. These two studies used data from the Manitoba Health – Public Health Agency of Canada (MB-PHAC) seroprevalence survey questionnaires (Appendices #1 - 3) and Manitoba Health mosquito surveillance data to analyze risk factors related to West Nile virus.

Manitoba is a central Canadian prairie province with a population of 1,165,000. The majority of individuals live in Winnipeg, the capital (750,000) or Brandon, the second largest city (40,000). The province is divided into 10 regional health authorities.

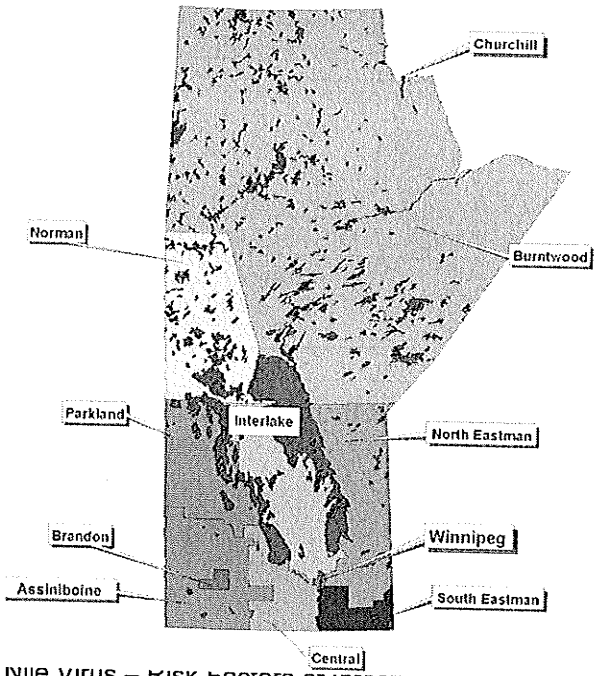


Figure #1:
Map of Manitoba regional health authorities

The case control studies were completed in the regional health authorities of Brandon (urban) and Assiniboine (rural). The Brandon regional health authority serves 48,000 people and the Assiniboine regional health authority serves 69,000 individuals, most who either live in small towns or other rural settings. The vast majority of individuals in these areas are employed either directly or indirectly in agriculture. 4 of the towns included in the case control study are within the Assiniboine regional health authority (Appendix #4).

The risk factors for West Nile virus (WNV) infection have been studied in other case-control and seroprevalence studies [1, 20-23]; however, risk factors for WNV disease have been less documented [24]. As the virus moves into different physical environments, animal reservoirs and arthropod populations and develops into genetically different strains of West Nile virus, it might also develop distinctive risk factors. Local risk factors may also vary due to exposure factors (i.e. the human and social environments differ). Thus, it is important to study which factors increase Manitobans' risk for this emerging disease.

The case control studies were used for two different comparisons, to determine respectively the risk factors for West Nile virus infection (case control study #1) and the risk factors for symptomatic West Nile virus disease (case control study #2). When sero-analysis was completed for the MB-PHAC Seroprevalence study, positive test results indicated that that individual had been exposed to the virus and had developed antibodies to the virus. As WNV had never been previously found in Manitoba and the participants had remained in their local community at least six weeks of the summer during 2003, the

exposure and subsequent infection was assumed to have occurred in Manitoba. This new emerging disease had moved through the eastern provinces of Canada and caused an outbreak in a new population.

1.2 Objectives

- to determine the risk factors for WNV infection in Southwestern Manitoba in the summer of 2003 (Appendix #5).
- the determine the risk factors for WNV disease in Southwestern Manitoba in the summer of 2003 (Appendix #6).

2. Review of Literature

2.1 General Literature – West Nile Virus

West Nile virus is an arbovirus (arthropod-borne virus) that infects birds, mammals, and humans [15, 25, 26]. It is transmitted from birds to humans via a vector. In North America, this arthropod vector is the mosquito. In Manitoba, as in other areas of North America, the type of mosquitoes that are part of this process are the *Culex* mosquitoes, specifically *Culex tarsalis* [4, 27-32]. *Aedes* mosquito species increase the level of the virus in the corvid and other bird populations in the affected areas [5, 6, 16]. As the mosquitoes become infected with the virus and retransmit it again and again in a small bird population, the level of viremia in that bird population escalates [7, 17, 33, 34]. Once the viremia has reached a high level, *Culex* mosquitoes, which feed on these birds as well as other mammals, begin to transmit the virus to other species. Horses, humans and other mammals are at risk for infection, with intermittent occurrences of serious disease consequences [8, 34-37].

West Nile virus was first documented in Uganda in 1937, in the West Nile area by Smithburn, Hughes and others [2]. It was one of the first arthropod-borne diseases to be recognized. Until the 1960's, the virus maintained endemic status in many areas and sporadically led to epidemics in rural areas in under-developed parts of the world [1, 31, 38]. Earlier epidemics, prior to the 1990's, had very little neurological syndromes associated with the disease. Since 1996, there have been four major epidemics: Romaina, Russia, Israel and North America [1, 39, 40]. The latest epidemic that is under study is the epidemic that

initially developed in New York City – the first experience of WNV on this continent. As the epidemics have continued to emerge and threaten new populations, it is obvious that the vectors and reservoir hosts necessary to maintain transmission of this disease have large territories across North America. *Culex* mosquitoes are also known for their close association with human dwellings, so it is not surprising then, that the humans epidemics have continued [17, 41].

The virus is in the flavivirus family. This is a family of zoonotic diseases (originated in animals and crossed the species barrier into human populations) in which there are many other well known viruses, such as Dengue, Japanese Encephalitis and Yellow Fever. WNV is a protein encapsulated RNA virion that may seem circular, but instead has faceted edges [42, 43] (Figure #2). As the virus' genetic material is comprised of RNA, not DNA, it can respond to environmental and evolutionally changes rapidly – causing significant shifts in genetic material in a short period of time [4, 44-46].

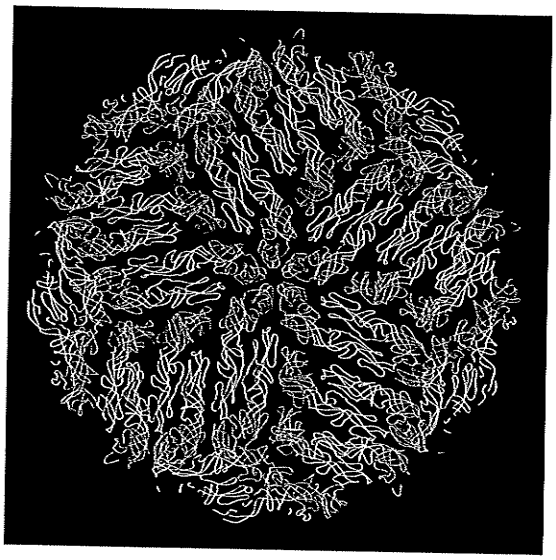


Figure #2: Structure of West Nile Virus

"This image shows the orientation of the envelope protein molecules that compose the surface of a West Nile virus particle. The major surface protein is composed of three domains color-coded pink, yellow and blue. The proteins self-assemble in a host cell, forming a well-organized geometric shape. Knowledge of the proteins' structure could help scientists in the effort to develop antiviral agents. (Purdue Department of Biological Sciences image)"

Yellow Fever, Japanese Encephalitis and WNV arboviruses are similar in that they are all transmitted by arthropods, where through a blood meal the arthropod ingests the virus. The virus then moves through the arthropod's digestive system and is absorbed into its circulatory system. In a competent vector, the virus is deposited in its salivary glands ten to fourteen days after ingestion [17, 47]. From here the virus can be transmitted to a new host by injection, passing from the arthropod into the new animal. Each species of mosquito has different abilities to absorb the virus, circulate the virus and accumulate the virus in the salivary glands; this leads to varying vector competencies. Depending on the geographical area, different arthropod species may be involved in this cycle. Additionally, different arthropods feed on different hosts. In Canada, *Aedes vexans*, *Culex pipiens* and *Culex restuans* mosquitoes feed on avian populations and *Culex tarsalis* mosquitoes feed on both birds and some mammal species [17, 37, 48, 49]. Vector competence in both bird feeding and mammalian feeding species is required for a human outbreak of West Nile virus.

Culex tarsalis mosquitoes, the species that transmit WNV in Manitoba, are not the most frequent mosquito to occur; yet, they are the species that displays the best vector competence for West Nile virus [17, 34, 50]. The rate of population growth of *Culex* mosquitoes is very dependent on weather [51], with temperatures likely having more effect than rainfall [52, 53]. When conditions in the areas where both humans and *Culex* come into contact are moderate and both populations increase their level of outdoor activity, then conditions for WNV

transmission develop. *Culex* mosquitoes have urbanized along side their human hosts as their breeding patterns involve spreading egg rafts in shallow basins, such as bird baths, old tires, discarded tins, and other human debris [33].

The avian species that are required to maintain the transmission loop of WNV are also experiencing the effects of this emerging virus. Discussion continues about the ways in which WNV moves throughout time and space, with different proponents arguing that the virus moves through migratory birds, non-migratory small animal reservoirs, vertical transmission, and/or over-wintering [7, 32, 48, 52, 54]. All might have an effect in Manitoba. Over-wintering, though, would be the least likely, due to the extreme weather that can occur in the Prairies [53]. Some bird species that became infected with WNV were severely affected by the emerging virus. They had no immune recognition of the virus and it decimated their population levels [55]. It is not yet documented whether the bird species in North America will develop immunity to the virus which could significantly change how the virus is transmitted, perhaps reducing the viral levels in *Culex* mosquito populations.

Other mammal populations are also at risk for WNV infections and their consequences. Equine encephalitis resulting from a WNV infection can have serious effects on livestock, at times reducing a herd of horses to a fraction of what it was. The odds of contracting WNV in horse herds that are non-vaccinated are three to 16 times that of horses that have been vaccinated and the death rate in non-vaccinated horses can reach 22% [56]. Equine infection with West Nile has serious fatal results in a significant number of cases [57, 58].

This can have devastating effects in the Canadian agricultural industry, which is currently also facing threats of Bovine Spongiform Encephalitis and Avian flu.

West Nile virus is a blood borne virus. It is transmitted by vector mosquitoes in the avian populations which contribute to the viremia amplification loop. Most often transmission is accomplished by a *Culex* mosquito, as the virus moves from bird into mammal via a blood meal. However, due to the fact that this virus is a blood borne virus, with a viremic stage in humans, there have been other, more infrequent, transmission routes [59-63]. Patients, who receive organs from live or cadaver donors, are at risk for contracting this virus if the donor organ is not tested for the presence of WNV. The virus can also be transmitted from mother to infant through the placenta, and patients who receive blood transfusions have also developed WNV disease symptoms when the disease passed from one individual to another through the blood donation system. Transmission of WNV has also been documented from mother to child through breastfeeding [64, 65]. West Nile virus and WNV IgM and IgG antibiotics were traced, in this case, in the mother, in the breast milk itself and later in the infant [65]. The infant did not show symptoms, however as WNV infection does not always cause symptomology, it is difficult to determine whether the maternal antibodies passed to the baby protected it. These are rare transmission routes for this emerging pathogen.

When a human is exposed to the WNV, the innate immune system, together with the adaptive immune system, attempts to reduce its impact on the body. Initially, the innate immune system would mount a generalized attack

against the invading foreign pathogen [58, 66]. The subsequent immune effects occur in the adaptive immune system, where West Nile specific antibodies are developed. Initially IgM is the first antibody produced; yet, as the immune system continues to struggle to control the pathogen, its antibody production becomes much more specific and potent, and IgG antibodies are produced [67]. Antibody memory is also developed to provide immunity to the patient. This memory also allows the West Nile antigen tests to detect for an immune response to WNV. Initially, levels of IgG are high, when there is still virus within the body of the individual who has been exposed. With time, these levels dissipate. Long term memory B cells (the cells that produce the antibodies IgG, IgE or IgA) are few in number, but do exist for a long period of time; for some diseases these can last a lifetime – protecting the individual from all further infections of that same pathogen.

The immune status of the participants in the MB-PHAC seroprevalence survey was determined using a micro-neutralization West Nile virus IgG assay at the National Microbiology Laboratory. This assay uses a medium on which West Nile antigens are adhered. When blood from individuals with IgG antibodies specifically for WNV is mixed with this medium, it results in a large clumping of the antibodies and the antigen-laced medium. This was tested against the appropriate controls and other flavivirus antigens (to ensure the cross-linking was a result of identifying WNV antibodies and not the other slightly related flavivirus antibodies, such as Dengue, Yellow Fever, or Japanese Encephalitis) [68]. When the analysis of the clumping levels was completed, and the results of the

WNV tests were at least four times greater than the results of the general flaviviruses, it was determined that the results were WNV positive.

West Nile virus infection does not frequently cause disease symptoms [19, 69-71]. In Manitoba, during the summer of 2003, approximately 3% of individuals who participated in a MB-PHAC seroprevalence survey tested positive for antibodies to the WNV [72]. This indicates that in those areas where West Nile existed, approximately 3% of individuals exposed became infected. However, it would be in error to state that all areas had the same rates of exposure or infection. Different topography in diverse areas of even one small corner of the province, can cause a large fluctuation in the numbers of mosquitoes and in the percentage of WNV infected mosquitoes [29]. As well, this says little about the populations of people that existed in these areas. Some may be at more risk for WNV infection due to health or demographic factors, while others might be at less risk due to personal protective behaviours [73-76].

Although very few individuals who are exposed to West Nile virus become infected [23], and even fewer develop disease symptoms related to their infection, this disease does warrant public health attention, as it does have serious, and sometimes fatal consequences [77]. The rates of neurological symptoms that have accompanied the North American epidemic of WNV have been above expectations, when compared to earlier outbreaks in other areas of the world [78, 79]. This indicates that the disease has either increased its level of morbidity or that the populations that are being exposed to the virus are reacting in ways that previously exposed populations did not. Nonetheless, the rates of

infection throughout the epidemic have remained relatively stable [80-82]. The increase in neurological outcomes related to this infection is not the only aspect of this epidemic that varies from previous outbreaks of West Nile in other areas of the world. The long-term sequelae of individuals who do become symptomatic with WNV disease and experience fevers, encephalitis and/or meningitis seem to be more complex and severe than previously expected as well [83-85].

West Nile virus is a worthwhile emerging disease to follow as it progresses across North America. It has dramatically affected summer public health messages and continues to be a public health department responsibility. Understanding the virus, its transmission cycles, its sporadic movement across the country, its vectors and its hosts will improve prevention efforts. As it is apparent that this disease is in North America to stay, it is imperative for health departments to research the risk factors of infection and disease, to ensure that their efforts at protecting their populations are targeted and appropriate.

2.2 Public Health and West Nile Virus

The public health implications of WNV disease in Canada may not be fully understood until the ranges of this disease are defined, the majority of the paths of transmission are found, and the abilities of the virus or mosquito species to over-winter or transfer vertically are determined. Immunology and infectious disease researchers have noted that exposure to WNV positive mosquitoes does increase one's risk of infection [74, 86, 87]. How public health officials can assist with the control of this virus and its vectors has long caused controversy [35, 88-90]. Public health departments in North America have been attempting to control

outbreaks and epidemics for centuries, and their responsibility continues with the emergence of WNV [91-93].

Public health agency responsibility with this emerging flavivirus disease focuses on vector or virus control as well as surveillance. As with other arboviruses, public health control concentrates on large populations, vector control, immunization and education of the public. Pesticides have been used to control arboviral vectors with varying success rates throughout history [94-98]. Large-scale spraying of insecticides has recently lost political favour, especially as more environmental research questions their use [89, 90, 96]. Rural populations have always been at a greater disadvantage for public health measures as their demographic concentration is so low that there is little benefit from environmental control efforts [99].

Public health agencies that are actively involved in surveillance for WNV since its emergence in North America in 1999 have relied on a variety of resources. Initially spurred on by the successes during Western Equine Encephalitis and Eastern Equine Encephalitis outbreaks, sentinel chickens were used [100, 101]. With greater study, it was discovered that other avian species were showing the effects of WNV infection significantly earlier than the sentinel chickens were testing positive [8, 102]. Crows and other corvids seemed to be especially susceptible to the virus, with nearly 100% fatality [103, 104]. Communities shifted their resources to find, count and test dead crows and other corvids [105, 106]. However, in rural environments the number of dead birds located and reported, was never as large as it appeared in the urban settings.

This was not due to fewer birds dying, but rather, likely due to the lower human population density resulting in fewer birds found [102, 107].

Manitoba has used mosquito pool testing for the last number of years as a sentinel for WNV outbreaks. Prior to 2003, WNV was found in some horses and dead birds, but no human cases were found [57]. The summer of 2003 showed a great increase in WNV positive dead birds, WNV positive mosquito pools and human cases [108]. Rural mosquito traps were also used to test for the density of *Culex tarsalis* in the trap areas and the level of infection in those populations.

When infected mosquito levels increased to the point where human risk of WNV exposure was significant, Manitoba Health initiated a control program for WNV. Pesticide use was introduced in areas where the mosquito traps indicated a high level of infected mosquitoes. Risk values were calculated based on the numbers of *Culex tarsalis* found in traps, the level of infection rates in those mosquitoes and other related factors. The maximum likelihood estimation is used to calculate the risk index formula related to infected mosquitoes [109].

Manitoba Health, like other North American public health departments, chose to also include various client education and encouragement strategies to assist with its risk management program. One strategy encouraged the public to follow proper personal protective behaviours. These included using DEET products when outside, wearing long sleeves and pants when outside at dawn and dusk, and reducing their time spent outdoors during times when mosquitoes were active [74, 110]. To reduce the number of breeding habitats for the *Culex*

tarsalis, people were encouraged to look for spots of standing water and to reduce these, if possible. As well, they were encouraged to ensure their windows had adequate netting, to prevent the mosquitoes from entering their homes [73, 75].

Although all public health departments may use the same strategies to educate the public about personal protective behaviours, not all segments of the population have the same WNV infection or disease risks. Age can influence some risk factors. Other factors change when health behaviours or underlying health status changes. Some segments of the population may increase their risk of WNV infection by the activities they choose. Personal opinions, though, do not have an effect on infection risk or disease rates; it is the behaviours that stem from these opinions that actually affect an individual's risk. To determine the WNV risk factors, research needs to focus on demographic differences, variations in health status, environmental exposure and behaviours that may increase or decrease the level of exposure.

Manitoba Health, the provincial public health department, has used some other strategies for controlling WNV outbreaks when they occur. They have used encouragement of removal of debris and birdbaths where mosquitoes can breed. In some communities, such as Winnipeg, the capital of Manitoba, public health departments have the enforcement capacities to order the removal of water breeding sites on private property. Manitoba Health and the health department in Winnipeg have the ability to order ground spraying with larvicides and adulticides and have used these strategies as necessary in the past.

2.3 Risk Factors for West Nile Infection

Becoming infected with WNV involves the mosquito inoculating a human with the virus. The virus moves into the blood stream of the new host, replicates and increases the level of the virus in the cells of the blood [61]. The host does not experience side effects during this time period (the incubation period). Once the virus reaches disease levels, the host experiences symptoms – headaches, malaise, fever and occasionally encephalitis [111]; however, in the case of WNV, these experiences are rare.

Risk factors for infection can be demographic-related, such as the area in which the population lives (address), their gender or their age [21, 74]. Most demographic risk factors are not modifiable and analyses need to appropriately adjust for these factors. Age is a noted risk factor for WNV infection [112, 113]. It has been reported that anyone over the age of 50 experiences an increase in risk for WNV infection, with the risk increasing with each decade beyond that [24, 39, 79]. Risks for WNV infection could also be related to health status. Risk factors related to health may or may not be modifiable (having cancer may not be modifiable, yet the drug used to treat the cancer may be modifiable). If certain health problems increase an individual's risk for WNV, it is advisable that public health education reach these individuals and their health care providers, so they can adjust their lifestyle, if possible, to reduce their risk. As well, public health education needs to make these individuals in the population more aware of their increased risk, so they might be able to modify other risk factors [110]. Health conditions that might be significant include: cancer, diabetes, heart disease,

asthma, emphysema, rheumatoid arthritis and Lupus, osteoarthritis, or Crohn's disease. Medications may also put a segment of the population at a greater risk of contracting a WNV infection [21, 74]. These risk factors are biologically plausible as some medications and certain medical conditions, such as cancer, impair the immune system, and therefore might alter an individual's ability to fight off this invading pathogen.

Risks for exposure can be environmental, such as the types and levels of mosquitoes in the area [36, 47, 48], the presence of standing water and weather systems that pass through the area [51, 52, 114], the level of vegetation and marshlands in the community, and the type of pesticide treatments that the local municipality has used, such as larvicides or adulticides [90, 115]. These risk factors would also explain the seasonality of the disease in North America, especially in the more northern regions, where environments change greatly throughout the year. As well, mosquitoes can not easily over-winter in many northern areas, due to temperature fluctuations and frost [53].

Risk factors that increase a person's exposure to the virus are also behavioral; the length of time the individual spends outdoors [50, 86], the type of activities he or she might be doing outdoors (working near animals or standing water), and attire or personal protective behaviours used when WNV is known to be in the area [71, 74, 116, 117] may influence risk. Individuals' activities might require a significant length of time outside at certain times of the day, which could increase their exposure to *Culex tarsalis* mosquitoes that are active from dusk, through the night until dawn. Other behaviour risk factors that might influence

one's exposure to West Nile are personal habits and recreational activities that require repeated time outside – this may appear to be simplistic; nevertheless, the more one repeatedly exposes oneself to infectious mosquitoes, the more likely one is to be bitten by one of these mosquitoes. This is a difficult risk factor to moderate in Manitoba, where the winters are long and cold and the summer is a time when families and individuals enjoy outdoor activities after working hours (usually toward dusk).

2.4 Risk Factors for West Nile Disease

Risk factors that increase a person's risk of developing WNV disease symptoms once they have been infected by the virus might be different than the risk factors for infection. These risk factors may be more personal, unique and non-modifiable [78, 79]. These risk factors could include gender, age, immune status, and other chronic conditions or diseases that would affect the individual's overall health status [61, 115, 118]. If one is less able to develop a strong immunological response to infection, WNV might be able to more severely affect this type of individual. Studies indicate there were different levels of risk for those who were older than 65 years of age or those who had a poor immune status [78]. There may be chronic diseases that increase an individual's risk as well.

Risk factors may also include the levels of infection that the mosquitoes are carrying in the surrounding environment. Certain diseases do manifest a dose effect (otherwise known as innoculum effect)– with increased dose of infection, an individual may have either an increased chance of developing

symptoms, or the symptoms they do develop may become increasingly severe [119]. Thus, the dose of inoculate that an individual who is exposed to the virus receives may influence the development of disease severity. This may also relate to the number of infected bites to which an individual is exposed, or how long the WNV season exists in their environment (some studies indicate more individuals will become infected and ill if the season is lengthened due to positive weather conditions for the mosquito vector) [120].

Although exposure is a primary risk factor for WNV infection, it is also a significant factor in the discussion of the risk factors for WNV disease. If individuals are not exposed to the virus at all because there are no competent vectors in their area, or the circumstances in their environment are not conducive to the spread of WNV in the animal populations, then humans will also not become infected, and thus have little to no risk of developing symptomatic WNV disease. Studies of other WNV outbreaks have determined that personal risk factors exist [51, 86]. There were different levels of risk for those who had WNV positive mosquitoes in their area compared with those who did not. People who had personal protective behaviors against mosquitoes were at a different risk level than those who did not [71]. The use of DEET has been noted as a protective activity in many previous publications [73, 75]. Wearing long sleeves and pants and reducing one's time spent outdoors when mosquitoes are active are other personal protective behaviours that reduced one's risk [74]. These activities would reduce one's infection risk, and possibly the risk of disease as

well, if they reduce the dose of the virus that was inoculated into the individual [86].

Research has also shown different levels of risk for those that needed to spend more time outdoors, thus increasing their virus exposure and perhaps their dose of virus [86]. These were individuals such as ranchers, gardeners and farmers. Certain populations, as well, chose to spend more time outdoors while others chose not to; thus they had different risk levels.

2.5 Case Control Design Literature

A case control study design is useful in this observational setting [121]. Case control studies are quite useful in situations where the disease under study is relatively rare, as is the case with both WNV infection and the even rarer diagnosed WNV disease symptoms [122]. The comparison of case and control groups was completed to determine what differences existed between the groups and how these differences might explain the presence of infection and disease [122, 123].

The disease was not previously known to occur in Manitoba; thus, temporality can easily be established. As this was the first exposure of Manitobans to this virus, comparisons can be made between those who were exposed and became infected. As well, comparisons could be made between those who were newly infected and symptom free to those who became ill and required medical attention. Using two different case control studies allowed for analyses to determine whether the frequencies of specific risk factors were higher in one or more particular groups for two different outcomes. The

biological plausibility of the risk factors contributing to the rate of infection and the rate of disease were also explored. The strength of association, another observational criteria for causation, was determined, as only when the risk factors were statistically significant ($p<0.05$) was the association considered. The case-control study design also allowed data regarding potential confounding variables to be collected and tested, and used to confirm or refute the original hypotheses [121]. Case control studies also allow for the assessment of not only individual risk factors, but combinations of risk factors, or even the interactions of risk factors [121].

Other reasons why a case-control study design was chosen included: cost, fewer subjects were required than a cohort study, and ease of study completion. The data was already in existence, so it lent itself to this type of study. The case-control study design also allows for multiple risk factors to be studied. Therefore the final assessment might guide the analysis to more potential results [121]. There are limitations to using this study design. These will be discussed in the Methods section of this document.

The study of WNV infection or disease risk factors would not be ethical in a prospective randomized trial, as it would be inappropriate to expose individuals to the virus, knowing that some might develop severe health complications as a result of the study. Only through a retrospective study, is it possible for the risk factors of WNV to be determined. Using a case-control method, the exposure, demographic and health condition differences between groups of individuals who

were or were not infected (study #1) and who became infected and were or were not diagnosed with WNV disease (study #2) can be shown [121].

The case-control design has been previously used in field studies on WNV disease [36, 81, 124, 125]. The case control study was useful in this case to determine what were the pertinent risk factors for the development of infection and disease [126]. The case definition was relatively tight with this study, as much research has already preceded this study and West Nile virus disease has been well documented [20, 127].

Descriptive analysis of a case-control study involves studying the group characteristics and determining odds ratios. Odds ratios are descriptive values that designate the increased (or decreased) odds or chance of an outcome with which this variable is associated [122]. In general, the variables were tested to see if they increased the individual's risk of developing a WNV infection or symptomatic disease, or whether they were protective and decreased one's risk. However, simple odds ratios do not tell a complete story, as the risks or benefits could specifically apply to one segment of the population only (e.g. the elderly, the individuals who spent much time outside, or individuals with diabetes). These types of confounders can not be controlled when using crude odds ratios [121].

In case control studies, confounders can be controlled using logistic regression [121, 126]. This type of regression is very useful for dichotomous outcomes. It also allows for many variables to be controlled within the same calculation. Confounders can thus be dealt with throughout the analysis, as long

as they continue to be forced into the model [121]. The final model that is chosen should include these forced variables as these were considered confounders [121]. Logistic regression also can allow for the analysis of any interactions that may be occurring in the model. On the other hand, the use of logistic regression also requires that the assumptions of this type of analysis are met by the data that is being tested. Matched case control studies use a matched logistic regression. A matched case control study uses controls that are specifically chosen to match certain variables for each case. Usually one to four controls are specifically chosen for each case, based on age, sex, vocation, location, or any variable that may seem to be critical to the analysis. However unmatched case controls analysis uses analysis to control for those variables and does not match the controls to specific cases. When there are ten times the numbers of controls than cases (the rule of ten), other ways of controlling for critical variables can be completed during the regression analysis. It has been determined that an unmatched logistic regression analysis can eliminate bias as well as a matched design and analysis [121, 128, 129], therefore this study uses logistic regression for multivariate analysis.

3. Methods and Study Design

3.1. Terms and Definitions

Infection with WNV involves the mosquito inoculating a human with the virus. Once infected with the virus, approximately 20% develop symptoms of fever [130], malaise and in severe cases, meningoencephalitis. Why certain individuals experience disease, and others do not, even though they have both been infected by WNV, is not well documented. Individuals infected with the virus who do experience symptoms, display diverse patterns of WNV disease. The immunology and virology of WNV is still being investigated [80, 131], however *West Nile virus disease*, for the purposes for this study, will be defined as a laboratory confirmed case of disease at the time of WNV disease-like symptoms that required medical attention. The cases of WNV disease used in these case-control studies were diagnosed by a physician and reported as positive WNV cases by the Public Health Department (Communicable Disease Unit) of Manitoba Health. None of the cases of disease used for this study were considered asymptomatic cases [132]. This definition of disease that must include medical intervention may allow cases of illness that are not identified to be included in the control groups. This is a major limitation of the examination of WNV disease and is further explained in the limitations of this study (#2).

The *"High-count towns"* variable utilized in these studies in Southwestern Manitoba is another term that requires definition. There were three towns (Deloraine, Killarney and Virden) within the group of five that were studied (Brandon, Minnedosa, Deloraine, Killarney and Virden) that had relatively higher

numbers of WNV infected *Culex tarsalis* mosquitoes trapped in the Manitoba Health mosquito surveillance traps housed near or within these towns. The study was designed to compare risk factors between individuals in and around towns with higher infected mosquito counts – higher peak risk indices (Killarney, Virden and Deloraine) and individuals in and around towns with lower infected mosquito counts – lower peak risk indices (Brandon and Minnedosa) (Appendix #1). Peak risk indices were obtained by Manitoba Health during the summer of 2003 based on values from mosquito traps in towns in many southwestern Manitoba towns.

Towards the end of autumn, when the complete tally of humans who had been confirmed to be infected with WNV was compiled, some of these towns also had higher numbers of human cases as well. *"High-count towns"*, though, is a relative term that requires the comparison of WNV-infected mosquito concentrations between towns. The two towns that served as towns with lower WNV infected *Culex tarsalis* rates were Brandon and Minnedosa. These two towns also had very low numbers of human infection cases in the summer of 2003. Individuals from the Assiniboine and Brandon health regions who experienced disease, as defined in the above paragraph, did not necessarily live <u>in</u> one of the five towns that were studied. The variable related to "town" was left as a missing value if the individual did not live in the municipality of one of the five towns that were used for the MB-PHAC seroprevalence study.

Demographic variables which were collected for use in the MB-PHAC seroprevalence study were: age, town address, gender, education, and occupation. The health status variables that were collected were:

1. The presence of a medical condition for which they received medical care.
2. The use of a medication that might reduce his/her body's ability to fight infection.
3. The presence of cancer, as the disease itself or its treatments might reduce his/her body's ability to fight infection.
4. as well as listings of specific medical conditions:
    a. Diabetes
    b. Heart disease
    c. Bronchitis
    d. Asthma
    e. Emphysema
    f. Rheumatoid arthritis and Lupus
    g. Osteoarthritis
    h. Ulcerative colitis
    i. Crohn's disease

There were many behavioral risk factors included in the MB-PHAC seroprevalence study that were analyzed (Appendix #1-3). As well, there were questions related to the WNV education that had been provided through many public health messages. It was hoped that the education had led to appropriate behavioral changes that might have protected individuals from WNV exposure.

Examples of these were using DEET, reducing time spent outside at dawn and dusk and eliminating standing water sources.

Maximum risk value as calculated for each town was a formulary computation of various variables to reach a single value for each town that was under study. The maximum likelihood estimation (MLE) is a statistical calculation. It is defined as the infection rate most likely observed given the testing results and an assumed probabilistic model (binomial distribution of infected individuals in a positive pool) [109]

The formula for calculating the MLE was as follows:

MLE = (1- (1- Y/X) 1/m)*1000, when Y is the number of positive pools, X is the number of pools tested, m is the pool size. This equation is only valid for a constant pool size. MLE for differing pool sizes is iterative and requires computer implementation (calculated by software). Finally the risk index was calculated using the following calculation: Risk index = (MLE * Average of *Cx. tarsalis* per trap)/1000 [109]. The risk indices were calculated weekly throughout the WNV season. The maximum risk index was the highest weekly risk index value for that community. Maximum risk values would be useful to use for assessment of viral load of the mosquitoes surrounding each town, however, as the values correspond to a specific town, maximum risk values were completely confounded by the variable of "town"; thus, little new information was provided by using this in the analysis.

3.2. Case Control Study #1

3.2.1 Study Design

This case-control study consisted of a secondary data analysis of the survey data from the MB-PHAC seroprevalence survey study of the WNV outbreak during the summer of 2003, in Southwestern Manitoba (Appendix #7, #8). Some WNV cases were originally reported to the Communicable Disease Unit of Manitoba Health. Other positive WNV cases, who did not experience significant disease symptoms during the outbreak of 2003, were discovered during the MB-PHAC seroprevalence survey. The cases had all been exposed and infected with West Nile virus. The controls had not and thus had no antibodies to WNV.

The survey questions covered the usual demographic data (age, address, gender and education), health status questions as well as questions about personal protective activities and behaviours that increased the risk for WNV exposure. This set of questions attempted to decipher what activities the individuals had been involved in both in their area of employment as well as their hobbies. The MB-PHAC seroprevalence study survey also asked opinion-related questions about mosquito control activities and public health education strategies. These were not used for the analysis of the risk factors of WNV infection.

3.2.2 Hypotheses

- Age, high levels of infected mosquitoes, length of time spent outside during the summer of 2003, and the nonuse of personal protective behaviors may be

significant risk factors associated with WNV infection in Southwestern Manitoba during the summer of 2003 (Appendix #5).

3.2.3 Case Definition

There were two types of individuals who made up the case sample in this study. All sero-positive individuals (34) identified through the MB-PHAC seroprevalence study were used. The individuals, who participated in that study, were chosen via random telephone number dialing, with the consenting individual with the nearest birth date in the household participating in the study. Individuals in the seroprevalence study had laboratory confirmation of their WNV sero-status. All participants received confirmation of their positive WNV status approximately 3 - 4 months after participating in the seroprevalence study survey, which occurred the spring of 2004.

The other component of the case group were 39 randomly chosen WNV positive individuals who had been listed as cases by the Communicable Disease Unit of Manitoba Health, after they were diagnosed and were provided treatment for WNV during the summer of 2003. These individuals knew of their status prior to answering the telephone questionnaire. Originally there were 67 possible participants. Public health nurses, familiar with each patient, obtained consent that allowed the study personnel to contact the 67 possible participants. A randomly generated list of case numbers was given to a study surveyor, who then contacted the patients individually in the order listed. The patients were allowed to decline participation once the surveyor had explained the study and what their involvement would entail. Once 40 patients consented, this portion of

the study was considered complete. Surveyors reached 39 individuals who had been case-listed with Manitoba Health.

3.2.4 Control Group Definition

All seronegative controls identified through the MB-PHAC seroprevalence study were used. The individuals who participated in this study were chosen via random telephone number dialing, with the consenting individual with the nearest birth date in the household participating in the study. The number of controls available for analysis was 1161 individuals (some of the 1195 individuals who participated in the MB-PHAC seroprevalence study tested sero-positive, and others were lost to follow-up or withdrew consent prior to the blood-draw).

Data for this study was collected as part of the MB-PHAC WNV seroprevalence study (the CHS student investigator was listed as a co-investigator for the ethics submission of the seroprevalence study). The seroprevalence study methodology, consents and surveys have been included as Appendices #1, #2, and #3 respectively.

The participation in the seroprevalence study was completely voluntary, and if individuals chose not to participate, the surveyor went to the next random telephone number. The target participatory group was 1500 people (approximately 300 each from Brandon, Minnedosa, Virden, Deloraine, and Killarney). Following the telephone interview, the consent form and blood test requisition form were mailed out to the seroprevalence participant and they were asked to attend a blood drawing clinic in their area. The lab technician at the blood draw clinic ensured that the participant had signed his or her consent form,

and then drew blood to test for WNV antibody levels. The consent, the tube of blood (labeled with the patient study number), and the lab requisition was forwarded to Cadham Lab. From there, the study tubes were sent to the National Microbiology Laboratory where testing for the IgG antibody levels was completed (micro-neutralization WNV IgG assay). The study coordinator for the study, based at Manitoba Health, collected the consent forms, and ensured that the database at Manitoba Health correctly linked the test results with the appropriate patient number. She also managed the database for Manitoba Health and guaranteed that the data was maintained in a confidential manner. The survey results and the lab results were tabulated and readied for analysis.

3.2.5 Descriptive, Univariate, & Bivariate Analysis

Descriptive statistics and univariate analysis were completed to assess the populations that participated in this case-control study. The age (continuous variable) and gender distributions were calculated, as well as population distributions over space (where the participants were situated in Assiniboine and Brandon Health districts). Frequency calculations of each risk factor were assessed. The frequency analysis of the case population and the control group were compared to determine how similar the control group and the case population were. If the control group did not match well with the case population, the risk factors that varied widely were identified. Further analyses were completed, if necessary, to determine the cause of any discrepancies.

Bivariate analysis was completed to determine the odds ratios relating WNV infection with a variety of exposures and risk factors. Using age as the risk

factor to calculate the power of this study, it was determined (Appendix #9) that with the available sample sizes of 40 cases and a pre-study potential of 1300 sero-negative controls, the study would have a power of 80% (1 – beta) to detect an odds ratio of between 2.0 and 3.0 for the case-control study #1 comparison, with an alpha error of 0.05.

3.2.6 Multivariate Analysis

The following risk factors for WNV were initially entered into a logistic regression model, with case/control status being the outcome of interest: town of residence, asthma, farming as an occupation (or grain farming and poultry farming specifically), walking-jogging, hours spent outside during the evening, hours spent outside during the night, total hours spent outside from dusk until dawn, burning a coil, burning candles and inspecting screens. These were the variables which had attained a significance level of $p<0.10$ on bivariate testing (not attaining statistical significance of $p<0.05$, however trending in that direction). Opinion-related variables (e.g. "Are you aware of how West Nile virus is spread?") and "fever during the summer of 2003" were not entered into the model as these were not plausible risk factors for WNV infection. Age, gender and town of residence were forced into the model at all stages to control for these variables. Once all variables were entered into the model, backwards stepwise logistic regression was used to arrive at a reduced model of significant risk factors ($p<0.05$), while controlling for age, gender and town of residence. Finally, removed variables were re-entered to ensure they remained insignificant.

3.3 Case Control Study #2

3.3.1 Study Design

This case-control study compared reported cases of WNV illness from the summer of 2003 to a similar number of controls who had been infected with WNV but were not diagnosed with WNV during the same period (Appendix #7, #8). Variables used in this analysis came from the MB-PHAC seroprevalence study. The survey questions covered the usual demographic data (age, address, gender and education), health status questions as well as questions about exposure behaviours and activities that increased the risk for WNV exposure. This last set of questions attempted to decipher which activities the individuals had been involved in both in their area of employment as well as their hobbies. Questions also were asked that focused on personal protective behaviours. A significant number of questions were asked about their opinion of West Nile control activities and how they received their public health education. These were not used for the analysis of the risk factors of WNV disease.

3.3.2 Hypotheses

- Age, chronic diseases and a weakened immune system may be significant risk factors associated with West Nile disease in southwestern Manitoba in the summer of 2003 (Appendix #6).

3.3.3 Case Definition

This case-control study examined thirty-nine cases of WNV disease (randomly chosen using a computerized random number generator from a numbered list of 67 confirmed cases reported to Manitoba Health from July to

September, 2003). The cases of WNV were already known to Public Health professionals in the rural health authorities of Assiniboine and Brandon. The cases were individuals who experienced signs and symptoms of West Nile disease and tested positive to an IgG micro-neutralization test completed by the National Microbiology Lab of Health Canada during the summer of 2003. These individuals experienced signs and symptoms related to their WNV infection that could be labeled as WNV disease. Their disease symptoms were significant enough that the medical professionals treating them tested their WNV antibody levels and subsequently treated them for WNV disease symptoms.

Manitoba Public Health staff asked all cases if they would consent to being approached to participate in the case-control study (as part of the larger MB-PHAC study). Surveyors, hired by the MB-PHAC seroprevalence study contacted potential participants if they verbally consented to hearing more about the study. Once 39 cases had consented and completed the telephone survey, no more cases were contacted. There was an attempt to arrive at a random, unbiased group of participating individuals.

The cases completed the same survey as the 1195 individuals who participated in the MB-PHAC seroprevalence study (Appendix #3). Since it was already documented through Manitoba Health that these patients were sero-positive, no blood work was required. The survey consisted of 45 mostly multiple-choice questions and took approximately 25 minutes to complete. Once the respondents completed the survey, the study consent form was mailed to them. They were required to sign and return it to the MB-PHAC study team. The

study coordinator ensured that the consents from the participating individuals were appropriately filed. The results from the surveys were entered into a secure, confidential database at Manitoba Health. Names were not stored with the data; instead, patient numbers were used and the results of the surveys were completely confidential. If signed consent forms were not returned, the data from the telephone questionnaires was removed from the database.

3.3.4 Control Group Definition

Individuals in the control group for the study of WNV disease risk factors (study #2) were infected with WNV during the summer of 2003. However, they were asymptomatic or did not experience WNV disease symptoms severe enough to require medical care and treatment. The number of controls per case was maximized. Prior to the MB-PHAC seroprevalence study, it was calculated that there would likely be 45 - 60 in this group (3.1% [CI 2.2 - 4.0%] of individuals in Oakville, ON [133] and 2.6% in New York, NY study [39]). Yet, the total number of seropositive individuals from the MB-PHAC seroprevalence survey was 34.

The individuals who participated in this study were chosen via random telephone number dialing, with the consenting individual with the nearest birth date in the household participating in the study. Therefore, the number of random controls available for analysis was 34 individuals (most of the 1195 individuals who participated in the study tested sero-negative, and others were lost to follow-up or withdrew consent prior to the blood-draw).

Data for this study was collected as part of the MB-PHAC WNV seroprevalence study (the CHS student investigator was listed as a co-investigator for the ethics submission of this study). The sero-prevalence study methodology, consents and surveys have been included in this proposal as Appendices #1, #2, and #3 respectively.

Participation was completely voluntary and if anyone chose not to participate, the surveyor went to the next random telephone number. The target participatory group was 1500 people (approximately 300 each from Brandon, Minnedosa, Virden, Deloraine, and Killarney). Following the telephone interview, the consent form and blood test requisition form were mailed out to the sero-prevalence participant and they were asked to attend a blood drawing clinic in their area. The lab technician at the blood draw clinic ensured that the participant had signed his or her consent form, and drew their blood to test for antibody levels to WNV. The consent, the tube of blood (labeled with the patient study number), and the lab requisition was forwarded to Cadham Lab. From there, the study tubes were sent to the National Microbiology Laboratory where testing for the IgG antibody levels occurred (micro-neutralization West Nile virus IgG assay). The study coordinator for the study, based at Manitoba Health, collected the consent forms, and ensured that the database at Manitoba Health correctly linked the test results with the appropriate patient number. The survey results and the lab results were tabulated and readied for analysis.

3.3.5 Descriptive, Univariate, & Bivariate Analysis

Descriptive statistics and univariate analysis was completed to assess the populations that participated in the case-control study #2. The age and gender distributions were calculated, as well as population distributions over space (where the participants were situated in Assiniboine and Brandon Health districts). Frequency calculations of each risk factor were assessed. The frequency analysis of the case population (39 individuals) and the control group (34 individuals) were compared to determine how similar the control group was to the case population. If the control group did not match well with the case population, the risk factors that varied widely were identified. Further analyses were completed if necessary to determine the cause of the discrepancies.

Bivariate analysis was completed to determine the odds ratios relating WNV disease with a variety of exposures and risk factors. Using age as the risk factor to calculate the power of this study, it was determined (Appendix #9) that with the available sample sizes of 40 cases and a pre-study potential of 45-60 sero-positive controls, the study would have a power of 80% (1 – beta) to detect an odds ratio of approximately 4.0 for the case-control study #2 comparison, with an alpha error of 0.05. However, due to the limited number of individuals who tested sero-positive for the West Nile virus antibody, this group was maximized at thirty-four.

3.3.6 Multivariate Analysis

The following risk factors for WNV were initially entered into a logistic regression model, with case/control status being the outcome of interest:

gardening, walking-jogging, and participating in other outdoor activities. These were the variables which had attained a significance level of <0.10 on bivariate testing (these variables had not reached a statistically significant level of $p<0.05$, however they were trending in that direction). Opinion-related variables (e.g. "Are you aware of how West Nile virus is spread?") and "fever during the summer of 2003" were not entered into the model as these were not plausible risk factors for WNV infection. In this case control study (#2) "inspecting screens" and town of residence were not collinear with other variables. Nonetheless, they were highly correlated and thus were not measuring one quantity alone (Appendix #19, 20). These were removed for analysis. Originally average WNV mosquito risk value and maximum WNV mosquito risk index values were intended to be used for analysis. Because they were based on the town of residence variable, and in this study the town of residence was highly correlated with other variables, these mosquito risk values could not be included. Age and gender were forced into the model at all stages to control for these variables. Once all variables were entered into the model, backwards stepwise logistic regression was used to arrive at a reduced model of significant risk factors ($p<0.05$), while controlling for age and gender. Finally, removed variables were re-entered to ensure they remained insignificant.

3.4 Limitations for Case Control Studies #1 & #2

Certain limitations are present with all case-control studies [134, 135]. All studies analyze numerous variables related to a particular outcome. If enough variables are studied, there is a risk of a statistically significant association based

on chance alone. However, if the sample size remains small, the study has limited power to discover significant variables (Appendix #9). If there were less than 1000 controls in case control study #1 or less than 70 cases, only significant odds ratio differences for variables great than 3 would be discovered (if 80% power and a α-value of 0.05 was required). Thus subtle differences between cases and control would not be found. As well, due to the "rule of 10" [135], very few variables can be included in the final model, especially if the sample size of the control group was less than 50. The rule of ten, when used in logistic regression with unmatched analysis, requires that 10 controls exist for each significant variable included in the final model.

Biases need to be controlled so that the risk of a significant association resulting from chance alone is reduced as much as possible. The common biases involved in case control studies are:

Sampling bias – individuals have unequal chances of being chosen for either the case or control groups.

Diagnostic access bias – not all individuals have equal access to diagnostic testing. This may have occurred as not all family and emergency department physicians would have the same comfort level with ordering the WNV antibody test and may have practices that rely more on their clinical assessment skills.

Diagnostic suspicion – not all diagnostic tests are viewed with the same confidence. As WNV had never entered the Manitoba population previously, this test had likely been ordered very rarely prior to the summer of 2003.

Selection bias – the subjects in the study are not similar enough to one another and to the larger population from which they are drawn. Controls or case samples do not appropriately represent the population at large and would confound the results of the study.

Non-response bias – the individuals who do not respond or are not home at the time of the telephone survey are not comparable to the group which does respond.

Volunteer bias – those that volunteer to participate in the survey are not comparable to the group that does not.

False control bias – the control group is not appropriately chosen and does not properly represent a sample without the exposure [122]. Further discussion about how this affects case control study #2 follows this list.

Measurement insensitivity bias – the instrument used to measure exposure and/or outcome does not have an accurate sensitivity. The WNV test used at the Public Health Agency of Canada is well developed. The test used to screen for IgG antibody was a gold standard ELISA based on the CDC format. The sensitivity is 95% or greater. The assay is however cross reactive with other flaviviruses, so second test was used to confirm that the serum neutralized WNV specific antigen (the plaque reduction neutralization test) [68].

Apprehension bias – individuals surveyed may have difficulties being completely forthcoming with their answers.

Differential recall bias – individuals who must remember actions or opinions from the past. These may not be completely accurate as the experiences in the past

or current knowledge will impact these memories (i.e. individuals with a certain outcomes will remember exposure related variables more significantly than those without that outcome) [134]. People who had been infected and became ill following the WNV outbreak, may remember activities they participated in differently than individuals who have never been ill. Memories of events that were seemly insignificant (such as checking for water in debris, using DEET or checking their window screens), which occurred up to 9 months previously would be very difficult to accurately remember, unless one believed they were directly related to a resulting serious illness.

Expectation bias – individuals participating will provide the interviewer with the response that they feel the interviewer expects.

Attention bias – questions at the beginning of a significantly long survey will be given more attention and thought than the questions at the end of a long survey, where the volunteer has lost interest.

As this study was a typical field epidemiology case-control study, many of these applied.

As well as the above limitations, there were specific limitations in the case control studies completed in this project. The definition of individuals who had been infected was relatively uncomplicated – the individuals either had antibodies to WNV or they didn't. If they had been infected, they had antibodies, if they had not been infected, they didn't. However, the definition of individuals who had been "diseased" by their infection was less simple to determine. Symptoms for WNV disease fall along a continuum. At one end are severe

expressions of disease such as encephalitis or meningitis. On the other side of the continuum are subtle symptoms such as headaches, fevers, and slight muscular pains. Thus determining who had disease and thus fell into one category versus another could be difficult. For the purposes of this project, diseased individuals must have been seen by medical staff and been ill enough to warrant being tested for WNV prior to the MB-PHAC seroprevalence survey. This definition issue may be a limitation of case control study #2.

3.5 Ethical Issues of the Studies

Participants were initially told about the study and were aware of what it entailed prior to completing the survey. They were aware that they were going to be asked to provide a blood sample if they participated in the study. Signed paper consent was required prior to the participant completing the blood draw. The University of Manitoba Bannatyne Campus Health Research Ethics Board reviewed the MB-PHAC seroprevalence study and the protocol was passed with minor revisions (H2003:168 date March 2, 2004). As well, the Health Canada Research Ethics Board reviewed and approved both the seroprevalence study and the case-control study (REB-2003-0059, date April 6, 2004). As the research study described was part of a Masters thesis project, it required individual ethics approval prior to commencement (H2003:168A, date June 24, 2004). Prior to completing any statistical analysis, the investigator confirmed with the study coordinator that each of the cases had returned a signed consent form. Thus, there were no anticipated ethical issues with this study.

3.6 Preliminary studies or pilot tests

There were no pilot studies related to the MB-PHAC seroprevalence study. Nevertheless, it had been modeled after the Oakville, Ontario sero-prevalence study [81]. The Oakville study was completed in 2002 – 2003, following an outbreak in the summer of 2002. The MB-PHAC study was also based on previous surveys and sero-prevalence studies that have been carried out in the United States [23, 28, 39, 74].

4. Results

4.1 Case Control Study #1 – Descriptive, Univariate & Bivariate Analysis

In this study, there were 73 cases (sero-positive individuals) and 1161 controls (sero-negative individuals). The following table describes the gender breakdown:

Table #1: Case Control Study #1 – Gender Breakdown of Cases and Controls

Variable		Case		Control	
		N=73	%	N=1161	%
Gender	male	19	26.0%	356	30.8%
	female	54	74.0%	800	69.2%

The study population came from the Regional Health Authorities of Assiniboine and Brandon. The City of Brandon, in the 2001 census, had 18,770 males (47.3%) and 20, 950 (52.7%) females [136]. General population statistics for the Assiniboine Health Region indicated they had a population gender breakdown of 34,765 males (49.7%) and 35,245 females (50.3%) [137]. So in both the case and the control groups, females were significantly over-represented. There was a significantly statistical likelihood that if you participated in the study you were a female ($p<0.001$). However, the differences of gender distribution between the cases and the control groups, was not significant ($p=0.39$, Chi square value = 0.736, 1df).

The epidemiological curve for this outbreak could not be determined from the data collected from the seroprevalence study. However, Manitoba Health provided epidemiological curves for the specific regional outbreaks for the

summer of 2003 (Appendix #10). There were only 8 cases in Brandon, so the Epi curve does not have the usual bell curve shape. However the Epi curve from the Assiniboine Health Region shows the expected outbreak distribution. These graphs do give some general ideas about the person, place and time variables that influenced this outbreak of West Nile virus in Southwestern Manitoba, during the summer of 2003.

When attempting to distribute the cases and controls into the township areas that were defined as "towns" of interest (Brandon, Minnedosa, Killarney, Virden and Deloraine), 97% of the cases actually lived in or very near (using the town's three digit telephone number) the 5 study towns. Cases were individuals who had become ill during the summer of 2003. These were thus defined prior to the MB-PHAC seroprevalence study. Controls were individuals who had all participated in the seroprevalence survey study so 100% of the controls are from a municipality of one of those five towns. All of the participants from the MB-PHAC seroprevalence study from each of these towns were used as controls for case control study #1.

Although exposure to mosquitoes does occur outside of one's residential area, there are few potential markers of geographical location for participants other than their home address.

Using their town as a marker, they were broken down into five groups:

Table #2: Case Control Study #1 – Geographical Breakdown of Cases and Controls

Town	Case	Control	Participants in Serosurvey	Total Population (2001)
Brandon	6	260	260	39,716
Minnedosa	9	162	162	2,426
Killarney	15	278	278	2,221
Virden	22	281	281	3,109
Deloraine	20	180	180	1,026

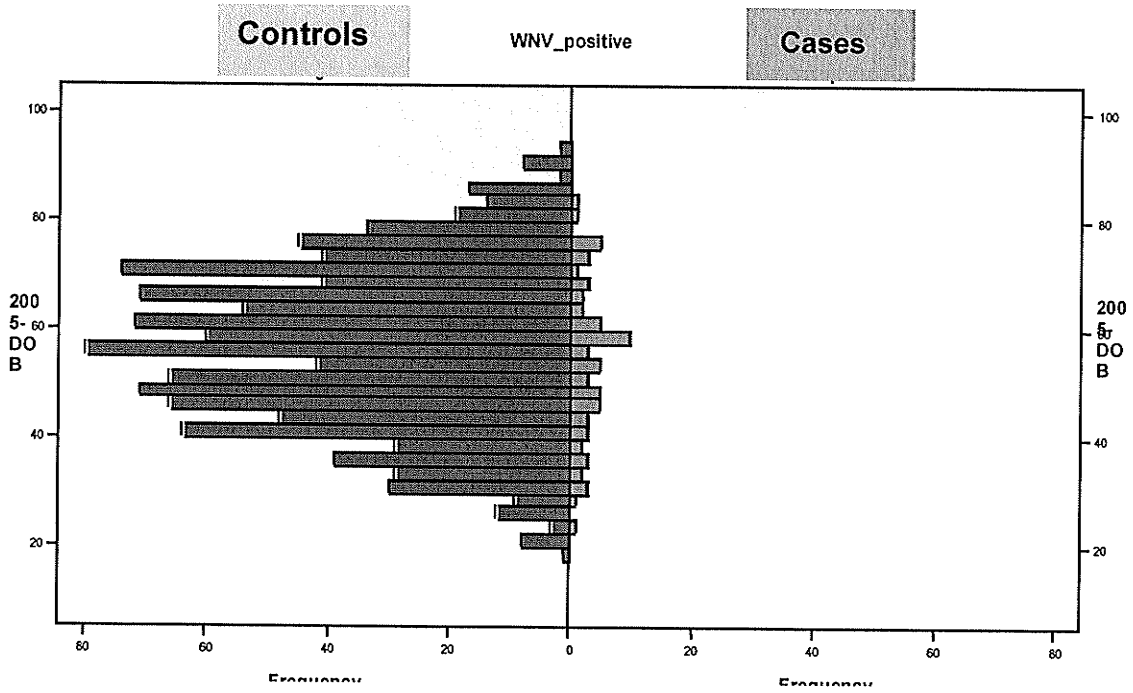
With this information, we can determine the percent of the population which participated in the survey (Of note: as the study design required an attempt for 300 participants from each community, in some smaller communities almost every single household was contacted. However in Brandon, a large city, this was not the case). In Brandon, 0.7% of the population participated, while in Minnedosa and Killarney, 6.7% and 12.5% respectively of the population participated. Virden had a participation rate of 9.0% and Deloraine 17.5%. Thus, over-sampling occurred in some communities and under-sampling occurred in others.

When the odds of WNV infection were studied for each town individually (cases compared to controls for those who lived in the town versus those who did not live in that town), Brandon had a low odds ratio (low chance of being a case), 0.31 (CL 0.12 – 0.74), whereas, Minnedosa, Killarney and Virden all had an odds ratio of around one (equal chance of becoming a control or a case). Their odds

ratios were 0.85 (CL 0.39 – 1.82), 0.81 (CL 0.43 – 1.49), and 1.34 (CL 0.78 – 2.31) respectively. Individuals from Deloraine had relatively higher odds of becoming infected with WNV (increase of the odds ratio above 1). Deloraine had an odds ratio of 2.02 (CL 1.14 – 3.55). It must be noted, however, that these odds ratios are not adjusted for age, gender, or any other variable studied.

The mean age of the case group was 54.32 with a range of 24-84 years of age (SD = 13.87). The mean age of the control group was 56.44 with a range of 19-95 years of age (SD = 15.02). There was no significant difference between the two groups.

Figure #3: Population Pyramid of Case Control Study #1



Education was one of the last variables discussed during the survey. After 25 minutes of participation in the survey, this question had a really low level of completion (52-68% completion rate). Due to the high level of missing values, it

could not be reliably used for any calculations. Occupation was another socio-economic variable included in the survey. This variable had an even lower response rate (23-31%, 23 of 73 cases and 266 of 1161 controls). Although general occupation was noted, some outdoor activities and occupations were specifically questioned (such as camping, fishing or farming). It did seem significant that 73% of the cases who did respond listed farming as their occupation. 52% of the controls listed farming as their occupation. Farming as an occupation was included in the analysis, as it seemed reasonable that it might affect one's exposure to WNV positive mosquitoes, yet results that include this variable need to be viewed with some caution, due to the low response rate for this question. With that said, the exposure risk variable that showed significance was working as a farmer, specifically as a grain or poultry farmer. These variables showed a significant increase in the chances of contracting a WNV infection (general farming 2.23, CL 1.21-4.07; grain farming 2.99, CL 1.56-5.67; poultry farming 8.03, CL 1.00-51.99).

The next set of variables that was explored was the health status indicators. These were all self reported variables, and no charts were screened to determine if some diagnoses were missed or other reports inaccurate.

Table #3: Case Control Study #1 – Health Status Indicators

Variable		Case	%	Control	%
Medical conditions	Present	5	6.8%	54	4.7%
	Not present	68	93.2%	1107	95.3%
Medication that affects the immune system	Present	26	35.6%	379	32.6%
	Not present	47	64.4%	782	67.4%
Cancer	Present	6	8.2%	72	6.2%
	Not present	67	91.8%	1089	93.8%

The health status variables had odds ratios that were not significant. The presence of a medical condition may show an odds of developing a WNV infection of 1.49 (CL 0.51 – 4.03). Using a medication that might negatively influence your body's ability to fight an infection showed an odds of 1.12 (CL 0.66 – 1.88), and having cancer, 1.33 (CL 0.50 – 3.33). These were all insignificant results, however did show some trends that they may increase one's odds of developing WNV infection. Further studies with great power may be needed to determine whether these positive ORs (OR>1.00) actually show an increase in the chances of a WNV infection.

When individual disease diagnoses were studied, most diagnoses had very few numbers of individuals. The diseases under study had been chosen as possible significant diagnoses which might influence the participant's WNV status. However, some diseases had no individuals report this diagnosis. Again, these diseases were all self-reported with no validation through medical records to confirm the findings.

Table #4: Case Control Study #1: Diseases Self-reported During the Serosurvey

Disease		Cases	%	Control	%
Diabetes	Present	1	1.4%	65	5.6%
	Not present	72	98.6%	1096	94.4%
Heart Disease	Present	8	11.0%	151	13.0%
	Not present	65	89.0%	1010	87.0%
Bronchitis	Present	1	1.4%	7	0.6%
	Not present	72	98.6%	1154	99.4%
Asthma	Present	6	8.2%	25	2.2%
	Not present	67	91.8%	1136	97.8%
Emphysema	Present	1	1.4%	2	0.2%
	Not present	72	98.6%	1159	99.8%
RA and Lupus	Present	5	6.8%	33	2.8%
	Not present	68	93.2%	1128	97.2%
OA	Present	3	4.1%	32	2.8%
	Not present	70	95.9%	1129	97.2%
Ulcerative Colitis	Present	0	0%	1	1.0%
	Not present	73	100%	1160	99.0%
Crohn's	Present	0	0%	0	0%
	Not present	73	100%	1161	100%

The following table indicates the odds ratios for each of these diseases. Some appear to trend towards being protective (the odds ratio is below 1.00, which indicates if one has this disease diagnosis, one has a lower odds of

developing a WNV infection) while others appear to trend towards increasing one's odds of developing a WNV infection. Only one variable is significant - asthma (no other variables were controlled for in this bivariate analysis). Things like age, gender and exposure to mosquitoes would still need to be controlled, prior to indicating that this variable actually increased a participant's likelihood of developing a WNV infection.

Table #5:

Case Control Study #1: Disease Diagnoses and Odds Ratios of WNV infection

Disease	Unadjusted Odds Ratio	Confidence Limits
Diabetes	0.23	0.01 – 1.59
Heart Disease	0.82	0.36 – 1.82
Bronchitis	2.29	CL invalid
Asthma	4.07	1.44 – 10.89
Emphysema	8.05	CL invalid
RA and Lupus	2.51	0.83 – 7.03
OA	1.51	0.36 – 5.33
Ulcerative colitis	-	-
Crohn's disease	-	-

Risk factors related to exposure that might show trends towards increases or decreases in one's odds of contracting a WNV infection using bivariate analysis are presented in Appendices #11 and #12. The recreational activities that were associated with an increase in odds of infection were the activities of walking or jogging. There also were personal protective behaviors that were

associated with an increase in one's chances of becoming infected with WNV: burning a coil or burning candles, and inspecting screens to ensure that they were intact. There was one personal protective behavior that decreased the odds of WNV infection: wearing long sleeves and pants while outdoors. In this bivariate analysis, age, gender or other variables could not be controlled.

The number of hours spent outdoors has significant face-validity as being a risk factor, so this variable was included in this study. The results of the survey responses are included in Appendix #13. There were some time periods that showed a significant increase in the odds of becoming infected with WNV (there was a significant difference in the amounts of time spent outdoors between the cases and the controls of this case control study). The hours spent outside during the evenings and the nights were significant. As well, the hours spent outdoors between dusk and dawn were calculated, and they were also significant. The most significant difference in the time spent outside between the cases and the control was the time spent outdoors at night.

So the descriptive and odds ratio data show that certain attributes were associated with an increase or decrease in a participant's likelihood of being infected with WNV. Noteworthy variables from this initial analysis were:

Demographic Factors:

1. Residence in a town with a high mosquito count
2. Or residence in Brandon (protective) and Deloraine (increased chances)

Health Factors:

3. Asthma

Exposure Factors:

4. Farming as an occupation
    - Or Grain farming and Poultry farming
5. Walking-jogging
6. Hours spent outside during the evening and hours spent outside during the night
    - Or total hours spent outside from dusk until dawn
7. Burning a coil
8. Burning candles
9. Inspecting screens

Case control study #1, the study of factors that influenced the odds of getting infected with WNV, did not meet its original targets of 1300 controls and 95 cases. Instead with 1161 controls and 73 cases, different sample sizes were developed. However, as sample sizes were only moderately different than originally planned, odds ratios of between 2.00 and 3.00 remained significant. The power of these new sample sizes allowed for significant odds ratios above 2.20 (power set at 80.0%, α=0.05, 20.00% exposure in the NOT ILL group, see Appendix #14).

4.2 Case Control Study #1 – Multivariate Analysis

Variables that showed a trend towards significance with single variable analysis using logistic regression were: residence in an "High-count Town" (p=0.019), asthma (p=0.004), emphysema (p=0.060), rheumatoid arthritis and lupus (p=0.047), an outside job (p=0.046), farming (p=0.046) [or grain farming

(p=0.0001), poultry farming (p=0.019) and market gardening-bees-beef (p=0.036)], walking or jogging as recreation (p=0.022), hours spent outside in the evening (p=0.049), using mosquito coils (p=0.004), burning candles (p=0.004), wearing long sleeves and pants (p=0.006), using DEET (p=0.014), and taking action on standing water (p=0.059).

When controlling for age, gender and residence in an "High-count Town", the individual variables that retained their trend towards significance (p<0.10) were: asthma (p=0.003), emphysema (p=0.087), rheumatoid arthritis and lupus (p=0.046), outside job (p=0.076), farming (p=0.09) or [grain farming (p=0.001), poultry farming (p=0.033) and market gardening-bees-beef (p=0.066)], walking or jogging as recreation (p=0.024), hours spent outside during the evening (p=0.064), burning a mosquito coil (p=0.003), burning candles (p=0.007), protecting oneself by avoiding areas with high numbers of mosquitoes (p=0.082), wearing long sleeves and pants (p<0.005), using DEET (p=0.011), and taking action on standing water (p=0.064).

The final model for case control study #1 offered the most information about the risk factors for WNV infection that significantly affected those in Southwestern Manitoba during the summer of 2003. These variables were associated with an increased chance of becoming infected. This was an associative relationship and no causal factors were discussed in this model.

Because farming was significant, and when tested only grain farmers within the farming categories was significant, it was difficult to determine which

model actually represented the more accurate picture. Both final models are shown here:

Figure #4:

Model for Case Control Study #1 – Using Farmer variable:

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
							Lower	Upper
gender	-.224	.299	.563	1	.453	.799	.445	1.436
age_appr	-.006	.009	.408	1	.523	.994	.976	1.012
High_count_towns	.627	.302	4.299	1	.038	1.872	1.035	3.385
asthma	1.513	.519	8.488	1	.004	4.541	1.641	12.569
RA/Lupus	1.141	.542	4.424	1	.035	3.129	1.081	9.057
farming	.928	.319	8.456	1	.004	2.530	1.353	4.730
Walk/jog	.611	.269	5.142	1	.023	1.842	1.086	3.123
Burn coil	.821	.390	4.424	1	.035	2.272	1.058	4.882
Burn candles	.698	.325	4.627	1	.031	2.010	1.064	3.797
long_sleeves	.323	.113	8.087	1	.004	1.381	1.105	1.725
Use DEET	.282	.104	7.391	1	.007	1.325	1.082	1.624
Constant	-5.652	.831	46.260	1	.000	.004		

Figure #5:

Model for Case Control Study #1 – Using Grain farmer variable:

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
							Lower	Upper
gender	-.233	.300	.603	1	.437	.792	.440	1.426
age_appr	-.007	.009	.505	1	.478	.993	.975	1.012
High_count_towns	.605	.303	3.982	1	.046	1.831	1.011	3.318
asthma	1.494	.525	8.085	1	.004	4.455	1.591	12.475
RA/Lupus	1.183	.543	4.747	1	.029	3.264	1.126	9.463
Walk/jog	.610	.270	5.117	1	.024	1.841	1.085	3.125
Burn coil	.798	.392	4.155	1	.042	2.221	1.031	4.785
Burn candles	.665	.324	4.210	1	.040	1.945	1.030	3.674
long_sleeves	.321	.114	8.009	1	.005	1.379	1.104	1.723
Use DEET	.289	.105	7.637	1	.006	1.335	1.088	1.640
Grain farming	1.186	.340	12.184	1	.000	3.274	1.682	6.373
Constant	-5.610	.826	46.167	1	.000	.004		

The following demographic factor was associated with an increase in the chance of developing a WNV infection:

- Living in a town with a high mosquito count (increased the odds by 1.83 times or 183%, CL 1.01 – 3.32).

The following health factors were associated with an increase in one's possibility for developing a WNV infection:

- Asthma (increased the odds by 4.5 times or 450%, CL1.59 – 12.48)
- Rheumatoid arthritis or lupus (increased the odds by 3.1 times or 310%, CL 1.13 – 9.46)

The following exposure factors increased one's odds for developing a WNV infection:

- Walking or jogging outside (increased the odds by 1.8 times or 180%, CL 1.09 – 3.13)
- Farming (increased the odds by 2.5 times or 250%, CL 1.35 – 4.73)
- or specifically grain farming (increased the odds by 3.3 times or 330%, CL 1.09 – 1.64).

The following behaviors were associated with an increased chance for WNV infection (that is, not protective in this study):

- Using a mosquito coil (associated with an increase in odds of 2.2 times)
- Burning citronella candles (associated with an increase in odds of 2 times)
- Wearing long sleeves and pants when outdoors (associated with an increase in odds of 1.4 times)
- Using DEET (associated with an increase in odds of 1.3 times)

These behaviors were used when mosquitoes were active in the area, thus these personal protective behaviours may have been confounded as they were used in areas of high levels of mosquitoes.

If the age variable was converted from a continuous into a categorical variable (all individuals younger than 54 in one category and all those over 55 in another), the age variable remained insignificant ($p = 0.564$, CL = 0.511 – 1.443). Thus in this particular case control study, age did not appear to have a significant association with WNV infection. This differs from previous literature.

Primary interactions were also studied. The interaction between variables labeled "High-count towns" and farming, "High-count towns" and DEET use, asthma and DEET use and finally Rheumatoid arthritis/Lupus and DEET use were tested. No primary interactions tested had any significance.

Case control study #1 analysis showed that there were numerous variables that influenced the odds of becoming infected with WNV. The risk factors that increased the possibility of WNV infection were the co-morbidities of asthma, Rheumatoid arthritis or Lupus, and recreational activities of walking or jogging, farming occupations (specifically grain farming), as well as living in a town with high mosquito counts and some personal protective behaviours that were used if mosquito levels were high.

4.3 Case Control Study #2 – Descriptive, Univariate & Bivariate Analysis

In case control study #2, there were 39 cases (sero-positive individuals who had been symptomatic, medically diagnosed prior to the study and required medical treatment for a WNV infection) and 34 controls (sero-positive individuals

who were not diagnosed until their participation in the MB-PHAC seroprevalence study). All of the participants in this study were sero-positive for WNV antibodies. However, only the cases had sought and received medical care for their diagnosis.

The table below shows the gender breakdown of this study:

Table #6: Case Control Study #2 – Gender Breakdown

Variable		Case		Control	
		N=39	%	N=34	%
Gender	male	11	28.2%	8	23.5%
	female	28	71.8%	26	76.5%

The study population for case control study #2 came from the Regional Health Authorities of Assiniboine and Brandon. In the City of Brandon, in the 2001 census, there were 18,770 males (47.3%) and 20, 950 (52.7%) females [136]. General population statistics for the Assiniboine Health Region indicate they had a population gender breakdown of 34,765 males (49.7%) and 35,245 females (50.3%) [137]. In both the case and the control groups, females were significantly over-represented. However, the differences of gender distribution between the case and the control groups were not significant (p=0.78).

The epidemiological curve for this outbreak could not be determined from the data collected from the Seroprevalence study, yet Manitoba Health provided an epidemiological curve for the outbreak in the summer of 2003 (Appendix #10). There were only 8 cases in Brandon, so the Epi curve does not have the usual bell curve shape. However the Epi curve from the Assiniboine Health Region

shows the expected outbreak distribution. These graphs give some general ideas about the person, place and time variables that were influenced during this outbreak of West Nile virus in Southwestern Manitoba, during the summer of 2003.

Table #7: Case Control Study #2 – Geographical Breakdown of Cases and Controls

Town	Cases	Total Population (2001)	Incidence of Cases (per 100,000)	Controls	Participants involved in Serosurvey	Seroprevalence
Brandon	6	39,716	15	0	260	0
Minnedosa	7	2,426	289	2	162	0.012
Killarney	3	2,221	135	12	278	0.043
Virden	10	3,109	322	12	281	0.043
Deloraine	12	1,026	1170	8	180	0.044

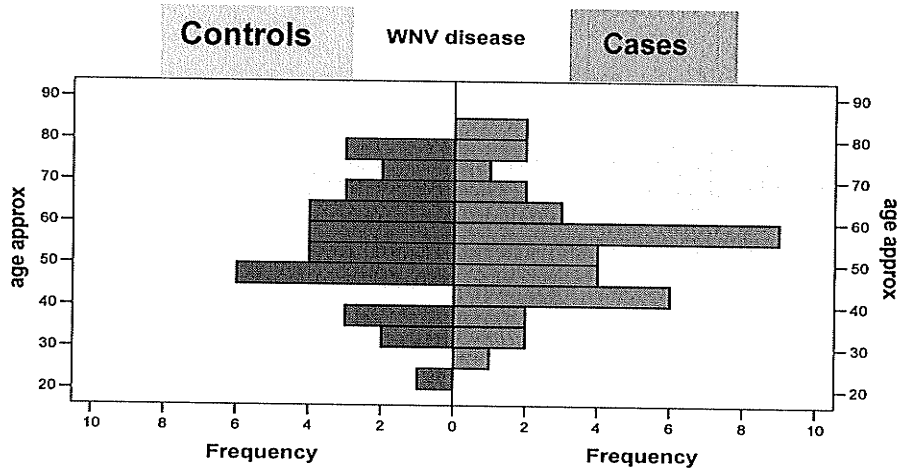
Brandon was unusual as there were only six cases and no controls from this area and it was a well populated area. Thus the incidence of disease in Brandon was very low. There were no individuals found to be seropositive in the 260 who were randomly chosen to participate in the seroprevalence study. However, because this was only a fraction of the total population of Brandon (0.6%), there may have been many others that were sero-positive, which were missed. Minnedosa had a high level of individuals who became infected and then developed symptomatic WNV disease (9/11 or 82%). Thus, Minnedosa's case incidence rate was much higher than Brandon's (289 per 100,000 individuals) on a per capita basis.

The odds of being diagnosed with symptomatic disease compared with asymptomatic infection varied from town to town. Brandon, due to the low

number of controls, had an undefined odds ratio. Minnedosa, due to the high number of cases and low number of controls had an odds ratio of being diagnosed with symptomatic disease of 3.50 (CL 0.59 – 26.58). Deloraine had an odds ratio close to one (1.18, CL 0.38 – 3.72), while Killarney and Virden appeared had a lower odds of developing symptomatic disease (Killarney's odds ratio was 0.57, CL 0.18 – 1.79, and Virden, 0.16, CL 0.03 – 0.71).

The mean age of the case group was 53.92 with a range of 29 to 84 years of age (SD = 13.69). The mean age of the control group was 54.81 with a range of 24-76 years of age. There was no significant difference between these two groups (SD = 13.81).

Figure #6: Population Pyramid of Case Control Study #2



Education was one of the last variables discussed in the survey. After 25 minutes of participation in the survey, this question had a really low level of completion. Due to the high level of missing values, it could not be reliably used

for any calculations. Occupation was another socio-economic variable included in the survey. This variable also had a lower response rate (26-33%, 13 of 39 cases and 9 of 34 controls). There was not a significant difference in the spread of reported occupations between what cases and controls who did participate in this question. Thus, it was not useful to add to the analysis.

The health status variables were the next set of variables that were examined. These were all self-reported through the seroprevalence study completed by MB-PHAC. No follow-up was completed of their medical history and their self-reports were taken at face value.

Table #8 – Case Control Study #2 – Health Status Indicators

Variable		Case	%	Control	%
Medical conditions	Present	15	38.5%	10	29.4%
	Not present	24	61.5%	24	70.6%
Medication that affects the immune system	Present	1	2.6%	3	8.8%
	Not present	38	97.4%	31	91.2%
Cancer	Present	4	10.3%	1	2.9%
	Not present	35	89.7%	33	97.1%

These health indicator variables did not show any significant differences between the case and control groups. The presence of a medical condition showed a trend towards increasing the odds of being diagnosed with symptomatic WNV

disease by 1.375 (CL 0.51 – 3.69). Using a medication that might affect one's immune system was not statistically significant with an odds ratio of 0.25 (CL 0.03 – 2.57). Having cancer was associated with a trend of increasing one's odds of developing WNV disease once infected by 3.54 (CL 0.38 – 33.41).

When individual diseases were studied within this very small sample size, many diseases were reported by only a few individuals. However, to determine if any of these diseases might affect one's likelihood of developing symptomatic WNV disease after becoming infected, all were included in the statistical analysis. Again these diseases were all self-reported with no triangulation by way of medical records to confirm the findings.

Table #9: Case Control Study #2: Diseases Self-reported During the Serosurvey

Disease		Cases	%	Control	%
Diabetes	Present	0	0%	0	0%
	Not present	39	100%	34	100%
Heart Disease	Present	1	2.6%	0	0%
	Not present	38	97.4%	34	100%
Bronchitis	Present	1	2.6%	0	0%
	Not present	38	97.4%	34	100%
Asthma	Present	2	5.2%	3	8.8%
	Not present	37	94.8%	31	91.2%
Emphysema	Present	0	0%	1	2.9%
	Not present	39	100%	33	97.1%
RA and Lupus	Present	0	0%	2	5.9%
	Not present	39	100%	32	94.1%
OA	Present	0	0%	0	0%
	Not present	39	100%	34	100%
Ulcerative Colitis	Present	0	0%	0	0%
	Not present	39	100%	34	100%
Crohn's	Present	0	0%	0	0%
	Not present	39	100%	34	100%

As noted above, there were few individuals in either the control or the case groups who had any specific medical conditions. The following table indicates the odds ratios for each of these diseases. As there were few data

elements to compare between the groups, for most of the diseases it was impossible to complete an odds ratio comparison. Although asthma appeared to be protective, it was not statistically significant.

Table #10:

Case Control Study #2: Disease Diagnoses and Odds Ratios of WNV Disease

Disease	Odds Ratio	Confidence Limits
Diabetes	-	-
Heart Disease	Undefined	-
Bronchitis	Undefined	-
Asthma	0.54	0.09 – 3.45
Emphysema	Undefined	-
RA and Lupus	Undefined	-
OA	-	-
Ulcerative colitis	-	-
Crohn's disease	-	-

Risk factors that were related to exposure variables are presented in Appendices #15 -#17. Risk factors related to exposure that showed some statistical significance were gardening, walking or jogging and inspecting screens for defects. These variables showed a significance difference between the control group (who were infected with WNV, but not previously diagnosed and not treated) and the case group (who had been previously diagnosed and treated for WNV disease symptoms). Two of these variables were recreation-related variables and one was considered a personal protective behavior. All were

associated with an increased chance of developing WNV disease. In this analysis, age and gender as well as other variables were not controlled. The variables related to the number of hours spent outside were also explored. There was no statistical difference between the groups related to time spent outdoors.

When the descriptive and bivariate data was reviewed, there were only four variables that were associated with a trend towards significance:

Demographic Factors

- Living in Virden was protective or individuals were les likely to be diagnosed(odds ratio of 0.16, CL = 0.03 – 0.71),

Exposure Factors

- Gardening, walking or jogging and inspecting one's household window screens increased the odds of developing WNV disease (odds ratios of 4.01 [CL = 1.14 – 14.09], 3.67 [CL = 1.22 – 11.04], and 6.81 [CL = 1.73 – 29.04] respectively).

Case control study #2, the study of factors that influenced the odds of getting symptomatic disease following a WNV infection, did not meet its original targets of 45 controls and 40 cases. Instead with 34 controls and 39 cases, different sample groupings were developed. As sample sizes were moderately different than originally planned, only odds ratios greater than 4.00 were now significant (power set at 80.0%, α=0.05, 20.00% exposure in the NOT ILL group, see Appendix #18). This was accomplished with the actual samples, only if the power was allowed to slip to 70% (β=0.30) instead of the usual 80%. Thus this

study had more limited power than expected, as the control group was smaller than anticipated.

4.4 Case Control Study #2 – Multivariate Analysis

When multivariate analysis was completed for each variable independently using logistic regression, while controlling for age and gender the following variables showed trends towards significance ($p<0.10$): looking for sources of standing water ($p=0.04$), taking action on the sources of standing water ($p=0.07$), gardening ($p=0.10$), walking-jogging ($p=0.03$), participating in other outdoor activities ($p=0.04$).

All variables in the final model are significant except for age and gender which have been forced into the model to control for those attributes. There was no collinearity in the model and no deviant residuals. This model offered the most significant information about the risk factors for WNV disease that affected individuals in Southwestern Manitoba during the summer of 2003. These represent associative relationships and no causal factors were discussed in the model.

Figure #7: Case Control Study #2, Final Model

Variables in the Equation

	B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
							Lower	Upper
gender	-.383	.595	.416	1	.519	.682	.212	2.187
age_approx	-.013	.019	.492	1	.483	.987	.951	1.024
walking	1.373	.633	4.707	1	.030	3.948	1.142	13.648
Constant	.505	1.469	.118	1	.731	1.658		

Age and gender were not significant. While controlling for age and gender, it was found that the activity of walking or jogging outside increased the odds of developing WNV disease symptoms (after being infected) by a factor of 3.95 or 395%.

If the age variable converted from a continuous to a categorical variable (all individuals younger than 54 in one category and all those over 55 in another), the age variable remained insignificant ($p = 0.78$, CL = 0.33 – 2.32). Thus age did not have a significant impact on the development of WNV disease in this case control study.

Primary interactions were also studied. The interaction between variables labeled "walking" and "age", "walking" and "gender", "walking" and residential location were tested. No primary interactions tested had any significance.

Case control study #2 analysis established that there was one variable that could influence the odds of becoming symptomatic with WNV disease following the development of a WNV infection. The risk factor that was associated with an increase in one's chances of developing WNV disease symptoms was the recreational activity of walking or jogging.

4.5 Comments from Participants

Following the MB-PHAC seroprevalence survey, there was an opportunity for the participants to provide the surveyor with some comments. Although these two case control studies utilize secondary analysis of the MB-PHAC study, the comments about the MB-PHAC study do speak to some study design issues of

these studies as well. The comments show evidence of the limitations of both of the case control studies.

This is a general synopsis of what the surveyors shared with the student researcher. Many participants found the survey too long, and their interest waned towards the end. Some participants found that there were too many choices for some of the questions (most answers were structured in a 5 choice sequence, e.g. all of the time, most of the time, some of the time, a little of the time, never). There were participants who had some trouble either understanding the wording of some questions, the grade level of the survey or the use of English as a second language. There were few participants who wished to leave comments in writing.

The only significant comment that approximately five patients stated following their survey participation, was that they had felt somewhat ill during the summer of 2003, and their doctor did not test them for West Nile virus. Although they did not receive medical care for their symptoms, they were never diagnosed and recovered well. As the comments were kept apart from the survey responses, it was impossible to determine if these patients were the individuals who tested positive for WNV antibodies during the MB-PHAC study.

5. Discussion

5.1 Case Control Study #1

5.1.1 Gender

The case control study #1 studied the risk factors for infection with WNV. The proportion of females who participated in the MB-PHAC seroprevalence study was much greater than the general population. When the 356 men and the 800 females who participated were compared to the general population levels in the two regional health authorities (according to the 2001 census there were 53535 males and 56195 females), the men were significantly under-represented. There was a greater likelihood that participants in the study were female. This was not the case with another WNV study that took place during the North American epidemic [112]. It is possible that gender affected responses that participants made during this study, thus controlling for gender throughout the analysis was warranted (occupations, hobbies or personal protective behaviors may be associated with one gender or another). When gender was controlled using regression analysis, it was no longer significant. Yet, due to the possibility of gender confounding other associations, it was necessary to force gender into regression models.

5.1.2 Residential location

Case control study #1 studied the risk factors that influenced WNV infection and location of residence was one of these risk factors. Town of residence (as determined by the first three digits of their telephone number) was not found to be an independent risk factor of WNV infection. The towns that

were chosen to participate in the MB-PHAC seroprevalence study were chosen because of their specific concentrations of infected *Culex tarsalis* mosquitoes. Comparisons were made between areas that had WNV-infected mosquito activity and areas that did not. The five towns that had mosquito traps were Brandon, Minnedosa, Killarney, Virden and Deloraine. Brandon and Minnedosa were chosen to represent areas that had low rates of WNV infected mosquitoes. These were compared to Killarney, Virden and Deloraine where there had been higher levels of WNV infected *Culex tarsalis* mosquitoes found in the town traps.

The gradient of levels of WNV infected mosquitoes did not necessarily correlate exactly with related levels of human disease incidence. The highest levels were consistent – Deloraine had the highest levels of WNV positive mosquitoes and positive mosquito pools. Deloraine had the highest human incidence of WNV infections as well, 1170 cases per 100,000 individuals. There was a medium level of incidence in Minnedosa, Killarney and Virden (289, 135 and 322 cases per 100,000 individuals respectively), even though Minnedosa had been classified as an area of lower infected mosquito activity. There was a low incidence of cases in Brandon (15 cases per 100,000 individuals).

Case control study #1 was designed to determine the risk factors related to becoming infected with WNV, and so grouping the participants according to whether their town was experiencing a high level of WNV infected mosquitoes or a low level of infected mosquitoes seemed scientifically and statistically appropriate. The variable "High-count town" was used to group Killarney, Virden and Deloraine, as these were the towns with the highest number of WNV infected

mosquitoes. However, with these three towns grouped for analysis, human incidence rates of 135, 322 and 1170 per 100,000 individuals respectively, were being compared to Brandon and Minnedosa incidence rates of 15 and 289 per 100,000 individuals. Thus, the use of groupings that related to the mosquito WNV infection rates may not actually be a relevant risk factor. Minnedosa was considered an area that had low WNV activity (due to the lower rates of WNV infected mosquitoes); nevertheless, its human incidence levels were comparable to Virden's once they were translated from crude numbers into incidence rates based on population levels in those communities.

There may have been altered rates of prevalence of WNV infected participants in the MB-PHAC study if the study populations in each community were relative to their population size. Then the under-sampling of Brandon and the over-sampling of communities such as Killarney and Deloraine might not have skewed results towards higher prevalence and the seroprevalence rates found for sero-positive individuals would represent the entire region. However, with the study design as it stands, it does show that different communities may have different ecosystems and thus diverse mosquito activities, human behaviours, weather and reservoir activities. One must also understand that human and mosquito populations do not stay within town borders for all of their activities and thus infections may have developed from a bite which took place far outside of their town of residence.

5.1.3 Age

In the descriptive analysis, age was not associated with an increase in one's odds for WNV infection. This is inconsistent with other research studies in this field [19, 74, 84, 112]. Once other variables were introduced using logistic regression, the variable age continued to not be significant with this participant population. Age can influence the activities one participates in, as well as how one understands new public health messages [24, 73, 75]. When logistic regression was used for analysis, age was forced into the model, not because it added to the final model, but rather because this controlled for age's influence on other risk factors.

5.1.4 Occupation and Education

It is regrettable that these two variables did not have sizeable data to use for analysis, either in descriptive or regression analysis. With the use of an education variable, further study might have been possible to determine if resources for public health education were required at different reading levels and whether individuals with certain levels of education were at more risk and were not aware of public health messages that were being delivered. Different socio-economic status indicators would also be useful to complete demographic analyses and would determine what type of population participated in the study. This would be especially useful when comparing the study results to regional averages in those areas.

Farming was associated with an increased odds of developing a WNV infection. However, as there had been such as low response rate to the question

regarding occupation, it was difficult to determine if farming was a significant risk factor. This issue also applies to the specific type of farming that was associated with an increased chance of infection, grain farming.

When logistic regression was applied to these variables, while forcing in age and gender (together with significant health status variables), it was found that being a farmer remained associated with significant odds for becoming infected with WNV. When specific types of farming were tested with the final model, only one type was found to remain significant, and that was grain farming. Farmers were generally at risk, even though the time spent outdoors variable was not significant. Why grain farmers were specifically at risk seemed somewhat perplexing. They work in relatively controlled conditions, with the majority of them working throughout the day in air-conditioned vehicles (trucks, combines, trackers etc.). They do need to leave these environments frequently to change machinery, check crops or fix things; however, during the summer, likely most of their work takes place during the daylight hours. However, WNV season becomes more active towards the end of summer and into autumn, when grain farmers are suddenly working extremely long hours into the evenings. Other types of farmers may not experience the sudden increase in outdoor activities that corresponds so well to the increase of WNV-infective mosquitoes.

Grain farmers' association with an increased odds for infection should be noted for public health education and they may need to be reached with appropriate materials, prior to harvest, when they are too busy to concern themselves with public health media releases or routine medical visits. It might

also be worthwhile for further studies to examine if farming remains a significant factor in larger, more powerful studies. In summary, although these occupations may have been associated with a greater chance of WNV exposure, it is difficult to confidently propose this without further study.

5.1.5 Health Status Indicators

Health status indicators including: self-reported cancer, medical conditions in general or the use of any immunosuppressive medication did not show significant association with WNV infection. However, there were specific diseases, when the participants were polled about what medical conditions they could report, that showed some significant differences between the cases and the controls. As there was a high level of participation in this section of the survey, there was enough power to show even slight differences between the two groups (cases and controls). Although diabetes has been shown to be a risk factor in previous seroprevalence studies [24, 138, 139], it was not present in significant numbers in this sample population and therefore, not significant in this study. Some other diseases that were considered scientifically probable influences of WNV infection were also not significant. Heart disease, bronchitis, emphysema, osteoarthritis, ulcerative colitis, and Crohn's disease were not significant in either the descriptive results, bivariate calculations (odds ratios) or the regression analyses. The Bonferroni correction was not used during bivariate analysis, as multiple comparisons between a series of paired values was not completed (e.g., Aa compared to Bb, Bb compared to Cc, and Cc then compared to Aa) [140]. Each disease was individually compared to WNV sero-status.

Asthma was significantly associated in both the descriptive and odds ratio results. It originally showed an odds of 4.01, meaning that if an individual was diagnosed with asthma, he/she had a 4 fold increase in the odds of developing a WNV infection. Once age, gender and other influencing variables were controlled for, using logistical regression, the odds of developing a WNV infection if one had asthma, increased to 4.50. This variable remains highly significant throughout the logistical regression ending in the final model with a significance of $p<0.005$. This is an association that has not been previous documented in the literature. The significance of this association is unclear. The number of individuals, with asthma who were found to be infected with WNV, was quite small (6), and there is a possibility that this variation occurred due to the low numbers of asthmatics in the control group. The proportion of controls with a history of asthma (2.2%) was lower than expected in the general population. Therefore, this could have biased the results in favor of an association with WNV infection. This association would need to be further studied. It should also be noted that this diagnosis was not associated with an increased chance of developing WNV disease.

Two other diseases that are immunologically related to asthma are Rheumatoid arthritis and Lupus. They were grouped into one risk factor for analysis in these case control studies. This factor did not show significance in bivariate analysis; however, once other variables, such as age and gender, were controlled, it became significant. After all significant variables in this case control study, as well as gender and age, were analyzed using logistical regression, this

risk factor (Rheumatoid arthritis and Lupus) showed a positive association with WNV infection. Rheumatoid arthritis, Lupus and asthma are diseases that are associated with certain unique shifts in the T cells of symptomatic individuals. The T helper cells of these individuals shift away from predominantly T type 1 helper cells to T type 2 helper cells. T type 2 helper cells are associated with auto-immune diseases, allergies and asthma [141-143]. However, causal relationships have not yet been found between the T cell changes and the associated diseases. Knowing that they do have immunological similarities, though, and discovering that Rheumatoid arthritis, Lupus and asthma may all significantly increase one's likelihood of WNV infection, might encourage further research in this area.

5.1.6 Exposure Risk Factors

Some activities were associated with increased odds of becoming infected with WNV, while others were associated with a decreased odds. Walking or jogging was significantly associated with the chance of becoming infected with WNV. This variable may have been significant for the simple reason that it required time spent out of doors (as time spent out doors was also significant in bivariate analysis).

Theoretically, the number of hours spent outdoors could greatly influence a participant's odds of becoming infected. If a participant spent a considerable time outside, they may have been exposed to virus-carrying mosquitoes [110, 113, 144], although this might not always have be the case. If communities used pesticide and the mosquito counts were low, then a long period of time spent

outdoors would not increase the likelihood of WNV infection. There may be few mosquitoes in the area, fewer infected mosquitoes or the individual may be wearing DEET, which protects the individual from mosquito bites. In summary, although hours spent outdoors may influence one's risk of contracting a WNV infection, this variable also depends on many other factors.

Regular walking or jogging was associated with an increase in the participants' chance of WNV infection, even when age and gender and other health status indicators were controlled, using logistic regression. These individuals likely had long standing habits of routine outdoor activity, which took place after or before work (at dusk and dawn). As well, if they were jogging at a sufficient pace, they would have been perspiring and it would have been difficult to maintain an appropriate level of mosquito repellent. There is no significant interaction between using DEET and jogging, so this conclusion can not be substantiated. However, it is plausible that the increased level of activity outdoors increased these individuals' exposure to WNV infection.

There were 3 activities in bivariate analysis and 4 activities in logistic regression analysis associated normally as protective behaviours that showed an increase odds of WNV infection with this study. They were burning a coil, burning citronella and other candles, and inspecting screens to ensure the mosquitoes could not get into the house (plus using DEET in the logistical regression analysis). These activities would be highly correlated with increases in mosquito activity or mosquito concentration levels (as the number of mosquitoes increases, and they become a nuisance, these activities may also

increase). Thus, these activities themselves are not plausible risk factors for the acquisition of WNV infection.

5.1.7 Limitations, confounding and biases of Case Control Study #1

There are several types of bias which could potentially influence the results of this study. Attempts were made during the MB-PHAC seroprevalence study to control sampling bias and selection bias. However, most respondents were women, so these biases may have influenced the results of this case control study which used the MB-PHAC data. The control group may not have been fully representative of the general population at risk for WNV infection.

There were other biases that may have had an effect on survey outcomes. Diagnostic access and diagnostic suspicion biases may have occurred during the summer of 2003 as some medical practitioners in Southwestern Manitoba were reluctant to test for West Nile virus, and others felt the diagnosis was not necessary unless the patient required hospitalization. Non-response bias was difficult to determine, as individuals who did not contribute were not available to compare to the participants. Likewise, volunteer bias was also difficult to determine, as non-participants were not available to complete comparisons. Since the survey was not measured for specificity or sensitivity, measurement insensitivity bias may have affected the results (if the survey did not actually measure what it was designed to measure). Attention bias was a factor during the MB-PHAC seroprevalence study. It resulted in significantly higher levels of missing values for the questions towards the end of the survey. Lastly, a significant bias that could not be easily controlled was differential recall bias.

Some of the cases were aware of their WNV diagnosis, which could have altered their responses to survey questions. This recall bias may have actually heightened the cases' memory of activities, whereas the controls may have had poor memory of events that took place the previous summer. This, however, could not be avoided with a retrospective field study design.

The limitation of small numbers of individuals having specific disease diagnoses may have had an impact on the significance of the results. It was unusual that there was such a low asthma rate in the control population (it was approximately a third of the known asthmatic rate for Canadian adults). As the controls have a low rate and the cases have a rate that is consistent with the Canadian average of approximately 6% [145], it could have distorted the results.

As the questions that were used during the survey were not overtly personal, apprehension bias was likely not a noteworthy issue during this study. As well, as individuals were not under pressure to answer questions and surveyors had no conflicts of interest with the participants, expectation bias likely had little impact on the final results. The control group was logically chosen. Cases were all WNV sero-positive and the controls were all sero-negative, therefore false control bias was unlikely.

The study was not able to determine exactly where in Manitoba cases had been infected and, thus, the student researcher was not able to complete any geographical or spatial analysis. The survey had a significant level of face-validity and looked reasonable as an approach to determine individual risk factors for West Nile infection. Concurrent validity was present as well, as the

questions about behaviours actually tested if the individuals surveyed acted on their opinions. In order to develop content validity, the committee responsible for the MB-PHAC seroprevalence survey's design employed previously used questions from other surveys that had resulted in publishable results related to risk factors. Predictive validity, which is validity of the instrument to be used as a predictive tool, was not measured.

Internal validity (whether the responses were accurate for the test groups) could not be scientifically determined with this study design, as the behaviours took place in the past, and there was no chart review of the participants to determine if they had given accurate information related to their health status. The results were likely not due to the effects of chance, bias or confounding due to poor study design or execution. Thus, a certain amount of internal validity did exist for this study; however, it could not be quantified.

The survey tool was based closely on previously validated WNV seroprevalence survey questionnaires (New York and Oakland, Ont.). However, as it had acceptable face-validity, the newly developed telephone survey was not pilot-tested for reliability or validity prior to its initiation in the MB-PHAC seroprevalence study. Nevertheless, prior to completing any analysis, the student researcher did test the tool for test-retest validity and content validity using volunteers outside of the study population. The survey instrument did show reliability when tested by this study researcher following the MB-PHAC seroprevalence study. When the questionnaire was repeated two weeks after initial use with a small group of volunteers, there was 94% test-retest reliability.

The results remained stable, consistent and were reproducible. Lastly, inter-rater reliability was checked with a high success rate (test scenarios were tested with the surveyors). As the data was entered directly into the database, there were no transcription errors. Nevertheless, there may have been inadvertent errors in data entry that were not correctable, even following data cleaning.

5.2 Case Control Study #2

5.2.1 Gender

Case control study #2 was a much smaller research study. In both the case and the control groups, the majority of the participants were females. This was significantly different than the actual population gender breakdown for both the Brandon and Assiniboine regions. Females were significantly over-represented in both of the study groups, and although that needs to be noted, the groups themselves were not significantly different.

In other seroprevalence studies, males had higher odds of becoming infected [112]. Thus it was interesting that in this case control study there was a high level of females who became sero-positive (in both the case and the control groups). In the MB-PHAC seroprevalence study there were 800 females who participated and completed their blood screening for WNV. It is likely that females were more open to participate in this survey as this was a rural community and the surveying took place after normal working hours. This could have been when men were still actively working on their rural occupations and women were home due to other commitments (families).

As was stated in the results, there were proportionally more females that participated in the serosurvey (70%), and thus it is not surprising that a greater number found to be seropositive were women. Because females were over-represented in the sero-survey, they were likewise over-represented in the positive tests from that study. The fact that so many females were involved in the study could alter some of the survey results. Occupations, recreational activities, and attitudes may be gender related. However, since the groups are not statistically different, both have proportionally high levels of females participating, and gender was controlled during the logistical regression, this difference from the general population is not disquieting.

5.2.2 Residential Location

Residential location indicates where these individuals may have spent more of their time, but as mosquitoes do not stay within the town limits, neither do humans. Activities beyond the small ecosystem surrounding their home were very likely. Many small ecozones are likely to have significantly different mosquito activity and rates of WNV infection. However, for this analysis, residential location was the only geographical variable that was easily captured. Participants in this study were included if they had not left their local community for more than six weeks. This did allow them to be away from their local community for up to 6 weeks, which is a significant portion of time. This was a significant limitation of the study.

Most WNV seroprevalence studies in the literature show population seroprevalence after outbreaks of approximately 3% [20, 71, 74, 81]. The

seroprevalence rate in the MB-PHAC study for Brandon and Minnedosa (0 and 1.2% respectively) may reflect that these communities did have lower rates of infected *Culex tarsalis* mosquitoes in their communities. However these rates are from the seroprevalence study and may not indicate the rates that actually exist in the populations as a whole. In the MB-PHAC study, Killarney, Virden and Deloraine had comparable rates of asymptomatic WNV infections due to WNV exposure (4.3, 4.2 and 4.4% respectively). These were the communities where the traps that Manitoba Health reviewed, to determine the mosquito population numbers and infectivity, showed increased numbers of infected *Culex tarsalis* mosquitoes.

5.2.3 Age

In the descriptive and bivariate analysis as well as in the multivariate analysis, age was not found to be statistically significant (i.e. it did not appear to have any effect on whether one developed WNV disease symptoms following WNV infection). This varies from some other publications which do show an increase in the severity of symptoms and sequelae with increased age [78, 83-85, 138].

5.2.4 Occupation and Education

It is regrettable that these two variables did not have useable data for either descriptive or regression analysis in this case control study, due to a low response rate. With responses that were given, there did not seem to be any significant difference between the case and control groups. With the use of these variables, further study might have been possible to determine if resources

for public health education were required at different reading levels and whether certain occupations were at more risk or were less aware of public health messages being delivered. Different socio-economic status indicators are also useful to complete demographic analyses and determine what type of population participated in the study, in order to compare to the regional averages in those areas.

5.2.5 Health Status Indicators

The hypothesis that personal health status would affect the development of WNV disease symptoms, once one had been infected with WNV, was not supported by the study findings. No health status indicators were significant in the analyses; this may be due to the limited sample sizes that were used. In this study, there were no health related factors that increased the likelihood that infection would progress to symptomatic disease, but very few illnesses were even present in the sample groups. Tests using primary interactions with health status indicators and multivariate analysis of health status indicators produced no significant results. This finding contradicts what some other researchers have found. Several researchers argue that with an increasing load of co-morbid diseases, WNV may have more serious outcomes [21, 83], while others have found specific diseases (such as diabetes) increase the likelihood of developing WNV disease symptoms [138, 139]. With the low sample size of this study, there was insufficient power to study the impact of co-morbidity. The initial power calculation required a larger control group (45-60, not 34) to be able to detect an odds ratio of 4.0, which is significantly high and not found with any variables

during this analysis. Further studies, with larger groups may have more significant health related results to discuss. Specifically areas that have shown some increase in risk in other studies should be further assessed with larger studies whenever possible.

5.2.6 Exposure Risk Factors

There was only one exposure variable that was significantly different between the case and control groups: walking/jogging. Exposure to mosquitoes during this activity would likely occur prior to or after working hours. These are also the times when mosquitoes carrying WNV are the most active. It had been hypothesized that there may be a dose-related response; either an increased number of bites from WNV infected mosquitoes or high load dose injected by an infectious mosquito into a human, which would then develop significant symptoms of WNV fever or encephalitis. However, this was not confirmed in this analysis. More research surrounding this theory would need to be conducted to support these conclusions.

5.2.7 Limitations, confounders and biases of Case Control Study #2

Certain limitations are present with all case-control studies. Two of these are recall and volunteer biases. As this study was a typical field epidemiology case-control study, these need to be recognized as potential limitations. Biases may have had an effect on the survey outcomes.

Diagnostic access and diagnostic suspicion biases may have occurred during the summer of 2003 as some medical practitioners in Southwestern Manitoba were reluctant to test for WNV and others felt that the specific

diagnosis was not necessary unless the patient required hospitalization. If physicians were not comfortable treating WNV disease, they may not have submitted samples for this test. As well, as little was known about how to treat the symptoms successfully, they may have decided that a positive test would not change their treatment choices, thus it was unnecessary. Therefore, some of the individuals classified as controls for this study may have actually been cases, if their doctors had completed the diagnostic testing as requested by the patient. The disease as a whole may have been under-diagnosed this summer as it had not previously been seen in Manitoba. However as most of these cases must have been mild (for if they had been hospitalized, further testing would have been completed), with the definition of case as specific as it stands, this particular limitation is not seriously critical to the results of this study.

Non-response bias was difficult to determine, as individuals who did not contribute, were not available to compare to the participants. Likewise, volunteer bias was difficult to determine, as non-participants were not available to complete comparisons. Attention bias was a factor during the MB-PHAC seroprevalence study, shown with the higher number of missing values for the questions towards the end of the survey. Lastly, recall bias was difficult to control as this was a retrospective study. As all of the cases were aware of their WNV diagnosis, this fact alone could have altered their responses to survey questions.

Sampling bias, popularity bias, and selection bias were attempted to be controlled when the MB-PHAC seroprevalence study was designed. As the questions that were used during the survey were not overtly personal,

apprehension bias was likely not a noteworthy issue during this study. Expectation bias was not an issue during the MB-PHAC study, as participants did not have any relationship with the surveyors.

There is some concern that a number of individuals, who had been classified in this study as controls (as they had not been diagnosed during the summer of 2003 and received medical attention), were actually cases. This misclassification risk may have been due to different medical care strategies used by specific physicians or different communities. This was the first year that WNV was present in the human population in Manitoba, so if some individuals only displayed evidence of fever, they may not have been diagnosed. Thus, for the purposes of this study, they would have been misclassified. There was no element of the study design that could control this.

The study was not able to determine where in Manitoba, the cases had been infected and the study researcher was not able to complete any geographical or spatial analysis. The rural areas of Manitoba each have a specific postal code for a large area, so using this as an address was not specific and did limit the study's conclusions and generalizations.

Although the survey tool was based closely on previously validated WNV seroprevalence questionnaires (New York and Oakland, Ont.), the newly developed telephone survey was not pilot-tested for reliability or validity prior to its initiation in the MB-PHAC study. The study questionnaire had acceptable face-validity and prior to completing any analysis the researcher tested the tool for test-retest validity and content validity using volunteers outside of the study

population. Inter-rater reliability was checked with a high success rate (test scenarios were tested with the surveyors). As the data was entered directly into the database, there were no transcription errors. However, there may still have been errors in data entry that were not correctable, even following data cleaning.

5.3 Significance of Studies

This study was practical and had immediate potential use, in terms of policy planning for the public health departments of Manitoba Health and Health Canada. Other provinces in Canada have had outbreaks of WNV since the summer of 2002 and they might benefit from understanding the presentation of WNV disease in a Canadian population. Manitoba was the first Prairie province to initiate both a seroprevalence study and case-control studies and all Prairie provinces have since commenced WNV seroprevalence studies. All public health professionals benefit when information is shared and emerging diseases are better understood. Additionally, since there have been very few published case-control studies with sero-positive individuals comparing the diseased and the non-diseased, this study will add to the growing mass of information that exists for WNV outbreaks in North America.

6. Conclusions

Individuals in southwestern Manitoba experienced an outbreak of West Nile disease during the summer of 2003. Different risk factors were associated with WNV infection and WNV disease. Asthma, rheumatoid arthritis/lupus, and walking/jogging were associated with an increased odds of developing a WNV infection, when controlling for location of residence, gender and age. Farming may also be associated with an increased odds of WNV infection. Walking/jogging was associated with an increased chance of developing WNV symptomatic disease, when controlling for gender and age.

When public health departments are preparing WNV education strategies and program plans, specific populations of individuals at risk may need to be targeted. As well, individuals who enjoy specific outdoor activities, such as walking or jogging, need to know that this may increase their risk for infection or developing WNV disease. Individuals who participate in such activities need to be strongly encouraged to use personal protective behaviours, especially if they live in rural areas, where large pesticide control measures are less effective.

As debates about the effectiveness of using large ground or aerial mosquito control programs in country areas continue and as environmental lobbyists persist to question the reliance on chemical methods of pest control, rural municipalities with arboviruses must attempt to think of solutions that may be significantly different than urban programs. Education, although beneficial, may not deal with the overwhelming environmental differences between these two settings.

Further research and business developments in mosquito control in these types of settings would be highly advantageous. Low levels of pesticides or herbicides routinely added to some crops might allow for the inclusion of chemicals that would assist with some mosquito control in rural areas. Although the addition of more chemical pollution in the environment may raise other issues, if determined to have a low or negligent impact, they may also assist with the growth of mosquito populations in ditches, where runoff from the fields would include these chemicals. As well, as new mosquito repellants are developed, which have less harmful effects on humans and other animals (e.g. permethrin, as it is used on mosquito netting), some farm apparel manufacturers might consider including this chemical in its outdoors wear. If this proves to be useful in reducing mosquito infections and has not harmful side-effects, public health departments may need to lobby governments to allow tax breaks for these specific businesses.

Research development is needed in many areas surrounding WNV and its emergence in North America. These two case control studies, which focus on the risks for WNV infection and WNV disease, indicate a few areas that could lead to future ventures. The areas of asthma, rheumatoid arthritis and lupus and how these diseases may or may not increase the odds of developing a WNV infection, needs to be further studied using case control studies, seroprevalence studies and basic science. Larger studies, also set in rural communities may definitively discover if farming is associated with an increased chance of WNV infection. Additionally, research that discovers why walking or jogging has been

associated with an increased odds of WNV disease would further expand the knowledge base that is just beginning to be developed about the North American WNV emergence.

7. References

1. Hayes, C.G., *West Nile virus: Uganda, 1937, to New York City, 1999.* Ann N Y Acad Sci, 2001. **951**: p. 25-37.
2. Smithburn, K.C., et al., *A neurotropic virus isolated from the blood of a native of Uganda.* American Journal of Tropical Medicine, 1940. **20**: p. 471 - 492.
3. Padbidri, V.S., et al., *A serological survey of arboviral diseases among the human population of the Andaman and Nicobar Islands, India.* Southeast Asian J Trop Med Public Health, 2002. **33**(4): p. 794-800.
4. Bakonyi, T., et al., *Novel flavivirus or new lineage of West Nile virus, central Europe.* Emerg Infect Dis, 2005. **11**(2): p. 225-31.
5. Kramer, L.D. and K.A. Bernard, *West Nile virus infection in birds and mammals.* Ann N Y Acad Sci, 2001. **951**: p. 84-93.
6. McLean, R.G., et al., *West Nile virus transmission and ecology in birds.* Ann N Y Acad Sci, 2001. **951**: p. 54-7.
7. Rappole, J.H. and Z. Hubalek, *Migratory birds and West Nile virus.* J Appl Microbiol, 2003. **94 Suppl**: p. 47S-58S.
8. McLean, R.G., et al., *West Nile virus in livestock and wildlife.* Curr Top Microbiol Immunol, 2002. **267**: p. 271-308.
9. Garmendia, A.E., H.J. Van Kruiningen, and R.A. French, *The West Nile virus: its recent emergence in North America.* Microbes Infect, 2001. **3**(3): p. 223-9.
10. Jacobson, E.R., et al., *West Nile virus infection in farmed American alligators (Alligator mississippiensis) in Florida.* J Wildl Dis, 2005. **41**(1): p. 96-106.
11. Root, J.J., et al., *Serologic evidence of exposure of wild mammals to flaviviruses in the central and eastern United States.* Am J Trop Med Hyg, 2005. **72**(5): p. 622-30.
12. Valiente Moro, C., C. Chauve, and L. Zenner, *Vectorial role of some dermanyssoid mites (Acari, Mesostigmata, Dermanyssoidea).* Parasite, 2005. **12**(2): p. 99-109.
13. Mumcuoglu, K.Y., et al., *Argasid ticks as possible vectors of West Nile virus in Israel.* Vector Borne Zoonotic Dis, 2005. **5**(1): p. 65-71.
14. Lawrie, C.H., et al., *Susceptibility of mosquito and tick cell lines to infection with various flaviviruses.* Med Vet Entomol, 2004. **18**(3): p. 268-74.
15. Gubler, D.J., *Human arbovirus infections worldwide.* Ann N Y Acad Sci, 2001. **951**: p. 13-24.
16. Kulasekera, V.L., et al., *West Nile virus infection in mosquitoes, birds, horses, and humans, Staten Island, New York, 2000.* Emerg Infect Dis, 2001. **7**(4): p. 722-5.
17. Turell, M.J., et al., *Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus.* J Med Entomol, 2001. **38**(2): p. 130-4.

18. *The buzz on West Nile.* Harv Health Lett, 2003. **28**(8): p. 7.
19. Ford-Jones, E.L., et al., *Human surveillance for West Nile virus infection in Ontario in 2000.* Cmaj, 2002. **166**(1): p. 29-35.
20. *Serosurveys for West Nile virus infection--New York and Connecticut counties, 2000.* MMWR Morb Mortal Wkly Rep, 2001. **50**(3): p. 37-9.
21. Aronson, S.M., *The hidden vulnerable among us.* Med Health R I, 2003. **86**(1): p. 3.
22. Bertrand, T. and U. Bandy, *West Nile virus surveillance and prevention.* Med Health R I, 2000. **83**(5): p. 160-1.
23. Hadler, J., et al., *West Nile virus surveillance in Connecticut in 2000: an intense epizootic without high risk for severe human disease.* Emerg Infect Dis, 2001. **7**(4): p. 636-42.
24. Nash, D., et al., *The outbreak of West Nile virus infection in the New York City area in 1999.* N Engl J Med, 2001. **344**(24): p. 1807-14.
25. Ilkal, M.A., et al., *Experimental studies on the susceptibility of domestic pigs to West Nile virus followed by Japanese encephalitis virus infection and vice versa.* Acta Virol, 1994. **38**(3): p. 157-61.
26. Shaman, J., J.F. Day, and M. Stieglitz, *Drought-induced amplification and epidemic transmission of West Nile virus in southern Florida.* J Med Entomol, 2005. **42**(2): p. 134-41.
27. Godsey, M.S., Jr., et al., *West Nile virus epizootiology in the southeastern United States, 2001.* Vector Borne Zoonotic Dis, 2005. **5**(1): p. 82-9.
28. Blackmore, C.G., et al., *Surveillance results from the first West Nile virus transmission season in Florida, 2001.* Am J Trop Med Hyg, 2003. **69**(2): p. 141-50.
29. Brownstein, J.S., et al., *Spatial analysis of West Nile virus: rapid risk assessment of an introduced vector-borne zoonosis.* Vector Borne Zoonotic Dis, 2002. **2**(3): p. 157-64.
30. Campbell, G.L., C.S. Ceianu, and H.M. Savage, *Epidemic West Nile encephalitis in Romania: waiting for history to repeat itself.* Ann N Y Acad Sci, 2001. **951**: p. 94-101.
31. Enserink, M., *Infectious disease. West Nile's surprisingly swift continental sweep.* Science, 2002. **297**(5589): p. 1988-9.
32. Miller, B.R., et al., *First field evidence for natural vertical transmission of West Nile virus in Culex univittatus complex mosquitoes from Rift Valley province, Kenya.* Am J Trop Med Hyg, 2000. **62**(2): p. 240-6.
33. Turell, M.J., et al., *Potential North American vectors of West Nile virus.* Ann N Y Acad Sci, 2001. **951**: p. 317-24.
34. Kilpatrick, A.M., et al., *West Nile virus risk assessment and the bridge vector paradigm.* Emerg Infect Dis, 2005. **11**(3): p. 425-9.
35. Rainham, D.G., *Ecological complexity and West Nile virus: perspectives on improving public health response.* Can J Public Health, 2005. **96**(1): p. 37-40.
36. Barker, C.M., W.K. Reisen, and V.L. Kramer, *California state Mosquito-Borne Virus Surveillance and Response Plan: a retrospective evaluation using conditional simulations.* Am J Trop Med Hyg, 2003. **68**(5): p. 508-18.

37. Bunning, M.L., et al., *Experimental infection of horses with West Nile virus and their potential to infect mosquitoes and serve as amplifying hosts.* Ann N Y Acad Sci, 2001. **951**: p. 338-9.
38. Murgue, B., et al., *West Nile in the Mediterranean basin: 1950-2000.* Ann N Y Acad Sci, 2001. **951**: p. 117-26.
39. Mostashari, F., et al., *Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey.* Lancet, 2001. **358**(9278): p. 261-4.
40. Platonov, A.E., *West Nile encephalitis in Russia 1999-2001: were we ready? Are we ready?* Ann N Y Acad Sci, 2001. **951**: p. 102-16.
41. Anderson, R., *Mosquito habitats in Manitoba*, M. Dyck, Editor. 2005: Winnipeg, MB.
42. Dokland, T., et al., *West Nile virus core protein; tetramer structure and ribbon formation.* Structure (Camb), 2004. **12**(7): p. 1157-63.
43. Mukhopadhyay, S., et al., *Structure of West Nile virus.* Science, 2003. **302**(5643): p. 248.
44. Gubler, D.J., *The global emergence/resurgence of arboviral diseases as public health problems.* Arch Med Res, 2002. **33**(4): p. 330-42.
45. Deubel, V., et al., *Variations in biological features of West Nile viruses.* Ann N Y Acad Sci, 2001. **951**: p. 195-206.
46. Weaver, S.C. and A.D. Barrett, *Transmission cycles, host range, evolution and emergence of arboviral disease.* Nat Rev Microbiol, 2004. **2**(10): p. 789-801.
47. Anderson, J.F., et al., *Characterization of West Nile virus from five species of mosquitoes, nine species of birds, and one mammal.* Ann N Y Acad Sci, 2001. **951**: p. 328-31.
48. Baqar, S., et al., *Vertical transmission of West Nile virus by Culex and Aedes species mosquitoes.* Am J Trop Med Hyg, 1993. **48**(6): p. 757-62.
49. Peel Region, O., *West NIle Virus in the Region of Peel 2002*, R.o.P.M. Government, Editor. 2002, Peel Municiple Government: Peel, Ontario.
50. White, D.J., *Vector surveillance for West Nile virus.* Ann N Y Acad Sci, 2001. **951**: p. 74-83.
51. Epstein, P.R., *West Nile virus and the climate.* J Urban Health, 2001. **78**(2): p. 367-71.
52. Spielman, A., *Structure and seasonality of nearctic Culex pipiens populations.* Ann N Y Acad Sci, 2001. **951**: p. 220-34.
53. Kay, B.H., I.D. Fanning, and P. Mottram, *Rearing temperature influences flavivirus vector competence of mosquitoes.* Med Vet Entomol, 1989. **3**(4): p. 415-22.
54. *Update: Surveillance for West Nile virus in overwintering mosquitoes--New York, 2000.* MMWR Morb Mortal Wkly Rep, 2000. **49**(9): p. 178-9.
55. Steele, K.E., et al., *Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York.* Vet Pathol, 2000. **37**(3): p. 208-24.

56. Schuler, L.A., et al., *Evaluation of an outbreak of West Nile virus infection in horses: 569 cases (2002).* J Am Vet Med Assoc, 2004. **225**(7): p. 1084-9.
57. Health, M., *West Nile Virus positive birds and horses in rural Manitoba*, P.H. Manitoba Health, Editor. 2002, Manitoba Health: WInnipeg.
58. Davidson, A.H., et al., *Immunologic responses to West Nile virus in vaccinated and clinically affected horses.* J Am Vet Med Assoc, 2005. **226**(2): p. 240-5.
59. Goldrick, B.A., *West Nile virus update: a new route of transmission is found.* Am J Nurs, 2003. **103**(5): p. 27.
60. Odelola, H.A. and O.O. Oduye, *West Nile virus infection of adult mice by oral route.* Arch Virol, 1977. **54**(3): p. 251-3.
61. Sampathkumar, P., *West Nile virus: epidemiology, clinical presentation, diagnosis, and prevention.* Mayo Clin Proc, 2003. **78**(9): p. 1137-43; quiz 1144.
62. *West Nile virus infection in organ donor and transplant recipients--Georgia and Florida, 2002.* MMWR Morb Mortal Wkly Rep, 2002. **51**(35): p. 790.
63. *Update: Investigations of West Nile virus infections in recipients of organ transplantation and blood transfusion--Michigan, 2002.* MMWR Morb Mortal Wkly Rep, 2002. **51**(39): p. 879.
64. Hayes, E.B. and D.R. O'Leary, *West Nile virus infection: a pediatric perspective.* Pediatrics, 2004. **113**(5): p. 1375-81.
65. *Possible West Nile virus transmission to an infant through breast-feeding--Michigan, 2002.* MMWR Morb Mortal Wkly Rep, 2002. **51**(39): p. 877-8.
66. Chen, Y.C., *Early induced IgM as a predictor and risk factor for severe disease outcome after West Nile virus infection.* Lancet Infect Dis, 2004. **4**(7): p. 394-5.
67. Hogrefe, W.R., et al., *Performance of immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays using a West Nile virus recombinant antigen (preM/E) for detection of West Nile virus- and other flavivirus-specific antibodies.* J Clin Microbiol, 2004. **42**(10): p. 4641-8.
68. Drebot, D.M., *Micro-neutralization West Nile virus IgG assay*, M. Dyck, Editor. 2004: Winnipeg.
69. Huhn, G.D., et al., *West Nile virus in the United States: an update on an emerging infectious disease.* Am Fam Physician, 2003. **68**(4): p. 653-60.
70. Kleinman, S., et al., *The 2003 West Nile virus United States epidemic: the America's Blood Centers experience.* Transfusion, 2005. **45**(4): p. 469-79.
71. Elliott, S., et al., *Results of a West NIle Virus Seroprevalence Survey, South Oakville, Ontario, 2003.* 2003, McMaster University: Guelph, ON.
72. Health, M., *Sero-survey indentifies WNV infection rates in Southwestern Manitoba*, M. Health, Editor. 2005, Manitoba Health: Winnipeg.
73. Wilson, S.D., M. Varia, and L.Y. Lior, *West Nile Virus: the buzz on Ottawa residents' awareness, attitudes and practices.* Can J Public Health, 2005. **96**(2): p. 109-13.

74. McCarthy, T.A., et al., *West Nile virus serosurvey and assessment of personal prevention efforts in an area with intense epizootic activity: Connecticut, 2000.* Ann N Y Acad Sci, 2001. **951**: p. 307-16.
75. Aquino, M., et al., *Protective behavior survey, West Nile virus, British Columbia.* Emerg Infect Dis, 2004. **10**(8): p. 1499-501.
76. Eidson, M., *"Neon needles" in a haystack: the advantages of passive surveillance for West Nile virus.* Ann N Y Acad Sci, 2001. **951**: p. 38-53.
77. Sampson, B.A. and V. Armbrustmacher, *West Nile encephalitis: the neuropathology of four fatalities.* Ann N Y Acad Sci, 2001. **951**: p. 172-8.
78. Pepperell, C., et al., *West Nile virus infection in 2002: morbidity and mortality among patients admitted to hospital in southcentral Ontario.* Cmaj, 2003. **168**(11): p. 1399-405.
79. Weiss, D., et al., *Clinical findings of West Nile virus infection in hospitalized patients, New York and New Jersey, 2000.* Emerg Infect Dis, 2001. **7**(4): p. 654-8.
80. *Provisional surveillance summary of the West Nile virus epidemic--United States, January-November 2002.* MMWR Morb Mortal Wkly Rep, 2002. **51**(50): p. 1129-33.
81. Ontario, P.o., *West Nile Virus Seroprevalence Study Report*, in *Ministry Reports*, M.o.H.a.L.-T. Care, Editor. 2003, Province of Ontario: Toronto.
82. Pile, J., *West Nile fever: here to stay and spreading.* Cleve Clin J Med, 2001. **68**(6): p. 553-60.
83. Ou, A.C. and R.C. Ratard, *One-year sequelae in patients with West Nile Virus encephalitis and meningitis in Louisiana.* J La State Med Soc, 2005. **157**(1): p. 42-6.
84. Klee, A.L., et al., *Long-term prognosis for clinical West Nile virus infection.* Emerg Infect Dis, 2004. **10**(8): p. 1405-11.
85. Rao, N., D. Char, and S. Gnatz, *Rehabilitation outcomes of 5 patients with severe West Nile virus infection: a case series.* Arch Phys Med Rehabil, 2005. **86**(3): p. 449-52.
86. Han, L.L., et al., *Risk factors for West Nile virus infection and meningoencephalitis, Romania, 1996.* J Infect Dis, 1999. **179**(1): p. 230-3.
87. Harrison, T.W., *West Nile encephalitis.* J Pediatr Health Care, 2002. **16**(6): p. 278-81.
88. Gould, L.H. and E. Fikrig, *West Nile virus: a growing concern?* J Clin Invest, 2004. **113**(8): p. 1102-7.
89. Shapiro, H. and S. Micucci, *Pesticide use for West Nile virus.* Cmaj, 2003. **168**(11): p. 1427-30.
90. Thier, A., *Balancing the risks: vector control and pesticide use in response to emerging illness.* J Urban Health, 2001. **78**(2): p. 372-81.
91. Tyshenko, M. and W. Leiss, *Improving risk communication of West Nile Virus, an emerging endemic disease*. 2004: Kingston, Ontario. p. 1-16.
92. Lopez, W. and J.R. Miller, *The legal context of mosquito control for West Nile Virus in New York City.* J Law Med Ethics, 2002. **30**(3 Suppl): p. 135-8.

93. Mullin, S., *Public health and the media: the challenge now faced by bioterrorism.* J Urban Health, 2002. **79**(1): p. 12.
94. Woodruff, A.W., *Achievements in tropical medicine during the past twenty-five years.* Br J Clin Pract, 1971. **25**(12): p. 529-35.
95. Miller, J.R., *The control of mosquito-borne diseases in New York City.* J Urban Health, 2001. **78**(2): p. 359-66.
96. Gratz, N.G. and W.C. Jany, *What role for insecticides in vector control programs?* Am J Trop Med Hyg, 1994. **50**(6 Suppl): p. 11-20.
97. Litsios, S., *Malaria control, the cold war, and the postwar reorganization of international assistance.* Med Anthropol, 1997. **17**(3): p. 255-78.
98. Hays, C.W., *The United States Army and malaria control in World War II.* Parassitologia, 2000. **42**(1-2): p. 47-52.
99. Barai, D., B. Hyma, and A. Ramesh, *The scope and limitations of insecticide spraying in rural vector control programmes in the states of Karnataka and Tamil Nadu in India.* Ecol Dis, 1982. **1**(4): p. 243-55.
100. Beckwith, W., S. Sirpenski, and D. Mayo, *Surveillance for avian-borne arboviruses in Connecticut, 2000.* Ann N Y Acad Sci, 2001. **951**: p. 336-7.
101. Cherry, B., et al., *Sentinel chickens as a surveillance tool for West Nile virus in New York City, 2000.* Ann N Y Acad Sci, 2001. **951**: p. 343-6.
102. Julian, K.G., et al., *Early season crow mortality as a sentinel for West Nile virus disease in humans, northeastern United States.* Vector Borne Zoonotic Dis, 2002. **2**(3): p. 145-55.
103. Lindsay, R., et al., *Rapid antigen-capture assay to detect west nile virus in dead corvids.* Emerging Infectious Diseases, 2003. **9**(11): p. 1406 - 1410.
104. Yaremych, S.A., et al., *West Nile virus and high death rate in American crows.* Emerg Infect Dis, 2004. **10**(4): p. 709-11.
105. Eidson, M., et al., *Crow deaths as a sentinel surveillance system for West Nile virus in the northeastern United States, 1999.* Emerg Infect Dis, 2001. **7**(4): p. 615-20.
106. Eidson, M., et al., *Dead bird surveillance as an early warning system for West Nile virus.* Emerg Infect Dis, 2001. **7**(4): p. 631-5.
107. Watson, J.T., et al., *Dead crow reports and location of human West Nile virus cases, Chicago, 2002.* Emerg Infect Dis, 2004. **10**(5): p. 938-40.
108. Manitoba Health, P.H., *West Nile virus 2003*, P.H. Manitoba Health, Editor. 2003: Winnipeg.
109. Zhang, L., *Risk Index Calculation*, M. Dyck, Editor. 2005: Winnipeg.
110. *Knowledge, attitudes, and behaviors about West Nile virus--Connecticut, 2002.* MMWR Morb Mortal Wkly Rep, 2003. **52**(37): p. 886-8.
111. Nedry, M. and C.R. Mahon, *West Nile virus: an emerging virus in North America.* Clin Lab Sci, 2003. **16**(1): p. 43-9.
112. Gaulin, C., et al., *Assessment of surveillance of human West Nile virus infection in Quebec, 2003.* Can Commun Dis Rep, 2004. **30**(11): p. 97-104.
113. Ruiz, M.O., et al., *Environmental and social determinants of human risk during a West Nile virus outbreak in the greater Chicago area, 2002.* Int J Health Geogr, 2004. **3**(1): p. 8.

114. Lopez, W., *West Nile virus in New York City.* Am J Public Health, 2002. **92**(8): p. 1218-21.
115. Petersen, L.R. and A.A. Marfin, *West Nile virus: a primer for the clinician.* Ann Intern Med, 2002. **137**(3): p. 173-9.
116. *Protection from West Nile virus.* Johns Hopkins Med Lett Health After 50, 2003. **15**(1): p. 1-2, 7.
117. Bender, K. and F.E. Thompson, Jr., *West Nile virus: a growing challenge.* Am J Nurs, 2003. **103**(6): p. 32-9; quiz 40.
118. Solomon, T., et al., *Natural and nosocomial infection in a patient with West Nile encephalitis and extrapyramidal movement disorders.* Clin Infect Dis, 2003. **36**(11): p. E140-5.
119. Ben-Nathan, D., et al., *West Nile virus neuroinvasion and encephalitis induced by macrophage depletion in mice.* Arch Virol, 1996. **141**(3-4): p. 459-69.
120. Guptill, S.C., et al., *Early-season avian deaths from West Nile virus as warnings of human infection.* Emerg Infect Dis, 2003. **9**(4): p. 483-4.
121. Schlesselman, J.J., *Case-Control Studies; Design, Conduct, Analysis.* 1982, New York: Oxford University Press.
122. Young, K., *Population Health: Concepts and methods*. 1998, New York, NY: Oxford.
123. Giesecke, J., *Modern Infectious Disease Epidemiology*. 2nd ed. 2002, London, England: Arnold Publishers.
124. Bernard, K.A. and L.D. Kramer, *West Nile virus activity in the United States, 2001.* Viral Immunol, 2001. **14**(4): p. 319-38.
125. Watts, D.M., et al., *Arthropod-borne viral infections associated with a fever outbreak in the northern province of Sudan.* J Trop Med Hyg, 1994. **97**(4): p. 228-30.
126. Hansson, L., *Statisical consideration in the analysis of matched case-control studies*, in *Social Sciences*. 2001, Uppsala University: Uppsala, Sweden. p. 33.
127. *Guidelines for surveillance, prevention, and control of West Nile virus infection--United States.* MMWR Morb Mortal Wkly Rep, 2000. **49**(2): p. 25-8.
128. Wacholder, S., et al., *Selection of controls in case-control studies. III. Design options.* Am J Epidemiol, 1992. **135**(9): p. 1042-50.
129. Wacholder, S., et al., *Selection of controls in case-control studies. I. Principles.* Am J Epidemiol, 1992. **135**(9): p. 1019-28.
130. Drebot, M.A. and H. Artsob, *West Nile virus. Update for family physicians.* Can Fam Physician, 2005. **51**: p. 1094-9.
131. *West Nile virus activity--eastern United States, 2001.* MMWR Morb Mortal Wkly Rep, 2001. **50**(29): p. 617-9.
132. Weiss, D.E., *Calculation of possible cases*, M. Dyck, Editor. 2005: Winnipeg.
133. Nosal, B. and R. Pellizzari, *West Nile virus.* Cmaj, 2003. **168**(11): p. 1443-4.

134. D'Agata, E.M., *Methodologic issues of case-control studies: a review of established and newly recognized limitations.* Infect Control Hosp Epidemiol, 2005. **26**(4): p. 338-41.
135. Hosek, R.S., W.D. Flanders, and A.J. Sasco, *Bias in case-control studies of screening effectiveness.* Am J Epidemiol, 1996. **143**(2): p. 193-201.
136. Canada, S., *Community Profiles - City of Brandon*, S. Canada, Editor. 2002, Statistics Canada: Ottawa, ON.
137. Canada, S., *Community Profiles - Assiniboine RHA*. 2002, Statistics Canada: Ottawa, ON.
138. Guarner, J., et al., *Clinicopathologic study and laboratory diagnosis of 23 cases with West Nile virus encephalomyelitis.* Hum Pathol, 2004. **35**(8): p. 983-90.
139. Sherman-Weber, S. and P. Axelrod, *Central diabetes insipidus complicating West Nile encephalitis.* Clin Infect Dis, 2004. **38**(7): p. 1042-3.
140. Hassard, T., *Understanding Biostatistics*. 1991, St. Louis: Mosby Year Book.
141. Yamada, R. and K. Ymamoto, *Recent findings on genes associated with inflammatory disease.* Mutat Res, 2005. **573**(1-2): p. 136-51.
142. Abbas, A.K. and A.H. Lichtman, *Basic Immunology*. 2nd ed. 2004, Philadelphia, PA: Elsevier.
143. Sharon, J.S., *Basic Immunology*. 1998, Baltimore: Williams and Wilkins.
144. Komar, N., *West Nile virus: epidemiology and ecology in North America.* Adv Virus Res, 2003. **61**: p. 185-234.
145. Association, B.C.L., *Asthma in Canada*, B.C.L. Association, Editor. May 3, 2005, British Columbia Lung Association: Vancouver.

Appendix 1:

Manitoba Canada

West Nile Virus Comparative Seroprevalence And Case Control Studies Protocol

Study Objectives:

The *primary objectives* of these studies are as follows:

- To compare the seroprevalences of West Nile Virus (WNV) antibody among people who live in five areas with different levels of mosquito activity and infection rates as indicated by ongoing mosquito monitoring in southwestern Manitoba by Manitoba Health during the summer of 2003.
- To determine the potential risk factors for infection in these populations
- To determine the risk factors for WNV disease among residents of Assiniboine by comparing, in a case control manner, those people with disease with those without infection and those with infection without disease.

Secondary objectives are as follows:

(1) To assess levels of knowledge, beliefs, and attitudes about WNV in this population;

(2) To determine the factors associated with risk reduction for WNV within this population.

Rationale:

West Nile Virus (WNV) is a mosquito-borne flavivirus that primarily infects birds, producing a transient high-titre viremia that allows transmission of the virus back to feeding mosquitoes in an amplifying cycle (1). Humans and other incidental hosts, such as horses, can become infected by bites from the amplifying mosquitoes, resulting in sporadic cases and sometimes outbreaks (2). Factors that determine the magnitude and severity of illness are not well understood, but appear to include the virulence of the WNV strain, the level of epizootic activity in the area and the immunological naivety of the exposed population (3).

Infection with WNV can present with non-specific viral symptoms such as fever, headache, gastrointestinal symptoms rash but can also lead to a

number of serious complications, including meningitis, encephalitis, meningoencephalitis, a polio-like syndrome, and neuropathy (4-6).

WNV was recently introduced to North America, where it was first detected during an epidemic of meningoencephalitis in the summer of 1999 in Queen's, New York (7). Evidence that WNV had arrived in Canada came in the summer of 2001 when active surveillance of the avian population indicated that several dead crows in southern Ontario tested positive for the virus. The first human case of WNV in Canada was reported in Peel in July of 2002. Since this initial human case multiple cases have been identified in Ontario and a seroprevalence survey conducted in the Oakville area of Ontario after the 2002 summer season indicated that 3.1% of a randomly selected population had antibody to the WNV.

Since then the WNV has spread rapidly across the prairies; initially in the corvid bird and equine populations in 2002, and into the human population in 2003. As of November 7, 2003 Manitoba has had 141 human cases of West Nile infection, 35 confirmed and 106 probable while Saskatchewan has been much more severely affected with 767 cases; 38 confirmed and 729 probable.

Results from these studies will be useful in informing policy decisions on issues relating to the importance / predictability of monitoring mosquito numbers and mosquito infection rates on human risk. The study may also identify modifiable risk factors for infection as well as evaluate the effectiveness of communication messages. The case control study may help in the understanding of the risk factors for infection and disease (febrile illness and neurological syndromes). Such a risk factor profile may be of use in assisting heath care providers in identifying high-risk subjects who could then be counseled vis-à-vis risk reduction strategies for personal protection.

Methods:

These studies will use a common methodology - a telephone survey questionnaire that will be administered to people living in five areas of southwestern Manitoba (Brandon, Minnedosa, Virden, Deloraine, and Killarney) Participants will be randomly selected from within randomly selected households on the basis of having the most recent birth date. This double-random selection process virtually ensures good age and gender representativeness in the final sample.

Participation will be restricted to respondents that are at least 18 years of age and French or English speaking. Residents who have spent more than six weeks out of their local area during the summer (July, August and

September) will be excluded from the study. Data will be collected in two forms. First, a brief (20-minute) survey will be conducted over the telephone by study personnel. This will include sociodemographic information, past health data, information about exposures to mosquitoes including their home environment, potential water reservoirs, exposure to mosquitoes, knowledge and beliefs about WNV, as well as preventive behaviours, such as use of mosquito repellent. Survey respondents will also be asked if they would agree to provide a blood sample in order to assess the presence and level of WNV antibodies. If the participant agrees, a laboratory requisition with a unique identifier number and a consent form will be mailed to the study participant after review of the consent form over the telephone. (Appendix B) The study participants will bring the laboratory requisition and the consent form with them when they present themselves to the laboratory for the blood sample. If required, the consent may be reviewed again with the participants.

For the seroprevalence survey, nurses or technologists trained in drawing blood specimens will obtain one specimen (10 milliliters of blood) from respondents who have provided written informed consent prior to obtaining blood. Sera will be tested for IgG and IgM antibodies to West Nile virus using an EIA test at the Cadham Provincial Laboratory and the National Microbiology Laboratory in Winnipeg.

The case control study will be a nested case control study. Initially, 40 patients with WNV disease will be selected from the Assiniboine RHA catchment area. Patients will initially be contacted by Manitoba Public Health and asked if they wish would be willing to have their names and contact numbers released to the study team. Verbal informed consent will be obtained for the release of their names. These patients will then be contacted by a member of the study team and, after informed verbal consent, complete the telephone survey questionnaire used in the comparative seroprevalence study. (Appendix A).

Analysis

Sample size

According to studies conducted in New York State, the initial screening seroprevalence of WNV in a naïve population is likely to be somewhere in the range of 0.46 – 2.6%. When corrected for specificity the seroprevalence was less than 0.01%. The seroprevalence in the Oakville area after a single WNV season was 3.1%. Provincial human surveillance information indicates very few cases in areas were mosquito counts are low (two of the 5 areas.) If we assume an α error of 0.05 and a β error of 0.20, then 1500 study subjects, 750 from the two areas of low mosquito counts and 750 from the three areas of high mosquito counts would permit the study to significantly detect a three-fold difference in seroprevalence.

For the case control study, each case will be age matched (± 5 years) with 3 controls drawn from the seroprevalence study.

Statistical analysis

The primary objectives of this survey are to determine the difference in prevalences of infection with WNV in areas in southwestern Manitoba with different mosquito numbers and infection rates. Univariate analysis will be conducted to assess potential risk factors using chi-square test to assess categorical variables and student's t-test to assess differences between infected and non-infected persons. Multi-variable analysis using logistic regression will also be performed using a backwards stepwise approach, selecting variables with a $p < 0.10$ or variables with important biological plausibility for inclusion into the model. The presence of detailed quantitative and qualitative mosquito data will be an important variable for mathematical modeling of risk. Another objective is to assess predictors of protective behavior against WNV. For example, respondents with frequent use of mosquito repellent will be compared with respondents with infrequent use. Variables of particular interest will be those addressing knowledge, attitudes, and beliefs about WNV. Both univariate and multivariable analysis, as described above, will be conducted. For the case control study similar statistical methodology will be used.

Ethical Considerations

The results of this study will be very useful in helping understand the important factors influencing the risk of human infection with WNV in Manitoba communities. In addition, this study will provide information on the levels of awareness and implementation of preventive measures, which will help guide public education planning. The study of people with the disease may help physicians understand what makes them particularly at risk of developing severe disease. It may be possible to reduce the risk of developing severe disease.

There are relatively few risks inherent in the survey portion, other than the potential of raising levels of concern and worry. All participants will have the opportunity to contact any of the investigators about the study should they have any concerns. For the seroprevalence study, there may be slight discomfort and bruising at the site where blood is drawn. They will also have the opportunity to receive their own results from blood samples (Appendix C), and have their meaning communicated to them by a qualified physician from the study team.

Dated: 24 November 2003

References:

1. Hubálek Z, Halouzka J. West Nile fever—a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis. 1999;5:643-50.
2. Petersen LR, Marfin AA. West Nile Virus: A Primer for the Clinician. Ann Intern Med 2002; 137:173-179.
3. Petersen LR, Roehrig JT. West Nile virus: a reemerging global pathogen. Emerg Infect Dis. 2001;7:611-4.
4. Asnis DS, Conetta R, Waldman G, Teixeira AA. The West Nile virus encephalitis outbreak in the United States (1999-2000): from Flushing, New York, to beyond its borders. Ann N Y Acad Sci. 2001;951:161-71.
5. Leis A. A., Stokic D. S., Polk J. L., Dostrow V., Winkelmann M. Poliomyelitis-like
Syndrome from West Nile Virus Infection N Engl J Med 2002; 347:1279-1280
6. Weiss D, Carr D, Kellachan J, Tan C, Phillips M, Bresnitz E, et al. Clinical findings of West Nile virus infection in hospitalized patients, New York and New Jersey, 2000. Emerg Infect Dis. 2001;7:654-8.
7. Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, et al. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med. 2001;344:1807-14.

Appendix #2 – Consent

Manitoba  Canada

RESEARCH PARTICIPANT INFORMATION AND CONSENT FORM

Title of Study: "The Comparative Seroprevalence of West Nile Virus (WNV) antibodies in people living in communities with high and low mosquito numbers and rates of infection and an examination of the risk factors for disease in patients living in Assiniboine Regional Health Authority area."

Principal Investigators:

Neil Simonsen
National Microbiology Laboratory
1015 Arlington St.
Winnipeg, MB R3E 3R2
Tel: 789-7054

Susan Roberecki
Manitoba Health
4th Floor – 300 Carlton Street
Winnipeg MB R3B 3M9
Tel. 788-6666

Elise Weiss
Manitoba Health / Brandon and Assiniboine RHAs
Unit A5 - 800 Rosser Avenue
Brandon, Manitoba
R7A 6N5

Co-Investigators:

Joel Kettner
Manitoba Health
4th Floor – 300 Carlton Street
Winnipeg MB R3B 3M9
Tel. 788-6666

Lawrence Elliott
Department of Community Health Sciences
Faculty of Medicine
University of Manitoba
S111 - 750 Bannatyne Avenue
Winnipeg, MB R3E 0W3
Tel: 789-3404

Sponsors: Manitoba Health
Health Canada
Assiniboine Regional Health Authority
Brandon Regional Health Authority

You are being asked to participate in a research study. Please take your time to review this consent form and discuss any questions you may have with the study staff. You may take your time to make your decision about participating in this study and you may discuss it with your friends, family or (if applicable) your doctor before you make your decision. This consent form may contain words that you do not understand. Please ask the study staff to explain any words or information that you do not clearly understand.

Purpose of Study

This research study is being conducted to study the relationship between mosquito exposure, human behaviours and activities and the risk of becoming infected with the West Nile Virus through a mosquito bite. This study will also try to understand why some people who are infected with the West Nile virus develop severe disease like inflammation of the brain and other nervous tissue.
A total of 1540 people will participate in this study.

Study procedures

There are two main procedures in these studies. The first is a telephone questionnaire that asks questions about area of residence, medical history, knowledge of West Nile Virus, and things you may have done to protect yourself from infection. The second procedure for those agreeing to participate is a blood sample of about 5 tablespoons that will be used to detect antibodies in the blood. The presence of these antibodies are a sign that you have been previously infected with the West Nile Virus. Those patients who already have had a documented West Nile Virus infection will only have the telephone questionnaire to complete.

Participation in the study will be for the time necessary to administer the questionnaire and draw the blood sample. When the results of the testing for West Nile Virus antibodies becomes available you will be notified of your result by mail and one of the study physicians will be available to answer any questions you may have about your testing results.

You can stop participating at any time. However, if you decide to stop participating in the study, we encourage you to talk to the study staff first.

Risks and Discomforts

There are relatively few risks in the questionnaire portion of this study, other than the potential of raising levels of concern and worry. You will have the opportunity to contact any of the investigators about the study should you have any concerns. For the seroprevalence study, there may be slight discomfort and bruising at the site where blood is drawn.

Benefits

The results of this study will be very useful in helping understand the important factors influencing the risk of human infection with WNV in Manitoba communities. In addition, this study will provide information on the levels of awareness and implementation of preventive measures, which will help guide public education planning. The study of people with the disease may help physicians understand what makes them particularly at risk of developing severe disease. It may be possible to reduce the risk of developing severe disease.

Costs

All the procedures, which will be performed as part of this study, are provided at no cost to you. The study doctors are not receiving professional fees to conduct this study. However, some of their time is paid for by Manitoba Health and Health Canada as part of the government's role in disease prevention.

Payment for participation

You will receive no payment or reimbursement for any expenses related to taking part in this study.

Confidentiality

Information gathered in this research study may be published or presented in public forums, however your name and other identifying information will not be used or revealed. At entry into the study you will be assigned a unique identification number that will be used to place your information into a database for analysis. Your name will not be part of this database but will be held separately until the results of the blood testing become available. Your name will then be linked to these results so that you may be informed of your infection status. Despite efforts to keep your personal information confidential, absolute confidentiality cannot be guaranteed. Your personal information may be disclosed if required by law.

Medical records that contain your identity will be treated as confidential in accordance with the Personal Health Information Act of Manitoba and the Privacy Act of Canada.

The University of Manitoba Health Research Ethics Board may review records related to the study for quality assurance purposes.

All records will be kept in a locked secure area and only those persons involved in the study will have access to these records. If any of your medical/research records need to be copied to any of the above, your name and all identifying information will be removed. No information revealing any personal information such as your name, address or telephone number will leave (Manitoba Health or Health Canada).

Voluntary Participation/Withdrawal from the Study

Your decision to take part in this study is voluntary. You may refuse to participate or you may withdraw from the study at any time. Your decision not to participate or to withdraw from the study will not affect your health care. If the study staff feel that it is in your best interest to withdraw you from the study, they will remove you without your consent.

We will tell you about any new information that may affect your health, welfare, or willingness to stay in this study.

You are not waiving any of your legal rights by signing this consent form nor releasing the investigator(s) or the sponsor(s) from their legal and professional responsibilities.

Questions

You are free to ask any questions that you may have about your treatment and your rights as a research participant. If any questions come up during or after the study you are free to contact one of the study investigators at the numbers noted above.

For 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.

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Statement of Consent

I have read this consent form. I have had the opportunity to discuss this research study with _____and or his/her study staff. I have had my questions answered by them in language I understand. The risks and benefits have been explained to me. I believe that I have not been unduly influenced by any study team member to participate in the research study by any statements or implied statements. Any relationship (such as employer, supervisor or family member) I may have with the study team has not affected my decision to participate. I understand that I will be given a copy of this consent form after signing it. I understand that my participation in this study is voluntary and that I may choose to withdraw at any time. I freely agree to participate in this research study.

I understand that information regarding my personal identity will be kept confidential, but that confidentiality is not guaranteed. I authorize the inspection of any of my records that relate to this study by The University of Manitoba Research Ethics Board for quality assurance purposes.

By signing this consent form, I have not waived any of the legal rights that I have as a participant in a research study.

Participant signature_____Date _____
(day/month/year)

Participant printed name: _____

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has knowingly given their consent

Printed Name: _____Date _____
(day/month/year)

Signature: _____

"Role in the study: _____

Appendix #3 – Survey
Questionnaire for Manitoba Health/Health Canada Seroprevalence And Case Control Study

Manitoba Canada

Questionnaire for Manitoba Health/Health Canada Seroprevalence And Case Control Study

Hello my name is _____ and I am calling on behalf of Manitoba Health, Health Canada, and the Assiniboine and Brandon Regional Health Authorities. We are conducting research on West Nile virus and would like to speak to the person living in your household who is over 18 years of age, with the birthday closest to today's date and who did not spend more than six weeks outside your local community during July, August and mid-September.

(If someone new:
Hello my name is _____ and I am calling on behalf of Manitoba Health, Health Canada, and the Assiniboine and Brandon Regional Health Authorities.)

As you may be aware, many people living in the three prairie provinces were infected with West Nile virus last year. Since most people infected by West Nile virus have no symptoms, many people may have been exposed to the virus and be unaware. To increase our understanding of West Nile virus, Manitoba Health in collaboration with Health Canada and Assiniboine and Brandon Regional Health Authorities, are studying several areas in south western Manitoba to determine why some people become ill with West Nile virus. A total of 1540 people will participate in this study.

Your number has been chosen by random telephone selection in the study areas of interest.

(OR, for the case control study:
Your name has been forwarded to us by the Assiniboine Regional Health Authority as someone who may wish to participate in a study on West Nile virus.)

This study will involve a telephone questionnaire survey lasting about twenty minutes and a blood sample. If you agree to participate in the questionnaire, a written consent form and a laboratory requisition with instructions on where to go to get your blood tested (*delete if case control)* will be sent to you by mail. The telephone questionnaire asks questions about area of residence, knowledge of West Nile virus, a brief medical history and things you may have done to protect yourself from infection.

A blood sample of about 2 teaspoons will be used to detect antibodies in the blood. The presence of these antibodies is a sign that you have been previously infected with the West Nile virus.

(*OR for the case control study, delete above paragraph),*

The answers you provide to these questions, as well as the results of your blood test *(delete if case control)* will remain strictly confidential. You may withdraw from the survey at any time and none of your information will be used in any subsequent analysis. At the end of the study, you will receive a letter outlining the results of your blood test and what the results mean. *(delete if*

case control study) If you have any questions about West Nile virus, you may contact Health Links at 1-888-315-9257.

Note: if respondent asks for more information about what the study is about, they can contact:

Kiri Shafto
West Nile Virus Sero Survey Coordinator
Manitoba Health
4thFloor-300 Carlton Street
Winnipeg, MB R3B 3M9
Tel. 788-6742

Dr. Neil Simonsen
National Microbiology Laboratory
1015 Arlington St.
Winnipeg, MB R3E 3R2
Tel. 789-7054

Dr. Susan Roberecki
Manitoba Health
4th Floor-300 Carlton Street
Winnipeg MB R3B 3M9
Tel. 788-6666

Dr. Elise Weiss
Manitoba Health / Brandon and Assiniboine RHAs
Unit A5 - 800 Rosser Avenue
Brandon, MB
R7A 6N5
Tel. 571-8395

Would you like to participate in the study?

No - Thank-you. Good-bye.

Yes – Thank you for agreeing to participate in the study.

Unique patient number _____ Date of Interview (DD/MM/YY)_____
Interviewer's Initials_____

We would first like to ask some general questions to help our study workers understand where you live and where you can be contacted in the future.

1. Your telephone number is _____ (for confirmation)

2. What is your name?_____

3. What is your gender?

1. Male
2. Female
3. Refused

4. What is your year of birth? 19____

5. What is your residential address?
   a. No. and street:_____
   b. Town/City/Village:_____
   c. Postal Code:_____
   d. Township and Range (rural):_____

6. Is your mailing address different from where you live? 1.Yes 2. No

7. If yes, what is your mailing address?
   a. Postal Box No.:_____
   b. Other:_____

   Alternatively, if don't know (a) or (b) or (a) or (b) is not applicable, ask:
   c. Distance and direction from nearest town/city:_____miles _____direction(N, S, E, W, NE, NW,etc).

8. Are you aware of how West Nile virus is spread? 1.Yes 2. No

9. If yes, Ask " How is it spread?"

Check all that apply

(Do not read this list)

____ mosquitoes
____ through blood
____ by birds
____ by handling dead birds
_____ by mosquitoes infected by biting birds
____ by close contact with a person who has West Nile virus infection
_____by not washing your hands
____ by Deer mice
_____ from horses

_____other

List _____

10. What is the best way to protect yourself against West Nile Virus?

Check all that apply (but do not read list)

___ use deet
___ reduce standing water
___ long sleeves and long pants
___ reduce time outside between dusk and dawn
___ fix screens
___ good hand washing
___ other (list) _____
___ don't know
___ refused

11. How worried were you last summer (July-Sept, 2003) about getting infected with West Nile virus?

1. Not worried at all
2. Somewhat worried
3. Very worried
8. Don't know
9. Refused

12. What effect did West Nile virus have on your summer plans in 2003? Would you say it had?

1. A big effect- we changed our plans
2. A medium effect - we considered it when making plans
3. A small effect - we took some precautions
4. No effect - it didn't influence our plans
5 Didn't have any plans
8. Don't know / Don't remember
9. Refused

Which of the following did you do in the summer of 2003?

13. Avoid areas where mosquitoes are likely to be a problem?

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

14. Avoid going outdoors in the early morning and evening

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

15. Wear long sleeves, long pants when outdoors

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

16. Wear DEET containing insect repellent when outdoors (i.e. Muskol, etc)

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

17. Wear non-DEET containing insect repellent when outdoors (i.e., citronella, etc)

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

18. Inspect and install / repair screens in your house

1. Always 2. Most of the time 3. Sometimes 4. Rarely 5. Never

19. Did you find any sources of standing water on your property ?
   A. Yes
   B. No
   C. Didn't look
   D. Don't know/Don't remember
   E. Refused
   F. Not applicable

20. If yes, did you take action to reduce sources of standing water on your property?

   1. Yes
   2. No
   8. Don't know/Don't remember
   9. Refused

21. Did you do anything else last summer to protect yourself and your family against being bitten by mosquitoes? (DON'T READ LIST, BUT CAN PROMPT WITH "ANYTHING ELSE" UNTIL THEY STOP OR SAY "NO"). CHECK ALL THAT APPLY.

___Swat/smack/slap mosquitoes
___Wear light coloured clothing
___Burn a coil
___Light a fire/smudge
___Do nothing
___Other (please specify)_____
___Burn candles (including citronella candles)
___Spray the area
___Use a bug zapper
___Use a mosquito magnet

22. If you did not use DEET repellents, why not? (CHECK ALL THAT APPLY, DO NOT READ CHOICES)

___Concern over pesticides
___Very low risk of West Nile infection
___Did not see any mosquitoes
___Concern over interaction with sunscreen
___Too much trouble
___ DEET adversely effects health
___Not applicable, did use DEET
___Other (specify):_____

23. Would you agree to the use of pesticides, such as larvicides to kill mosquito larvae, in your area to reduce the number of mosquitoes?

1. Yes
2. No
8. Don't Know
9. Refused

24. Would you agree to the use of pesticides, such as mosquito fogging to kill adult mosquitoes, in your area to reduce the number of mosquitoes?

1. Yes
2. No
8. Don't Know
9. Refused

25. (IF "NO" to #23 or24), Why not? (DO NOT READ LIST, CHECK ALL THAT APPLY)

___No insects/mosquitoes
___Child's health
___Subject's health
___Pet's health
___Livestock health
___Organic farming/agricultural reasons
___Concerned about the environment
___Unsafe/hazardous
___Too expensive
___Other (specify)_____

26. How worried are you about getting infected with West Nile virus next summer?

1. Not worried at all
2. Somewhat worried
3. Very worried
8. Don't know
9. Refused

Now I would like to ask you a few questions about your personal health:

27. Were you diagnosed by a blood test with West Nile virus infection last summer?

1. Yes
2. No
3. Don't Know
4. Refused

28. Were you ill with a fever in July, August or September of 2003?

1. Yes
2. No

8. Don' t Know/Don' t Remember
9. Refused

29. Do you have any medical conditions for which you require regular medical care and/or treatment (i.e., diabetes)?

1. Yes
2. No
8. Don't Know
9. Refused

30. If yes, please list. (CHECK ALL THAT APPLY).

___Diabetes
___Heart Disease
___Chronic Bronchitis
___Asthma
___Emphysema
___Cystic Fibrosis
___Ulcerative Colitis
___Regional Enteritis/Crohn's disease
___Rheumatoid Arthritis
___Osteoarthritis

Other (specify)_____

31. Do you have cancer or other conditions such as blood disorders or being an organ or bone marrow recipient that might affect your ability to fight infection?

1. Yes
2 No
8. Don't Know
9. Refused

32. Are you taking any medication that may affect your ability to fight infection? This may include steroids like prednisone, cortisone, chemotherapy treatments for cancer or other diseases? (NOTE TO SURVEYOR—STEROID PUFFERS DO NOT COUNT)

1. Yes
2. No
8. Don't Know
9. Refused

The next few questions have to do with your outdoor activity. Take your time to think carefully about your general pattern of being outdoors. This may include when you're around your home, at the park or at work, or any other time spent outdoors.

33. Do you spend time outside as a result of your occupation/job?

1. Yes

2. No
3. Not Applicable (unemployed, retired) go to question #36.
9. Refused

34. If yes, what type of occupation do you have (Do not read options)

1____Agriculture/farming
2____Manufacturing and processing
3____Construction
4____Natural and applied sciences and related occupations (i.e., natural resources)
5____Art, culture, recreation and sport
6____Trades, transport and equipment operators and related occupations
7____Sales and service occupations
8____Health
9____Social Science, education, government service and religion
10___Retail and Wholesale trade
11___Public utilities (Hydro, telephone, water, cable)
12.__Other (specify)_____

35. (If respondent names Agriculture/farming) Please indicate which agricultural/farming activities you are involved in.

1.___Grain farming
2.___Animal husbandry
3.___Market gardening
4.___Poultry farming, including geese/turkeys
5.___Beekeeper
6.___Beef farmer

36. What type of recreational activities did you participate in **regularly** last summer? (CHECK ALL THAT APPLY)

1. Gardening
2. Golfing
3. Camping
4. Walking / Jogging
5. Fishing / Boating
6. Cottaging
7. Other sports
8. Other (list)_____

37. On a typical day this past summer, how much time did you spend outdoors during the following time periods?(4-8 AM; 8AM-5PM MEANS AFTER 8AM, i.e., 8:15am, etc.)

Early morning	(4am to 8am)	____hours
Daytime	(8am to 5 pm)	____hours
Evening	(5pm to 9pm)	____hours
Night time	(9pm to 4 am)	____hours

38. Do you recall being bitten by mosquitoes last summer?

1. Never
2. Rarely
3. Frequently
8. Don' t Know/Don' t Remember
9. 9. Refused

39. Where were you exposed to the most mosquitoes last summer:

1. In your yard
2. Within 2 km of your house
3. Other
8. Don' t Know/Don' t Remember
9. Refused

40. Are there woods or other heavy vegetation within 100m of where you spend most of your outdoor time?

1.Yes
2. No
8. Don' t Know/Don' t Remember
9. Refused

41. Is there a marsh or other body of standing water within 100 m of where you spend most of your outdoor time?

1.Yes
2.No
8. Don' t Know
9. Refused

42. What sources did you and your family rely on most for information and updates on West Nile Virus? (Do NOT read options) (Check all that apply)

1.pamphlets/posters
2. news (tv, radio, newspaper)
3. internet
4. friend/family
5. doctors/health care worker
6. other
(specify):_____
7. Health Links
8. Don't know/don't remember
9. Refused

43. In your opinion, how important a health issue is West Nile virus ....

1. Not at all important
2. Somewhat important
3. Very important
8. Don't know
9. Refused

44. This past summer, do you remember receiving any information about West Nile virus and/or about how to avoid mosquito bites from the following sources (READ EACH LINE AND CHOICES):

Source:	1.Yes	2. No	8. Don't Know	9. Refused
Newspaper				
Radio				
TV				
Internet				
Neighbors/friends/ acquaintances				
Doctors/health care professionals				
Government				
Child's School				
Pamphlets / Posters				
Health Links				

I just have one more question before we finish.

45. What is the highest level of formal education you have completed? (DO NOT READ, CHECK ONLY ONE OF THE FOLLOWING. FILL IN GRADE IF APPLICABLE).

____Grade _____
____High School Diploma/ G.E.D.
____Some trade, technical, vocational, or business college
____Some (Community) College, CEGEP
____Some University

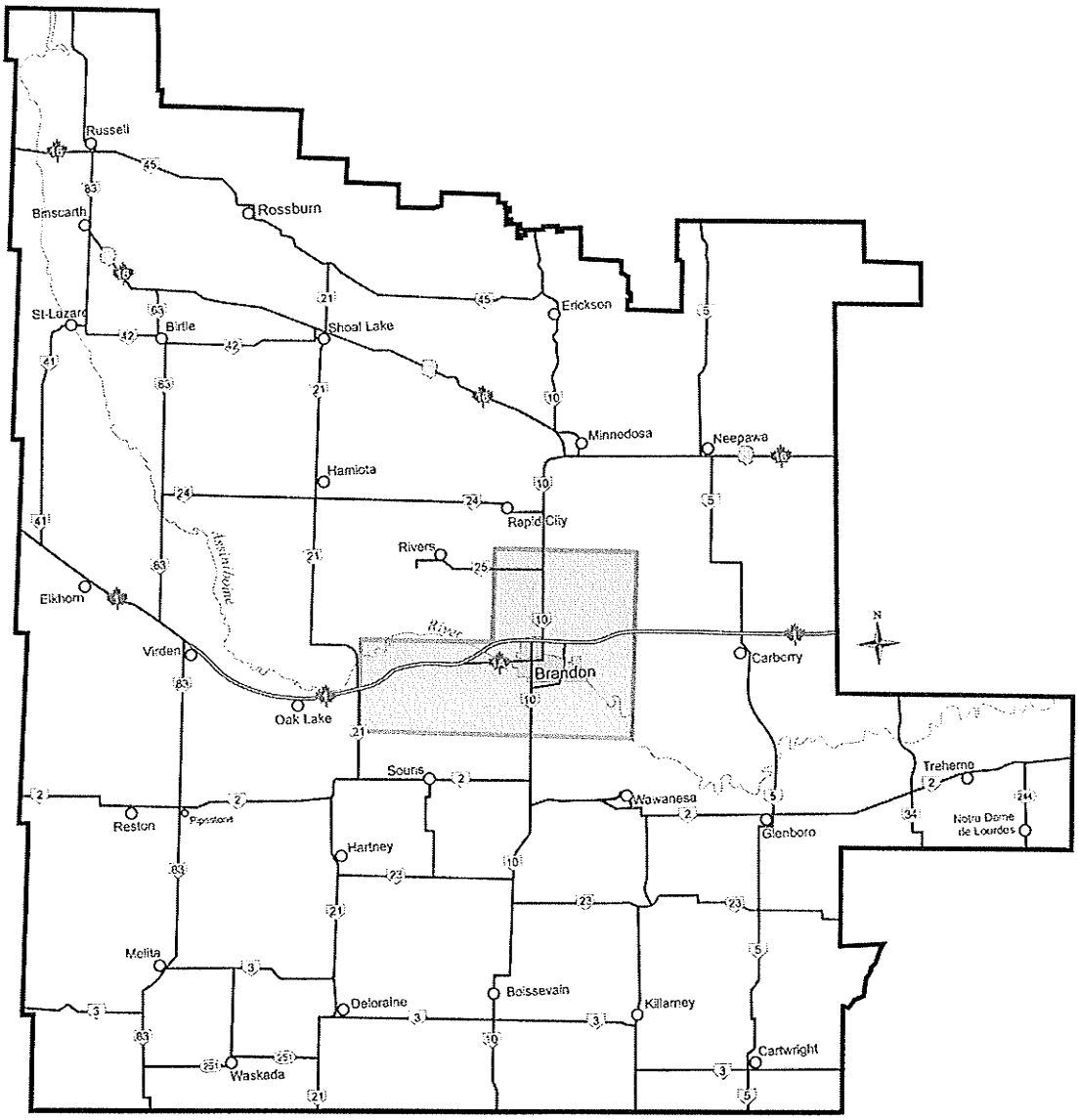
____Diploma or certificate from trade, technical, or vocational school or business college
____Diploma or certificate from (Community) College, CEGEP
____Bachelor's or undergraduate Degree (BA, BSc, LLB)
____Master's Degree (MA, MSc, Med)
____Degree in Medicine, Dentistry, Chiropractic, Veterinary Medicine, or Optometry (MD, DDS, DC, DMD, DVM, OD).
____Earned Doctorate (PhD, DSc, Ded)
____Refused
____Other (Specify):_____

Do you have any additional comments at this time?

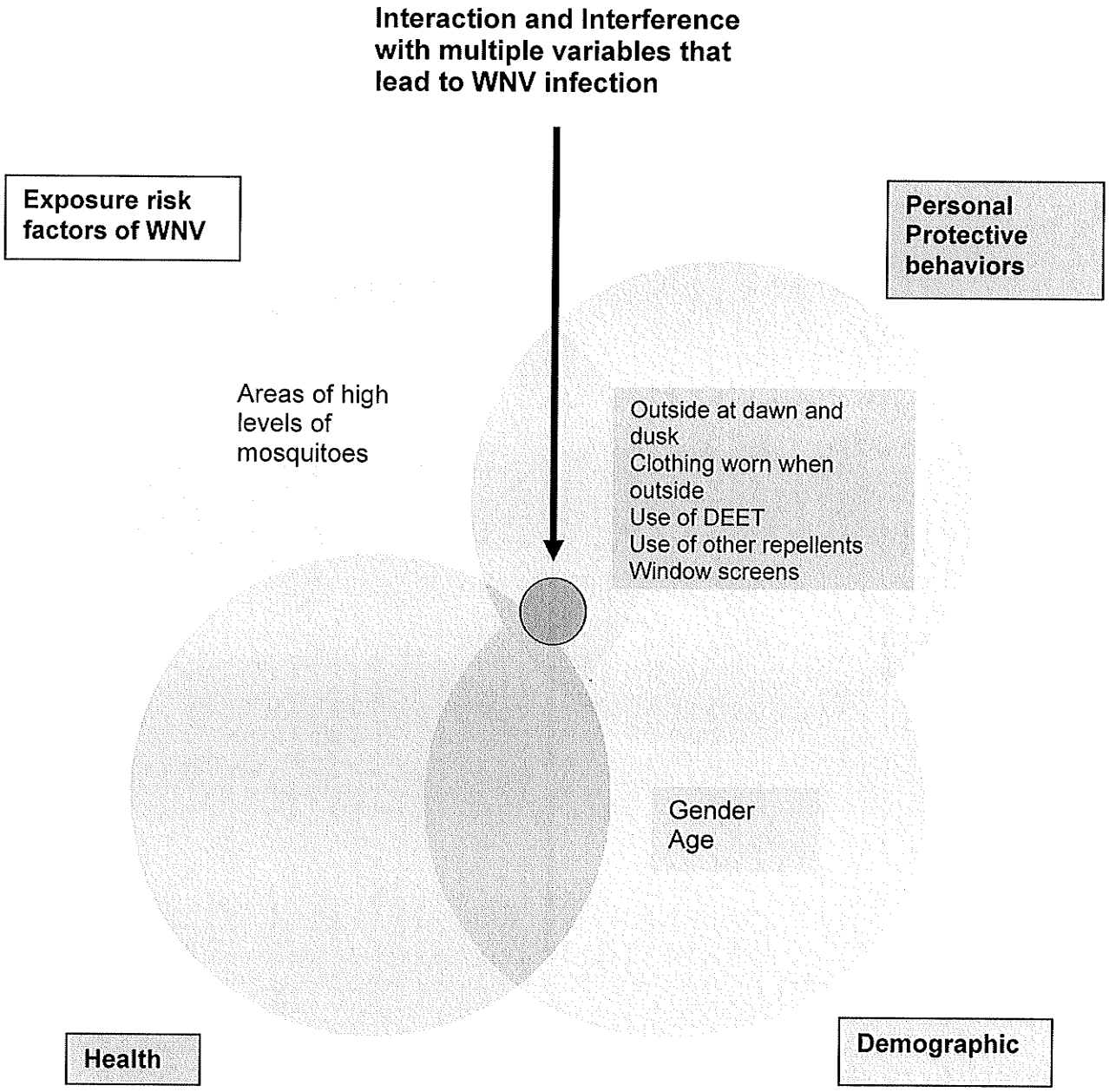
Closing:

I'D LIKE TO THANK YOU FOR YOUR TIME. You will be receiving information in the mail on where to go to get your blood sample, a laboratory requisition and a consent form to sign. Please bring this information with you when getting your blood sample. Do you have any questions? THANK YOU AGAIN FOR PARTICIPATING IN THIS VERY IMPORTANT STUDY. If you have any questions about West Nile Virus, please call Health Links at 1-888-315-9257. If you have questions about the study please, call any of the study investigators listed in the informed consent document.
GOODBYE.

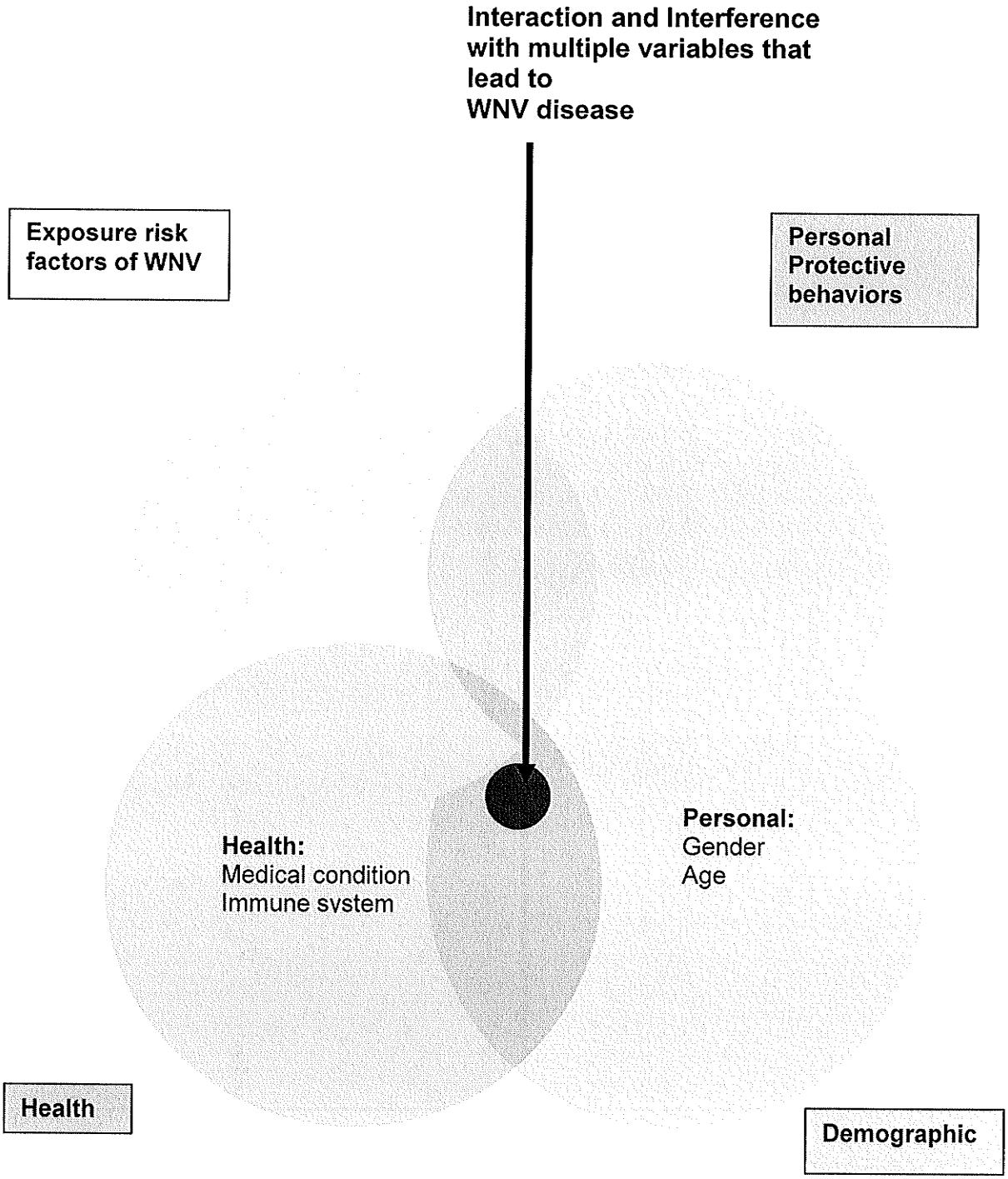
Appendix #4:
Map of Assiniboine RHA



Appendix #5
Risk factors analyzed for West Nile Virus infection, Case Control Study #1

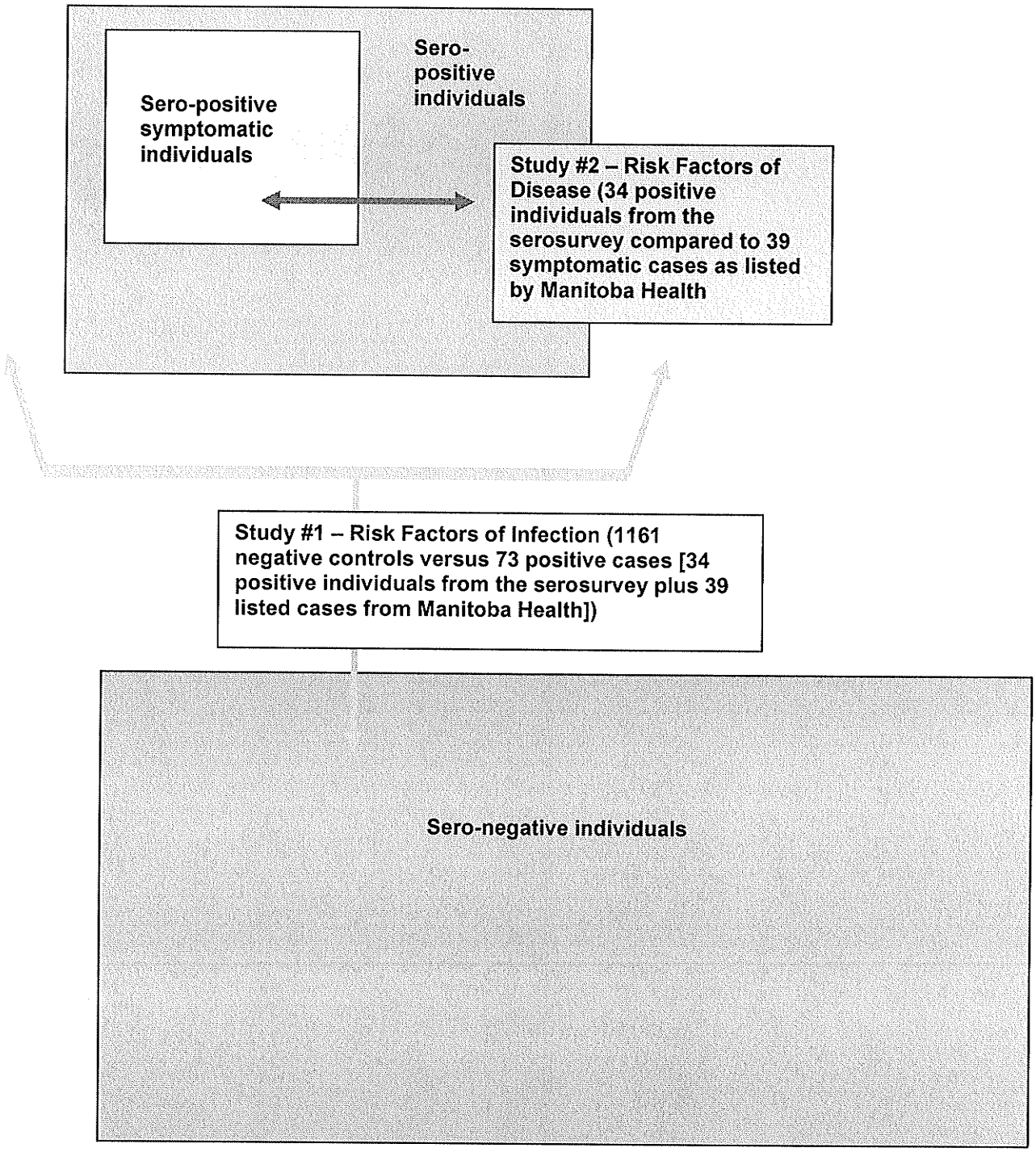


Appendix #6
Risk factors analyzed for West Nile Virus disease, Case Control Study #2

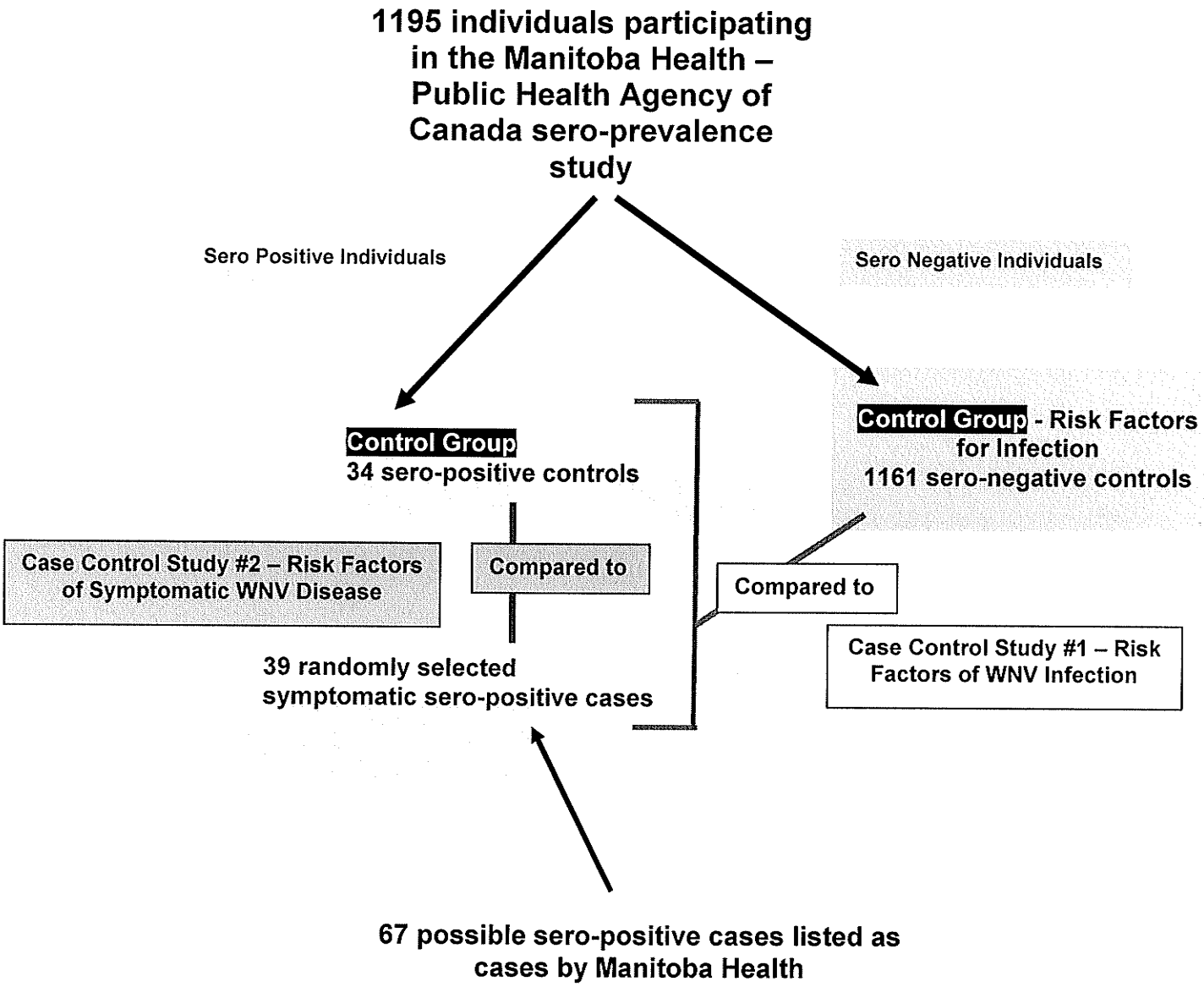


Appendix #7: Study Design Layout

This is a diagram of the overall study design, indicating that it is a nested study.



Appendix #8 – Study Design Details



Appendix #9 - Sample Size and Power Calculations

In the Oakville study, 65% of the population that tested positive for West Nile virus antibodies were fifty-five years of age or older. The city of Oakville (as of the 2001 census, StatsCanada) had a population in which 20% of the population was aged 55 years or older. Using this data to assist in the estimation of appropriate sample sizes, the following power and sample size calculations were made:

For the infection risk factor analysis (40 cases, 45 anticipated positive controls vs. 1300 anticipated negative controls) - an odds ratio between 2 and 3 will be able to be determined as significant:

Unmatched Case-Control Study (Comparison of ILL and NOT ILL)
Sample Sizes for 20.00 % Exposure in NOT ILL Group

		NOT ILL	Exposure	Odds	Sample Size		
Conf.	Power	:ILL	in ILL	Ratio	NOT ILL	ILL	Total
95.00 %	80.00 %	1300:85	42.86 %	3.00	535	35	570

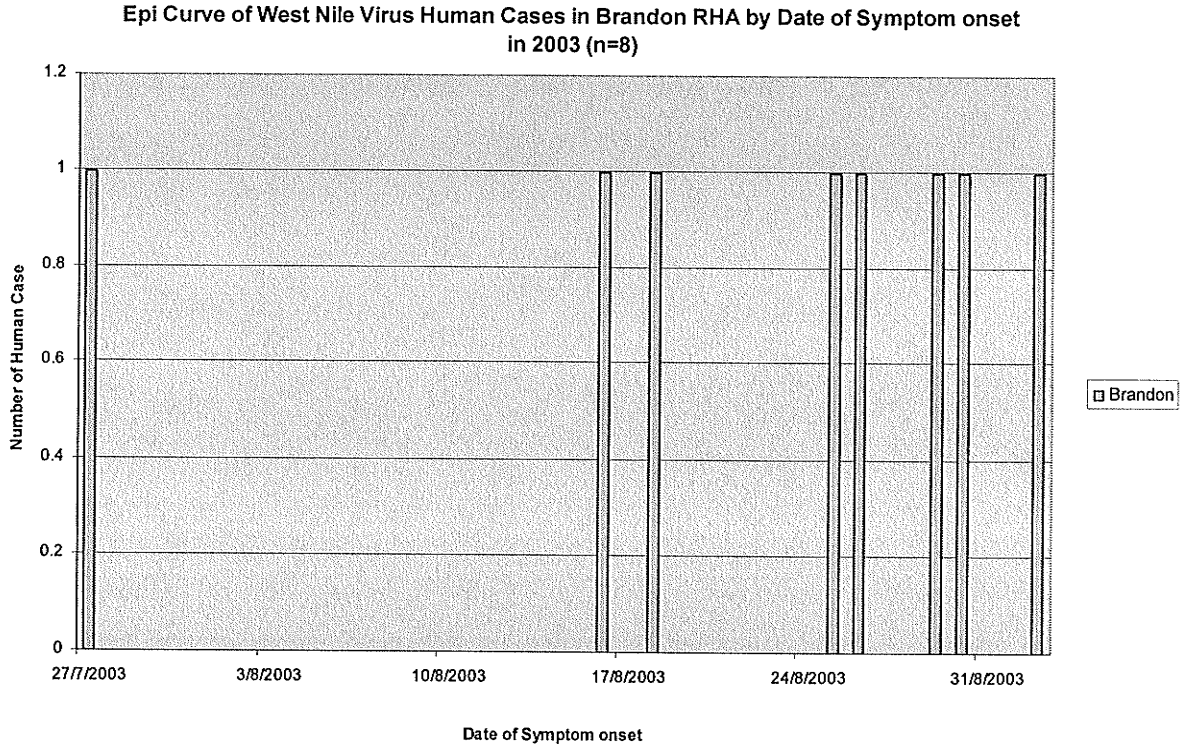
For the disease risk factor analysis (45 controls anticipated and 40 cases) - an odds ratio of 4.00 will be able to be determined as significant:

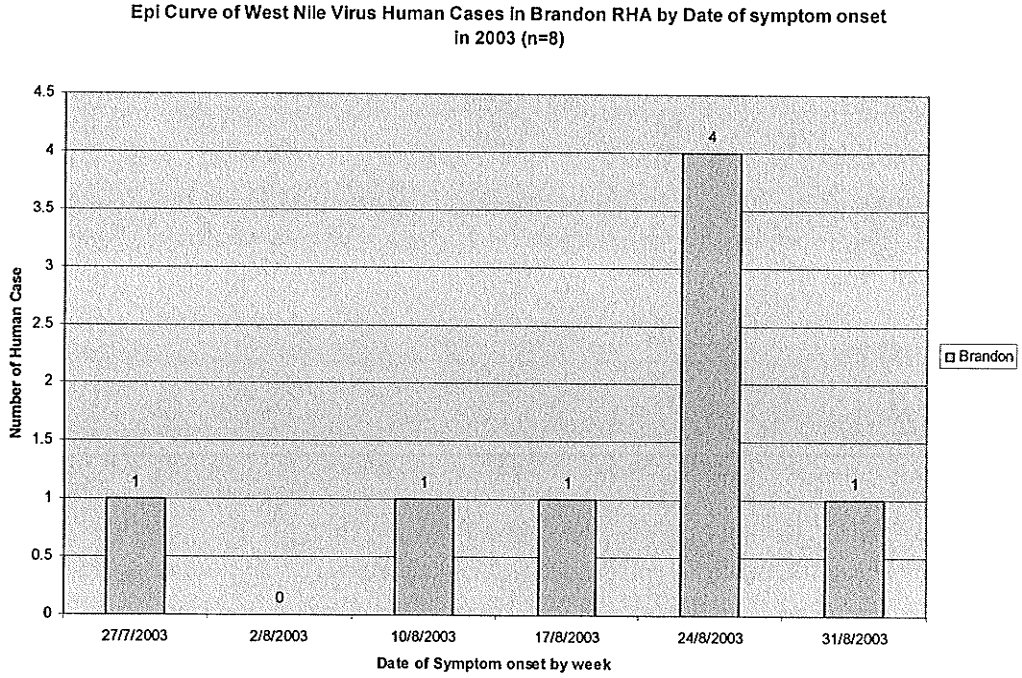
Unmatched Case-Control Study (Comparison of ILL and NOT ILL)
Sample Sizes for 20.00 % Exposure in NOT ILL Group

		NOT ILL	Exposure	Odds	Sample Size		
Conf.	Power	:ILL	in ILL	Ratio	NOT ILL	ILL	Total
95.00 %	80.00 %	1:1	50.00 %	4.00	45	45	90

Appendix #10: Epidemiological Curves

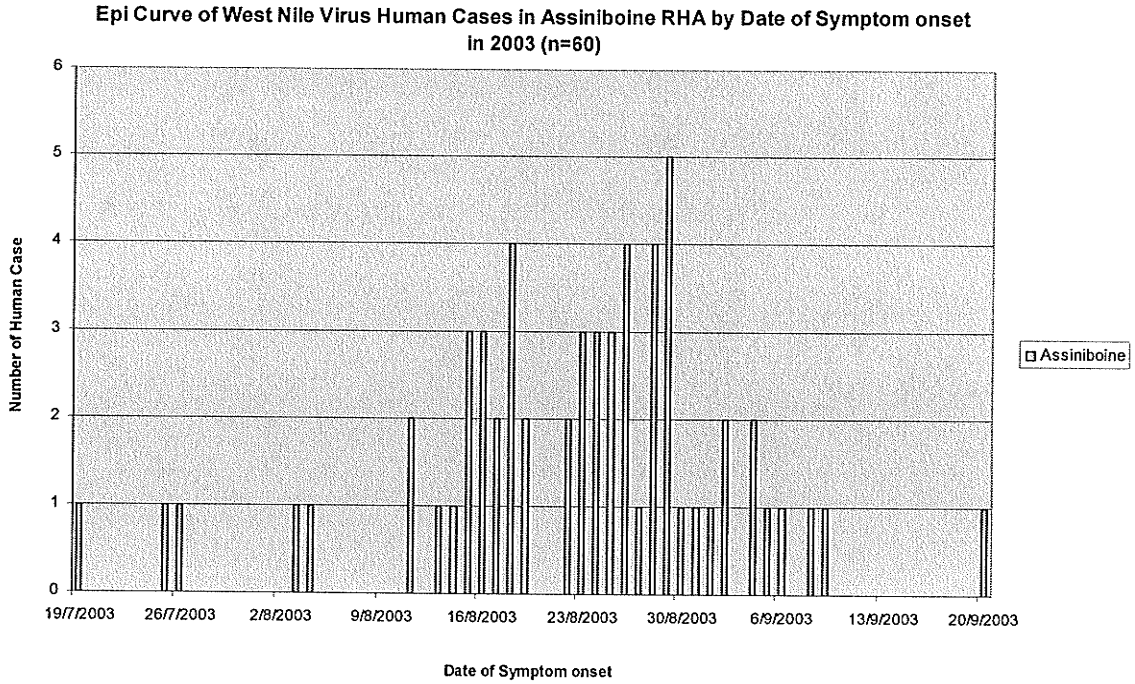
(provided by the WNV unit at Manitoba, September, 2005)

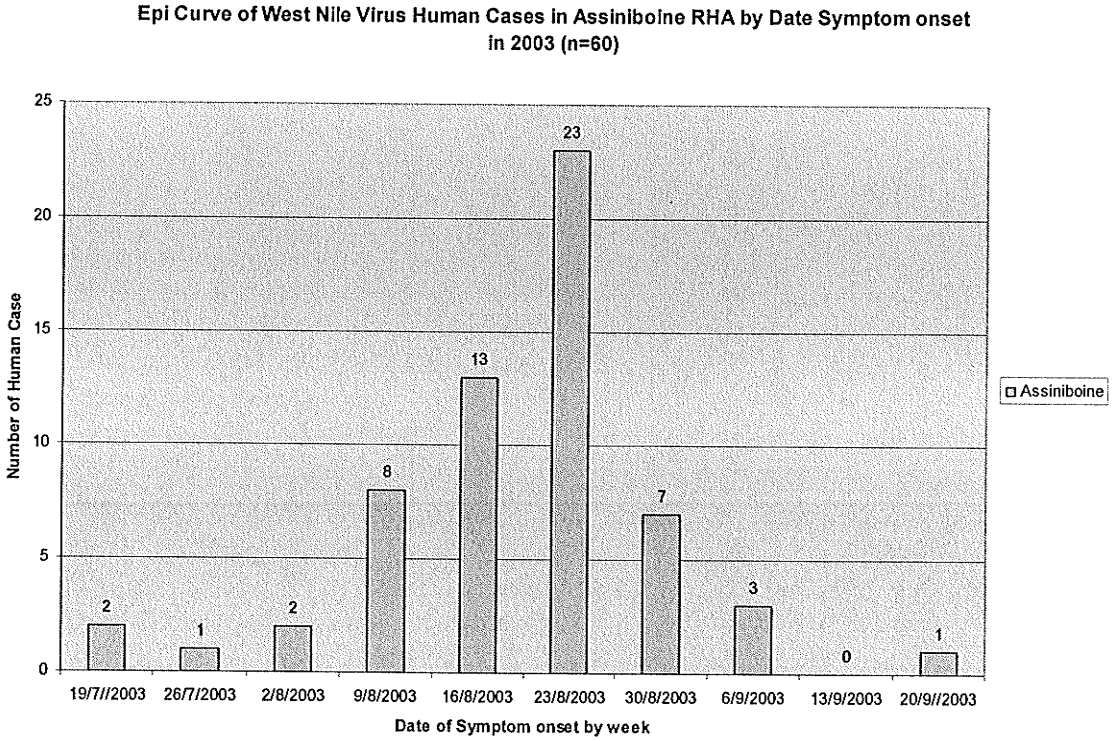




Appendix #10 continued : Epidemiological Curves

(provided by the WNV unit at Manitoba, September, 2005)





Appendix #11: Case Control Study #1
Exposure Risk Factors – Frequency of Survey Replies

EXPOSURE		Present or not?	Cases		Controls	
outside job?		no	45	62.5%	835	72.2%
		yes	27	37.5%	322	27.8%
type of job	**INDICATORS**					
	1=farmer	no	56	77.0%	1024	88.2%
		yes	17	23.0%	137	11.8%
farm	**INDICATORS**					
	grain	no	58	80.0%	1070	92.2%
		yes	15	20.0%	91	7.8%
	animals	no	71	97.3%	1132	97.5%
		yes	2	2.7%	29	2.5%
	market garden	no	73	100.0%	1157	99.7%
		yes	0	0.0%	4	0.3%
	poultry	no	71	97.3%	1157	99.7%
		yes	2	2.7%	4	0.3%
	beekeeper	no	73	100.0%	1160	99.9%
		yes	0	0.0%	1	0.1%
	beef	no	65	89.2%	1102	94.9%
		yes	8	10.8%	59	5.1%
recreation	**INDICATORS**					
	gardening	no	59	81.1%	881	75.9%
		yes	14	18.9%	280	24.1%
	golfing	no	58	79.7%	987	85.0%
		yes	15	20.3%	174	15.0%
	camping	no	53	73.0%	932	80.3%
		yes	20	27.0%	229	19.7%
	walking/jogging	no	24	32.4%	539	46.4%
		yes	49	67.6%	622	53.6%
	fishing/boating	no	65	89.2%	960	82.7%
		yes	8	10.8%	201	17.3%
	cottage	no	66	90.5%	1034	89.1%
		yes	7	9.9%	127	10.9%
	other	no	56	77.0%	866	74.6%
		yes	17	23.0%	295	25.4%
marsh		no	37	51.4%	492	42.4%
		yes	36	48.6%	669	57.6%
forest/woods		no	0	0.0%	1161	100.0%
		yes	73	100.0%	0	0.0%
protection actions done the summer of 2003						
	INDICATORS					
	swat mos	no	72	98.6%	1090	93.9%
		yes	1	1.4%	71	6.1%

Appendix #11 cont.

Behavior	Present?	Cases		Controls	
light clothing	no	70	95.9%	1021	87.9%
	yes	3	4.1%	140	12.1%
burn coil	no	59	85.1%	1128	97.2%
	yes	11	14.9%	33	2.8%
light a smudge	no	70	95.9%	1138	98.0%
	yes	3	4.1%	23	2.0%
burn candles	no	55	75.7%	1091	94.0%
	yes	18	24.3%	70	6.0%
spray the area	no	72	98.6%	1141	98.3%
	yes	1	1.4%	20	1.7%
bug zapper	no	72	98.6%	1131	97.4%
	yes	1	1.4%	30	2.6%
bug magnet	no	73	100.0%	1158	99.7%
	yes	0	0.0%	3	30.0%
did you avoid mos	no	40	56.9%	534	46.5%
	yes	31	43.1%	614	53.5%
did you avoid dawn/dusk	no	49	67.1%	680	59.0%
	yes	24	32.9%	472	41.0%
did you use long sleeves	no	36	49.3%	344	30.0%
	yes	37	50.7%	811	70.0%
did you use DEET	no	32	43.8%	402	34.8%
	yes	41	56.2%	752	65.2%
did you use insect repell	no	66	90.4%	942	82.2%
when you didn't use deet?	yes	7	9.6%	204	17.8%
did you inspect screens	no	16	22.9%	490	44.2%
	yes	54	77.1%	619	55.8%
did you look for standing H20	no	45	60.8%	677	58.3%
	yes	28	39.2%	484	41.7%
did you act on H20	no	60	82.4%	832	71.7%
	yes	13	17.6%	329	28.3%

hours outside

# answered	73	98.60%	1137	97.90%
early am	mean= 0.53	range= 0-4	mean= 0.52	range= 0-20
# answered	73	98.60%	1152	99.20%
daytime	mean= 4.05	range= 0-9	mean= 3.68	range= 0-30
# answered	73	98.60%	1157	99.70%
evening	mean= 2.82	range= 1-4	mean= 2.29	range= 0-41
# answered	73	98.60%	1137	97.90%
night	mean= .90	range= 0-5	mean= .6	range= 0-30
# answered	73	98.60%	1129	97.20%
dawn to dusk	mean= 4.26	range= 0-41	mean=3.41	range= 1-10

Appendix #12: Case Control Study #1
Exposure Risk Factors – Odds ratios and Confidence Limits

Variable	Subcategory	Odds Ratio	CL
Outside job?	-	1.56	0.92 – 2.62
Farmer?	-	2.23	1.21 – 4.07
If farmer…	Type?		
	Grain	2.99	1.56 – 5.67
	Animals	1.08	CL invalid
	Market gardening	0	0 – 24.43
	Poultry	8.03	1.00 – 51.99
	Beekeeper	0	0 – 274.77
	Beef	2.26	0.96 – 5.16
Recreation	Type?		
	Gardening	0.73	0.39 – 1.38
	Golfing	1.44	0.76 – 2.68
	Camping	1.51	0.85 – 2.64
	Walking-jogging	1.81	1.07 – 3.07
	Fishing-boating	0.58	0.25 – 1.27
	Cottage	0.35	0.35 – 1.97
	Other	0.88	0.48 – 1.57
Marsh	-	0.7	0.42 – 1.14
Forest-wood	-	Undefined	
Protection actions	Type used?		
	Swat mosquitoes	0.21	0.01 – 1.43
	Light clothing	0.31	0.08 – 1.03
	Burn coil	5.97	2.70 – 12.98
	Light a smudge	2.09	0.49 – 7.56
	Burn candles	5.01	2.68 – 9.30
	Spray area	0.78	CL invalid
	Bug zapper	0.52	0.03 – 3.61
	Bug magnet	0	0 – 35.60
Avoid mosquitoes?	-	0.66	0.40 – 1.09
Avoid dawn & dusk?	-	0.71	0.41 – 1.20
Wear long sleeves and pants?	-	0.44	0.26 – 0.72
Use DEET?	-	0.68	0.41 – 1.13
Use non-DEET insect repellant?	-	0.49	0.20 – 1.13
Inspect screens?	-	2.67	1.47 – 4.93
Look for standing water?	-	0.90	0.54 – 1.50
Remove standing water?	-	0.54	0.28 – 1.03

Appendix #13:
Case Control Study #1
Time Spent outdoors

hours outside			CASES		CONTROLS	
Early AM	# answered	%	73	98.60%	1137	97.90%
Daytime	# answered	%	73	98.60%	1152	99.20%
Evening	# answered	%	73	98.60%	1157	99.70%
Night	# answered	%	73	98.60%	1137	97.90%
Dusk to Dawn	# answered	%	73	98.60%	1129	97.20%

Time spent outdoors (in hours)

	Cases		Controls		T test
	Mean	Range	Mean	Range	
Early AM	0.53	0 – 4	0.52	0 – 20	0.934
Daytime	4.05	0 – 9	3.68	0 – 30	0.221
Evening	2.82	1 – 4	2.29	0 – 41	0.0001
Night	0.90	0 – 5	0.60	0 - 30	0.014
Dusk to dawn	4.26	0 – 41	3.41	1 - 10	0.001

Appendix #14 – Actual Power of Case Control Study #1

For the infection risk factor analysis (39 cases and 1161 negative controls) - an odds ratio between 2 and 3 will be able to be determined as significant:

Unmatched Case-Control Study (Comparison of ILL and NOT ILL)
Sample Sizes for 20.00 % Exposure in NOT ILL Group

		NOT ILL	Exposure	Odds	Sample Size		
Conf.	Power	:ILL	in ILL	Ratio	NOT ILL	ILL	Total
95.00%	80.00%	1161:73	33.33 %	2.00	1464	92	1556
95.00%	**80.00%**	**1161:73**	**34.43 %**	**2.10**	**1273**	**80**	**1353**
95.00%	**80.00%**	**1161:73**	**42.86 %**	**3.00**	**557**	**35**	**592**

Appendix #15:
Case Control Study #2 – Frequency of Survey Results

EXPOSURE			CASES		CONTROLS	
outside job?		0=no	14	45.2	12	57.1
		1=yes	17	54.8	9	42.9
type of job	**INDICATORS**					
	farmer					
	0=no		29	74.4	29	82.9
		1=yes	10	25.6	6	17.1
farm	**INDICATORS**					
	grain					
	0=no		31	79.5	29	82.9
		1=yes	8	20.5	6	17.1
	animals					
	0=no		38	97.4	34	97.1
		1=yes	1	2.6	1	2.9
	market garden					
	0=no		39	100	35	100
		1=yes	0	0	0	0
	poultry					
	0=no		37	94.9	35	100
		1=yes	2	5.1	0	0
	beekeeper					
	0=no		39	100	35	100
		1=yes	0	0	0	0
	beef					
	0=no		35	89.7	32	91.4
		1=yes	4	10.3	3	8.6
recreation	**INDICATORS**					
	gardening					
	0=no		4	10.3	11	31.4
		1=yes	35	89.7	24	68.6
	golfing					
	0=no		25	64.1	26	74.3
		1=yes	14	35.9	9	25.7
	camping					
	0=no		27	69.2	24	68.6
		1=yes	12	30.8	11	31.4
	walking/jogging					
	0=no		6	15.4	14	40
		1=yes	33	84.6	21	60
	fishing/boating					
	0=no		35	89.7	30	85.7
		1=yes	4	10.3	5	14.3
	cottage					
	0=no		36	92.3	30	85.7
		1=yes	3	7.7	5	14.3
	other					
	0=no		31	79.5	23	65.7
		1=yes	8	20.5	12	34.3
marsh		0=no	15	38.5	19	59.4
		1=yes	24	61.5	13	40.6
forest/woods		0=no	0	0	0	0
		1=yes	39	100	34	100

APPENDIX #15 continued...

protection actions done the summer of 2003

INDICATORS		CASES		CONTROLS	
swat mos	0=no	39	100	34	97.1
	1=yes	0	0	1	2.9
light clothing	0=no	30	76.9	27	77.1
	1=yes	9	23.1	8	22.9
burn coil	0=no	37	94.9	34	97.1
	1=yes	2	5.1	1	2.9
light a smudge	0=no	37	94.9	34	97.1
	1=yes	2	5.1	1	2.9
nothing	0=no	19	48.7	22	62.9
	1=yes	20	51.3	13	37.1
burn candles	0=no	30	76.9	27	77.1
	1=yes	9	23.1	8	22.9
spray the area	0=no	39	100	34	97.1
	1=yes	0	0	1	2.9
bug zapper	0=no	39	100	34	97.1
	1=yes	0	0	1	2.9
bug magnet	0=no	39	100	35	100
	1=yes	0	0	0	0
did you avoid mos	0=no	26	66.6	14	45.2
	1=yes	13	33.3	17	54.8
did you avoid dawn/dusk	0=no	28	73.7	19	59.4
	1=yes	10	26.3	13	40.6
did you use long sleeves	0=no	22	56.4	14	43.8
	1=yes	17	43.6	18	56.2
did you use DEET	0=no	17	43.6	15	46.9
	1=yes	22	56.4	17	53.1
did you use insect repell when you didn't use deet?	0=no	34	87.2	30	93.8
	1=yes	5	12.8	2	6.2
did you inspect screens	0=no	4	10.3	14	43.8
	1=yes	35	89.7	18	56.2
did you look for standing H20	0=no	19	48.7	23	71.9
	1=yes	20	51.3	9	28.1
did you act on H20	0=no	22	75.9	5	45.5
	1=yes	7	24.1	6	54.5

Appendix #16: Case Control Study #2
Exposure Risk Factors – Odds ratios and Confidence Limits

Variable	Subcategory	Odds Ratio	CL
Outside job?	-	2.07	0.76 – 5.63
Farmer?	-	1.67	0.54 – 5.19
If farmer...	Type?		
	Grain	1.55	0.46 – 5.27
	Animals	0.90	0.05 – 14.86
	Market gardening	NA	-
	Poultry	Undefined	-
	Beekeeper	NA	-
	Beef	1.22	0.25 – 5.87
Recreation	Type?		
	Gardening	4.01	1.14 – 14.09
	Golfing	1.62	0.59 – 4.40
	Camping	0.97	0.36 – 2.79
	Walking-jogging	3.67	1.22 – 11.04
	Fishing-boating	0.69	0.16 – 2.79
	Cottage	0.50	0.11 – 2.23
	Other	0.50	.017 – 1.41
Marsh	-	2.34	0.90 – 6.08
Forest-wood	-	NA	-
Protection actions	Type used?		
	Swat mosquitoes	Undefined	-
	Light clothing	1.01	0.34 - 3.00
	Burn coil	1.84	0.16 – 21.20
	Light a smudge	1.84	0.16 – 21.20
	Burn candles	1.01	0.34 – 3.00
	Spray area	Undefined	-
	Bug zapper	Undefined	-
	Bug magnet	NA	-
Avoid mosquitoes?	-	0.41	0.14 – 1.21
Avoid dawn & dusk?	-	0.56	0.18 – 1.74
Wear long sleeves and pants?	-	0.60	0.21 – 1.71
Use DEET?	-	1.14	0.40 – 3.25
Use non-DEET insect repellant?	-	2.21	0.34 – 17.88
Inspect screens?	-	6.81	1.73 – 29.04
Look for standing water?	-	2.69	0.90 – 8.24
Remove standing water?	-	0.27	0.05 – 1.41

Appendix #17

Case Control Study #2, Hours Spent Outside.

1 case did not respond to this question, all controls completed this question.

	Cases (n=39-1)		Controls (n=34)		T test
	Mean	Range	Mean	Range	
Early AM	0.59	0 – 4	0.47	0 – 2	0.52, NS
Daytime	4.21	0 – 9	3.81	0 – 9	0.51, NS
Evening	2.77	1 – 4	2.88	1 – 4	0.65, NS
Night	1.03	0 – 5	0.78	0 – 3	0.30, NS
Dusk to Dawn	4.39		4.13		0.57, NS

Appendix #18 – Actual Power of Case Control Study #2

For the infection risk factor analysis (39 cases and 34 controls) - an odds ratio above 4.00 will be able to be determined as significant:

Unmatched Case-Control Study (Comparison of ILL and NOT ILL)
Sample Sizes for 20.00 % Exposure in NOT ILL Group

		NOT ILL	Exposure	Odds	Sample Size		
Conf.	Power	:ILL	in ILL	Ratio	NOT ILL	ILL	Total
95.00%	80.00%	39:34	42.86%	3.00	77	67	144
95.00%	80.00%	39:34	50.00%	4.00	48	42	90
95.00%	**80.00%**	**39:34**	**55.56%**	**5.00**	**36**	**31**	**67**
95.00%	70.00%	39:34	42.86%	3.00	55	63	118
95.00%	60.00%	39:34	42.86%	3.00	45	52	97
95.00%	**70.00%**	**39:34**	**50.00%**	**4.00**	**35**	**40**	**75**

Appendix #19:
Case Control Study #2:
Correlation of the variable of checking the window screens

With assessment of screens variable included in analysis:

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1(a)	gender	.799	1.307	.374	1	.541	2.224
	age_approx	-.123	.067	3.361	1	.067	.884
	Highcount_town	-3.411	1.879	3.295	1	.069	.033
	screens	-1.575	.839	3.522	1	.061	.207
	stand_water	-1.483	1.854	.640	1	.424	.227
	action_water	2.704	1.573	2.954	1	.086	14.933
	gardening	-2.689	2.568	1.097	1	.295	.068
	walking	4.330	1.857	5.434	1	.020	75.924
	other	-1.182	1.756	.453	1	.501	.307
	time_outside	-.056	.340	.028	1	.868	.945
	Constant	10.682	7.805	1.873	1	.171	43545.890

a Variable(s) entered on step 1: gender, age_approx, High-count_town, screens, stand_water, action_water, gardening, walking, other, time_outside.

Gardening and walking each had odds ratios approximately 4.00, however with logistic regression, the odds ratios diverge greatly and the slopes become opposite (which doesn't make logical sense).

With assessment of screens variable removed:

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1(a)	gender	.115	1.224	.009	1	.925	1.121	.102	12.356
	age_approx	-.095	.062	2.327	1	.127	.910	.806	1.027
	Highcount_town	-2.054	1.338	2.357	1	.125	.128	.009	1.765
	stand_water	-.505	1.403	.130	1	.719	.603	.039	9.440
	action_water	2.458	1.426	2.971	1	.085	11.677	.714	190.953
	gardening	.451	1.473	.094	1	.760	1.569	.088	28.128
	walking	2.520	1.277	3.895	1	.048	12.423	1.018	151.660
	other	-2.668	1.684	2.509	1	.113	.069	.003	1.883
	time_outside	-.160	.292	.299	1	.584	.853	.481	1.510
	Constant	3.112	5.048	.380	1	.538	22.477		

a Variable(s) entered on step 1: gender, age_approx, High-count_town, stand_water, action_water, gardening, walking, other, time_outside.

This appears to have more accurate and logical results.

Appendix #20:
Case Control Study #2 – Correlation of High count towns with other personal protective variables

wnv worry * Affected_town Crosstabulation

			Affected_town		
			0	1	Total
wnv worry	0	Count	18	30	48
		% within wnv worry	37.5%	62.5%	100.0%
		% within Affected_town	100.0%	56.6%	67.6%
		% of Total	25.4%	42.3%	67.6%
	1	Count	0	23	23
		% within wnv worry	.0%	100.0%	100.0%
		% within Affected_town	.0%	43.4%	32.4%
		% of Total	.0%	32.4%	32.4%
Total		Count	18	53	71
		% within wnv worry	25.4%	74.6%	100.0%
		% within Affected_town	100.0%	100.0%	100.0%
		% of Total	25.4%	74.6%	100.0%

It was also correlated with the diagnosis of WNV:

WNV disease * Affected_town Crosstabulation

			Affected_town		
			0	1	Total
WNV disease	0	Count	3	32	35
		% within WNV disease	8.6%	91.4%	100.0%
		% within Affected_town	15.8%	58.2%	47.3%
		% of Total	4.1%	43.2%	47.3%
	1	Count	16	23	39
		% within WNV disease	41.0%	59.0%	100.0%
		% within Affected_town	84.2%	41.8%	52.7%
		% of Total	21.6%	31.1%	52.7%
Total		Count	19	55	74
		% within WNV disease	25.7%	74.3%	100.0%
		% within Affected_town	100.0%	100.0%	100.0%
		% of Total	25.7%	74.3%	100.0%