Identifying Comorbid Risk Factors of West Nile Neuroinvasive Disease in the Ontario Population, 2002-2012, Using Laboratory and Health Administrative Data

Jessica Sutinen

Thesis submitted to the University of Ottawa in partial Fulfillment of the requirements for the Master's of Science in Epidemiology

School of Epidemiology and Public Health
Faculty of Medicine
University of Ottawa

Abstract

Background/Objectives:

West Nile neuroinvasive disease (WNND) is a severe neurological illness that develops in approximately 1% of individuals infected with West Nile virus (WNV). Manifesting most frequently as encephalitis (WNE), meningitis (WNM), or acute flaccid paralysis (WNP), there is no cure for WNND beyond supportive care and rehabilitation, and death or permanent disability are common outcomes. As the virus arrived in North America less than 20 years ago, determinants of severe disease progression following infection are still being explored. This project is the first to examine comorbid conditions as risk factors of WNND in Ontario using a population-based study design. As prevention is the only avenue of defence against WNND, identifying comorbid risk factors of WNND would allow for public health prevention campaigns targeted to high-risk groups. The main objectives of this thesis were to explore whether pre-existing chronic diseases were associated with the development of WNND, or any of its three manifestations (i.e., encephalitis, meningitis, acute flaccid paralysis).

Methods:

This was a retrospective, population-based study including all Ontario residents with a confirmed diagnosis of WNV infection between January 1, 2002 and December 31, 2012. A cohort of individuals with WNV was identified from a provincial laboratory database and individually-linked to health administrative databases. In the WNV cohort, individuals with WNND and 13 comorbid conditions were identified using algorithms based on ICD-10-CA diagnostic codes. Incidence of WNND following WNV infection was then compared among

individuals with and without comorbid conditions using relative risks estimated by log binomial regression. Additionally, risk ratios were calculated for associations between specific comorbid conditions and WNND neuroinvasive manifestation (i.e., encephalitis, meningitis, acute flaccid paralysis). Finally, associations between Charlson Comorbidity Index (CCI) scoring and development of WNND was examined through calculation of relative risk using log binomial regression.

Results/Potential Impact:

Risk factors for WNND included male sex (aRR: 1.21; 95% CI: 1.00-1.46) in addition to the combined effect of hypertension and increasing age (5-year intervals) (aRR: 1.16; 95% CI: 1.08-1.24); WNND was also associated with increasing CCI scores; individuals in low, medium, and high categories had increased risk compared to individuals with a score of zero, but the greatest risk was in the high CCI category (aRR: 3.45; 95% CI: 2.25-4.83) Male sex (aRR: 1.32; 95% CI: 1.00-1.76), increasing age (aRR: 1.02; 95% CI: 1.02-1.03), and being immunocompromised (aRR: 2.61; 95% CI: 1.23-4.53) were associated with development of WNE. No risk factors were identified for WNM and WNP. Identification of comorbid risk factors of WNND will allow public health officials to identify high-risk groups and to develop prevention strategies targeted for vulnerable individuals.

Acknowledgements

I am grateful to many individuals for the completion of this thesis. First, my thesis supervisor, Dr. Manisha Kulkarni, who accepted an anthropologist as a research assistant, and then as a graduate student, a few years ago. Without that opportunity, I might not have become an epidemiologist. As a thesis supervisor, Manisha has been knowledgeable, patient, and ingenuitive, and I am fortunate to have been a part of her lab. Second, I am grateful to Dr. Deshayne Fell, who, as my co-supervisor, helped me navigate health administrative data, SAS coding, and ICES. Her knowledge and experience helped me feel confident in my decisions as I progressed with my analysis. Third, I must thank Dr. Beate Sander, my co-supervisor, whose previous work on West Nile virus in Ontario laid the foundation for this thesis, and which gave me a very interesting thesis topic to research. Beate's knowledge of existing data and how to properly describe health administrative data in a publication have made writing this thesis easier. Additionally, I am grateful to Ewa Sucha, the ICES analyst that helped me create the dataset and who was always available to help me with a problem or to share SAS coding tips. I am also grateful to Dr. Marie-Hélène Roy-Gagnon and Dr. Tim Ramsay, both instructors of statistics and modelling during my degree, who were always open to meeting with me and discussing analytical challenges that I was facing. Finally, I am grateful to members, past and present, of the INSIGHT Lab – kind, supportive, knowledgeable, and all-around good people.

Thank you.

Table of Contents

List of Figures and Tables

Figures

Tables

List of Acronyms

AP	Proportion attributable to interaction
CCI	Charlson Comorbidity Index
CDC	(U.S.) Centers for Disease Control and Prevention
CHF	Congestive heart failure
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CRD	Chronic renal disease
CSF	Cerebral spinal fluid
DAD	Discharge Abstract Database
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
IKN	ICES key number
NACRS	National Ambulatory Care Reporting System
ODB	Ontario Drug Benefit programme
OHIP	Ontario Health Insurance Plan
PHO	Public Health Ontario
PHOL	Public Health Ontario Laboratories
PNS	Peripheral nervous system
RERI	Relative excess risk due to interaction
RPDB	Registered Persons Database
TLR	Toll-like receptor
WNE	West Nile encephalitis
WNF	West Nile fever
WNM	West Nile meningitis
WNND	West Nile neuroinvasive disease
WNP	West Nile acute flaccid paralysis
WNV	West Nile virus

Statement of Originality

As part of the requirements for the University of Ottawa's Master of Science degree in epidemiology, this thesis represents original work by the author.

CHAPTER 1: Introduction

1.1 *Project Rationale, Overview, and Objectives*

West Nile neuroinvasive disease (WNND) is a severe illness caused by West Nile virus (WNV) infection that progresses to involve the neurological system. Inflammation of the brain parenchyma (encephalitis) and meninges (meningitis), as well as disruption of specialised spinal cord cells (acute flaccid paralysis), can lead to severe outcomes like death, coma, paralysis, or permanent disability, with most individuals requiring extended hospitalisation and post-illness rehabilitation. No cure for WNND currently exists and there is no licensed vaccine available for prevention of WNV infection in humans. Approximately 1% of WNV infections progress to WNND. A number of published risk factor studies from the United States and Europe report an association between comorbid conditions and WNND development following WNV infection.(1-6) However, this association has not been studied in Canada. As climate change continues to warm the planet, the ranges of the mosquitoes that serve as vectors of WNV will expand in Canadian provinces and breeding seasons will lengthen, allowing for more of the population to be exposed to WNV vectors and increasing the seasonal transmission period, highlighting the need for studies on WNV in Canadian populations.

The Province of Ontario has been recording laboratory-confirmed WNV infections since 2002, creating a population-based cohort from which it is possible to examine risk factors for WNND. This laboratory data can be deterministically linked to health administrative data held by ICES (formerly known as the Institute for Clinical Evaluative Sciences) that contains information on the disease status of individuals in the WNV cohort.

Associative studies on comorbid risk factors and development of WNND have not been conducted in Ontario, which has a distinct, multi-ethnic population with multiple factors that affect comorbidity. Additionally, it is Canada's largest and most densely populated province, its population is rapidly ageing, meaning that more of the population will be living with chronic disease, and its warm, temperate climate in spring and summer is conducive to WNV transmission. Examining the association between comorbid conditions and WNND in the Ontario population will inform potential risk factors for WNND in this population, allowing for identification of high-risk groups and public health prevention strategies, and contribute to the growing body of literature on comorbid risk factors of WNND, the majority of which originates from the United States.

The objectives of this thesis project were developed to explore the association between comorbid risk factors and the development of WNND in the Ontario population. Specifically, we examined whether having one or more of 13 common chronic diseases was associated with neuroinvasive disease following WNV infection, and whether specific comorbid conditions were associated with different manifestations of WNND (i.e., encephalitis, meningitis, acute flaccid paralysis). Additionally, multimorbidity, or living with two or more conditions, and its association with WNND was explored.

This thesis has six chapters. Following this introduction (Chapter 1), Chapter 2 provides a literature review describing WNV global and North American emergence and WNV and WNND incidence in Canada with particular emphasis on Ontario. It then details the transmission cycle and human infection from virus entry to illness and recovery. Lastly, potential risk factors of WNND are discussed, ranging from genetics and age to living with chronic disease. A methods

section follows in Chapter 3, describing the study population and design, WNND case definition and health administrative data sources, outcome variables, and the descriptive and analytical statistics used in this study. Chapter 4 comprises the results, which first presents the descriptive portion of the study and describes WNND incidence in Ontario, followed by an analytical results section that presents regression modelling results. A discussion follows in Chapter 5, contextualising the descriptive and analytical results of the previous chapter as well as describing study limitations and strengths. Lastly, Chapter 6 presents a brief conclusion to this thesis.

CHAPTER 2: Literature Review

2.1 West Nile Virus Global Emergence

West Nile virus was first isolated in Uganda in 1937(7), and has since spread to every inhabited continent, becoming one of the most wide-spread mosquito-borne illnesses. Following isolation in Uganda, the virus was not detected again until the 1950s in Egypt and Israel(8), where a series of outbreaks led to epidemiological and ecological studies that established the transmission cycle and clinical characteristics of infection.(9, 10) Serosurveys along the Egyptian Nile suggested seroprevalence rates approaching 60%, with higher rates among older children and adults while young children more frequently experienced symptoms of a non-specific viral fever.(10) Similar seroprevalence rates were detected in several central African countries, suggesting widespread endemicity. Viewed as a mild and self-limiting illness up to this point, an outbreak in Israel in 1957 changed perceptions of WNV infection severity: out of 49 elderly nursing home residents with suspected WNV infection, 12 developed meningoencephalitis and four with unconfirmed WNV infection, but suffering from diffuse encephalitis, died.(11, 12) Patients in subsequent outbreaks in France and South Africa during the 1960s and 1970s also presented with neurological symptoms, yet progression to neuroinvasive disease was infrequent.(13, 14) During the mid-1990s, WNV outbreaks throughout Europe and the Middle East began occurring more frequently and had higher rates of neurological involvement and/or higher fatality rates: notable are the 1996 Romanian epidemic and the 1999 Russian epidemic. In the former, 90% of almost 400 serologically confirmed or suspected cases of WNV involved neurological symptoms, with epidemiologists describing an increased susceptibility to infection and death in older individuals(15); and in the

latter, approximately 50% of meningoencephalitis cases (n=84) resulted in death, and 75% of deaths occurred in individuals >60 years old.(16)

The virus strains responsible for these epidemics belonged to lineage 1, one of eight WNV lineages, and one of the two major lineages responsible for epidemics in humans. Lineage 1 is wide-spread and has caused epidemics in Africa, the Middle East, Europe, Asia, Oceania, and North America. Strains of the second major WNV lineage, lineage 2, have caused significant epidemics since the mid-2000s in Europe and South Africa.(17-20)

West Nile virus was first detected in North America during the 1999 New York City epidemic. How it was introduced to the western hemisphere remains unknown, but genomic sequencing demonstrated comparable homology between the New York strain and a strain circulating in Israel.(21) Additionally, its epidemiological and clinical characteristics were similar to epidemics caused by lineage 1 strains in Europe and the Middle East: neurological involvement was common (63% of detected cases) and the case-fatality rate was high (12% of detected cases); and older individuals were disproportionately affected (median age of 71 years).(22) By 2004, human cases had been reported in all of the contiguous 48 states, bringing the total number of American WNV cases (since 1999) to over 10 000; more than 5 000 of these cases were diagnosed as WNND.(23) However, the true rate of WNND in individuals with WNV is not 50% - the symptoms of WNND are more severe than those of non-neurological WNV infection, leading more individuals to seek professional medical care when suffering from neurological disease.

In Canada, the first human cases were reported in Ontario and Québec in 2002.(24) Clinical characteristics of infection in Canada were similar to those reported in the United States

and characteristic of lineage 1 strains: there were considerable numbers of neuroinvasive disease cases and some infections resulted in death.(25, 26) In Central America, Mexico reported evidence of equine WNV infections in 2002 and identified its first human case in 2004.(27, 28) The virus spread throughout the Caribbean and Central and South America during the mid-2000s, with many countries reporting human or animal seropositivity or infected mosquitoes.(29-38) However, these countries report very low incidence of human WNV infections and WNND compared to the United States and Canada. In addition to fewer resources and a lack of WNV surveillance in many Central and South American countries, researchers have suggested that pre-existing neutralising anti-bodies against another *Flavivirus* might offer partial immunity(39, 40), and that strains circulating in Mexico and Central and South America may have undergone a mutation resulting in attenuation of their pathogenicity.(41)

2.2 West Nile Virus Emergence in Canada and Severe Disease in Ontario

West Nile virus was first detected in Ontario in 2001 from mosquito pools within the Greater Toronto Area and in a dead bird from Windsor.(42, 43) In 2002, the first human cases identified in Canada were reported from Ontario and Québec, with three additional provinces (Manitoba, Saskatchewan, and Nova Scotia) reporting WNV-infected dead birds.(43, 44) The following year, despite a low number of reported cases in Ontario, the Prairie Provinces experienced a human WNV epidemic: Alberta and Manitoba reported 275 and 143 cases, respectively, while Saskatchewan reported 937 (an attack rate of 93/100 000).(45-47) British Columbia did not detect any WNV activity until 2009, when four human cases were identified, as well as equine infections and positive mosquito pools.(48) The cooler climate of the Atlantic

provinces has inhibited WNV's eastward expansion; as of 2019, New Brunswick and Nova Scotia are the only Atlantic provinces that have reported human WNV cases, although Prince Edward Island has identified WNV in dead birds.(49, 50) Despite the virus' broad distribution across Canada, Ontario and Québec appear to be the most consistently affected.(51)

In Ontario, human infections were initially limited to southern areas of the province, where the warmer climate is more favourable to natural WNV circulation amongst avian hosts and mosquitoes, but in 2006, a case was reported in Sudbury, indicating that the virus had spread into Northern Ontario.(52) As of 2017, human cases have been reported as far north as Timiskaming and the virus has been detected in birds in Thunder Bay.(53, 54) Despite the northward expansion of the virus, its activity remains highest on the western shore of Lake Ontario and in Windsor-Essex County.(42)

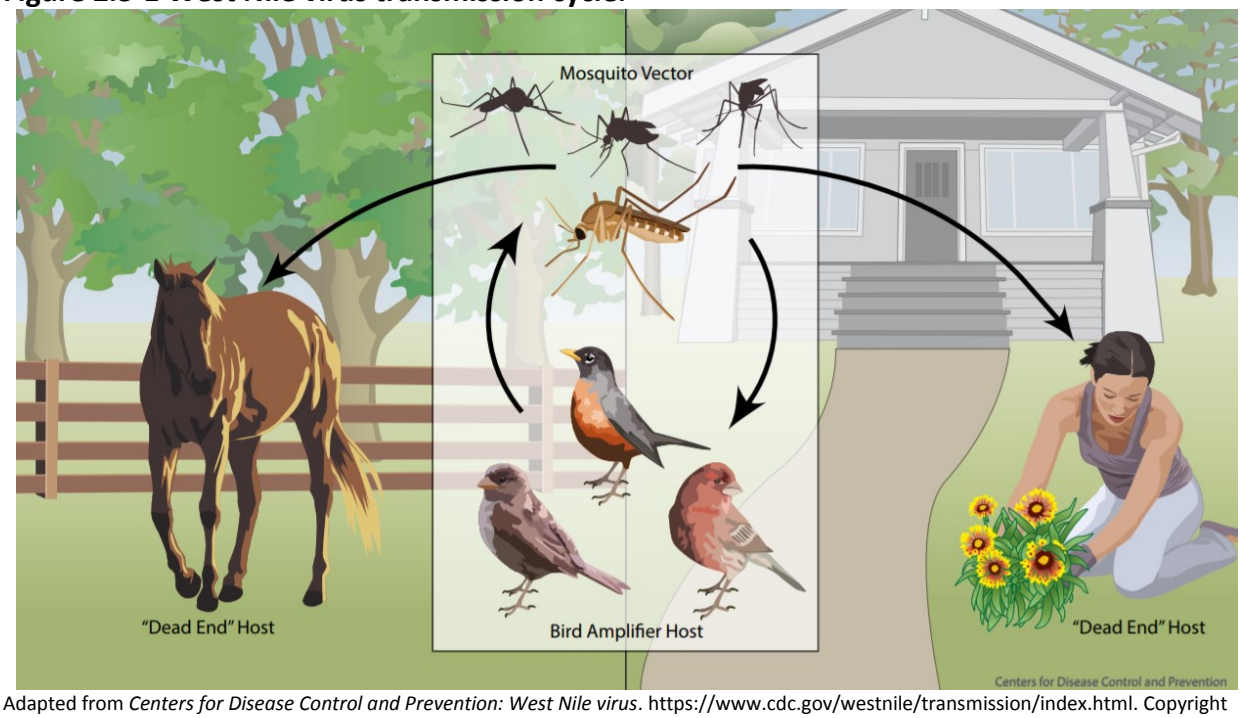
In 2002 and 2012, Ontario experienced WNV epidemics that resulted in incidence rates of 3.3 and 2.0 cases per 100 000 population, respectively.(55) During the Southern Ontario epidemic of 2002, a study on WNV-related hospitalisations in the Toronto area identified 64 cases, 62 of which were WNND.(56) The majority of patients were >50 years of age and male, and 50% had underlying chronic conditions. Ten percent of those with WNND died, and 72% of those who survived required rehabilitation or support at home following discharge. At one hospital, the most common manifestation of WNND was encephalitis (78.6%, n=11) with subsequent neuromuscular involvement.(57) During the 2012 epidemic, a small case series study (n=7) on patients presenting with WNND at a hospital in Hamilton also reported that the majority of patients (71.4%, n=5) were older individuals (i.e., >65 years of age) and suffered from encephalitis.(58) The case fatality ratio was 14.3% (n=1).

2.3 Transmission Cycle

In North America, WNV is maintained in an enzootic cycle with birds as the primary reservoir and mosquitoes as vectors. The cycle is initiated when infected mosquitoes feed on non-immune birds. Over 110 species of song birds, owls, shorebirds, and hawks are susceptible to infection, with most bird hosts surviving and developing long-lasting immunity.(59, 60) Infected birds that develop high-titre viraemia are essential to the maintenance of the enzootic cycle by transmitting the virus to feeding mosquitoes; in North America, American robins and corvids are crucial in the sylvatic maintenance of WNV.(61, 62) Corvids, in particular, are among the most susceptible to infection(63), developing severe disease and exhibiting significant mortality(64) – their 'die-offs' serve as sentinel surveillance indicators for human infection.(65) Horizontal transmission between birds has been documented(66), but the principal mode of transmission is vector-borne. The virus has been detected in 10 different mosquito genera in the United States and Canada(67), but members of the *Culex* genus (particularly *Culex pipiens*, *Culex quinquefasciatus*, and *Culex tarsalis*) are considered the primary vectors in the enzootic cycle and in spillover events.(61, 68-70) Many *Culex* mosquitoes are ornithophilic, and shift their feeding preferences to humans and mammals in late summer and early fall, accounting at least partially for the increase in human WNV cases during this period.(71) Potential bridge vectors between the avian-*Culex* cycle and mammals include members of the *Aedes* and *Ochlerotatus* genera, which have exhibited high efficiency as vectors under laboratory conditions.(68) Although WNV infections occur frequently in mammals, the majority of non-avian species are incapable of amplifying the virus to sufficient titres to transmit to feeding mosquitoes (two exceptions are the American alligator(72), and the Russian lake frog(73)).

Humans and horses are common dead-end hosts – both can develop severe disease and have high fatality rates(74-79), but cannot otherwise contribute to the transmission cycle. WNV transmission to humans is predominantly vector-borne, although alternative routes have been documented.

Figure 2.3-1 West Nile virus transmission cycle.



Adapted from *Centers for Disease Control and Prevention: West Nile virus*. https://www.cdc.gov/westnile/transmission/index.html. Copyright (2018) by Centers for Disease Control and Prevention. Adapted with permission.

2.4 Alternative Transmission Routes in Humans

The primary mode of transmission of WNV in humans is through a mosquito vector, although infections via non-mosquito-borne routes have been reported and include: vertical transmission through birth or breastfeeding (suggested) and horizontal transmission through blood and organ donation.(80-83)

Vertical transmission is a common route of infection for many viruses, but appears to be rare with WNV.(82) An infant who tested positive for WNV at birth in New York in 2002 remains the only confirmed case of vertical transmission; associated chorioretinal scarring and central nervous system malformations prompted the CDC to suggest women take precautions against WNV infection during pregnancy.(84) A longitudinal study following pregnant women infected with WNV found that a small percentage of infants may have acquired the virus congenitally, but overall, the risk of this mode of transmission seems low.(82) Additionally, transmission through breastfeeding may be possible, but rare, and has not been conclusively determined.(83)

A more commonly reported alternative route of transmission is through donated blood and organs. In 2002, 23 individuals were infected following blood transfusions from viraemic donors(85), and four individuals were infected following organ transplants from one donor(80), prompting Canadian Blood Services to begin WNV screening of donated blood in 2003.(86)

2.5 Mechanisms of Infection

Following virus entry via mosquito inoculation in a human host, Langerhans cells, dermal dendritic cells, and epidermal keratinocytes are likely targets for initial WNV replication.(87-89) Cell-surface glycosaminoglycans, ubiquitous across cell types, provide attachment contact for WNV virions, but specific co-receptors have not been identified.(90) Although WNV exhibits wide tissue tropism, its cellular tropism may be limited by co-receptors expressed on its replication targets (endothelial cells, monocytes, neurons, dendritic cells, macrophages, and non-migrating skin cells).(88, 91) Replication occurs in the lymph nodes, potentially as a result of dendritic migration or an unidentified haematogeneous entry mechanism(92), resulting in

viraemia and secondary infection of visceral organs, the more permissive of which serve as primary sites of replication in peripheral tissue.(93) Infection and dissemination are limited by early innate immune responses, particularly interferon type I response(94-96), and innate and adaptive immune cell effector functions; of particular importance are γ/δ T cells, which produce elevated levels of interferon-γ(97), and IgM-secreting B-cells.(98) Once WNV enters the central nervous system (CNS), approximately 6-8 days after infection(93), $CD8^+$ T cells, neutrophils, $CD4^+$ T cells, macrophages, and microglials are critical in controlling infection and clearing the virus from the CNS.(99-104)

The mechanism of CNS entry by WNV is not well understood. Viral determinants, particularly structural proteins constituting receptor binding domains on the virus envelope, are implicated in WNV's neuroinvasiveness(105-107), possibly because they enhance endothelial cell binding to viral epitopes.(108) Routes of entry, particularly haematogenous(108-113) and transneural(114-117), are common research foci, although mechanisms employing multiple routes have been proposed.(118-120)

A haematogenous mechanism may be the most probable route - more rapid CNS entry by WNV is correlated with higher levels of viraemia.(121) Proposed mechanisms include: infection of cerebral endothelial cells and pericytes, which may permit viral transcytosis across these cell types into the brain(110, 122); overexpression of vasoactive cytokines (e.g., tumour necrosis factor) and matrixins, which can destabilise tight junction proteins, leading to a weakening of the blood-brain barrier and permitting virus entry into the cerebral parenchyma(123-125); and infected leukocyte trafficking across the blood-brain barrier, as a

result of degraded junctions and help from upregulated cell adhesion molecules(126), a function of which is control of leukocyte movement during inflammatory processes.(127)

Transneural routes of infection have also been hypothesised as potential mechanisms of entry, with WNV migrating to the CNS from olfactory nerves or the peripheral nervous system (PNS). Olfactory receptor neuron axons are connected to the olfactory bulb via olfactory ensheathing cells, which permit particles ≤100 nm (e.g., WNV) into the CNS.(128) Additionally, the virus could infect the ensheathing cells directly, and upon release, could reach the olfactory bulb.(129) Alternatively, the virus may be transported from the peripheral nervous system (PNS) to the CNS; the PNS directly enervates a wide variety of tissue types and lacks the immunological barriers of the CNS, allowing for greater susceptibility to viral infection. WNV may enter the PNS by directly infecting nerve endings in the tissue and entering the CNS via retrograde axonal transport(114, 115), preferentially infecting motor neurons.(115)

Once the virus has entered the CNS, neurological disease can develop in the brain parenchyma and the meninges of the spinal cord. The inflammation and neuronal loss caused by the disease, whether through viral pathogenicity or immune response, results in WNND. The virus infects a range of residential CNS cell types including neurons, astrocytes, microglials, and macrophages, all of which have varying levels of susceptibility to infection dependent on intrinsic defense. Immune response in the CNS is induced following infection of residential cells and the entry of inflammatory cells (i.e., neutrophils, microglia, macrophages, and effector $CD4^+$ and $CD8^+$ T cells) into this immunoprivileged area.

2.6 Illness and Recovery

Infection with WNV is predominantly asymptomatic, but approximately 20% of infected individuals develop West Nile fever (WNF), a non-neuroinvasive, self-limiting illness that presents with non-specific symptoms of viral infection including fever, fatigue, headache, and nausea. It is considered a relatively mild illness, although not without complications, and most individuals fully recover. However, an estimated ~1% (130) of all infections result in WNND, which generally manifests as West Nile encephalitis (WNE), West Nile meningitis (WNM), or West Nile acute flaccid paralysis (WNP), although combinations of disease type (e.g., WNE and WNP) can occur.(131) WNND infections are severe and can lead to permanent disability, coma, and death; neuroinvasive infections are more likely to develop in older individuals, or those with pre-existing conditions.(3, 6, 79, 132-136) Clinical management of most WNV infections is supportive, with most individuals requiring care related to pain control and dehydration.(137)

In general, many individuals with diagnosed WNV infections, particularly those who develop WNND, require hospitalisation.(134) Recovery is often lengthy, with most individuals requiring rehabilitation(138), and the development of persistent, long-term symptoms is common.(134, 139-141) These sequelae range from functional and physical to cognitive and psychological and they appear to affect individuals with WNND and WNF differently – functional and physical sequelae are more common in individuals with WNND, while cognitive and psychological symptoms seem to affect individuals with WNF and WNND equally.(140, 142-146)

Few individuals are capable of returning to independent, unassisted living immediately following hospitalisation with WNV, particularly if they developed WNND(6, 56, 147); discharge

to a rehabilitation hospital, nursing home, or a home with help following hospitalisation is common, although some patients do not return to premorbid functional status despite rehabilitation.(148)

2.6.1 West Nile Fever

West Nile Fever is the most common clinical syndrome of WNV, developing in approximately 20% of infected individuals. It is distinct from the more severe manifestations of WNND because it does not involve the nervous system. WNF is considered a benign, self-limiting illness, with non-specific symptoms of viral infection developing after 2-14 days. The most commonly experienced symptoms are fatigue, fever, headache, myalgia, and nausea, although a wide range of symptoms have been reported and include (but are not limited to) arthralgia, diarrhoea, chills, maculopapular rash, and sensitivity to light.(139, 149, 150) Illness severity is typically milder in younger individuals.(136)

WNF is associated with significant short- and long-term morbidity. However, compared to WNND, WNF results in fewer hospitalisations and a shorter length of stay, with fatigue, muscle weakness, and myalgia among the most reported persistent symptoms.(135, 139) One Canadian study reported delayed recall in 50% of patients between 2-4 years post-illness(146), suggesting that neuropsychiatric impairment may be the longest-lasting class of symptoms of WNV infection. Sequelae develop in most individuals, with one study finding that almost two-thirds of patients experienced symptoms 30 days after illness onset.(139) WNF sequelae can be debilitating, and prolonged absence from school/work following infection is not uncommon.(147) Older age is a risk factor for poorer outcomes (e.g., delayed recovery, longer rehabilitation periods, death)(148, 151), and although mortality rates are very low, WNF may

exacerbate underlying conditions, resulting in significant mortality in older adults and the immunocompromised.(148, 152)

2.6.2 West Nile Encephalitis

If the virus bypasses the blood-brain barrier and enters the brain, any acute inflammation of the parenchyma is termed West Nile encephalitis. WNE is the most common manifestation of WNND, comprising ~60% of all WNND cases(131, 153, 154), and develops more frequently in older and immunocompromised individuals.(155) Illness severity ranges from mild (e.g., confusion) to severe (e.g., death, coma)(155), with many patients presenting with symptoms that overlap with those of WNF and other manifestations of WNND (e.g., headache, nausea, fatigue, stiff neck, dizziness, etc.).(142, 150) Some patients initially present with an altered mental state – confusion, or a change in the level of consciousness – before more severe neurological signs or symptoms develop.

Individuals with acute WNE suffer higher rates of persistent neurological abnormalities and longer hospital stays (i.e., mean length of stay, 8-25 days)(156, 157) compared to those with WNF or WNM.(158) Although the movement disorders characteristic of WNE are generally transient, disappearing after a few days or weeks(159, 160), tremors(161), and tandem gait and strength/reflex abnormalities have been recorded years post-illness.(158) For example, Weatherhead et al. found that 17% of WNE patients in their sample continued to experience tandem gait abnormalities 8-11 years post-infection.(158) WNE can be severely debilitating, with rehabilitation and assisted living/care frequently needed – less than 50% of patients are discharged into their homes(6, 162, 163), and one study reported 65% of patients required rehabilitation post-illness.(5) Mortality is high, ranging from 3-29%(6, 162, 163) in-hospital and

during acute illness, and is elevated in older adults (~46%).(151) Long-term mortality rates (e.g., 1-year post-illness) have been compared to those of patients with severe common chronic diseases.(138)

2.6.3 West Nile Meningitis

West Nile meningitis is the acute inflammation of the meninges of the brain and/or spinal cord following infection with WNV. It is the second most common manifestation, developing in ~40% of WNND cases(131), and is associated with a more favourable outcome than WNE, with most individuals recovering fully.(155) Rapid onset of fever and headache, pleocytosis in the CSF, meningeal signs (e.g., nuchal rigidity, nausea, Kernig's sign, Brudzinski's signs), and photophobia and/or phonophobia(155), are clinical characteristics that define WNM. Patients often present with symptoms that overlap with those of WNF and other WNND manifestations, with the most frequently experienced symptoms including headache, nausea, fever, neck/back pain, myalgia, photophobia, and vomiting.(142, 150)

WNM develops more frequently in younger individuals, with one study finding median ages of 35 and 70 in patients with WNM and WNE, respectively.(161) It is also associated with better survival rates, slightly fewer hospitalisations (81% of WNM cases hospitalised vs. 82% [WNP] and 86% [WNE])(79), and shorter lengths of stay than other manifestations of WNND.(4, 157, 162, 164) In-hospital and acute mortality is low (<2%)(162), and when not accompanied by an altered mental state, prognosis is generally favourable(137), although recovery and rehabilitation for some may be prolonged and last >12 months.(147) Individuals hospitalised with WNM may have the highest home discharge rates.(163, 165)

2.6.4 West Nile Acute Flaccid Paralysis

Acute flaccid paralysis presents similarly to poliomyelitis and likely results from infection of the anterior horn cells of the spinal cord.(160, 166) WNP is the least common manifestation of WNND and more commonly affects younger individuals (e.g., in one study, 75% of patients were ≤61 years old).(167) Within 48 hours of symptom onset, asymmetric limb paralysis/weakness (or, less commonly, symmetric quadriplegia) can develop.(168) Facial nerve palsy(169), dysphagia, and dysarthria are also associated with WNP.(167) Respiratory failure from intercostal and diaphragmatic muscle paralysis can occur, with patients requiring intubation and mechanical ventilation.(168, 170)

Similarly to WNE, WNP is a severe illness with complicated recovery. Acute mortality with respiratory involvement, which necessitates intubation and mechanical ventilation, was as high as 50% in one study.(169) Compared to patients with WNE, WNM, and WNF, patients with WNP have the longest mean hospital stay (11-68 days(136, 171, 172)), yet frequently have poor physical recovery – in one study, no patients had become ambulatory or made full recovery of strength despite acute inpatient rehabilitation by 6-month follow-up.(171) Discharge to long-term care facilities is common, with some patients continuing physical therapy following discharge home.(173) However, most patients do make improvements in functional and strength outcomes, with complete recovery possible, but uncommon, 1-year post-illness.(174) In comparison, mental health outcomes are generally favourable.(172)

2.7 Risk Factors of West Nile Neuroinvasive Disease

Disease progression to WNND is influenced by a range of individual host risk factors including genetic variants, chronic diseases, sex, and age, the latter being the most consistently identified across studies. While genetic predisposition and age and their involvement in the progression to WNND has been explored in the literature, the associations between WNND and sex and comorbid conditions remain less clear and reporting on these two risk factors remains predominantly descriptive.

2.7.1 Genetic Variants

Several genomic determinants for development of WNND following infection with WNV have been identified. Specific human leukocyte antigen alleles have been found to be more associated with severe WNV infection, suggesting that these alleles may in part determine disease progression.(175) Genetic variation in the interferon pathway may also influence individual susceptibility to development of WNND: a single mutation in the gene OAS1 was found to be associated with an increased risk of WNE and WNP.(176) Additionally, although once viewed as potentially unimportant during infection, variation in chemokine receptor CCR5, specifically homozygosity in *CCR5Δ32*, was found to be associated with more aggressive disease and multisystem symptomology, including neurologic involvement, in patients with WNV.(177)

2.7.2 Age

Older age is a well-established risk factor for severity of disease during viral infection and is one of the most frequently reported risk factors for the development of WNND. Changes in immune function and immune cell populations due to ageing affect each step of the WNV

infection process, some of which may be critical for neurological involvement. Several studies have examined age-related effects on WNV immune response and have shown increased dysfunction of the immune system, particularly concerning inflammation, T-cell functionality, and identification of foreign antigens.(178-182) These age-related changes are important because an altered inflammatory response may affect the permeability of the blood-brain barrier, which is implicated in the development of WNND.

Epidemiological studies frequently report age as one of the most important risk factors in the development of WNND. One nationwide study in the United States reported that individuals aged 40-49 were 2.8 (95% CI: 2.5-3.2) times as likely to develop WNND compared to those <40 years of age(183), while two focussing on WNV patients in Illinois and Colorado reported that those >50 years of age had 3.3 (95% CI: 2.6-4.3) and 2.7 (95% CI: 1.2-6.5) times the risk of developing WNND than those ≤50 years of age.(6, 157) Huhn et al. comprehensively reported WNND risk ratios for seven different age groups for patients during the 2002 Illinois epidemic(157): compared to individuals age 0-19 years, individuals between 20-29 years were 2.4 (95% CI: 1.5-3.8) times more likely to develop WNND, while those between 30-39 years saw their risk nearly triple in comparison to individuals in their twenties (6.1 [95% CI: 4.2-9.1]); the age groups 40-49 years and 50-59 years were associated with comparable risk ratios for WNND at 10.0 (95% CI: 6.9-14.5) and 12.2 (95% CI: 8.4-17.8), respectively; and the oldest age groups (60-69 years, 70-79 years, and ≥80 years) were associated with the highest risks of neuroinvasive illness with risk ratios of 18.0 (95% CI: 12.3-26.3), 22.9 (95% CI: 15.7-33.4), and 27.5 (95% CI: 18.6-40.8), respectively.

2.7.3 Sex

Sex is a biological determinant of immune system response, with females generally mounting stronger responses to infection than males.(184) Genetic variations resulting from sex-based chromosomal differences are responsible but have not been explored with WNV. In a multi-state study conducted in 2002, males had a 10-80% increased risk of developing WNND, depending on age.(183) Additionally, males had 1.57 (95% CI: 1.18-2.09) times the odds of developing neurological disease in a 2005 study in California(132), and 3.1 (95% CI: 1.5-6.4) times the odds in a 2012 epidemic in Greece.(1)

2.7.4 Comorbid Conditions

The association between comorbidity and severe outcomes of viral infection is well-documented, with varying mechanisms of pathology affecting the immune system's ability to fight infection, leading to severe illness and/or death.(185-193) The majority of severe outcomes associated with WNV occur when the virus enters the CNS and causes inflammation in the brain parenchyma (WNE) and/or meninges (WNM) or, in the case of acute flaccid paralysis, infects the anterior horn cells of the spinal cord (WNP).(160, 166) Although the exact mechanisms by which WNV enters the CNS have not been determined, comorbid conditions affecting haematogenous and transneuronal pathways of entry are likely to increase the risk of developing WNND. Studies examining the potential relationship between chronic disease and WNND have been exploratory in nature and have focused on prevalent, major chronic and infectious diseases such as diabetes, cardiovascular disease, chronic obstructive pulmonary disease, cancer, HIV, and chronic renal disease. These conditions cause chronic inflammation

and/or immune suppression, both believed to be important factors mediating WNV entry into the CNS.

Chronic disease prevalence is high in Canada, with one in five people living with at least one major chronic disease (i.e., cardiovascular disease, chronic renal disease, diabetes, or cancer).(194) Multi-morbidity is also common: 3.6% of Canadian adults age 20 years or older have two or more major chronic diseases, which nearly quadruples (11.7%) for Canadians aged 65 years and older.(194) Ontario's ageing population, projected to have nearly a quarter of its citizens reach age 65 years and older by 2041(195), will experience a dramatic increase in chronic disease morbidity and multi-morbidity. A higher prevalence of chronic disease will result in a population with increased susceptibility to the development of severe outcomes from viral infections like WNV.

A number of studies from the United States have explored the effects of chronic disease on WNV infection and have helped identify specific conditions that may be risk factors for WNND. A West Nile virus epidemic in Colorado, responsible for nearly 1/3 of all reported WNV infections in the United States in 2003, resulted in a large number of individuals developing WNND.(6) A study on a subset of the infected cohort reported that having cancer was associated with 6.6 (95% CI: 1.6-27.5) and 7.5 (95% CI: 1.2-45.4) times the odds of developing WNM and WNE, respectively, while patients who had undergone chemotherapy had 7.7 (95% CI: 1.5-40.0) and 25.9 (95% CI: 4.2- 159.7) times the odds (respectively).(5) Cancer was also associated with 2.7 (95% CI: 1.5-5.1) times the odds of developing WNE in an ArboNET (national arbovirus surveillance system managed by the CDC) enhanced population-based surveillance study of West Nile virus in 19 states between 2008 and 2010.(2)

Hypertension has also been reported as a risk factor for WNND. In a subset of WNV-positive individuals in the 2003 Colorado epidemic, those with hypertension had 2.1 (95% CI: 1.0-4.6) times the odds of developing WNE.(5) A matched case-control study also reported an increased odds ratio for WNE in Houston, Texas patients with hypertension (4.0; 95% CI: 1.5-10.4)(3); and hypertensive patients included in the ArboNET study had 1.8 (95% CI: 1.3-2.6) times the odds of developing WNE, and 1.6 (95% CI: 1.1-2.3) times the odds of developing WNM.(2)

Also reported as a WNND risk factor is diabetes; it was associated with 1.8 (95% CI: 1.3-2.6) times the odds of WNE in the ArboNET study and 2.6 (95% CI: 1.0-6.5) times the odds in a subset of WNV-positive individuals during the 2003 Colorado epidemic.(2, 5) Another study of a WNV-positive cohort from the 2003 Colorado epidemic reported an odds ratio for WNE of 4.1 (95% CI: 1.2-13.6) for diabetes.(6)

Individuals with kidney disease also have increased odds of disease progression to WNND: kidney disease patients in the ArboNET enhanced surveillance cohort had 2.9 (95% CI: 1.3-6.3) times the odds of developing encephalitis (2); and during the 2003 Colorado epidemic, individuals with kidney disease had 24.9 (95% CI: 4.7-132.5) times the odds of disease progression to WNE.(5)

Additionally, immunocompromising conditions (including HIV, chemotherapy, organ transplant, use of immune suppression drugs, etc.) and cardiovascular disease were associated with 5.6 (95% CI: 2.1-14.9) and 28.3 (95% CI: 5.9-134.9) times the odds of developing WNE in a case control study on patients from Houston, Texas.(3)

CHAPTER 3: Methods

3.1 Source Population, Study Population, and Study Design

Ontario is the most populous province in Canada; its 14.5 million residents comprise almost 40% of the nation's total population.(196) Demographically, the median age in Ontario is 40.7 years, and the largest age group is 15-64 years, which represents just over two-thirds of the population.(195, 196) The province's population is rapidly ageing; seniors (≥65 years) currently represent 16.7% of all Ontario residents and account for a larger proportion of the population than those aged 0-14 years.(195) Projections suggest that seniors will comprise nearly a quarter of Ontario's population by 2041.(195) Ontario's ageing population is an important factor in the current and future epidemiology of WNND; older individuals are more likely to develop neurological illness following WNV infection than younger individuals.(1, 6, 132, 157, 183)

Geographically, more than 90% of the province's population is concentrated in Southern Ontario, a region 139 000 km^2 in size that stretches eastwards from Windsor to Ottawa and extends north to Parry Sound. The warmer climate of Southern Ontario is more favourable to natural WNV circulation amongst avian hosts and mosquitoes, and the majority of WNV infections in the province have been recorded in this region.

This study used a retrospective, population-based cohort design using province-wide data on all incident confirmed and probable WNV infections identified in Ontario between January 1st, 2002 and December 31st, 2012.

3.2 West Nile Virus Case Definitions, Public Health Ontario Cohort, and Data Sources

West Nile virus infection is a reportable disease in Ontario, and all reported laboratory-confirmed, probable, and suspected cases since 2002 are recorded in a database maintained by Public Health Ontario (PHO). Health care professionals who suspect a patient may have been infected with WNV must submit samples (blood, plasma, serum, or cerebral spinal fluid [CSF]) to the Public Health Ontario Laboratory (PHOL) for serological or molecular testing. However, patients with WNV infection predominantly present with symptoms of a mild, non-specific viral illness, and it is likely that many symptomatic WNV infections go undetected in patients seeking care from health professionals.

This study used an already existing cohort of Ontario residents with laboratory-confirmed WNV infection (hereafter referred to as the WNV cohort).(197) Briefly, individual WNV cases between January 1st, 2002 and December 31st, 2012 were identified in PHOL data according to the 2017 diagnostic test case definitions to create a cohort of confirmed and probable WNV cases in Ontario. To reduce potential misclassification of the study outcome, only individuals with confirmed or probable WNV infections were included in this study. PHOL uses the definitions for confirmed and probable WNV cases developed by the Ontario Ministry of Health and Long-Term Care.(198) The definitions are based on diagnostic test criteria and are displayed in Table 3.2-1.

The WNV cohort was then deterministically linked to provincial health administrative and demographic datasets held by ICES. ICES is a prescribed entity under Ontario's Personal Health Information Privacy Act and is specially designated to receive and use publicly funded administrative health services data for the Ontario population since 1986. ICES research focuses

Table 3.2-1 Confirmed and probable diagnostic test criteria used to identify WNV infections in the PHOL data.*

West Nile Virus Confirmed Case Diagnostic Test Criteria **At least one of the following:** A significant (i.e., fourfold or greater) rise in WNV neutralizing antibody titres (using a PRNT or other kind of neutralization assay) in paired acute and convalescent sera, or cerebrospinal fluid (CSF) **OR** Isolation of WNV from, or demonstration of WNV antigen or WNV- specific genomic sequences using an assay verified for clinical testing in tissue, blood, CSF or other body fluids **OR** Demonstration of flavivirus antibodies in a single serum sample using a WNV immunoglobulin M (IgM) enzyme-linked immuno-sorbent assay (ELISA) confirmed by the detection of WNV specific antibodies using a PRNT (acute or convalescent serum sample) **OR** Demonstration of WNV antibodies in a single CSF sample using a WNV immunoglobulin M (IgM) enzyme-linked immuno-sorbent assay (ELISA) **OR** A significant (i.e., fourfold or greater) rise in flavivirus haemagglutination inhibition (HI) titres in paired acute and convalescent sera or demonstration of a seroconversion using a WNV Immunoglobulin G (IgG) ELISA AND the detection of WN specific antibodies using a PRNT (acute or convalescent serum sample).
West Nile Virus Probable Case Diagnostic Test Criteria** **At least one of the following:** Detection of flavivirus antibodies in a single serum sample using a WNV IgM ELISA3 without confirmatory neutralization serology (e.g., PRNT) **OR** A significant (i.e., fourfold or greater) rise in flavivirus HI titres in paired acute and convalescent sera or demonstration of a seroconversion using a WNV IgG ELISA3 **OR** A titre of >1:320 in a single WNV HI test, or an elevated titre in a WNV IgG ELISA, with a confirmatory PRNT result **OR** Demonstration of Japanese encephalitis (JE) serocomplex-specific genomic sequences in blood by nucleic acid amplification test (NAAT) screening on donor blood, by Blood Operators in Canada.

*Definitions for confirmed and probable WNV cases were developed by the Ontario Ministry of Health and Long-Term Care.
**Case definitions for probable WNV infections prior to 2014 included clinical criteria in addition to laboratory test criteria.

primarily on evaluating health care delivery and population outcomes by accessing its holdings of health-related and demographic data. These include, but are not limited to, clinical and administrative databases, population-based health surveys, and patient records.

In this study, ICES-held data were used to ascertain study outcomes and prevalent comorbid conditions in the WNV cohort, as well as to supply additional demographic information (i.e., age and sex). Specifically, the ICES data holdings used were (Table 3.2-2): Canadian Institute for Health Information's Hospital Discharge Abstract Database (DAD) and National Ambulatory Care Reporting System (NACRS), Ontario Health Insurance Plan claims database (OHIP), Ontario Drug Benefit programme (ODB), Registered Persons Database (RPDB), as well as several ICES-acquired cohorts/registries and ICES-derived cohorts.

Table 3.2-2 ICES data holdings and data used in this study.

Data Holding	Description	Data Provided
Registered Persons Database (RPDB)	Information on individuals registered under the Ontario Health Insurance Plan (OHIP) and those eligible for the Ontario Drug Programme	Date of birth, date of death, sex
Discharge Abstract Database (DAD)	Clinical and administrative information on hospital discharges from all provincial and territorial acute care hospitals	Ascertainment of outcomes: WNND Ascertainment of: CRD, MS, Alzheimer's disease/dementia, stroke
National Ambulatory Care Reporting System (NACRS)	Information on all hospital-based and community-based ambulatory care (day surgeries, outpatient and community-based clinics, emergency departments)	Ascertainment of outcomes: WNND Ascertainment of: CRD, MS, Alzheimer's disease/dementia, stroke
Ontario Health Insurance Plain (OHIP) Claims Database	Information on OHIP billing claims	Ascertainment of outcomes: WNND Ascertainment of: CRD, MS, Alzheimer's disease/dementia, stroke
Ontario Drug Benefits Programme (ODB)	Information on drug claims history of ODB recipients (for this study period, ≥65 years)	Ascertainment of: Alzheimer's disease/dementia
ICES special registries – Ontario Cancer Registry	Information on all Ontario residents diagnosed with cancer (exception: basal cell and squamous cell skin cancers), or who have died from cancer	Ascertainment of: cancer
ICES derived cohorts	Information on chronic conditions in Ontario in cohorts developed at ICES using linked data algorithms	Ascertainment of: asthma, CHF, COPD, HIV, hypertension, diabetes, rheumatoid arthritis
ICES health services – Canadian Organ Replacement Registry	Information on groups of individuals in Canada who have had similar health services experiences	Ascertainment of: organ transplant

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; HIV, human immunodeficiency virus; MS, multiple sclerosis.

3.3 Data Linkage – PHOL and ICES

PHOL data were used to create the study cohort and contributed only infection status (i.e., that a case was a confirmed or probable WNV infection). The ICES holdings (i.e., DAD, NACRS, ODB, OHIP, RPDB, and special registries and cohorts) provided comorbid condition and demographic information for the individuals in the study cohort. Data sharing and privacy agreements between PHO and ICES for this study allowed for linkage between the PHOL data and the ICES-held data. ICES assigns each Ontario resident with provincial health care coverage a unique, encrypted identifier, the ICES key number (IKN), which is generated from an individual's Ontario health card number using a secure algorithm. Upon receipt of the PHOL data at ICES, the Ontario health card numbers were replaced with IKNs, allowing for deterministic linkage between ICES-held data and PHOL data.

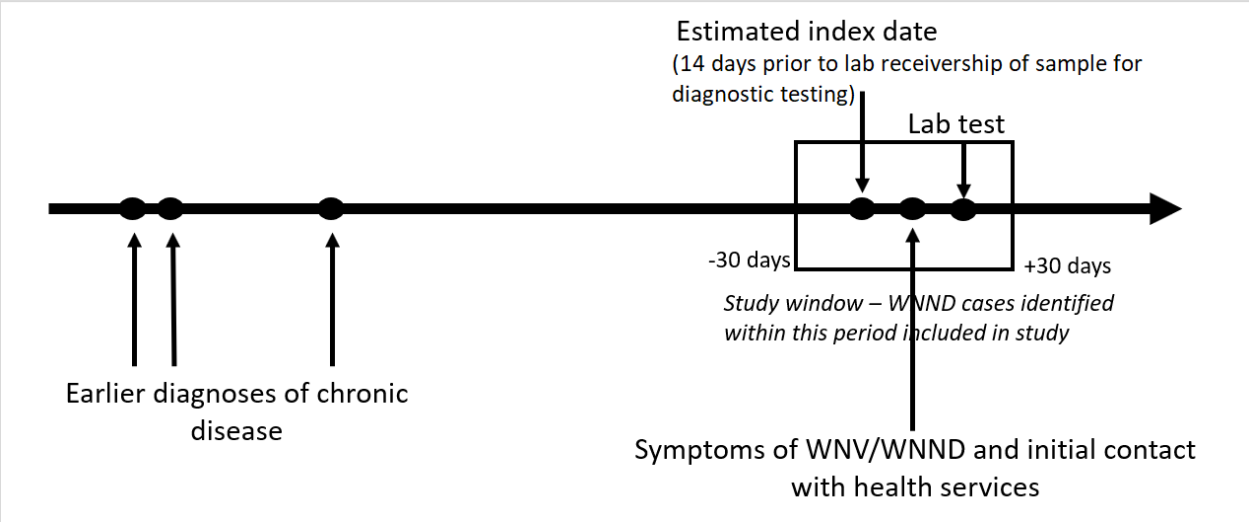
3.4 Outcome Variable: West Nile Neuroinvasive Disease

The outcomes of interest were neurological conditions that comprise WNND, which is the collective term for the severe neurological illnesses caused by WNV infection and includes encephalitis, meningitis, and acute flaccid paralysis. Diagnosis of WNND is not straightforward - encephalitis and meningitis (inflammation of the brain and meninges, respectively) are common immune responses to illnesses with neurological involvement; these conditions, as well as acute flaccid paralysis, have multiple aetiologies (both viral and non-viral), so their diagnosis alone does not establish WNV as the causative agent. The non-specific nature of WNV pathogenicity, with or without neurological involvement, makes diagnosis with a WNV aetiology difficult. A study examining rates of encephalitis in Canada between 1994 and 2008 reported that nearly 50% of encephalitis hospitalisations had unknown aetiologies; and it also

identified spatio-temporal clusters [of undiagnosed encephalitis hospitalisations] in the summer and autumn months that could potentially signify arboviral (e.g., WNV) infections.(199)

We identified WNND outcomes in our study cohort by searching for any records with at least one diagnostic code indicating encephalitis, meningitis, acute flaccid paralysis, or a combination of these conditions in the OHIP, NACRS, or DAD databases (see Appendix A for diagnosis codes used in definition) within ±30 days of the WNV infection index date (estimated as 14 days prior to earliest recorded laboratory date). Individuals with ≥2 occurrences of identical diagnostic codes within two years prior to the index date were excluded in order to include only incident WNND cases in the analysis. Using algorithms developed with ICD-10 codes can be an effective method for identifying cases of disease in health administrative data.(200-202) Unfortunately, this algorithm has not been validated and its sensitivity and specificity when applied to health administrative data are unknown. Fifty-two individuals with encephalitis, meningitis, and/or acute flaccid paralysis were excluded from the study because they violated this inclusion criterion (i.e., they had repeated encephalitis, meningitis, and/or acute flaccid paralysis diagnostic codes within two years prior to index date).

Figure 3.4-1 Study design diagram showing study window for identification of WNND cases within the WNV cohort.



3.5 Independent Variables, Case Data Provenience, and Algorithm Look-Back Windows

Thirteen chronic conditions were evaluated as independent risk factors for WNND in this study. Ten were selected based on their use in similar studies examining associations between comorbid conditions and WNND (i.e., cancer, congestive heart failure [CHF], chronic obstructive pulmonary disease [COPD], chronic renal disease [CRD], diabetes, human immunodeficiency virus [HIV], hypertension, [recipient of] organ transplant, rheumatoid arthritis, and stroke); and three were added based on clinician advice due to their high prevalence in the Ontario population and/or association with neurotropic disease (i.e., Alzheimer's disease and/or dementia, asthma, and multiple sclerosis [MS]).

We also evaluated the association between multimorbidity and WNND using the Charlson Comorbidity Index. The Charlson Comorbidity Index (CCI) is a method used to capture the effects of comorbid diseases an individual may have (in addition to the disease of interest) on a specific outcome (e.g., mortality). It is a standardised metric that considers the combined

effects of multiple chronic diseases and allows for meaningful comparison between multimorbid groups or individuals. Seventeen conditions (i.e., myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, rheumatologic disease, peptic ulcer disease, mild liver disease, diabetes or diabetes with chronic complication, haemiplegia or paraplegia, renal disease, any malignancy [including leukemia and lymphoma], moderate or severe liver disease, metastatic solid tumour, AIDS/HIV) are included in the index, each with a value assigned, and the sum of these values produces the index score. Scores range from 0 (no comorbidity detected) to 33 (high comorbidity). In general, higher CCI scores are predictive of poorer outcomes. CCI scores are often categorised before inclusion in regression analyses, and this study adopted a similar scoring system to those in existing publications. Four categories were created, ranging from no comorbidities to severe: 0 – no comorbidities; 1-3 low; 4-6 medium; 7+ high.

Comorbid conditions in the study cohort were identified in ICES-held data by: 1) extracting records of prevalent/incident cases from pre-existing disease-specific registries or ICES-derived cohorts; or 2) applying algorithms designed to identify cases in health administrative data through the satisfaction of multiple criteria. The latter method is more susceptible to misclassification and was only used when a pre-existing registry or cohort did not exist. Validated algorithms, which are algorithms tested against a gold standard (usually medical chart reviews), have been published for CRD (203), MS (204), and Alzheimer's/dementia (205) and were used in this study. The stroke algorithm was developed for this study by an ICES analyst with input from a stroke clinician scientist. Table 3.6-1 displays

the definitions for each of the comorbid conditions and provides validation measures, where available.

Some chronic conditions were subject to 'look-back windows', the period between outcome diagnosis and how far into a patient's past medical history an algorithm could search to satisfy its case criteria. In this study, look-back windows were five years (except for stroke and cancer). For example, for a patient with MS to be identified by the algorithm, they needed to have either one hospitalisation or five physician billings in a four-year window during the five years prior to WNND diagnosis. The look-back window for stroke was reduced to six months to account for post-stroke immunodepression.(206) Additionally, for cancer diagnoses, which were identified using clinically confirmed diagnoses from the Ontario Cancer Registry and not an algorithm, a look-back window of two years was used on the suggestion of an oncologist for similar reasons.

3.6 Missing Data

Missing data was only relevant for age, sex, and time-related variables (e.g., index date) because a lack of a diagnostic code for any comorbid condition in the database was interpreted as indication that the individual did not have the condition, not as an indication of missing information. Time-related variables were requisite components of the inclusion criteria; therefore, no individuals had missing time-related information. Individuals with missing information on sex, age, or time-related variables were deleted from the dataset under the assumption that data were missing-at-random; and because the percentage of patients with missing information was <5%, data imputation was not used.

Table 3.6-1 Definitions of the 13 chronic conditions included in this study and their provenience.

Comorbidity	Provenience	Registry/Cohort Name or Algorithm Publication	Sensitivity (SN) and Specificity (SP)
Asthma	ICES-derived cohort	Ontario Asthma Dataset	(Sensitive cohort) Adults (18+) SN 80.6%, SP 81.4% Children (<18) SN 89%, SP 72%; established from (207-209)
Alzheimer's/dementia	Validated algorithm	2016 publication, see (205)	SN 79.3%, SP 99.1%
Cancer	Registry	Ontario Cancer Registry	N/A[a]
CHF	ICES-derived cohort	Ontario Congestive Heart Failure Dataset	Adults (40+) SN 84.8%, SP 97%; established from (210)
COPD	ICES-derived cohort	Ontario Chronic Obstructive Pulmonary Disease Dataset	(Sensitive cohort) Adults (35+) SN 85%, 78.4% SP; established from (211)
CRD	Validated algorithm	2013 publication, see(203)	SN 32.7%, SP >94.0%
Diabetes	ICES-derived cohort	Ontario Diabetes Dataset	(Sensitive cohort) Children ≤18 SN 82.8%, 98.9% SP Adults SN 90%, SP 97.7%; established from (212, 213)
HIV	ICES-derived cohort	Ontario HIV Dataset	SN 96.2%, SP 99.6%; established from (214)
Hypertension	ICES-derived cohort	Ontario Hypertension Dataset	Adults (18+) SN 72%, SP 95%; established from(215, 216)
Multiple sclerosis	Validated algorithm	2015 publication, see (204)	SN 87.4%, SP 100%
Organ transplant recipient	Registry	Canadian Organ Replacement Registry	N/A[a]
Rheumatoid arthritis	ICES-derived cohort	Ontario Rheumatoid Arthritis Dataset	Ages 15+ SN 78%, SP 100%; established from (217, 218)
Stroke	Algorithm	-[b]	N/A

[a] Sensitivity and specificity values were not available for all conditions.

[b] Algorithm developed by ICES analyst and stroke clinician scientist using Canadian Stroke Strategy Case Definitions.

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; HIV, human immunodeficiency virus; MS, multiple sclerosis.

3.7 Statistical Analyses

3.7.1 Descriptive

Descriptive statistics were used to describe the burden of WNV and WNND in Ontario between 2002 and 2012. Ontario population denominators were obtained from Statistics Canada Population Estimates(196) and used to calculate cumulative incidence rates of WNV infection per 100 000 population over the 11-year period, and by year, age, and sex. Simple counts and proportions were used to describe demographic and health-related baseline characteristics of the study population, overall and by specific WNND manifestation. Similarly, cumulative incidence of WNND overall and by specific WNND manifestation were computed. Baseline characteristics of individuals with and without WNND (and specific manifestations) were compared using chi-square or Fisher's exact tests for categorical variables and t-tests for continuous variables.

3.7.2 Analytical

Binary outcome modelling was used in this study to explore the associations between potential risk factors for developing WNND or one of its manifestations. This study used a population cohort, so relative risks could be calculated. Additionally, WNND outcome prevalence in the study population was >10%, so more frequently used modelling methods like logistic regression could not be used. Instead, a log binomial model, which calculates relative risks and can be used on higher prevalence outcomes, was used.

Log binomial regression belongs to a class of regression models called 'generalised linear models' that have three components: 1) the random component, which is the response variable's probability distribution; 2) the systematic component, which compromises the

explanatory variables and any relationships among them (e.g., interactions); and 3) the link function, which specifies the link between the random and systematic components.(219) The link function permits generalisation of linear models for different types of response variables and ensures linearity and constrains predictions within a range of possible values. Generalised linear model assumptions include: data are independently distributed; the dependent variable assumes a normal distribution or an exponential distribution; the transformed response assumes a linear relationship with explanatory variables; and errors are independent.(219)

Associations between outcomes and independent study variables were first assessed using chi-square and Fisher's exact tests. Those variables associated with the outcome at a level of $p<0.20$ were entered into a full multivariable model. We then used backward selection to remove non-significant variables ($p>0.05$) one at a time from the model and the impact on the model and remaining independent variables was assessed. If the removal of any variable altered another independent variable's coefficient by more than 10%, it was considered a confounder and was retained in the model. We used likelihood ratio tests to compare model fit between the larger and nested models (i.e., whenever a variable was removed), but if the variable removed was identified as a confounder, the variable was retained even if the likelihood ratio test indicated a better fit. Removal of variables from the model stopped when: 1) likelihood ratio tests indicated that variable removal no longer benefitted overall model fit, or 2) confounding prevented further removal of any variables. Statistical interactions between age and sex with comorbid conditions were added to the WNND model and included if the interaction term was statistically significant (i.e., $p<0.05$). This was only performed with the overall WNND outcome because statistical power was considerably reduced in models of

individual WNND manifestations. Since individuals living with comorbid conditions often have multiple comorbidities, which can create collinearity problems in data analysis, collinearity was assessed in the WNND, WNE, WNM, and WNP models by adding Hessian weights to the preliminary effects model and then running the model in PROC REG to calculate variance inflation factors. A variance inflation factor of 10 or more indicated collinearity and would result in removal of the variable from the model, but collinearity was not detected for any of the models.

Finally, two study variables, HIV and recipient of organ transplant, were combined during modelling to form a variable representing immunocompromised immune status because both had relatively small cell numbers.

CHAPTER 4: Results

4.1 Descriptive Analysis

4.1.1 Characteristics of the Study Cohort and WNV Infection

Of the 2 036 confirmed and probable WNV cases in the WNV cohort occurring between 2002 and 2012, 1 937 (95.1%) had a valid IKN and were successfully linked with ICES-held databases. Subsequent to linkage, 53 individuals (2.7%) were excluded because of missing information on sex (n=48) and/or age (n=8), resulting in a final sample size of n=1884.

The cumulative incidence of WNV infection in Ontario between 2002 and 2012 was 1.3 per 100 000 population (Figure 4.1-1). In general, WNV incidence was highest among individuals between the ages of 50 and 80 years of age, and lowest in children and adolescents (Figure 4.1-2). Females between adolescence and late middle age (~50 years) had higher WNV incidence than males, although after age 65, males had a much higher incidence than females.

Slightly more than 40% (n=777/1 884) of the cohort were infected in either 2002 or 2012, with less than 3.0% of infections occurring during 2004 and 2007 (Figure 4.1-1). The WNV transmission season is generally between May and October in Ontario, and most infections occurred in August and September (58.3%, n= 1 099/1 884). There were no statistically significant sex-related differences in infection rates between 2002 and 2012 by year or by month. Slightly over half of the cohort was female (52.4%, Table 4.1-1). The age at time of infection ranged between less than 1 year and 94 years, and the average age at time of infection was 49.3 years (SD± 18.1). Almost half of the cohort was living with at least one comorbid condition (47.1%, n=887/1884), the most common being hypertension (30.6%, n=576/1884), asthma (13.9%, n=261/1884), and diabetes (11.7%, n=221/1884).

Figure 4.1-1 Number of confirmed and probable WNV cases and cumulative incidence per 100 000 population by year, Ontario, 2002-2012.

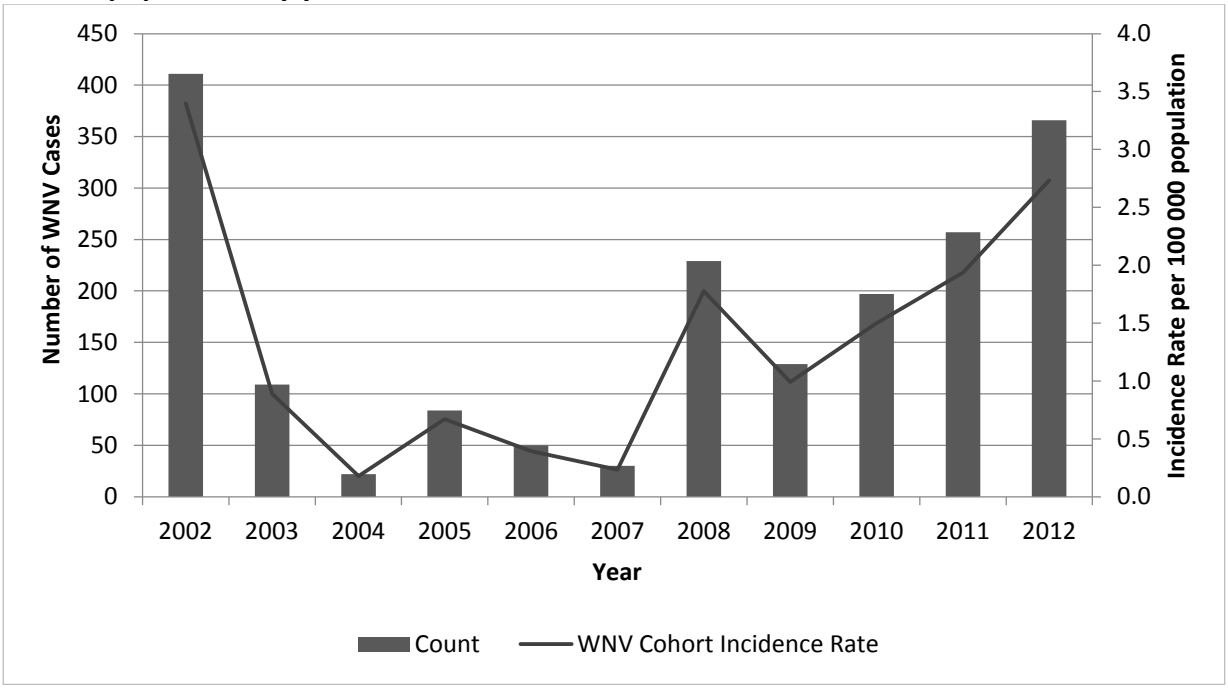


Figure 4.1-2 Eleven-year WNV cumulative incidence rate by age and sex, Ontario, 2002-2012.

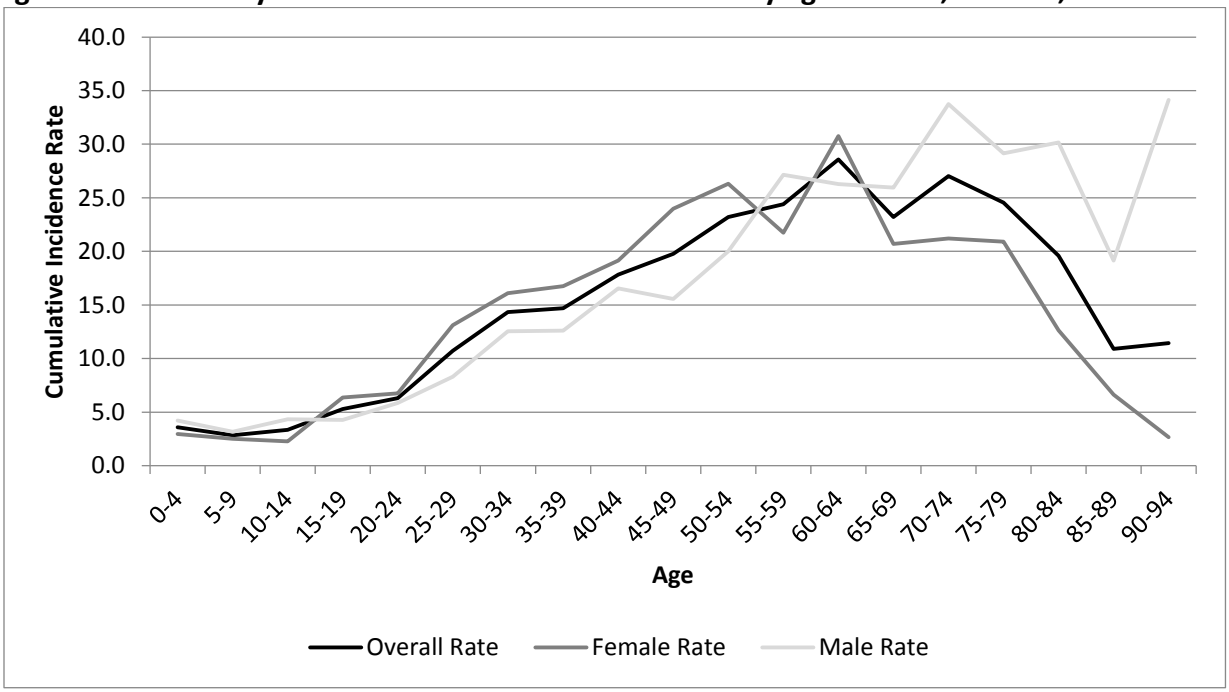


Table 4.1-1 Characteristics of the study population, Ontario, 2002-2012.

Characteristic[a]	Number (n=1 884)	Proportion (%)
Sex		
Female	988	52.4
Male	896	47.6
Age at time of WNV infection (age categories; years)		
Under 18	95	5.0
18-44	616	32.7
45-64	791	42.0
65-79	312	16.6
80 and older	70	3.7
Age at time of WNV infection (mean ±SD; years)	49.3 (±18.1)	-
Any chronic condition		
Yes	887	47.1
No	997	52.9
Charlson Comorbidity Index score		
0: no comorbidities	1459	77.4
1-3: low	320	17.0
4-6: medium	73	3.9
7 +: high	32	1.7
Alzheimer's disease and/or dementia	11	0.6
Asthma	261	13.9
Cancer (within two years prior to WNND diagnosis)	36	1.9
CHF	53	2.8
COPD	134	7.1
CRD	39	2.1
Diabetes	221	11.7
HIV	10	0.5
Hypertension	576	30.6
Recipient of organ transplant	7	0.4
Rheumatoid arthritis	36	1.9
Stroke (within six months prior to WNND diagnosis)	26	1.4
Any West Nile neuroinvasive disease (WNND)[b]	345	18.3
West Nile encephalitis (WNE)	176	9.3
West Nile meningitis (WNM)	145	7.7
West Nile acute flaccid paralysis (WNP)	66	3.5

[a] MS removed from this table because cell counts were <6.

[b] Number of patients with WNND, any manifestation/s. For example, a patient with WNE and WNM would be counted here only once. The rows (manifestations) below are not mutually exclusive – a patient with WNE and WNM would be added to the separate counts for both manifestations.

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; HIV, human immunodeficiency virus; MS, multiple sclerosis.

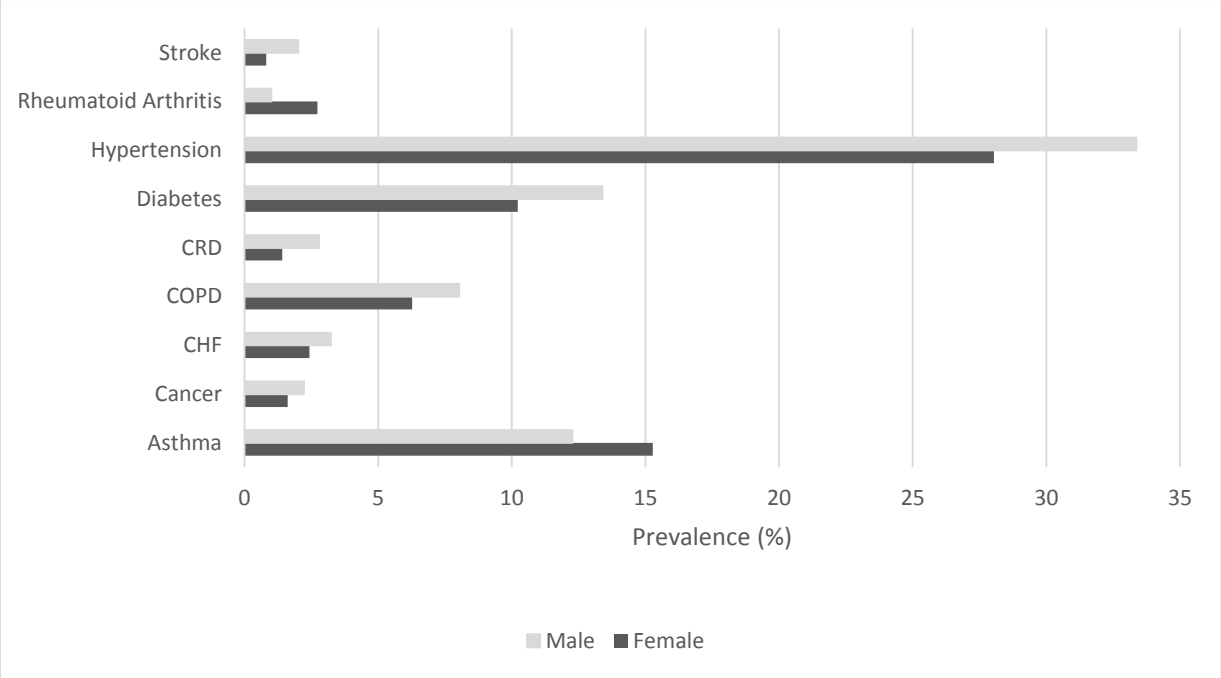
As displayed in Figure 4.1-3, there were differences in the prevalence of chronic disease by sex. For most conditions, prevalence was higher in males with exception of autoimmune-related conditions (i.e., rheumatoid arthritis, asthma, multiple sclerosis, and receiving an organ transplant).

4.1.2 West Nile Neuroinvasive Disease

The overall cumulative incidence of any WNND from 2002-2012 was 18.3% (95% CI:16.6-20.1), with most individuals experiencing only one manifestation (87.8%, n=303/345 [Table 4.1-2]). There were no records of all three neuroinvasive manifestations (i.e., WNE, WNM, WNP) developing in a single individual. In crude analyses, development of WNND was significantly associated with sex (X^2=6.84, p=0.0089), with a higher cumulative incidence in males (20.8%, 95% CI: 18.2-23.6) compared to females (16.1%, 95% CI: 13.9-18.5). Age was significantly related to development of WNND (*t*=-3.91, p=0.0001): the mean age in individuals who did not develop WNND was 48.4 years, whereas the mean age among those who did develop WNND was 53.2 years. Significant differences also existed between age groups (X^2=63.85, p<0.0001): the highest WNND incidence was in individuals 65-79 years of age (29.2%, 95% CI: 24.2-34.6), and 80 years of age and older (37.1%, 95% CI: 25.9-49.5).

Having at least one comorbid condition was significantly associated with WNND (X^2=11.63, p=0.0007), with a higher cumulative incidence of WNND (21.5%, 95% CI: 18.9-24.4) in individuals with ≥1 comorbidity compared to those (15.4%, 95% CI: 13.3-17.8) who did not have any comorbid conditions. The incidence of WNND was highest among individuals with CHF (39.6%, 95% CI: 26.5-54.0), CRD (28.2%, 95% CI: 15.0-44.9), and diabetes (28.1%, 95% CI: 22.2-34.5).

Figure 4.1-3 Comorbidity prevalence in the study population by sex.*



*Small cell counts preclude the inclusion of HIV, MS, Alzheimer's disease and/or dementia, and receiving an organ transplant in this figure.
Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; HIV, human immunodeficiency virus; MS, multiple sclerosis.

Table 4.1-2 Characteristics of individuals with WNND in the WNV cohort, Ontario, 2002-2012.

Characteristic[c]	WNND Yes (%)[a,d] n=345	WNND No (%)[a,d] n=1 539	Cumulative incidence per 100 (95% CI)[b]	p-value	Test statistic (X^2)
Sex					
Female	159 (46.1)	829 (53.5)	16.1 (13.9-18.5)	0.0089	6.84
Male	186 (53.9)	710 (45.8)	20.8 (18.2-23.6)		
Age at time of WNND development (age categories; years)					
Under 18	27 (7.8)	68 (4.4)	28.4 (19.6-38.6)		
18-44	81 (23.5)	535 (34.8)	13.1 (10.6-16.1)		
45-64	120 (34.8)	671 (43.6)	15.2 (12.7-17.9)	<0.0001	63.85
65-79	91 (26.4)	221 (14.4)	29.2 (24.2-34.6)		
80 and older	26 (7.5)	44 (2.9)	37.1 (25.9-49.5)		
Age at WNND development (mean, SD; years)	53.2 (21.3)	48.4 (17.2)	-	0.0001	-3.91**
Charlson Comorbidity Index Score					
0: no comorbidities	204 (59.1)	1 255 (81.6)	14.0 (12.2-15.9)		
1-3: low	95 (27.5)	225 (14.6)	29.7 (24.7-35.0)	<0.0001	394.30
4-6: medium	29 (8.4)	44 (2.9)	39.7 (28.5-51.9)		
7+: high	17 (4.9)	15 (1.0)	53.1 (34.7-70.9)		
Any chronic condition					
Yes	191 (55.4)	696 (45.2)	21.5 (18.9-24.4)	0.0007	11.63
No	154 (44.6)	843 (57.8)	15.4 (13.3-17.8)		
Asthma	45 (13.0)	216 (14.0)	17.2 (12.9-22.4)	0.6300	0.23
Cancer	9 (2.6)	27 (1.8)	25.0 (12.1-42.2)	0.3000	1.10
CHF	21 (6.1)	32 (2.1)	39.6 (26.5-54.0)	<0.0001	16.56
COPD	33 (9.6)	101 (6.6)	24.6 (17.6-32.8)	0.0586	3.85
CRD	11 (3.2)	28 (1.8)	28.2 (15.0-44.9)	0.1065	2.61
Diabetes	62 (18.0)	159 (10.3)	28.1 (22.2-34.5)	<0.0001	15.89
Hypertension	132 (38.3)	444 (28.8)	22.9 (19.5-26.6)	0.0006	11.76
Immunocompromised	7 (2.0%)	10 (1.0%)	41.2 (18.4-67.1)	0.0235	0.02*
Rheumatoid Arthritis	7 (2.0)	29 (1.9)	19.4 (8.2-36.0)	0.8592	0.03
Stroke	9 (2.6)	17 (1.1)	34.6 (17.2-55.7)	0.0400	0.02*

[a] column percentages

[b] row percentages

[c] MS and Alzheimer's disease/dementia were removed from this table because cell counts were <6.

[d] comorbid conditions are not mutually exclusive

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

* Fisher's exact test

** T-test

Most WNND cases occurred between May and October (88.1%, n=304/345) during the WNV transmission season in Ontario, with most cases identified in August and September (71.9%, n=248/345). The number of WNND cases in a year was significantly associated with the number of WNV infections in a year (X^2=50.14, p<0.0001); and years with the most WNND cases between 2002 and 2012 were the epidemic years of 2002 (33.3%, n=115/345) and 2012 (18.3%, n=63/345). The case definition specified a ±30-day window from the WNV infection index date (estimated as 14 days prior to laboratory receivership date): in this analysis, cases occurred from -30 days before the estimated index date to 29 days after, with the majority of WNND cases identified 5-14 days post-index date (Figure 4.1-4). The average amount of time between the index date and presentation with neurological symptoms was 7.8 days.

4.1.3 West Nile Encephalitis

West Nile encephalitis was the most frequently identified manifestation of WNND, with a cumulative incidence of 9.3% (95% CI: 8.1-10.8) over the 11-year study period and developing in 51.0% (n=176/345) of individuals with WNND (Table 4.1-3). In crude analyses, WNE was significantly associated with sex (X^2=7.52, p=0.0061), with a higher cumulative incidence in males (11.3%, 95% CI: 9.3-13.5) compared to females (7.6%, 95% CI: 6.0-9.4). Age was also associated with encephalitis (t=-5.27, p<0.0001), with a mean age of 57.4 years in individuals who developed WNE, whereas the mean age among those who did not develop WNE was 48.4 years. Additionally, there were significant differences between age groups (X^2=100.75, p<0.0001): the highest cumulative incidences were in individuals 65-79 years of age (19.9%, 95% CI: 15.6-24.7) and 80 years of age and older (30.0%, 95% CI: 19.6-42.1).

Figure 4.1-4 Time in days between WNV infection index date and presentation with neurological symptoms of WNND, Ontario, 2002-2012.

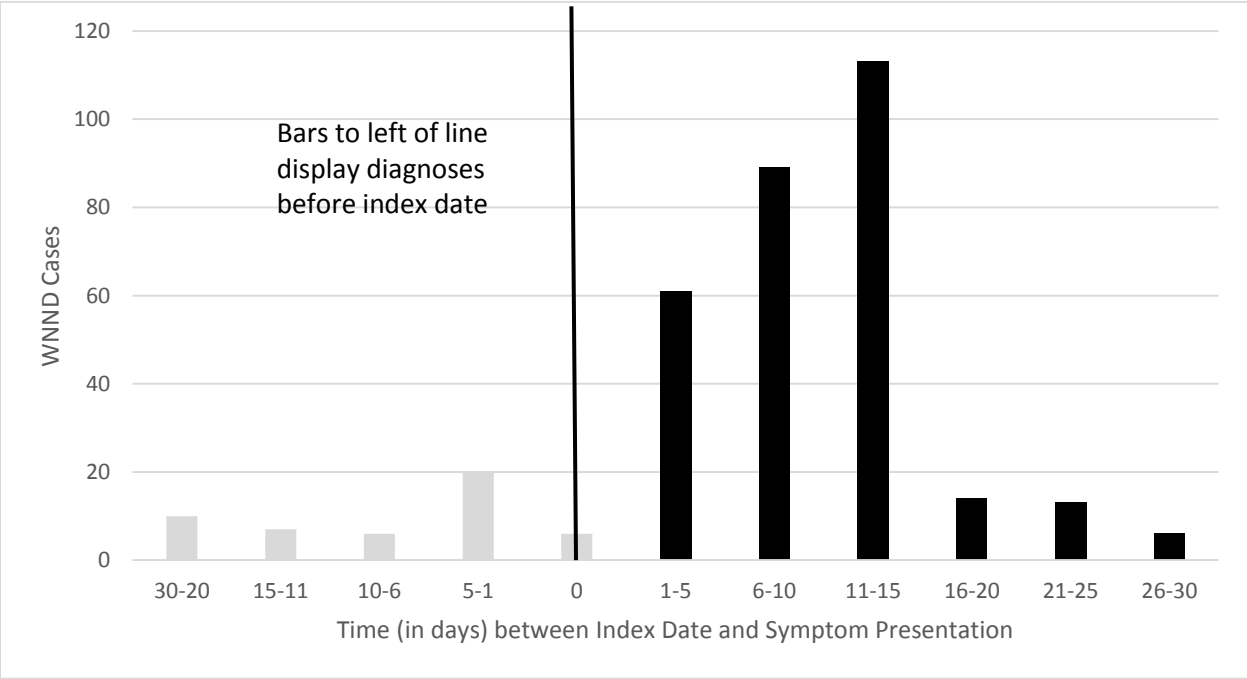


Table 4.1-3 Characteristics of individuals with WNE in the WNV cohort, Ontario, 2002-2012.

Characteristic[c]	WNE Yes (%)[a,d] n=176	WNE No (%)[a,d] n=1 708	Cumulative incidence per 100 (95% CI)[b]	p-value	Test statistic (X^2)
Sex					
Female	75 (42.6)	913 (53.5)	7.6 (6.0-9.4)	0.0061	7.52
Male	101 (57.4)	795 (46.5)	11.3 (9.3-13.5)		
Age at time of WNE development (age categories; years)					
Under 18	13 (7.4)	82 (4.8)	13.9 (7.5-22.3)		
18-44	32 (18.2)	584 (34.2)	5.2 (3.4-7.3)	<0.0001	100.75
45-64	48 (27.3)	743 (43.5)	6.1 (4.5-8.0)		
65-79	62 (35.2)	250 (14.6)	19.9 (15.6-24.7)		
80 and older	21 (11.9)	49 (2.9)	30.0 (19.6-42.1)		
Age at WNE development (mean, SD; years)	57.4 (22.1)	48.4 (17.5)		<0.0001	-5.27**
Any chronic condition					
Yes	106 (60.2)	781 (45.7)	12.0 (9.9-14.3)	0.0002	13.47
No	70 (39.8)	927 (54.3)	7.0 (5.5-8.8)		
Asthma	24 (13.6)	237 (13.9)	9.2 (6.0-13.4)	0.9302	0.01
CHF	15 (8.5)	38 (2.2)	28.3 (16.8-42.4)	<0.0001	<0.0001*
COPD	19 (10.8)	115 (6.7)	14.2 (8.8-21.3)	0.0459	3.99
CRD	10 (5.7)	29 (1.7)	25.6 (13.0-42.1)	0.0023	0.00*
Diabetes	39 (22.2)	182 (10.7)	17.6 (12.9-23.3)	<0.0001	20.39
Hypertension	77 (43.2)	499 (29.2)	13.4 (10.7-16.4)	<0.0001	15.88
Stroke	6 (3.4)	20 (1.2)	23.1 (9.0-43.7)	0.0288	0.02*

[a] column percentages

[b] row percentages

[c] being immunocompromised, Alzheimer's disease/dementia, MS, rheumatoid arthritis, and cancer were removed from this table because cell counts were <6.

[d] comorbid conditions are not mutually exclusive

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

* Fisher's exact test

** T-test

Having at least one comorbid condition was significantly associated with developing WNE (X^2=13.47, p=0.0002): among individuals with at least one comorbidity, WNE cumulative incidence was 12.0% (95% CI: 9.9-14.3) compared with 7.0% (95% CI: 5.5-8.8) in those with no comorbidities. The incidence of WNE was highest among individuals with CHF (28.3%, 95% CI: 16.8-42.4), CRD (25.6%, 95% CI: 13.0-42.1), and stroke (23.1%, 95% CI: 9.0-43.7).

The majority of WNE cases occurred in the months of August and September (73.3%, n=129/176). The number of WNE cases in a year and the number of WNV cases in a year were significantly associated (X^2=61.94, p<0.0001), and over half (51.1%, n=90/176) of all identified cases of WNE were in the epidemic years of 2002 (39.8%, n=70/176) and 2012 (11.9%, n=21/176).

4.1.4 West Nile Meningitis

West Nile meningitis was the second most common manifestation with a cumulative incidence of 7.7% (95% CI: 6.5-9.0) over the 11-year study period, and it developed in 42.0% (n=145/354) of individuals with WNND (Table 4.1-4). There were no significant sex- or age-related differences in individuals with WNM. Unlike WNE, having at least one comorbid condition was not associated with the development of WNM. However, WNM, like WNE, was identified most frequently (77.2%, n=112/145) in August and September. It was also identified most frequently during the epidemic years 2002 (40.0%, n=58/145) and 2012 (24.8%, n=36/145).

Table 4.1-4 Characteristics of individuals with WNM in the WNV cohort, Ontario, 2002-2012.

Characteristic[c]	WNM Yes (%)[a,d] n=145	WNM No (%)[a,d] n=1739	Cumulative incidence per 100 (95% CI)[b]	p-value	Test statistic (X^2)
Sex					
Female	76 (52.4)	912 (52.4)	7.7 (6.1-9.5)	0.9944	0.00
Male	69 (47.6)	827 (47.6)	7.7 (6.1-9.6)		
Age at WNM development (mean, SD; years)[e]	48.7 (19.7)	49.3 (18.0)	-	0.6806	0.41**
Any chronic condition					
Yes	78 (53.8)	809 (56.5)	8.8 (7.0-10.9)	0.0919	2.84
No	67 (46.2)	930 (53.5)	6.7 (5.2-8.5)		
Asthma	21 (14.5)	240 (13.8)	8.0 (5.1-12.0)	0.8194	0.05
COPD	13 (9.7)	121 (7.0)	9.7 (5.3-16.0)	0.3662	0.82
Diabetes	18 (12.4)	203 (11.7)	8.1 (4.9-12.6)	0.7901	0.07
Hypertension	47 (32.4)	529 (30.4)	8.2 (6.1-10.7)	0.6166	0.25

[a] column percentages

[b] row percentages

[c] being immunocompromised, MS, CHF, stroke, CRD, Alzheimer's disease/dementia, rheumatoid arthritis, and cancer were removed from this table because cell counts were <6.

[d] comorbid conditions are not mutually exclusive

[e] Age categories removed from table because most age groups had cell sizes <6.

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

* Fisher's exact test

** T-test

4.1.5 West Nile Acute Flaccid Paralysis

West Nile acute flaccid paralysis was the least frequently identified manifestation of WNND with a cumulative incidence of 3.5% (95% CI: 2.7-4.3) over the 11-year study period, and developing in 19.1% (n=65/345) of individuals with WNND (Table 4.1-5). In crude analyses, development of WNP was not associated with sex, but the majority of the cohort with WNP was 45 years old or older (72.7%, 95% CI: 60.4-83.0). Having at least one chronic condition was not associated with WNP. Small cell sizes for many conditions precluded any meaningful calculation of cumulative incidence for individual chronic conditions. Similarly to WNE, and WNM, WNP was identified most frequently in August and September (45.5%, n=30/66).

4.1.6 Multiple Manifestations

The most common WNND co-development was WNE/WNM (9.3% of WNND infections, n=32/345) while WNE/WNP and WNM/WNP were infrequently identified (<3.0% of WNND infections). There were no sex- or age-associated differences in individuals who developed WNE/WNM. Cell counts were too small for WNE/WNP and WNM/WNP to determine whether there were any significant relationships with age or sex. Additionally, cell counts were too small to meaningfully examine temporal relationships.

Table 4.1-5 Characteristics of individuals with WNP in the WNV cohort, Ontario, 2002-2012.

Characteristic[c]	WNP Yes (%)[a,d] n=66	WNP No (%)[b,d] n=1818	Cumulative incidence per 100 (95% CI)[b]	p-value	Test statistic (X^2)
Sex					
Female	29 (43.9)	959 (52.7)	31.7-56.7	0.2083	0.1985
Male	37 (56.1)	860 (47.3)	43.3-68.3		
Age at WNP development (age categories; years)					
44 and under	18 (27.3)	693 (38.1)	17.0-39.6	0.0742	3.1886
45 and older	48 (72.7)	1 125 (61.9)	60.4-83.0		
Age at WNP development (mean, SD; years)	53.2 (21.5)	49.1 (18.0)		0.1352	-1.51**
Any chronic condition					
Yes	37 (56.1)	850 (46.8)	4.2 (3.0-5.7)		
No	29 (43.9)	968 (53.2)	2.9 (2.0-4.1)	0.1368	2.21
Asthma	6 (9.1)	255 (14.0)	2.3 (0.8-4.9)	0.2722	1.21
Diabetes	12 (18.2)	209 (11.5)	5.4 (2.8-9.3)	0.0861	2.95
Hypertension	28 (42.4)	548 (30.1)	4.9 (3.3-6.9)	0.0260	4.96

[a] column percentages

[b] row percentages

[c] being immunocompromised, MS, COPD, CHF, stroke, CRD, Alzheimer's disease/dementia, rheumatoid arthritis, and cancer were removed from this table because cell counts were <6.

[d] comorbid conditions are not mutually exclusive

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

* Fisher's exact test

** T-test

4.2 Risk Factor Analysis

4.2.1 West Nile Neuroinvasive Disease

Results of univariate regression analyses indicated that CHF, COPD, immunocompromised, stroke, diabetes, hypertension, age, and sex were significantly associated with the WNND outcome ($p<0.05$). To build the multivariate model, we started by including all demographic variables and comorbid conditions associated with the outcome at $p<0.20$ in unadjusted analyses.

After removal of non-significant variables and controlling for confounding, CHF, diabetes, hypertension, immunocompromised, chronic renal disease, stroke, and covariates age and sex were retained in the final parsimonious model (adjusted RRs in Table 4.2-1). CHF was associated with a 55% (adjusted RR [aRR]: 1.55; 95% CI: 1.00-2.25) relative increase in risk of developing WNND, and age and sex were also significantly associated with the outcome: being male was associated with a 23% relative increase in risk of developing WNND (aRR: 1.23; 95% CI: 1.02-1.49) compared to females, and each one-year increase in age was associated with a 1% relative increase in risk of WNND (aRR: 1.01; 95% CI: 1.00-1.02). No other conditions were significantly associated with the outcome.

Testing for statistical interactions between age and sex with the other retained variables yielded one significant statistical interaction between hypertension and age. The inclusion of the product term also improved model fit based on a likelihood ratio test comparing the model with the term to the smaller model ($p=0.0056$). The model including the interaction term is shown in Table 4.2-2. For every five-year increase in age in an individual with hypertension, the risk of developing WNND increased by 16% (aRR: 1.16; 95% CI: 1.08-1.24). Calculation of the

relative excess risk due to interaction (RERI) and the proportion of the combined effect that can be attributed to interaction (AP) indicated departure from additivity: RERI=0.963 (95% CI: 0.698-1.228) and AP=0.833 (95% CI:0.662-1.004). Therefore, 83% of WNND developments in hypertensive individuals are attributable to the interaction with age. Additionally, the immunocompromised variable became significant (p=0.0055): immunocompromised individuals had a 126% increase in risk (aRR: 2.26; 95% CI: 1.12-3.68) for developing WNND compared to those with uncompromised immune status. Male sex was associated with a 21.0% (aRR: 1.21; 95% CI: 1.00-1.46) percent higher risk of WNND than female sex. CHF was not significant in this model.

Table 4.2-1 Unadjusted and adjusted relative risks for WNND.

Characteristic	Unadjusted RR, 95% CI	p-value	Adjusted RR, 95% CI[a]	p-value
Sex				
Female	Ref.		Ref.	
Male	1.29 (1.07-1.56)	0.0091	**1.23 (1.02-1.49)**	0.0346
Age at time of WNND development (continuous variable, years)	1.01 (1.01-1.02)	<0.0001	**1.01 (1.00-1.02)**	0.0041
Alzheimer's disease and/or dementia	2.00 (0.71-3.63)	0.0852	-	-
Asthma	0.93 (0.69-1.22)	0.6318	-	-
Cancer	1.38 (0.70-2.26)	0.2769	-	-
CHF	2.24 (1.52-3.05)	<0.0001	**1.55 (1.00-2.25)**	0.0314
COPD	1.38 (0.99-1.85)	0.0430	-	-
CRD	1.56 (0.87-2.43)	0.0884	0.75 (0.41-1.25)	0.3061
Diabetes	1.65 (1.29-2.07)	<0.0001	1.25 (0.95-1.63)	0.0983
Immunocompromised	2.27 (1.11-3.61)	0.0052	1.95 (0.96-3.19)	0.0231
Hypertension	1.41 (1.16-1.70)	0.0005	1.05 (0.84-1.32)	0.6640
MS	1.37 (0.09-4.00)	0.7190	-	-
Rheumatoid Arthritis	1.06 (0.48-1.89)	0.8583	-	-
Stroke	1.91 (1.01-3.01)	0.0178	1.46 (0.78-2.30)	0.1611

[a] Only statistically significant variables (as based on likelihood ratio tests) are included in the adjusted model. Additionally, model is adjusted for identified confounders and covariates (age and sex).

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

4.2.2 West Nile Encephalitis

Univariate analysis of study variables identified nine (CHF, COPD, diabetes, hypertension, immunocompromised, CRD, stroke, age, and sex) that were significantly associated with WNE development ($p<0.05$). After removal of non-significant variables and controlling for confounding, CHF, diabetes, immunocompromised, and covariates age and sex were retained in the final multivariate model (Table 4.2-3). Being immunocompromised was associated with the development of WNE with an adjusted relative risk of 2.61 (95% CI: 1.23-4.53), and each one-year increase in age was associated with a 2% (aRR: 1.02; 95% CI: 1.02-1.03) increase in risk. In addition, being male was associated with a 32% (aRR: 1.32; 95% CI: 1.00-1.76) increase in risk. CHF and diabetes were not significantly associated with WNE.

4.2.3 West Nile Meningitis

Univariate analyses yielded no significant associations ($p<0.05$) between study variables and the development of WNM (Table 4.2-4). Study variables associated with the outcome at $p<0.20$ were included in the model (i.e., cancer, Alzheimer's disease and/or dementia, and immunocompromised) in addition to the covariates age and sex. Removal of non-significant variables ($p>0.05$) from the model did not result in significant estimates for any of the included variables (Table 4.2-4).

Table 4.2-2 Results of multivariate regression analysis for WNND including the interaction term.

Characteristic	Adjusted RR, 95% CI	p-value
CHF	1.42 (0.93-2.02)	0.0780
Diabetes	1.23 (0.94-1.60)	0.1239
Hypertension	0.18 (0.07-0.45)	0.0003
Hypertension*Age	**1.16 (1.08-1.24)**	<0.0001
Immunocompromised	**2.26 (1.12-3.68)**	0.0055
CRD	0.66 (0.36-1.08)	0.1270
Stroke	1.49 (0.80-2.30)	0.1337
Male Sex	**1.21 (1.00-1.46)**	0.0529
Age	1.00 (1.00-1.01)	0.5989

Abbreviations: CHF, congestive heart failure; CRD, chronic renal disease.

Table 4.2-3 Unadjusted and adjusted relative risks for WNE.

Characteristic	Unadjusted RR, 95% CI	p-value	Adjusted RR, 95% CI	p-value
Sex				
Female	Ref.		Ref.	
Male	1.48 (1.12-1.98)	0.0065	**1.32 (1.00-1.76)**	0.0535
Age at time of WNE development (continuous variable, years)	1.03 (1.02-1.04)	<0.0001	**1.02 (1.02-1.03)**	<0.0001
Alzheimer's Disease/Dementia	0.97 (0.06-3.71)	0.9772	-	-
Asthma	0.98 (0.63-1.45)	0.9303	-	-
Cancer	1.50 (0.56-3.03)	0.3351	-	-
CHF	3.22 (1.94-4.84)	<0.0001	1.47 (0.85-2.39)	0.1424
COPD	1.58 (0.98-2.39)	0.0426	-	-
CRD	2.85 (1.51-4.63)	0.0002	-	-
Diabetes	2.14 (1.52-2.93)	<0.0001	1.33 (0.91-1.88)	0.1220
Immunocompromised	3.88 (1.72-6.64)	<0.0001	**2.61 (1.23-4.53)**	0.0020
Hypertension	1.77 (1.33-2.34)	<0.0001	-	-
MS[b]	-	-	-	-
Rheumatoid Arthritis	1.19 (0.38-2.62)	0.7103	-	-
Stroke	2.52 (1.01-4.61)	0.0114	-	-

[a] Only statistically significant variables (as based on likelihood ratio tests) are included in the model. Additionally, model is adjusted for identified confounders and covariates (age and sex).

[b] Model failed to converge.

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

Table 4.2-4 Unadjusted and adjusted relative risks for WNM.

Characteristic	Unadjusted RR, 95% CI	p-value	Adjusted RR, 95% CI	p-value
Sex				
Female	Ref.		Ref.	
Male	1.00 (0.73-1.37)	0.9944	0.99 (0.72-1.36)	0.9616
Age at time of WNM development (continuous variable, years)	1.00 (0.99-1.01)	0.6783	1.00 (0.99-1.01)	0.5115
Alzheimer's Disease/Dementia	2.38 (0.43-6.18)	0.1783	2.68 (0.47-7.30)	0.1350
Asthma	1.05 (0.66-1.60)	0.8192	-	-
Cancer	1.83 (0.68-3.71)	0.1518	1.93 (0.71-3.93)	0.1235
CHF	0.98 (0.31-2.21)	0.9670	-	-
COPD	1.29 (0.71-2.12)	0.3627	-	-
CRD	0.33 (0.02-1.40)	0.2610	-	-
Diabetes	1.07 (0.64-1.66)	0.7897	-	-
Immunocompromised	2.32 (0.61-5.32)	0.1124	2.36 (0.62-5.43)	0.1059
Hypertension	1.09 (0.77-1.51)	0.6162	-	-
MS	3.26 (0.21-9.63)	0.1738	-	-
Rheumatoid Arthritis	1.08 (0.28-2.68)	0.8845	-	-
Stroke	0.50 (0.03-2.07)	0.4764	-	-

[a] Only statistically significant variables (as based on likelihood ratio tests) are included in the model. Additionally, model is adjusted for identified confounders and covariates (age and sex).

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

4.2.4 West Nile Acute Flaccid Paralysis

Univariate regression analyses yielded three study variables with significant ($p<0.05$) associations with the outcome (Alzheimer's/dementia, hypertension, and stroke). All study variables associated with WNP at $p<0.20$ were included in the multivariate model (i.e., Alzheimer's disease and/or dementia, CHF, diabetes, hypertension, and stroke) in addition to the covariates age and sex. Removal of non-significant variables from the model ($p>0.05$) did not yield significant estimates for any of the variables included in the model (Table 4.2-5).

4.2.5 Charlson Comorbidity Index Scoring and WNND

This model included the Charlson Comorbidity Index Score variable and the two covariates, age and sex. Both the independent variable and age were significantly associated with developing WNND (Table 4.2-6). Regression analysis results indicate that for each 1-unit increase in comorbidity scoring, there was a 14% (aRR:1.14; 95% CI: 1.03-1.17) increase in the risk of developing WNND, while age was associated with an adjusted relative risk of 1.01 (95% CI: 1.00-1.01). After categorising the CCI variable (0: null CCI score; 1-3: low CCI score; 4-6: medium CCI score; 7+: high CCI score), results suggested an increase in risk for individuals with any CCI score not equal to zero (Table 4.2-7). Individuals with a low score had a 98% (aRR: 1.98; 95% CI: 1.57-2.48) relative increase in risk of WNND compared to those with a CCI score of 0, while those with medium and high scoring had a 163% (aRR: 2.63; 95% CI: 1.86-3.56) and a 245% (aRR: 3.45; 95% CI: 2.25-4.83) relative increase in risk of WNND, respectively, compared to those with a CCI score of 0. Age remained associated with WNND with an adjusted relative risk of 1.01 (95% CI: 1.00-1.01).

Table 4.2-5 Adjusted and unadjusted relative risks for WNP.

Characteristic	Unadjusted RR, 95% CI	p-value	Adjusted RR, 95% CI	p-value
Sex				
Female	Ref.		Ref.	
Male	1.41 (0.87-2.29)	0.1613	1.31 (0.81-2.14)	0.2684
Age at time of WNP development (continuous variable, years)	1.01 (1.00-1.03)	0.0717	1.00 (0.99-1.02)	0.6502
Alzheimer's Disease/Dementia	5.32 (0.95-14.14)	0.0103	2.93 (0.49-9.61)	0.1343
Asthma	0.74 (0.31-1.49)	0.4404	-	-
Cancer	0.00 (0.00-0.00)	0.9997	-	-
CHF	2.23 (0.70-5.15)	0.1066	1.21 (0.34-3.29)	0.7408
COPD	1.07 (0.38-2.36)	0.8814	-	-
CRD	0.73 (0.04-3.15)	0.7494	-	-
Diabetes	1.67 (0.86-2.96)	0.0983	1.22 (0.59-2.34)	0.5686
Immunocompromised	0.00 (0.00-0.00)	0.9997	-	-
Hypertension	1.67 (1.03-2.69)	0.0349	1.34 (0.74-2.40)	0.3300
MS	0.00 (0.00-0.00)	0.9996	-	-
Rheumatoid Arthritis	0.00 (0.00-0.00)	0.9997	-	-
Stroke	3.40 (0.87-8.39)	0.0279	2.09 (0.51-5.71)	0.2167

[a] Only statistically significant variables (as based on likelihood ratio tests) are included in the model. Additionally, model is adjusted for identified confounders and covariates (age and sex).

Abbreviations: CHF, congestive heart failure; COPD, chronic obstructive pulmonary disease; CRD, chronic renal disease; MS, multiple sclerosis.

Table 4.2-6 Unadjusted and adjusted relative risks for WNND, CCI score as a continuous variable.

Characteristic	Unadjusted RR, 95% CI	p-value	Adjusted RR, 95% CI	p-value
CCI score	1.16 (1.13-1.18)	<0.0001	**1.14 (1.03-1.17)**	<0.0001
Age	1.01 (1.01-1.02)	<0.0001	**1.01 (1.00-1.01)**	0.0256
Sex				
Female	Ref.		Ref.	
Male	1.29 (1.07-1.56)	0.0091	1.19 (0.99-1.44)	0.0628

Abbreviations: CCI, Charlson Comorbidity Index.

Table 4.2-7 Unadjusted and adjusted relative risks for WNND, CCI score as a categorical variable.

Characteristic	Unadjusted RR, 95% CI	p-value	Adjusted RR, 95% CI	p-value
CCI score				
Null (0)	Ref.		Ref.	
Low (1-3)	1.86 (1.51-2.27)	<0.0001	**1.98 (1.57-2.48)**	<.0001
Medium (4-6)	2.28 (1.64-3.00)	<0.0001	**2.63 (1.86-3.56)**	<.0001
High (7+)	3.0 (2.02-4.02)	<0.0001	**3.45 (2.26-4.83)**	<.0001
Age	1.01 (1.01-1.02)	<0.0001	**1.00 (1.00-1.01)**	0.2366
Sex				
Female	Ref.		Ref.	
Male	1.29 (1.07-1.56)	0.0091	1.18 (0.98-1.43)	0.0758

Abbreviations: CCI, Charlson Comorbidity Index.

CHAPTER 5: Discussion

5.1 Results Discussion

Identifying comorbid risk factors of WNND is important to allow for medical and public health recognition of groups at high-risk for neuroinvasive involvement following WNV infection and to help inform prevention strategies targeted towards susceptible groups. Despite the virus' arrival in North America nearly two decades ago, only some studies have explored the relationship between comorbid conditions and the development of neuroinvasive disease from an epidemiological standpoint, and the majority are based on American populations. This study is the first to examine comorbid conditions as WNND risk factors in a Canadian population; specifically, it explored whether 13 common comorbid conditions and Charlson Comorbidity Index scores were associated with the development of WNND. The relationship between comorbidity and the development of specific WNND manifestations (WNE, WNM, WNP) was also explored. Additionally, this study described WNND incidence in Ontario between 2002 and 2012.

Briefly, between 2002 and 2012, the cumulative incidence of WNND in individuals with laboratory-confirmed WNV infection was 18.3%. WNND developed more frequently in males and in older individuals (~60+ years). WNND (any manifestation) was associated with being immunocompromised, male sex, higher CCI scoring, and an interaction between hypertension and age. WNE was associated with male sex, older age, and being immunocompromised. WNM and WNP were not significantly associated with any of the study variables in multivariate modelling.

Results of the descriptive analysis corroborate other published results suggesting that WNND rates vary geographically. In Ontario, the average annual incidence of WNND between 2002-2012 was 0.25/100 000 population, with peaks in 2002 (0.95/100 000 population) and 2012 (0.47/100 000 population). The 11-year average annual incidence was similar to what was reported in Alberta around this period, but lower than in Manitoba and Saskatchewan.(220-222) Rates of WNND appear to be higher in Ontario than in Québec.(221) Elevated or decreased WNND rates reflect geographic and temporal fluctuations in WNV rates, with WNV average annual incidence in the Prairie provinces generally higher than in Ontario or Québec during the last two decades.

In the United States, an average annual WNND incidence of 0.40/100 000 was reported by the CDC for 47 states reporting between 1999 and 2008, with a peak in 2002 (1.02/100 000 population) and 2003 (0.99/100 000 population).(79) The highest incidence of WNND in the United States is in the West Central and Mountain regions, and may be due to the efficiency of *Culex tarsalis* as a WNV vector(68, 79, 223); a similar phenomenon may be occurring in Canada where Ontario's main vectors of WNV are *Culex restuans* and *Culex pipiens*, while in the Prairie provinces, it is *Culex tarsalis.*(224)

Similarly to other published epidemiological results on West Nile neuroinvasive disease, males and older individuals were more susceptible to development of neurological disease in this study: males accounted for 53.9% of cases, and the median age of infection with WNND was 56 years.(1, 132, 183) In the CDC report on WNND in 47 states, 58% of cases were males and the median age of disease was 57 years.(79) On a regional scale, 66% of all WNND cases reported in North Dakota between 2002 and 2007 were male(225), while a similar proportion

(62.4%) of male cases was reported during the 2012 Dallas, Texas epidemic. A median age disease development of 61 years was also reported for this outbreak.(226) Outside of North America, the increased susceptibility to WNND of males and older individuals has been reported from Romania (between 2012 and 2017, 55.3% of all Romanian WNND cases were males and the median age of disease was 64 years)(227), Italy (median age of WNND development was 69 years)(77), and Greece (in 2012, 65% of cases were male and the median age of disease was 70 years).(1) Genetic, hormonal, and environmental mediators that differ between the sexes result in decreased susceptibility to many infectious diseases in females compared to males. The immune response in females, in general, is faster and stronger than it is in males.(228, 229) However, human behaviour is also a factor– during the 2012 Greek epidemic, males living in rural areas where at increased risk of developing WNND, but in urban areas, the difference in risk between the sexes was non-significant.(1)

The results of this study corroborate results from published literature regarding measures of association and WNND and comorbid conditions. Hypertension, being immunocompromised, male sex, and older age have been identified as risk factors for WNND in several American studies(1-3, 5, 6, 132, 157, 183): the former two, either directly or through related treatments, are known immune system disruptors; and males generally exhibit increased susceptibility to viral infection than females, and experience more severe illness(184); and older age is associated with decreased immune system function.(178-182) Whatever the mechanism of immune dysfunction, these comorbid conditions and individual host characteristics increase the risk of WNV entering the CNS and causing severe neurological disease.

Hypertension was the most common chronic condition in our WNV cohort, and through effect measure modification, increased the risk of WNND by 16.0% for every five-year increase in age. Hypertension, a known disruptor of blood-brain barrier integrity, promotes permeability, potentially allowing larger molecules, like WNV virions, to enter the neurological system, whether through direct perfusion or trafficking of inflammatory cells.(115-119) Ageing is associated with increased immune dysfunction manifesting as, among others, chronic low-grade inflammation and a decreased ability to fight infection.(230) The effect measure modification identified between these two risk factors may have arisen from elements common to both (e.g., chronic low-grade inflammation), or a combination of hypertension and ageing factors leading to a cascade of physiological events conducive to the development of WNND. Additionally, hypertension is a major risk factor for many chronic health conditions, some of which were not included in this study. It is possible that a mediating condition, developed from pathology promoted by hypertension, is responsible, or partially responsible, for the association identified in regression modelling.

Being immunocompromised was also identified as a risk factor in this analysis, increasing the risk of developing WNND by 161%. However, this result was limited by low statistical power due to small cell sizes (17 individuals in this study were immunocompromised). Immunocompromising conditions have been identified as a risk factor for severe neurological disease following infection with WNV in a Texas population, with immune-deficient individuals experiencing 5.6 (95% CI: 2.1-14.9) times the odds of developing WNE.(150) Additionally, a 2004 study from Toronto estimated an increased risk of 40% of WNND (meningoencephalitis) in WNV-infected individuals living with an organ transplant.(231) Being immunocompromised

confers an increased risk of CNS infection by an opportunistic agent (e.g., WNV) because the immune system is unable to mount an appropriate response to foreign antigens. Additionally, some conditions may exacerbate immune system vulnerability by actively targeting immune defenses: for example, HIV viral proteins affect disrupt blood-brain barrier permeability by reducing expression of tight junction proteins and increasing localised inflammation.(232)

Although there were fewer males in the WNV cohort, more males developed WNND, and the regression modelling results identified increases in risk of WNND and WNE of 21.0% and 33.0%, respectively, for males. These results corroborate Greek and American studies which found increased risk (10.0-80.0%) and odds ratios (1.57, 3.10) for WNND in males relative to females.(1, 132, 183)

Older age has been identified as a risk factor for WNND in multiple studies but could not be examined independently in the WNND and WNE multivariate models of this study due to its significant interactions with hypertension (for WNND) and being immunocompromised (for WNE).(6, 157, 183) Age was on the cusp of significance for both WNM and WNP as an independent variable in the multivariate analyses, and its lack of significance may be due to small cell sizes.

Several chronic conditions were associated with WNND, WNE, and WNP in univariate analyses, but were not significant when included in multivariate modelling. Surprisingly, diabetes, chronic renal disease, and congestive heart failure (cardiovascular disease), all previously identified as risk factors for WNND in American epidemiological studies, were not identified as risk factors in this study's multivariate analyses. Diabetes has been identified as a risk factor in two different cohorts of WNV-infected individuals and three different

epidemiological studies.(2, 5, 6) Chronic renal disease has also been identified as a risk factor in two different WNV cohorts – it may not have been identified as a risk factor in this analysis due to small cell sizes (i.e., 11 individuals with chronic renal disease developed WNND). Lastly, cardiovascular disease has been identified as a risk factor of WNE but was not associated with WNND in this study (3); however, this analysis explored congestive heart failure, and not the larger disease category of cardiovascular disease, and only 21 individuals were diagnosed with CHF.

Additionally, CCI scores above zero were associated with the development of WNND, with relative risk increasing as categorical severity rose. Relationships between individuals living with comorbid conditions and infectious disease severity have been previously documented, with higher CCI scores associated with intensive care unit admission and death in individuals with influenza.(233, 234) Interestingly, several comorbid conditions in this study that were significant in univariate or multivariate modelling are included in the CCI (i.e., CHF, diabetes, HIV [immunocompromised in this study], and CRD). However, mechanisms of WNV progression to WNND are not well studied in the context of contributing comorbid conditions, so without a better understanding of how WNV enters the CNS and how this is facilitated by existing conditions, it is difficult to discern whether the CCI, as a whole, is a useful predictor, or whether the effects of specific conditions included in the CCI are contributing to its predictive ability. For a particular disease (e.g., WNND), holistically the CCI may not be important, but the underlying diseases contributing to a specific score probably are.

Development of WNND is a severe disease progression leading to neurological involvement that, although more common in older individuals or those living with a chronic

disease, is not limited to the elderly or sick. Case fatality rates vary (e.g., 9% in the US between 1999 and 2008(79), 25.5% between 2016 and 2017 in Romania(235), 18.6% in Illinois in 2002(157)), but demonstrate that death is not uncommon. Survivors can expect a lengthy, complicated recovery that often necessitates rehabilitation and is frequently accompanied by long-term sequelae that negatively impact quality of life.(134, 138) Costs are significant as well: a 2019 costing study from Québec suggested that during an epidemic year, direct and indirect costs of WNV for the province could reach ~$1.7 million (236); and for an WNV-infected person in Ontario, direct healthcare costs over one year were estimated at $13 648.(197)

The consequences of WNV infections progressing to WNND in Ontario could become more significant in the coming years. The province's population is ageing, and more people will be living with chronic disease and multimorbidity. The results of this study, and of others, suggest that individuals living with comorbid conditions are at greater risk of developing WNND. Additionally, global warming is affecting Ontario's climate, with projections suggesting a provincial average increase in temperature of 2.3°C in the next 10 years under a high emissions scenario.(237) This could lengthen mosquito breeding season and extend the WNV transmission period in Ontario, exposing more Ontarians to WNV-infected vectors each year.

The severity of WNND, the lack of a cure, the strain on health care resources, Ontario's changing climate and demographics, and the current gap in understanding of how the virus progresses to neurological disease necessitate prevention strategies that target the first step in the disease chain: infection with WNV. Public health strategies that preclude infection with WNV are currently best practice for preventing WNND and can be tailored to target vulnerable groups with identified risk factors, such as those living with comorbid conditions and the

elderly. However, it should be taken into account that attitudes towards prevention measures differ between different demographics, with studies suggesting that, in general, older individuals and females are more likely to practice personal protective behaviours.(238-240)

In Ontario, a formal provincial WNV prevention and control strategy critically includes dissemination of information at multiple stakeholder levels (e.g., physicians, community-based organisations, individual residents).(241) Under the *Health Protection and Promotion Act*, if there is sufficient risk of WNV transmission [in a jurisdiction], several measures to decrease risk may be taken by public health units, including: chemical control (e.g., use of larvicides); source reduction (e.g., flushing storm drains, storing tires where they will not collect water) (242); and public education and outreach.(241) For the latter, the Ministry of Health and Long-Term Care focuses on promotion of education/awareness of personal protection against mosquito bites, which includes measures such as wearing long-sleeved shirts or pants, using insect repellant, and reducing the amount of time spent outside at dawn and dusk. The effectiveness of similar WNV public health prevention strategies was examined in Colorado following a severe outbreak of WNV with significantly different rates of WNND in two adjacent cities.(243) The study suggests that personal protection measures, even in municipalities with established mosquito control programmes, may influence disease rates; and public health authorities should promote personal protection measures in addition to reducing risk through maintenance of mosquito control programmes. A stakeholder decision analysis from Québec reinforces the suggestions from the Colorado study – use of larvicides to control mosquito populations combined with public health promotion of personal protective measures were the most acceptable and effective interventions under [that study's] current, and future, transmission risks.(244)

Human vaccination against WNV is not discussed in Ontario's prevention plan because no human WNV vaccines are available, although four effective equine WNV vaccines have been developed.(245) There are licensed human vaccines against other mosquito-borne viruses in the *Flaviviridae* family (i.e., Dengue fever, Yellow fever, Japanese encephalitis), some of which offer lifelong immunity after only one dose.(245) To date, only six candidate WNV vaccines have been evaluated in clinical trials, with two vaccines entering phase II trials; promisingly, one candidate had a high seroconversion rate and was safe and effective in different age groups using a single dose.(246) However, none have yet entered phase III trials. Costing studies suggest that once a human WNV vaccine is available, targeted vaccination programmes will be more cost-effective than universal vaccination.(247) Those at highest risk for infection and for development of WNND should be targeted, namely, the elderly and those living with comorbid conditions.

5.2 Strengths and Limitations

This study had a number of strengths including a population-based cohort design, laboratory confirmation of West Nile virus infections, temporal ordering of variables, and deterministic linkage of health information across multiple databases. Using population-level data allowed for the estimation of relative risks during risk factor analysis and estimation of incidence rates on a provincial scale, and laboratory diagnostic test confirmation of WNV infection decreased the chances of misclassification. Temporal ordering of variables (i.e., chronic disease occurred *before* WNND) aids in interpretation of results. Lastly, the ability to deterministically link patient health information from different health administrative databases

allowed for low false-match rates, and the use of multiple datasets allowed for a richer and more complete health picture of individuals in this cohort.

This study is also subject to several limitations. One major limitation in this study was the lack of an existing Canadian ICD-10 diagnosis code for WNND. In order to identify individuals with WNND in the PHOL cohort, an algorithm was developed containing ICD-10 diagnosis codes for encephalitis, meningitis, and acute flaccid paralysis not specific to WNV infection but that were recorded within ±30 days of the estimated index date. Additionally, the algorithm is not validated and its sensitivity and specificity when applied to health administrative data is unknown. The lack of a validated diagnosis for WNND introduces the possibility of outcome misclassification; specifically, we may have classified some individuals without WNND as having WNND and vice versa. Differential misclassification may be present due to the window of time used in our definition (±30 days from estimated index date). Some individuals may have developed unrelated neuropathy prior to WNV infection, but due to our definition, were identified as having WNND. This may have biased estimates away from the null.

The inclusion of a specific WNND code in Canadian ICD manuals would improve identification of individuals with WNND from hospital administrative data. The American ICD-10 manual includes a code specifying encephalitis resulting from WNV infection (i.e., A92.31), although one does not exist for meningitis or acute flaccid paralysis. Additionally, the relative rarity of symptoms developing from a WNV infection, or the non-specific flu-like symptoms of WNF, may mean that WNV as an aetiologic cause of encephalitis, meningitis, or acute flaccid paralysis may not be considered in many cases. For example, a 2012 study found increased

arboviral encephalitis hospitalisation rates in Ontario in summer and autumn, and a corresponding increase in summer and autumn hospitalisation rates of encephalitis with unknown aetiologies.(199) Greater health care professional awareness of the symptoms of WNV infection, particularly when these occur during the summer and autumn months, may improve proper diagnosis of the disease.

Another limitation is the low sensitivity and specificity of some algorithms used to identify patients with chronic disease in derived and acquired cohorts. Unlike registries (e.g., Ontario Cancer Registry), which are populated using multiple data points (e.g., hospital records, pathology reports, etc.) and thus more accurately identify individuals with disease, the algorithms are developed to identify disease from health administrative records only. Although many algorithms have been validated, low sensitivity and specificity remain a challenge. Additionally, only 13 chronic diseases were examined as risk factors for WNND. The independent variables were selected due to their prevalence in the Ontario population and whether or not an existing registry or cohort was available at ICES. Many chronic diseases were not included in this analysis, and additionally, multimorbidity is complex, and many chronic diseases act as mediators or interact with each other in manners that were not considered (i.e., mediation analysis was not performed and interaction was only examined between chronic disease variables and the covariates age and sex).

Small cell numbers were also a challenge in this analysis. Only 345 individuals in the cohort developed WNND, and consequently, there were very small case numbers for some comorbid conditions (e.g., HIV) which precluded specific assessment when numbers were <6 due to privacy regulations. Therefore, it is likely that some effects were over- or under-

estimated in the calculation of relative risks. Expanding the study cohort to include other Canadian provinces would reduce small cell sizes and allow for a more robust estimation of risk.

Lastly, a time-to-event analysis (Cox proportional hazards analysis) would have shed additional light on the relationship between comorbid conditions and WNND and its manifestations. However, this was not possible because the index date (date of infection with WNV by mosquito inoculation) was estimated (i.e., two weeks prior to lab receivership).

5.3 Future Directions

Identifying risk factors of WNND is of public health importance because it allows for the identification of high-risk groups and, consequently, targeted prevention programmes. Due to the complexity of chronic disease pathology and multimorbid mechanisms of interaction and mediation, future WNND risk factor analyses should examine comorbid interactions and conduct mediation analyses in addition to including more comorbid conditions as independent variables. These approaches would better disentangle the effects of related chronic diseases. For example, hypertension has been identified in some studies as a risk factor of WNND, while in others related comorbid conditions were identified (e.g., CHF), but not hypertension. Chronic disease is very common in Ontario, and it would be inefficient, financially and logistically, to target all individuals living with one or more chronic diseases in a prevention campaign. Understanding which chronic diseases are true risk factors of WNND, which are mediating factors, or which interact with other comorbid conditions to increase risk, is important for developing appropriate intervention strategies.

CHAPTER 6: Conclusion

This study is the first to identify comorbid risk factors of WNND in a Canadian population. The results of this study are consistent with those in published studies on WNND risk factors from the United States and abroad. Additionally, this study examined the relationship between Charlson Comorbidity Index scoring and development of WNND and suggests an association between higher CCI scores and progression to neuroinvasive disease following WNV infection. These results will add to the growing body of literature on WNND risk factors and can inform public health prevention programmes on at-risk groups. Future work should focus on examining the mediation pathways and interactions of comorbid conditions so at-risk groups may be better defined and targeted for public health prevention programmes. Additionally, considering more chronic diseases as risk factors and increasing the study sample size will allow for a more robust examination.

References

1. Pervanidou D, Detsis M, Danis K, Mellou K, Papanikolaou E, Terzaki I, et al. West Nile virus outbreak in humans, Greece, 2012: third consecutive year of local transmission. Euro Surveill. 2014;19(13).
2. Lindsey NP, Staples JE, Lehman JA, Fischer M. Medical risk factors for severe West Nile Virus disease, United States, 2008-2010. Am J Trop Med Hyg. 2012;87(1):179-84.
3. Murray KO, Koers E, Baraniuk S, Herrington E, Carter H, Sierra M, et al. Risk factors for encephalitis from West Nile Virus: a matched case-control study using hospitalized controls. Zoonoses Public Health. 2009;56(6-7):370-5.
4. Murray K, Baraniuk S, Resnick M, Arafat R, Kilborn C, Cain K, et al. Risk factors for encephalitis and death from West Nile virus infection. Epidemiol Infect. 2006;134(6):1325-32.
5. Patnaik JL, Harmon H, Vogt RL. Follow-up of 2003 Human West Nile Virus Infections, Denver, Colorado. Emerg Infect Dis. 2006;12(7):1129-31.
6. Bode AV, Sejvar JJ, Pape WJ, Campbell GL, Marfin AA. West Nile virus disease: a descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003. Clin Infect Dis. 2006;42(9):1234-40.
7. Smithburn KC, Hughes TP, Burke AW, Paul JH. A Neurotropic Virus Isolated from the Blood of a Native of Uganda. Am J Trop Med Hyg. 1940;s1-20(4):471-92.
8. Melnick JL, Paul JR, Riordan JT, Barnett VH, Goldblum N, Zabin E. Isolation from human sera in Egypt of a virus apparently identical to West Nile virus. Proc Soc Exp Biol Med. 1951;77(4):661-5.
9. Work TH, Hurlbut HS, Taylor RM. Indigenous wild birds of the Nile Delta as potential West Nile virus circulating reservoirs. Am J Trop Med Hyg. 1955;4(5):872-88.
10. Hurlbut HS, Rizk F, Taylor RM, Work TH. A study of the ecology of West Nile virus in Egypt. Am J Trop Med Hyg. 1956;5(4):579-620.
11. Spigland I, Jasinska-Klingberg W, Hofshi E, Goldblum N. Clinical and laboratory observations in an outbreak of West Nile fever in Israel in 1957. Harefuah. 1958;54(11):275-80.
12. Hayes CG. West Nile virus: Uganda, 1937, to New York City, 1999. Ann N Y Acad Sci. 2001;951:25-37.
13. Panthier R. Epidemiology of the West Nile virus: study of an outbreak in Camargue. I. Introduction. Ann Inst Pasteur. 1968;114(4):518-20.
14. McIntosh BM, Jupp PG, Dos Santos I, Meenehan GM. Epidemics of West Nile and Sindbis viruses in South Africa with Culex (Culex) univittatus Theobald as vector. S Afr J Sci. 1976;72(10):295-300.
15. Tsai TF, Popovici F, Cernescu C, Campbell GL, Nedelcu NI. West Nile encephalitis epidemic in southeastern Romania. Lancet. 1998;352(9130):767-71.
16. Platonov AE, Shipulin GA, Shipulina OY, Tyutyunnik EN, Frolochkina TI, Lanciotti RS, et al. Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. Emerg Infect Dis. 2001;7(1):128-32.
17. Danis K, Papa A, Theocharopoulos G, Dougas G, Athanasiou M, Detsis M, et al. Outbreak of West Nile virus infection in Greece, 2010. Emerg Infect Dis. 2011;17(10):1868-72.
18. Platonov AE, Karan LS, Shopenskaia TA, Fedorova MV, Koliasnikova NM, Rusakova NM, et al. Genotyping of West Nile fever virus strains circulating in southern Russia as an epidemiological investigation method: principles and results. Zh Mikrobiol Epidemiol Immunobiol. 2011(2):29-37.
19. Donadieu E, Bahuon C, Lowenski S, Zientara S, Coulpier M, Lecollinet S. Differential virulence and pathogenesis of West Nile viruses. Viruses. 2013;5(11):2856-80.
20. Venter M, Swanepoel R. West Nile virus lineage 2 as a cause of zoonotic neurological disease in humans and horses in southern Africa. Vector Borne Zoonotic Dis. 2010;10(7):659-64.

21. Giladi M, Metzkor-Cotter E, Martin DA, Siegman-Igra Y, Korczyn AD, Rosso R, et al. West Nile encephalitis in Israel, 1999: the New York connection. Emerg Infect Dis. 2001;7(4):659-61.
22. 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(24):1807-14.
23. Chancey C, Grinev A, Volkova E, Rios M. The global ecology and epidemiology of West Nile virus. Biomed Res Int. 2015;2015:376230-.
24. Public Health Agency of Canada. West Nile virus and other mosquito-borne diseases national surveillance report [Internet]. 2018 [cited Mar 10 2019] [Available from: https://www.canada.ca/en/public-health/services/diseases/west-nile-virus/west-nile-virus-other-mosquito-borne-disease.html#a2008_12.
25. Pepperell C, Rau N, Krajden S, Kern R, Humar A, Mederski B, et al. West Nile virus infection in 2002: morbidity and mortality among patients admitted to hospital in southcentral Ontario. CMAJ. 2003;168(11):1399-405.
26. Tellez-Zenteno J, Hunter G, Ronquillo L, Haghir E. Neuroinvasive West Nile Virus Disease in Canada: The Saskatchewan Experience. Can J Neurol Sci. 2013;40:580-4.
27. Estrada-Franco JG, Navarro-Lopez R, Beasley DWC, Coffey L, Carrara A-S, Travassos da Rosa A, et al. West Nile virus in Mexico: evidence of widespread circulation since July 2002. Emerg infect Dis. 2003;9(12):1604-7.
28. Elizondo-Quiroga D, Davis CT, Fernandez-Salas I, Escobar-Lopez R, Velasco Olmos D, Soto Gastalum LC, et al. West Nile Virus isolation in human and mosquitoes, Mexico. Emerg Infect Dis. 2005;11(9):1449-52.
29. Cruz L, Cardenas VM, Abarca M, Rodriguez T, Reyna RF, Serpas MV, et al. Short report: serological evidence of West Nile virus activity in El Salvador. Am J Trop Med Hyg. 2005;72(5):612-5.
30. Morales-Betoulle ME, Komar N, Panella NA, Alvarez D, Lopez MR, Betoulle JL, et al. West Nile virus ecology in a tropical ecosystem in Guatemala. Am J Trop Med Hyg. 2013;88(1):116-26.
31. Hobson-Peters J, Arevalo C, Cheah WY, Blitvich BJ, Tan CS, Sandis A, et al. Detection of antibodies to West Nile virus in horses, Costa Rica, 2004. Vector Borne Zoonotic Dis. 2011;11(8):1081-4.
32. Pauvolid-Correa A, Morales MA, Levis S, Figueiredo LT, Couto-Lima D, Campos Z, et al. Neutralising antibodies for West Nile virus in horses from Brazilian Pantanal. Mem Inst Oswaldo Cruz. 2011;106(4):467-74.
33. Mattar S, Edwards E, Laguado J, Gonzalez M, Alvarez J, Komar N. West Nile virus antibodies in Colombian horses. Emerg Infect Dis. 2005;11(9):1497-8.
34. Adrian Diaz L, Komar N, Visintin A, Dantur Juri MJ, Stein M, Lobo Allende R, et al. West Nile virus in birds, Argentina. Emerg Infect Dis. 2008;14(4):689-91.
35. Bosch I, Herrera F, Navarro JC, Lentino M, Dupuis A, Maffei J, et al. West Nile virus, Venezuela. Emerg Infect Dis. 2007;13(4):651-3.
36. Mazzei M, Savini G, Di Gennaro A, Macchioni F, Prati MC, Guzman LR, et al. West Nile seroprevalence study in Bolivian horses, 2011. Vector Borne Zoonotic Dis. 2013;13(12):894-6.
37. Pupo M, Guzman MG, Fernandez R, Llop A, Dickinson FO, Perez D, et al. West Nile Virus infection in humans and horses, Cuba. Emerg Infect Dis. 2006;12(6):1022-4.
38. Komar O, Robbins MB, Klenk K, Blitvich BJ, Marlenee NL, Burkhalter KL, et al. West Nile virus transmission in resident birds, Dominican Republic. Emerg Infect Dis. 2003;9(10):1299-302.
39. Elizondo-Quiroga D, Elizondo-Quiroga A. West Nile virus and its theories, a big puzzle in Mexico and Latin America. J Glob Infect Dis. 2013;5(4):168-75.
40. Tesh RB, Travassos da Rosa AP, Guzman H, Araujo TP, Xiao SY. Immunization with heterologous flaviviruses protective against fatal West Nile encephalitis. Emerg Infect Dis. 2002;8(3):245-51.

41. Osorio JE, Ciuoderis KA, Lopera JG, Piedrahita LD, Murphy D, Levasseur J, et al. Characterization of West Nile viruses isolated from captive American Flamingoes (Phoenicopterus ruber) in Medellin, Colombia. Am J Trop Med Hyg. 2012;87(3):565-72.
42. Giordano BV, Kaur S, Hunter FF. West Nile virus in Ontario, Canada: A twelve-year analysis of human case prevalence, mosquito surveillance, and climate data. PLoS One. 2017;12(8):e0183568.
43. Drebot MA, Lindsay R, Barker IK, Buck PA, Fearon M, Hunter F, et al. West Nile virus surveillance and diagnostics: A Canadian perspective. Can J Infect Dis. 2003;14(2):105-14.
44. Public Health Agency of Canada. Assessment of Surveillance of Human West Nile Virus Infection in Quebec, 2003 [Internet]. 2003 [cited Mar 16 2019]. [Available from: https://www.canada.ca/en/public-health/services/reports-publications/canada-communicable-disease-report-ccdr/monthly-issue/2004-30/assessment-surveillance-human-west-nile-virus-infection-quebec-2003.html.
45. Government of Manitoba. 2003 Surveillance for West Nile virus in Manitoba [Internet]. 2005 [cited Mar 17 2019]. [Available from: https://www.gov.mb.ca/health/wnv/stats2003.html.
46. Government of Alberta. West Nile virus and surveillance [Internet]. 2019 [cited Mar 17 2019]. [Available from: https://www.alberta.ca/west-nile-virus-surveillance.aspx.
47. Schellenberg TL, Anderson ME, Drebot MA, Vooght MT, Findlater AR, Curry PS, et al. Seroprevalence of West Nile virus in Saskatchewan's Five Hills Health Region, 2003. Can J Public Health. 2006;97(5):369-73.
48. British Columbia Centre for Disease Control. West Nile Virus Activity in British Columbia: 2009 Surveillance Program Results. 2009. [Available from: http://www.bccdc.ca/resource-gallery/Documents/Statistics%20and%20Research/Statistics%20and%20Reports/Epid/Vector-bourne/WNV/2009/WNVSurveillanceresults2009v2.pdf.
49. Government of Canada. 2018 West Nile virus weekly surveillance and monitoring (Week 45) [Internet]. 2018 [cited Mar 17 2019]. [Available from: https://www.canada.ca/en/public-health/services/diseases/west-nile-virus/surveillance-west-nile-virus/west-nile-virus-weekly-surveillance-monitoring.html.
50. Government of Canada. West Nile virus weekly surveillance and monitoring: Year-to-date total of West Nile virus clinical cases and asymptomatic infections, as of October 26, 2019 [Internet]. 2019 [cited Jan 19 2020]. [Available from: https://www.canada.ca/en/public-health/services/diseases/west-nile-virus/surveillance-west-nile-virus/west-nile-virus-weekly-surveillance-monitoring.html.
51. Government of Canada. West Nile virus and other mosquito-borne diseases national surveillance reports [Internet]. 2018 [cited Mar 17 2019]. [Available from: https://www.canada.ca/en/public-health/services/diseases/west-nile-virus/west-nile-virus-other-mosquito-borne-disease.html.
52. Public Health Sudbury and Districts. Health Unit reports second confirmed human case of West Nile virus [Internet]. 2017 [cited Mar 16 2019]. [Available from: https://www.phsd.ca/alerts/health-unit-reports-second-confirmed-human-case-west-nile-virus.
53. Government of Canada. West Nile virus and other mosquito-borne diseases national surveillance report — July 30 to August 5, 2017 (Week 31) [Internet]. 2017 [cited Mar 16 2019]. [Available from: https://www.canada.ca/en/public-health/services/publications/diseases-conditions/west-nile-virus-surveillance/2017/west-nile-virus-other-mosquito-borne-disease-national-surveillance-report-july-30-august-5-2017-week-31.html.
54. Government of Canada. West Nile virus and other mosquito-borne diseases in Canada: Annual national surveillance report -2017 [Internet]. 2018 [cited Jan 19 2020] [Available from: https://www.canada.ca/en/public-health/services/publications/diseases-conditions/west-nile-virus-surveillance/2017/annual-national-surveillance-report.html.

55. Public Health Ontario. Vector-borne diseases 2012 summary report [Internet]. 2013 [cited Feb 06 2020] [Available from: https://www.publichealthontario.ca/-/media/documents/vector-borne-diseases-2012.pdf?la=en.
56. Pepperell C, Rau N, Krajden S, Kern R, Humar A, Mederski B, et al. West Nile virus infection in 2002: morbidity and mortality among patients admitted to hospital in southcentral Ontario. CMAJ: Canadian Medical Association Journal. 2003;168(11):1399-405.
57. Burton JM, Kern RZ, Halliday W, Mikulis D, Brunton J, Fearon M, et al. Neurological manifestations of West Nile virus infection. Can J Neurol Sci. 2004;31(2):185-93.
58. Jiao L, Main C. A brief report of West Nile Virus neuroinvasive disease in the summer of 2012 in Hamilton, Ontario. Can J Infect Dis Med Microbiol. 2014;25(1):24-6.
59. Komar N, Burns J, Dean C, Panella NA, Dusza S, Cherry B. Serologic evidence for West Nile virus infection in birds in Staten Island, New York, after an outbreak in 2000. Vector Borne Zoonotic Dis. 2001;1(3):191-6.
60. Nemeth NM, Oesterle PT, Bowen RA. Humoral immunity to West Nile virus is long-lasting and protective in the house sparrow (Passer domesticus). Am J Trop Med Hyg. 2009;80(5):864-9.
61. Apperson CS, Hassan HK, Harrison BA, Savage HM, Aspen SE, Farajollahi A, et al. Host feeding patterns of established and potential mosquito vectors of West Nile virus in the eastern United States. Vector Borne Zoonotic Dis. 2004;4(1):71-82.
62. Kilpatrick AM, Daszak P, Jones MJ, Marra PP, Kramer LD. Host heterogeneity dominates West Nile virus transmission. Proc Biol Sci. 2006;273(1599):2327-33.
63. Reed KD, Meece JK, Henkel JS, Shukla SK. Birds, migration and emerging zoonoses: west nile virus, lyme disease, influenza A and enteropathogens. Clin Med Res. 2003;1(1):5-12.
64. Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. Epidemiology and Transmission Dynamics of West Nile Virus Disease. Emerging Infectious Diseases. 2005;11(8):1167-73.
65. Eidson M, Komar N, Sorhage F, Nelson R, Talbot T, Mostashari F, 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):615-20.
66. Banet-Noach C, Simanov L, Malkinson M. Direct (non-vector) transmission of West Nile virus in geese. Avian Pathol. 2003;32(5):489-94.
67. Centers for Disease Control and Prevention. Mosquito species in which West Nile virus has been detected, United States, 1999-2017 [Internet]. 2017 [cited Oct 14 2017]. [Available from: https://stacks.cdc.gov/view/cdc/46971/Share.
68. Turell MJ, Dohm DJ, Sardelis MR, Oguinn ML, Andreadis TG, Blow JA. An update on the potential of north American mosquitoes (Diptera: Culicidae) to transmit West Nile Virus. J Med Entomol. 2005;42(1):57-62.
69. Turell MJ, Sardelis MR, Dohm DJ, O'Guinn ML. Potential North American vectors of West Nile virus. Ann N Y Acad Sci. 2001;951:317-24.
70. Kilpatrick AM, Kramer LD, Campbell SR, Alleyne EO, Dobson AP, Daszak P. West Nile Virus Risk Assessment and the Bridge Vector Paradigm. Emerg Infect Dis. 2005;11(3):425-9.
71. Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. PLoS Biol. 2006;4(4):e82.
72. Klenk K, Snow J, Morgan K, Bowen R, Stephens M, Foster F, et al. Alligators as West Nile virus amplifiers. Emerg Infect Dis. 2004;10(12):2150-5.
73. Kostiukov MA, Gordeeva ZE, Bulychev VP, Nemova NV, Daniiarov OA. [The lake frog (Rana ridibunda)--one of the food hosts of blood-sucking mosquitoes in Tadzhikistan--a reservoir of the West Nile fever virus]. Med Parazitol (Mosk). 1985(3):49-50.

74. van den Hurk AF, Hall-Mendelin S, Webb CE, Tan CS, Frentiu FD, Prow NA, et al. Role of enhanced vector transmission of a new West Nile virus strain in an outbreak of equine disease in Australia in 2011. Parasit Vectors. 2014;7:586.
75. Epp T, Waldner C, West K, Townsend H. Factors associated with West Nile virus disease fatalities in horses. Can Vet J. 2007;48(11):1137-45.
76. Ward MP, Levy M, Thacker HL, Ash M, Norman SK, Moore GE, et al. Investigation of an outbreak of encephalomyelitis caused by West Nile virus in 136 horses. J Am Vet Med Assoc. 2004;225(1):84-9.
77. Rizzo C, Salcuni P, Nicoletti L, Ciufolini MG, Russo F, Masala R, et al. Epidemiological surveillance of West Nile neuroinvasive diseases in Italy, 2008 to 2011. Euro Surveill. 2012;17(20).
78. Kopel E, Amitai Z, Bin H, Shulman LM, Mendelson E, Sheffer R. Surveillance of West Nile virus disease, Tel Aviv district, Israel, 2005 to 2010. Euro Surveill. 2011;16(25).
79. Lindsey NP, Staples JE, Lehman JA, Fischer M. Surveillance for human West Nile virus disease - United States, 1999-2008. MMWR Surveill Summ. 2010;59(2):1-17.
80. Iwamoto M, Jernigan DB, Guasch A, Trepka MJ, Blackmore CG, Hellinger WC, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. N Engl J Med. 2003;348(22):2196-203.
81. Ravindra KV, Freifeld AG, Kalil AC, Mercer DF, Grant WJ, Botha JF, et al. West Nile virus-associated encephalitis in recipients of renal and pancreas transplants: case series and literature review. Clin Infect Dis. 2004;38(9):1257-60.
82. O'Leary DR, Kuhn S, Kniss KL, Hinckley AF, Rasmussen SA, Pape WJ, et al. Birth outcomes following West Nile Virus infection of pregnant women in the United States: 2003-2004. Pediatrics. 2006;117(3):e537-45.
83. Hinckley AF, O'Leary DR, Hayes EB. Transmission of West Nile virus through human breast milk seems to be rare. Pediatrics. 2007;119(3):e666-71.
84. Alpert SG, Fergerson J, Noel LP. Intrauterine West Nile virus: ocular and systemic findings. Am J Ophthalmol. 2003;136(4):733-5.
85. Pealer LN, Marfin AA, Petersen LR, Lanciotti RS, Page PL, Stramer SL, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. N Engl J Med. 2003;349(13):1236-45.
86. Cameron C, Reeves J, Antonishyn N, Tilley P, Alport T, Eurich B, et al. West Nile virus in Canadian blood donors. Transfusion. 2005;45(4):487-91.
87. Byrne SN, Halliday GM, Johnston LJ, King NJ. Interleukin-1β but not tumor necrosis factor is involved in West Nile virus-induced Langerhans cell migration from the skin in C57BL/6 mice. J Invest Dermatol. 2001;117(3):702-9.
88. Lim PY, Behr MJ, Chadwick CM, Shi PY, Bernard KA. Keratinocytes Are Cell Targets of West Nile Virus In Vivo. J Virol. 2011;85(10):5197-201.
89. Johnston LJ, Halliday GM, King NJ. Langerhans cells migrate to local lymph nodes following cutaneous infection with an arbovirus. J Invest Dermatol. 2000;114(3):560-8.
90. Lee E, Hall RA, Lobigs M. Common E protein determinants for attenuation of glycosaminoglycan-binding variants of Japanese encephalitis and West Nile viruses. J Virol. 2004;78(15):8271-80.
91. Pierson T, Diamond M. Flaviviruses. In: Knipe D, Howley P, editors. Fields Virology. Philadelphia, PA: Wolters Kluwer/Lippencott Williams & Wilkins; 2013. p. 747-94.
92. Bardina SV, Brown JA, Michlmayr D, Hoffman KW, Sum J, Pletnev AG, et al. Chemokine Receptor Ccr7 Restricts Fatal West Nile Virus Encephalitis. J Virol. 2017;91(10).
93. Suthar MS, Diamond MS, Gale Jr M. West Nile virus infection and immunity. Nat Rev Micro. 2013;11(2):115-28.
94. Hoffman KW, Sachs D, Bardina SV, Michlmayr D, Rodriguez CA, Sum J, et al. Differences in Early Cytokine Production Are Associated With Development of a Greater Number of Symptoms Following West Nile Virus Infection. J Infect Dis. 2016;214(4):634-43.

95. Tobler LH, Cameron MJ, Lanteri MC, Prince HE, Danesh A, Persad D, et al. Interferon and interferon-induced chemokine expression is associated with control of acute viremia in West Nile virus-infected blood donors. J Infect Dis. 2008;198(7):979-83.
96. Ramos HJ, Lanteri MC, Blahnik G, Negash A, Suthar MS, Brassil MM, et al. IL-1beta signaling promotes CNS-intrinsic immune control of West Nile virus infection. PLoS Pathog. 2012;8(11):e1003039.
97. Wang T, Scully E, Yin Z, Kim JH, Wang S, Yan J, et al. IFN-γ-Producing γδ T Cells Help Control Murine West Nile Virus Infection. J Immunol. 2003;171(5):2524-31.
98. Diamond MS, Sitati EM, Friend LD, Higgs S, Shrestha B, Engle M. A critical role for induced IgM in the protection against West Nile virus infection. J Exp Med. 2003;198(12):1853-62.
99. Ben-Nathan D, Huitinga I, Lustig S, van Rooijen N, Kobiler D. West Nile virus neuroinvasion and encephalitis induced by macrophage depletion in mice. Arch Virol. 1996;141(3-4):459-69.
100. Bai F, Kong KF, Dai J, Qian F, Zhang L, Brown CR, et al. A paradoxical role for neutrophils in the pathogenesis of West Nile virus. J Infect Dis. 2010;202(12):1804-12.
101. Sitati EM, Diamond MS. CD4+ T-cell responses are required for clearance of West Nile virus from the central nervous system. J Virol. 2006;80(24):12060-9.
102. Brien JD, Uhrlaub JL, Nikolich-Zugich J. West Nile virus-specific CD4 T cells exhibit direct antiviral cytokine secretion and cytotoxicity and are sufficient for antiviral protection. J Immunol. 2008;181(12):8568-75.
103. Shrestha B, Pinto AK, Green S, Bosch I, Diamond MS. CD8+ T cells use TRAIL to restrict West Nile virus pathogenesis by controlling infection in neurons. J Virol. 2012;86(17):8937-48.
104. Shrestha B, Diamond MS. Role of CD8+ T cells in control of West Nile virus infection. J Virol. 2004;78(15):8312-21.
105. Beasley DW, Barrett AD. Identification of neutralizing epitopes within structural domain III of the West Nile virus envelope protein. J Virol. 2002;76(24):13097-100.
106. Deubel V, Fiette L, Gounon P, Drouet MT, Khun H, Huerre M, et al. Variations in biological features of West Nile viruses. Ann N Y Acad Sci. 2001;951:195-206.
107. Chambers TJ, Halevy M, Nestorowicz A, Rice CM, Lustig S. West Nile virus envelope proteins: nucleotide sequence analysis of strains differing in mouse neuroinvasiveness. J Gen Virol. 1998;79 (Pt 10):2375-80.
108. Verma S, Lo Y, Chapagain M, Lum S, Kumar M, Gurjav U, et al. West Nile virus infection modulates human brain microvascular endothelial cells tight junction proteins and cell adhesion molecules: Transmigration across the in vitro blood-brain barrier. Virology. 2009;385(2):425-33.
109. Garcia-Tapia D, Loiacono CM, Kleiboeker SB. Replication of West Nile virus in equine peripheral blood mononuclear cells. Vet Immunol Immunopathol. 2006;110(3-4):229-44.
110. Hasebe R, Suzuki T, Makino Y, Igarashi M, Yamanouchi S, Maeda A, et al. Transcellular transport of West Nile virus-like particles across human endothelial cells depends on residues 156 and 159 of envelope protein. BMC Microbiol. 2010;10:165.
111. Medigeshi GR, Hirsch AJ, Brien JD, Uhrlaub JL, Mason PW, Wiley C, et al. West nile virus capsid degradation of claudin proteins disrupts epithelial barrier function. J Virol. 2009;83(12):6125-34.
112. Shen J, SS TT, Schrieber L, King NJ. Early E-selectin, VCAM-1, ICAM-1, and late major histocompatibility complex antigen induction on human endothelial cells by flavivirus and comodulation of adhesion molecule expression by immune cytokines. J Virol. 1997;71(12):9323-32.
113. Xu Z, Waeckerlin R, Urbanowski MD, van Marle G, Hobman TC. West Nile virus infection causes endocytosis of a specific subset of tight junction membrane proteins. PLoS One. 2012;7(5):e37886.
114. Samuel MA, Wang H, Siddharthan V, Morrey JD, Diamond MS. Axonal transport mediates West Nile virus entry into the central nervous system and induces acute flaccid paralysis. Proc Natl Acad Sci U S A. 2007;104(43):17140-5.

115. Wang H, Siddharthan V, Hall JO, Morrey JD. West Nile virus preferentially transports along motor neuron axons after sciatic nerve injection of hamsters. J Neurovirol. 2009;15(4):293-9.
116. Nir Y, Beemer A, Goldwasser RA. West Nile Virus infection in mice following exposure to a viral aerosol. Br J Exp Pathol. 1965;46(4):443-9.
117. McMinn PC, Dalgarno L, Weir RC. A comparison of the spread of Murray Valley encephalitis viruses of high or low neuroinvasiveness in the tissues of Swiss mice after peripheral inoculation. Virology. 1996;220(2):414-23.
118. Hunsperger EA, Roehrig JT. Temporal analyses of the neuropathogenesis of a West Nile virus infection in mice. J Neurovirol. 2006;12(2):129-39.
119. Roe K, Kumar M, Lum S, Orillo B, Nerurkar VR, Verma S. West Nile virus-induced disruption of the blood-brain barrier in mice is characterized by the degradation of the junctional complex proteins and increase in multiple matrix metalloproteinases. J Gen Virol. 2012;93(Pt 6):1193-203.
120. Brown AN, Kent KA, Bennett CJ, Bernard KA. Tissue tropism and neuroinvasion of West Nile virus do not differ for two mouse strains with different survival rates. Virology. 2007;368(2):422-30.
121. Johnson RT, Mims CA. Pathogenesis of viral infections of the nervous system. New England Journal of Medicine. 1968;278(2):84-92.
122. Liou ML, Hsu CY. Japanese encephalitis virus is transported across the cerebral blood vessels by endocytosis in mouse brain. Cell Tissue Res. 1998;293(3):389-94.
123. Chambers TJ, Diamond MS. Pathogenesis of flavivirus encephalitis. Adv Virus Res. 2003;60:273-342.
124. Wang T, Town T, Alexopoulou L, Anderson JF, Fikrig E, Flavell RA. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. Nat Med. 2004;10(12):1366-73.
125. Verma S, Kumar M, Gurjav U, Lum S, Nerurkar VR. Reversal of West Nile virus-induced blood-brain barrier disruption and tight junction proteins degradation by matrix metalloproteinases inhibitor. Virology. 2010;397(1):130-8.
126. Dai J, Wang P, Bai F, Town T, Fikrig E. Icam-1 participates in the entry of west nile virus into the central nervous system. J Virol. 2008;82(8):4164-8.
127. Sigmundsdottir H, Butcher EC. Environmental cues, dendritic cells and the programming of tissue-selective lymphocyte trafficking. Nat Immunol. 2008;9(9):981-7.
128. Oberdorster G, Sharp Z, Atudorei V, Elder A, Gelein R, Kreyling W, et al. Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol. 2004;16(6-7):437-45.
129. van Riel D, Verdijk R, Kuiken T. The olfactory nerve: a shortcut for influenza and other viral diseases into the central nervous system. J Pathol. 2015;235(2):277-87.
130. Sejvar JJ. West Nile Virus Infection. Microbiol Spectr. 2016;4(3).
131. Davis LE, DeBiasi R, Goade DE, Haaland KY, Harrington JA, Harnar JB, et al. West Nile virus neuroinvasive disease. Ann Neurol. 2006;60(3):286-300.
132. Jean CM, Honarmand S, Louie JK, Glaser CA. Risk factors for West Nile virus neuroinvasive disease, California, 2005. Emerg Infect Dis. 2007;13(12):1918-20.
133. Lindsey NP, Sejvar JJ, Bode AV, Pape WJ, Campbell GL. Delayed mortality in a cohort of persons hospitalized with West Nile virus disease in Colorado in 2003. Vector Borne Zoonotic Dis. 2012;12(3):230-5.
134. Murray KO, Garcia MN, Rahbar MH, Martinez D, Khuwaja SA, Arafat RR, et al. Survival analysis, long-term outcomes, and percentage of recovery up to 8 years post-infection among the Houston West Nile virus cohort. PLoS One. 2014;9(7):e102953.
135. Patel H, Sander B, Nelder MP. Long-term sequelae of West Nile virus-related illness: a systematic review. Lancet Infect Dis. 2015;15(8):951-9.
136. Yeung MW, Shing E, Nelder M, Sander B. Epidemiologic and clinical parameters of West Nile virus infections in humans: a scoping review. BMC Infect Dis. 2017;17:609.

137. Hayes EB, Sejvar JJ, Zaki SR, Lanciotti RS, Bode AV, Campbell GL. Virology, Pathology, and Clinical Manifestations of West Nile Virus Disease. Emerg Infect Dis. 2005;11(8):1174-9.
138. Sejvar JJ. The long-term outcomes of human West Nile virus infection. Clin Infect Dis. 2007;44(12):1617-24.
139. Watson JT, Pertel PE, Jones RC, Siston AM, Paul WS, Austin CC, et al. Clinical characteristics and functional outcomes of West Nile Fever. Ann Intern Med. 2004;141(5):360-5.
140. Cook RL, Xu X, Yablonsky EJ, Sakata N, Tripp JH, Hess R, et al. Demographic and clinical factors associated with persistent symptoms after West Nile virus infection. Am J Trop Med Hyg. 2010;83(5):1133-6.
141. Carson PJ, Konewko P, Wold KS, Mariani P, Goli S, Bergloff P, et al. Long-term clinical and neuropsychological outcomes of West Nile virus infection. Clin Infect Dis. 2006;43(6):723-30.
142. Sejvar JJ, Curns AT, Welburg L, Jones JF, Lundgren LM, Capuron L, et al. Neurocognitive and functional outcomes in persons recovering from West Nile virus illness. J Neuropsychol. 2008;2(Pt 2):477-99.
143. Murray KO, Resnick M, Miller V. Depression after infection with West Nile virus. Emerg Infect Dis. 2007;13(3):479-81.
144. Loeb M, Hanna S, Nicolle L, Eyles J, Elliott S, Rathbone M, et al. Prognosis after West Nile virus infection. Ann Intern Med. 2008;149(4):232-41.
145. Nolan MS, Hause AM, Murray KO. Findings of long-term depression up to 8 years post infection from West Nile virus. J Clin Psychol. 2012;68(7):801-8.
146. Samaan Z, McDermid Vaz S, Bawor M, Potter TH, Eskandarian S, Loeb M. Neuropsychological Impact of West Nile Virus Infection: An Extensive Neuropsychiatric Assessment of 49 Cases in Canada. PLoS One. 2016;11(6):e0158364.
147. Klee AL, Maidin B, Edwin B, Poshni I, Mostashari F, Fine A, et al. Long-term prognosis for clinical West Nile virus infection. Emerg Infect Dis. 2004;10(8):1405-11.
148. Berner YN, Lang R, Chowers MY. Outcome of West Nile fever in older adults. J Am Geriatr Soc. 2002;50(11):1844-6.
149. Ferguson DD, Gershman K, LeBailly A, Petersen LR. Characteristics of the rash associated with West Nile virus fever. Clin Infect Dis. 2005;41(8):1204-7.
150. Murray KO, Baraniuk S, Resnick M, Arafat R, Kilborn C, Shallenberger R, et al. Clinical investigation of hospitalized human cases of West Nile virus infection in Houston, Texas, 2002-2004. Vector Borne Zoonotic Dis. 2008;8(2):167-74.
151. Emig M, Apple DJ. Severe West Nile virus disease in healthy adults. Clin Infect Dis. 2004;38(2):289-92.
152. Sejvar JJ, Lindsey NP, Campbell GL. Primary causes of death in reported cases of fatal West Nile Fever, United States, 2002-2006. Vector Borne Zoonotic Dis. 2011;11(2):161-4.
153. Brilla R, Block M, Geremia G, Wichter M. Clinical and neuroradiologic features of 39 consecutive cases of West Nile Virus meningoencephalitis. J Neurol Sci. 2004;220(1-2):37-40.
154. 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(4):654-8.
155. Sejvar JJ. Clinical Manifestations and Outcomes of West Nile Virus Infection. Viruses. 2014;6(2):606-23.
156. Ford-Jones EL, Fearon M, Leber C, Dwight P, Myszak M, Cole B, et al. Human surveillance for West Nile virus infection in Ontario in 2000. CMAJ. 2002;166(1):29-35.
157. Huhn GD, Austin C, Langkop C, Kelly K, Lucht R, Lampman R, et al. The emergence of west nile virus during a large outbreak in Illinois in 2002. Am J Trop Med Hyg. 2005;72(6):768-76.

158. Weatherhead JE, Miller VE, Garcia MN, Hasbun R, Salazar L, Dimachkie MM, et al. Long-Term Neurological Outcomes in West Nile Virus–Infected Patients: An Observational Study. Am J Trop Med Hyg. 2015;92(5):1006-12.
159. Robinson RL, Shahida S, Madan N, Rao S, Khardori N. Transient parkinsonism in West Nile virus encephalitis. Am J Med. 2003;115(3):252-3.
160. Sejvar JJ, Leis AA, Stokic DS, Van Gerpen JA, Marfin AA, Webb R, et al. Acute Flaccid Paralysis and West Nile Virus Infection. Emerg Infect Dis. 2003;9(7):788-93.
161. Sejvar JJ, Haddad MB, Tierney BC, Campbell GL, Marfin AA, Van Gerpen JA, et al. Neurologic manifestations and outcome of West Nile virus infection. Jama. 2003;290(4):511-5.
162. Yeung MW, Tomlinson G, Loeb M, Sander B. Health-related quality of life in persons with West Nile virus infection: a longitudinal cohort study. Health Qual Life Outcomes. 2017;15(1):210.
163. Bhangoo S, Chua R, Hammond C, Kimmel Z, Semenov I, Videnovic A, et al. Focal neurological injury caused by West Nile virus infection may occur independent of patient age and premorbid health. J Neurol Sci. 2005;234(1-2):93-8.
164. LaBeaud AD, Lisgaris MV, King CH, Mandalakas AM. Pediatric West Nile virus infection: neurologic disease presentations during the 2002 epidemic in Cuyahoga County, Ohio. Pediatr Infect Dis J. 2006;25(8):751-3.
165. Tyler KL, Pape J, Goody RJ, Corkill M, Kleinschmidt-DeMasters BK. CSF findings in 250 patients with serologically confirmed West Nile virus meningitis and encephalitis. Neurology. 2006;66(3):361-5.
166. Glass JD, Samuels O, Rich MM. Poliomyelitis Due to West Nile Virus. N Engl J Med. 2002;347(16):1280-1.
167. Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Ewing D, Mazowiecki M, et al. West Nile virus-associated flaccid paralysis. Emerg Infect Dis. 2005;11(7):1021-7.
168. Sejvar JJ, Leis AA, Stokic DS, Van Gerpen JA, Marfin AA, Webb R, et al. Acute Flaccid Paralysis and West Nile Virus Infection. Emerging Infectious Diseases. 2003;9(7):788-93.
169. Sejvar JJ, Bode AV, Marfin AA, Campbell GL, Pape J, Biggerstaff BJ, et al. West Nile Virus-associated flaccid paralysis outcome. Emerg Infect Dis. 2006;12(3):514-6.
170. Alker A. West Nile virus-associated acute flaccid paralysis. BMJ Case Reports. 2015;2015.
171. Marciniak C, Sorosky S, Hynes C. Acute flaccid paralysis associated with West Nile virus: motor and functional improvement in 4 patients. Arch Phys Med Rehabil. 2004;85(12):1933-8.
172. Johnstone J, Hanna SE, Nicolle LE, Drebot MA, Neupane B, Mahony JB, et al. Prognosis of West Nile virus associated acute flaccid paralysis: a case series. J Med Case Rep. 2011;5:395.
173. Jeha LE, Hanes GP, Sila CA, Lederman RJ, Isada CM, Gordon SM. Long-term outcome of patients with West Nile virus infection. Infect Dis Clin Pract (Baltim MD). 2005;13(3):101-3.
174. Sejvar JJ, Marfin AA. Manifestations of West Nile neuroinvasive disease. Rev Med Virol. 2006;16(4):209-24.
175. Lanteri MC, Kaidarova Z, Peterson T, Cate S, Custer B, Wu S, et al. Association between HLA class I and class II alleles and the outcome of West Nile virus infection: an exploratory study. PLoS One. 2011;6(8):e22948.
176. Bigham AW, Buckingham KJ, Husain S, Emond MJ, Bofferding KM, Gildersleeve H, et al. Host genetic risk factors for West Nile virus infection and disease progression. PLoS One. 2011;6(9):e24745.
177. Lim JK, McDermott DH, Lisco A, Foster GA, Krysztof D, Follmann D, et al. CCR5 Deficiency is a Risk Factor for Early Clinical Manifestations of West Nile Virus Infection, but not for Infection per se. J Infect Dis. 2010;201(2):178-85.
178. Jing Y, Shaheen E, Drake RR, Chen N, Gravenstein S, Deng Y. Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. Hum Immunol. 2009;70(10):777-84.

179. Panda A, Qian F, Mohanty S, van Duin D, Newman FK, Zhang L, et al. Age-associated decrease in TLR function in primary human dendritic cells predicts influenza vaccine response. J Immunol. 2010;184(5):2518-27.
180. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired Toll-like receptor expression and function in aging. J Immunol. 2002;169(9):4697-701.
181. Mo R, Chen J, Han Y, Bueno-Cannizares C, Misek DE, Lescure PA, et al. T cell chemokine receptor expression in aging. J Immunol. 2003;170(2):895-904.
182. Brien JD, Uhrlaub JL, Hirsch A, Wiley CA, Nikolich-Zugich J. Key role of T cell defects in age-related vulnerability to West Nile virus. J Exp Med. 2009;206(12):2735-45.
183. O'Leary DR, Marfin AA, Montgomery SP, Kipp AM, Lehman JA, Biggerstaff BJ, et al. The epidemic of West Nile virus in the United States, 2002. Vector Borne Zoonotic Dis. 2004;4(1):61-70.
184. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016;16(10):626-38.
185. Van Kerkhove MD, Vandemaele KA, Shinde V, Jaramillo-Gutierrez G, Koukounari A, Donnelly CA, et al. Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. PLoS Med. 2011;8(7):e1001053.
186. Kristensen K, Hjuler T, Ravn H, Simoes EA, Stensballe LG. Chronic diseases, chromosomal abnormalities, and congenital malformations as risk factors for respiratory syncytial virus hospitalization: a population-based cohort study. Clin Infect Dis. 2012;54(6):810-7.
187. Badawi A, Ryoo SG. Prevalence of comorbidities in the Middle East respiratory syndrome coronavirus (MERS-CoV): a systematic review and meta-analysis. Int J Infect Dis. 2016;49:129-33.
188. Mattner F, Sohr D, Heim A, Gastmeier P, Vennema H, Koopmans M. Risk groups for clinical complications of norovirus infections: an outbreak investigation. Clin Microbiol Infect. 2006;12(1):69-74.
189. Economopoulou A, Dominguez M, Helynck B, Sissoko D, Wichmann O, Quenel P, et al. Atypical Chikungunya virus infections: clinical manifestations, mortality and risk factors for severe disease during the 2005-2006 outbreak on Reunion. Epidemiol Infect. 2009;137(4):534-41.
190. Massard J, Ratziu V, Thabut D, Moussalli J, Lebray P, Benhamou Y, et al. Natural history and predictors of disease severity in chronic hepatitis C. J Hepatol. 2006;44(1 Suppl):S19-24.
191. Echevarria-Zuno S, Mejia-Arangure JM, Mar-Obeso AJ, Grajales-Muniz C, Robles-Perez E, Gonzalez-Leon M, et al. Infection and death from influenza A H1N1 virus in Mexico: a retrospective analysis. Lancet. 2009;374(9707):2072-9.
192. Moss WJ, Fisher C, Scott S, Monze M, Ryon JJ, Quinn TC, et al. HIV type 1 infection is a risk factor for mortality in hospitalized Zambian children with measles. Clin Infect Dis. 2008;46(4):523-7.
193. Nicholson KG, Kent J, Hammersley V, Cancio E. Risk factors for lower respiratory complications of rhinovirus infections in elderly people living in the community: prospective cohort study. BMJ. 1996;313(7065):1119-23.
194. Public Health Agency of Canada. Health Status of Canadians 2016 [Internet]. 2016 [cited Nov 19 2017]. [Available from: https://www.canada.ca/content/dam/hc-sc/healthy-canadians/migration/publications/department-ministere/state-public-health-status-2016-etat-sante-publique-statut/alt/pdf-eng.pdf.
195. Ontario Ministry of Finance. Ontario Population Projections Update, 2017-2041 [Internet]. 2018 [cited Nov 25 2019]. [Available from: https://www.fin.gov.on.ca/en/economy/demographics/projections/.
196. Statistics Canada. Table 17-10-0005-01. Populations estimates on July 1st, by age and sex (table). [Internet]. 2019 [cited Jan 07 2019]. [Available from: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=1710000501.

197. Shing E, Wang J, Nelder MP, Parpia C, Gubbay JB, Loeb M, et al. The direct healthcare costs attributable to West Nile virus illness in Ontario, Canada: a population-based cohort study using laboratory and health administrative data. BMC Infect Dis. 2019;19(1):1059.
198. Ontario Ministry of Health and Long-Term Care. Appendix B: Provincial Case Definitions for Reportable Diseases - Disease: West Nile Virus. 2017.
199. Kulkarni MA, Lecocq AC, Artsob H, Drebot MA, Ogden NH. Epidemiology and aetiology of encephalitis in Canada, 1994-2008: a case for undiagnosed arboviral agents? Epidemiol Infect. 2013;141(11):2243-55.
200. Nakhla M, Simard M, Dube M, Larocque I, Plante C, Legault L, et al. Identifying pediatric diabetes cases from health administrative data: a population-based validation study in Quebec, Canada. Clin Epidemiol. 2019;11:833-43.
201. Tan C, Hansen M, Cohen G, Boyle K, Daneman N, Adhikari NK. Accuracy of administrative data for identification of patients with infective endocarditis. Int J Cardiol. 2016;224:162-4.
202. Myers RP, Shaheen AA, Fong A, Wan AF, Swain MG, Hilsden RJ, et al. Validation of coding algorithms for the identification of patients with primary biliary cirrhosis using administrative data. Can J Gastroenterol. 2010;24(3):175-82.
203. Fleet JL, Dixon SN, Shariff SZ, Quinn RR, Nash DM, Harel Z, et al. Detecting chronic kidney disease in population-based administrative databases using an algorithm of hospital encounter and physician claim codes. BMC Nephrol. 2013;14:81.
204. Widdifield J, Ivers NM, Young J, Green D, Jaakkimainen L, Butt DA, et al. Development and validation of an administrative data algorithm to estimate the disease burden and epidemiology of multiple sclerosis in Ontario, Canada. Mult Scler. 2015;21(8):1045-54.
205. Jaakkimainen RL, Bronskill SE, Tierney MC, Herrmann N, Green D, Young J, et al. Identification of Physician-Diagnosed Alzheimer's Disease and Related Dementias in Population-Based Administrative Data: A Validation Study Using Family Physicians' Electronic Medical Records. J Alzheimers Dis. 2016;54(1):337-49.
206. Westendorp WF, Nederkoorn PJ, Vermeij JD, Dijkgraaf MG, van de Beek D. Post-stroke infection: a systematic review and meta-analysis. BMC Neurol. 2011;11:110.
207. Gershon AS, Guan J, Wang C, To T. Trends in asthma prevalence and incidence in Ontario, Canada, 1996-2005: a population study. Am J Epidemiol. 2010;172(6):728-36.
208. To T, Dell S, Dick PT, Cicutto L, Harris JK, MacLusky IB, et al. Case verification of children with asthma in Ontario. Pediatr Allergy Immunol. 2006;17(1):69-76.
209. Gershon AS, Wang C, Guan J, Vasilevska-Ristovska J, Cicutto L, To T. Identifying patients with physician-diagnosed asthma in health administrative databases. Can Respir J. 2009;16(6):183-8.
210. Schultz SE, Rothwell DM, Chen Z, Tu K. Identifying cases of congestive heart failure from administrative data: a validation study using primary care patient records. Chronic Dis Inj Can. 2013;33(3):160-6.
211. Gershon AS, Wang C, Guan J, Vasilevska-Ristovska J, Cicutto L, To T. Identifying individuals with physcian diagnosed COPD in health administrative databases. Copd. 2009;6(5):388-94.
212. Guttmann A, Nakhla M, Henderson M, To T, Daneman D, Cauch-Dudek K, et al. Validation of a health administrative data algorithm for assessing the epidemiology of diabetes in Canadian children. Pediatr Diabetes. 2010;11(2):122-8.
213. Lipscombe LL, Hwee J, Webster L, Shah BR, Booth GL, Tu K. Identifying diabetes cases from administrative data: a population-based validation study. BMC Health Serv Res. 2018;18(1):316.
214. Antoniou T, Zagorski B, Loutfy MR, Strike C, Glazier RH. Validation of case-finding algorithms derived from administrative data for identifying adults living with human immunodeficiency virus infection. PLoS One. 2011;6(6):e21748.

215. Tu K, Chen Z, Lipscombe LL. Prevalence and incidence of hypertension from 1995 to 2005: a population-based study. Cmaj. 2008;178(11):1429-35.
216. Tu K, Campbell NR, Chen ZL, Cauch-Dudek KJ, McAlister FA. Accuracy of administrative databases in identifying patients with hypertension. Open Med. 2007;1(1):e18-26.
217. Widdifield J, Bombardier C, Bernatsky S, Paterson JM, Green D, Young J, et al. An administrative data validation study of the accuracy of algorithms for identifying rheumatoid arthritis: the influence of the reference standard on algorithm performance. BMC Musculoskelet Disord. 2014;15:216.
218. Widdifield J, Bernatsky S, Paterson JM, Tu K, Ng R, Thorne JC, et al. Accuracy of Canadian health administrative databases in identifying patients with rheumatoid arthritis: a validation study using the medical records of rheumatologists. Arthritis Care Res (Hoboken). 2013;65(10):1582-91.
219. McCullagh P, Nelder J. Generalized Linear Models. 2nd ed. London: Chapman and Hall; 1989.
220. Government of Manitoba. Surveillance Statistics for West Nile virus in Manitoba [Internet]. 2019 [cited Nov 19 2019]. [Available from: https://www.gov.mb.ca/health/wnv/stats.html.
221. Government of Alberta. Alberta West Nile Virus summary report 2003-2011 [Internet]. 2012 [cited Nov 19 2019]. [Available from: https://open.alberta.ca/publications/alberta-west-nile-virus-summary-report.
222. Government of Saskatchewan. West Nile Virus Surveillance Report, 2019: August 24 [Internet]. 2018 [cited Nov 20 2019]. [Available from: https://publications.saskatchewan.ca/#/products/102393.
223. Lindsey NP, Kuhn S, Campbell GL, Hayes EB. West Nile virus neuroinvasive disease incidence in the United States, 2002-2006. Vector Borne Zoonotic Dis. 2008;8(1):35-9.
224. Chen CC, Jenkins E, Epp T, Waldner C, Curry PS, Soos C. Climate change and West Nile virus in a highly endemic region of North America. Int J Environ Res Public Health. 2013;10(7):3052-71.
225. Borchardt SM, Feist MA, Miller T, Lo TS. Epidemiology of West Nile virus in the highly epidemic state of North Dakota, 2002-2007. Public Health Rep. 2010;125(2):246-9.
226. Chung WM, Buseman CM, Joyner SN, Hughes SM, Fomby TB, Luby JP, et al. The 2012 West Nile encephalitis epidemic in Dallas, Texas. JAMA. 2013;310(3):297-307.
227. Tsai CL, Delclos GL, Huang JS, Hanania NA, Camargo CA, Jr. Age-related differences in asthma outcomes in the United States, 1988-2006. Ann Allergy Asthma Immunol. 2013;110(4):240-6, 6.e1.
228. Markle JG, Fish EN. SeXX matters in immunity. Trends Immunol. 2014;35(3):97-104.
229. Klein SL, Marriott I, Fish EN. Sex-based differences in immune function and responses to vaccination. Trans R Soc Trop Med Hyg. 2015;109(1):9-15.
230. Franceschi C, Campisi J. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J Gerontol A Biol Sci Med Sci. 2014;69 Suppl 1:S4-9.
231. Kumar D, Drebot MA, Wong SJ, Lim G, Artsob H, Buck P, et al. A seroprevalence study of west nile virus infection in solid organ transplant recipients. Am J Transplant. 2004;4(11):1883-8.
232. Spindler KR, Hsu TH. Viral disruption of the blood-brain barrier. Trends Microbiol. 2012;20(6):282-90.
233. Segaloff HE, Petrie JG, Malosh RE, Cheng CK, McSpadden EJ, Ferdinands JM, et al. Severe morbidity among hospitalised adults with acute influenza and other respiratory infections: 2014-2015 and 2015-2016. Epidemiol Infect. 2018;146(11):1350-8.
234. Gutierrez-Gonzalez E, Cantero-Escribano JM, Redondo-Bravo L, San Juan-Sanz I, Robustillo-Rodela A, Cendejas-Bueno E, et al. Effect of vaccination, comorbidities and age on mortality and severe disease associated with influenza during the season 2016-2017 in a Spanish tertiary hospital. J Infect Public Health. 2019;12(4):486-91.
235. Popescu CP, Florescu SA, Cotar AI, Badescu D, Ceianu CS, Zaharia M, et al. Re-emergence of severe West Nile virus neuroinvasive disease in humans in Romania, 2012 to 2017-implications for travel medicine. Travel Med Infect Dis. 2018;22:30-5.

236. Ouhoummane N, Tchouaket E, Lowe AM, Fortin A, Kairy D, Vibien A, et al. Economic Burden of West Nile Virus Disease, Quebec, Canada, 2012-2013. Emerg Infect Dis. 2019;25(10):1943-50.
237. McDermid J, Fera S, Hogg A. Climate change projections for Ontario: An updated synthesis for policymakers and planners [Internet]. 2015 [cited Nov 19 2019]. [Available from: http://www.climateontario.ca/MNR_Publications/CCRR-44.pdf.
238. Trumbo CW, Harper R. Perceptual influences on self-protective behavior for West Nile virus, a survey in Colorado, USA. BMC Public Health. 2015;15:557.
239. McCarthy TA, Hadler JL, Julian K, Walsh SJ, Biggerstaff BJ, Hinten SR, 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;951:307-16.
240. Herrington JE, Jr. Pre-West Nile virus outbreak: perceptions and practices to prevent mosquito bites and viral encephalitis in the United States. Vector Borne Zoonotic Dis. 2003;3(4):157-73.
241. Ontario Ministry of Health and Long Term Care. West Nile Virus Preparedness and Prevention Plan [Internet]. 2019. [Available from: http://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/reference/WNV_plan_en.pdf.
242. Government of Ontario. Health Protection and Promotion Act, R.S.O. 1990, c.H.7. Available from: https://www.ontario.ca/laws/statute/90h07.
243. Gujral IB, Zielinski-Gutierrez EC, LeBailly A, Nasci R. Behavioral risks for West Nile virus disease, northern Colorado, 2003. Emerg Infect Dis. 2007;13(3):419-25.
244. Hongoh V, Campagna C, Panic M, Samuel O, Gosselin P, Waaub JP, et al. Assessing Interventions to Manage West Nile Virus Using Multi-Criteria Decision Analysis with Risk Scenarios. PLoS One. 2016;11(8):e0160651.
245. Kaiser JA, Barrett ADT. Twenty Years of Progress Toward West Nile Virus Vaccine Development. Viruses. 2019;11(9).
246. Dayan GH, Bevilacqua J, Coleman D, Buldo A, Risi G. Phase II, dose ranging study of the safety and immunogenicity of single dose West Nile vaccine in healthy adults >/= 50 years of age. Vaccine. 2012;30(47):6656-64.
247. Shankar MB, Staples JE, Meltzer MI, Fischer M. Cost effectiveness of a targeted age-based West Nile virus vaccination program. Vaccine. 2017;35(23):3143-51.

Appendix

ICD-10-CA codes used to identify cases of WNND in the WNV cohort.

Manifestation	ICD-10-CA/ CCI Code	Description
WNM	A878	Other viral meningitis
	A879	Meningitis, viral
	G020	Meningitis in viral diseases classified elsewhere
	G028	Meningitis in other specified infectious and parasitic diseases classified elsewhere
	G030	Nonpyogenic meningitis
	G031	Chronic meningitis
	G038	Meningitis due to other specified causes
	G039	Meningitis, unspecified
WNP	A801	Acute paralytic poliomyelitis, wild virus, imported
	A802	Acute paralytic poliomyelitis, wild virus, indigenous
	A803	Acute paralytic poliomyelitis, other and unspecified
	A804	Acute nonparalytic poliomyelitis
	A809	Acute poliomyelitis, unspecified
	A818	Other atypical virus infections of central nervous system
	A819	Atypical virus infection of central nervous system, unspecified
	B91	Sequelae of poliomyelitis
	G373	Acute transverse myelitis in demyelinating disease of central nervous system
	G378	Other specified demyelinating diseases of central nervous system
	G379	Demyelinating disease of central nervous system, unspecified
	G610	Guillain-Barré syndrome
	G8100	Flaccid hemiplegia of dominant side
	G8101	Flaccid hemiplegia of non-dominant side
	G8109	Flaccid hemiplegia of unspecified [unilateral] side
	G8110	Spastic hemiplegia of dominant side
	G8111	Spastic hemiplegia of non-dominant side
	G8119	Spastic hemiplegia of unspecified [unilateral] side
	G8190	Hemiplegia of unspecified type of dominant side
	G8191	Hemiplegia of unspecified type of non-dominant side
	G8199	Hemiplegia of unspecified type of unspecified [unilateral] side
	G830	Diplegia of upper limbs

	G831	Monoplegia of lower limb
	G8320	Monoplegia of upper limb on dominant side
	G8321	Monoplegia of upper limb on non-dominant side
	G8322	Monoplegia of upper limb on unspecified [unilateral] side
	G833	Monoplegia, unspecified
WNE	A838	Other mosquito-borne viral encephalitis
	A839	Mosquito-borne viral encephalitis, unspecified
	A852	Arthropod-borne viral encephalitis, unspecified
	A858	Other specified viral encephalitis
	A86	Unspecified viral encephalitis
	B941	Sequelae of viral encephalitis
	G040	Acute disseminated encephalitis
	G048	Other encephalitis, myelitis and encephalomyelitis
	G049	Encephalitis, myelitis and encephalomyelitis, unspecified
	G051	Encephalitis, myelitis and encephalomyelitis in viral diseases classified elsewhere
	G052	Encephalitis, myelitis and encephalomyelitis in other infectious and parasitic diseases classified elsewhere
	G058	Encephalitis, myelitis and encephalomyelitis in other diseases classified elsewhere
	G934	Encephalopathy, unspecified

OHIP diagnosis codes used to identify WNND in the WNV cohort.

Manifestation	OHIP Diagnosis Code	Description
WNM	036	Meningococcal infection or meningitis
	321	Meningitis due to other organisms
WNP	045	Acute poliomyelitis
	356	Idiopathic peripheral neuritis
	358	Myoneural disorders
	359	Muscular dystrophies
WNE		
	062	Mosquito-borne viral encephalitis
	323	Encephalitis, encephalomyelitis