Powassan Virus and Other Tick-Borne Pathogens from Wildlife and Companion Animals in Southern Ontario

by

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POWASSAN VIRUS AND OTHER TICK-BORNE PATHOGENS FROM WILDLIFE AND COMPANION ANIMALS IN SOUTHERN ONTARIO

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University of Guelph, 2017

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The objectives of the present research were to survey potential vertebrate host and tick species for evidence of infections with POWV and other arthropod-borne pathogens including West Nile virus, Heartland virus, Anaplasma phagocytophilum, Babesia microti, Borrelia miyamotoi, B. burgdorferi and Ehrlichia chaffeensis in mammalian wildlife, dogs and ticks in southern Ontario. During the summers of 2015-2016, ticks and tissues were collected from carcasses of free-ranging, medium-sized mammals; blood and ticks were collected from live-trapped mammals, and ticks removed from dogs were collected from local veterinary clinics. Albeit rarely, evidence of the presence of POWV was found both by polymerase chain reaction in ticks and serological tests in groundhogs (Marmota monax) and striped skunks (Mephitis mephitis). Evidence of A. phagocytophilum and B. burgdorferi was also rarely detected in Ixodes scapularis ticks. These findings emphasize the importance of both broad and targeted surveillance strategies for investigating emerging tick-borne diseases.
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DECLARATION OF WORK

I declare that all work in this thesis was completed by me, except for the items listed below:

Colleagues in Dr. Robbin Lindsay’s Laboratory at the Public Health Agency of Canada’s National Microbiology Laboratory in Winnipeg performed the diagnostic testing of tick extracts (for Powassan virus, *Anaplasma phagocytophilum, Babesia microti, Borrelia miyamotoi, B. burgdorferi* and *Ehrlichia chaffeensis*) and serum samples (for Powassan virus and West Nile virus).

Dr. Angela M. Bosco-Lauth at the Department of Biomedical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University in Fort Collins, Colorado performed the diagnostic testing of serum samples for Heartland virus.
# TABLE OF CONTENTS

ABSTRACT .......................................................................................................................... ii  
ACKNOWLEDGEMENTS ........................................................................................................ iii  
DECLARATION OF WORK ...................................................................................................... v  
LIST OF TABLES .................................................................................................................. viii  
LIST OF FIGURES ................................................................................................................ ix  
LIST OF SYMBOLS, ABBREVIATIONS AND NOMENCLATURE ........................................... x  

CHAPTER 1: LITERATURE REVIEW & RESEARCH OBJECTIVES ........................................ 1  
1.1 Tick-Borne Viral Zoonoses .............................................................................................. 1  
1.2 History and Global Distribution of Powassan Virus ......................................................... 2  
1.3 Clinical Manifestations of POWV in Humans and Other Animals ...................................... 5  
1.3.1 Human ....................................................................................................................... 5  
1.3.2 Animal ....................................................................................................................... 8  
1.4 Phylogeny of Powassan Prototype Virus and Deer Tick Virus ............................................ 10  
1.5 Transmission Ecology – Vector and Host Dynamics .......................................................... 11  
1.5.1 Vector ....................................................................................................................... 11  
1.5.2 Potential Vertebrate Reservior Hosts ........................................................................... 13  
1.6 Conclusions ................................................................................................................... 16  
1.7 Study Rationale and Objectives ....................................................................................... 17  
1.8 References ..................................................................................................................... 18  

CHAPTER 2: TICKS AND TICK-BORNE PATHOGENS INVOLVING WILDLIFE AND  
COMPANION ANIMALS IN SOUTHERN ONTARIO, CANADA .............................................. 33  
2.1 Abstract ......................................................................................................................... 33  
2.2 Introduction ..................................................................................................................... 34
Materials and Methods ................................................................. 35

Results ............................................................................................ 37

Discussion ....................................................................................... 37

References ....................................................................................... 42

CHAPTER 3: SURVEY OF POWASSAN VIRUS AND OTHER ARBOVIRUSES FROM WILDLIFE IN SOUTHERN ONTARIO .......................................................... 50

Abstract ......................................................................................... 50

Introduction ..................................................................................... 51

Materials and Methods .................................................................. 52

Results ............................................................................................ 55

Discussion ....................................................................................... 56

References ....................................................................................... 61

CHAPTER 4: SUMMARY DISCUSSION AND CONCLUSIONS ....................... 68

Study Limitations ........................................................................... 70

Future Studies ............................................................................... 73

Conclusions .................................................................................... 74

References ....................................................................................... 75
LIST OF TABLES

Table 1.1. Published human cases of Powassan virus from 1958 to present with location and demographic data, clinical signs, and references. ................................................................. 25

Table 2.1. Tick species and number opportunistically collected from free-ranging wildlife and companion animals in southern Ontario between May 2015 and May 2017 and in archived tick samples collected from 2011-2013. .................................................................................................. 46

Table 2.2. Tick species and number collected from 334 parasitized wildlife and companion animals by species between May 2015 and December 2016 and in archived tick samples collected from 2011-2013. .................................................................................................. 47

Table 3.1. Species of origin and number of wildlife tissue samples tested for Powassan virus RNA by reverse transcriptase polymerase chain reaction from May to October, 2015-2016. .......................................................... 65

Table 3.2. Number of wildlife serum samples tested by species from May—October in 2015 and 2016 and tested for Powassan virus (POWV), West Nile virus (WNV) and Heartland virus (HRTV). .......................................................................................................................... 66
LIST OF FIGURES

Figure 2.1. The distribution of tick samples opportunistically collected between May 2015 until May 2017 from free-ranging wildlife and dogs in southern Ontario, Canada. .......................... 49

Figure 3.1. The distribution of Powassan virus- and West Nile virus-positive samples by plaque reduction neutralization test in southern Ontario, Canada. Serum samples were collected from carcasses and live-trapped mammals from May-September in 2015 and 2016. .......................... 67
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation and Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>95% CI</td>
<td>95% confidence interval</td>
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<td>cELISA</td>
<td>competitive enzyme linked immune sorbent assay</td>
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<td>DTV</td>
<td>Deer tick virus</td>
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<td>Canadian Wildlife Health Cooperative</td>
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<td>HRTV</td>
<td>Heartland virus</td>
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<td>polymerase chain reaction</td>
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<td>POWV</td>
<td>Powassan virus</td>
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<td>POWV-p</td>
<td>Powassan virus prototype</td>
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<tr>
<td>PRNT</td>
<td>plaque reduction neutralization test</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>reverse transcriptase polymerase chain reaction</td>
</tr>
<tr>
<td>SGE</td>
<td>salivary gland extract</td>
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<tr>
<td>WNV</td>
<td>West Nile virus</td>
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</table>
CHAPTER 1: LITERATURE REVIEW & RESEARCH OBJECTIVES

1.1 Tick-Borne Viral Zoonoses

The number of new zoonotic diseases (or zoonoses) has increased significantly since the
1940’s with vector-borne diseases accounting for 23% of emerging infectious diseases (Jones et
al. 2008). Vector-borne pathogens are transmitted by a variety of hematophagous female insects
(e.g., mosquitoes, ticks, midges). When they feed by ingesting blood meals, there is an
opportunity for pathogen transmission to hosts, including zoonotic pathogens (Vasconcelos and
Calisher 2016). This transmission cycle is the basis for the maintenance of arthropod-borne
viruses (i.e., arboviruses) within vertebrate hosts, which may include wildlife and humans.

In Canada, arboviruses are concerning because they have the potential to spread over
large distances via infectious arthropod vectors through international movement or northward
spread from more southern, endemic areas (e.g., the United States). In addition, environmental
changes due to urbanization, climate change or agricultural expansion may impact movements
and geographic range of wildlife species that may serve as reservoir hosts, as well as arthropod
vectors (Kulkarni et al. 2015).

As a blood-feeding ectoparasite, ticks may spread a greater diversity of zoonotic,
infectious agents than other arthropod vectors (Jongehan and Uilenberg 2009). Tick-borne
pathogens are of global importance and may cause high morbidity and mortality, including with
potential impacts on livestock production (Jongehan and Uilenberg 2009). The northward
expansion and establishment of ticks in Canada is predicted to continue, leading to the likely
emergence, establishment and expanded range of zoonotic pathogens, including Lyme disease,
anaplasmosis, babesiosis, and ehrlichiosis, and also involving members of the tick-borne
encephalitis serocomplex, such as Powassan virus (POWV; Family Flaviridae, Genus
Flavivirus) (Ogden et al. 2006a, 2015; Kulkarni et al. 2015; Clow et al. 2016).

Reports of tick-borne pathogens in humans are increasing in northern latitudes (De la
Fuente and Estrada-Peña 2012). For example, the average number of human POWV cases
reported per year in eastern Canada and the northeastern United States has nearly doubled from
0.7 cases/year to 1.3 cases/year between the years of 1958-1998 and 1999-2005, respectively
(Hinten et al. 2008). Powassan virus can cause severe neurologic disease associated with
encephalitis in humans and horses, and appears to have a high case fatality rate in humans with no currently available vaccines or specific drug therapies (Little et al. 1985; Hinten et al. 2008).

Powassan virus is maintained in nature between ticks (e.g., Ixodes scapularis, I. cookei and I. marxi) and wild mammals, such as groundhogs (Marmota monax), striped skunks (Mephitis mephitis) and white-footed mice (Peromyscus leucopus) (Ko 1971; Hinten et al. 2008). Despite the detection of human POWV cases in central-eastern Canada and the northeastern and upper midwestern United States, the impacts of POWV on public health are likely underestimated. Further, knowledge gaps exist in its ecology and epidemiology, such as the level of involvement of various tick and wildlife species in natural transmission cycles (Wilson et al. 1979; Fitch and Artsob 1990; Ebel et al. 2001; Piantadosi et al. 2015). Further, the extent of the current geographic distribution of these potential or known tick vector species and the range of potential vertebrate hosts is not well understood. Such information is critical to understanding the risks of POWV infection to humans and animals, as well as for monitoring for future changes in virus distribution and transmission dynamics in association with environmental and socioeconomic factors. This review outlines the current state of knowledge surrounding POWV and its global distribution, clinical manifestation in humans and animals, and the host-virus-vector transmission triad.

1.2 HISTORY AND GLOBAL DISTRIBUTION OF POWASSAN VIRUS

Powassan virus is a positive sense, single-stranded, 10.8-kilobase, RNA virus (Mandl et al. 1993). Powassan virus is part of Casal’s group B arboviruses, which includes antigenically similar viruses such as West Nile virus, Russian spring-summer encephalitis virus (RSSE), Silverwater virus, St. Louis virus and Japanese encephalitis virus (McLean and Donohue 1959). Powassan virus was first isolated from a 5 year-old boy from Powassan Ontario, Canada, who died in September 1958 due to encephalitis (McLean and Donohue 1959).

Initial studies to help delineate the geographic distribution and transmission dynamics of POWV included serological testing of both wildlife and human samples in northern Ontario. Small mammals and ticks were also targeted, since other group B arboviruses (e.g., RSSE and Silverwater virus) were known to be maintained in nature through a transmission cycle that involved ticks and small- or medium-sized mammals (McLean and Donohue 1959; McLean and Larke 1963). Serosurveillance at the location of the first known POWV case (i.e., Powassan,
Ontario), revealed neutralizing and complement-fixing antibodies to POWV in healthy family members of the deceased child (1/6; 16.7%), as well as in wild animals such as eastern chipmunks (*Tamias striatus*) (4/13; 30.8%) and red squirrels (*Sciurus vulgaris*) (1/9; 11.1%) (McLean et al. 1960). Furthermore, neutralizing antibodies to POWV were detected in humans in Ontario from the regions of North Bay, Manitoulin and Sault Sainte Marie (McLean et al. 1960). Extensive serological sampling efforts involving both humans and animals in Ontario throughout the 1960s and 1970s indicated that POWV was present in multiple townships and cities across both northern and southern Ontario (McLean and Donohue 1959; McLean et al. 1960, 1962, 1964a, 1964b, 1967; Artsob et al. 1986). Ultimately, based on the isolation of POWV from three of 60 pools of *I. cookei* removed from groundhogs and the presence of naturalizing antibodies in groundhogs born within the trapping season (summer 1966), McLean et al. (1967) proposed that POWV was maintained in nature between groundhogs and *I. cookei* ticks. Subsequently, POWV surveillance studies have focused on small- to medium-sized mammals (both wild and domestic) and ticks.

POWV was first detected in the United States in 1964 in ticks (*I. cookei* and an unidentified species) and tissues of two groundhogs trapped in St. Lawrence County, New York (Whitney and Jamnback 1965). Retrospectively, POWV was determined to be the identity of a virus isolated from *Dermacentor andersoni* ticks in Colorado in 1952, suggesting that this virus is also present in the southwestern United States (Thomas et al. 1960). These two isolates of POWV from ticks further supported the hypothesis that ticks were the main POWV vector species and that groundhogs were the corresponding reservoir host species.

The second human case of POWV was documented in New Jersey in 1970 and was the first recognized non-fatal case of POWV encephalitis. Further serology, virus isolation and human case studies conducted in the United States between 1965 and 1980 revealed evidence of POWV in the states of California, Connecticut, Maine, Massachusetts, New York and Pennsylvania (Whitney and Jamnback 1965; Rossier et al. 1974; Smith et al. 1974; Deibel et al. 1979; Main et al. 1979; Johnson 1987). The increasing detection of POWV over a large geographic range fueled continued public health concern over this rarely detected and poorly understood virus.

However, the geographic range of POWV is not limited to North America. The first isolation of POWV in Russia was from *Haemaphysalis longicornis (neumanni)* in 1972 (Lvov et
al. 1974; Leonova et al. 2009). Since then, it has been isolated from *Dermacentor silvarum* ticks (1975 and 1978), and from the serum of humans (1978, 1991 and 2006) and the brain of a human that died of POWV encephalitis in 1979 (Leonova et al. 2009). These isolation events led to the conclusion that POWV is endemic to far eastern Russia and transmission most likely involves *Haemaphysalis longicornis (neumanni)* ticks and medium-sized mammals (Leonova et al. 2009).

Currently, POWV has been detected across much of Canada, the United States, and Russia (Deardorff et al. 2013). Human cases of POWV encephalitis have been diagnosed in the eastern Canadian provinces of Ontario, Quebec and New Brunswick (McLean and Donohue 1959; Wilson et al. 1979; Partington et al. 1980; Fitch and Artsob 1990; Kolski et al. 1998; Gholam et al. 1999). In addition, anti-POWV antibodies in wildlife have been detected in the Canadian provinces of Alberta and British Columbia (McLean et al. 1968, 1970, 1971; Zarnke and Yuill 1981). In the United States, human cases of POWV have been detected in the states of Maine, Massachusetts, Michigan, Minnesota, New Hampshire, New Jersey, New York, Pennsylvania, Vermont, Virginia, and Wisconsin (Goldfield et al. 1973; Rossier et al. 1974; Smith et al. 1974; Deibel et al. 1979; Embil et al. 1983; Hinten et al. 2008; Tavakoli et al. 2009; Hicar et al. 2011; Birge and Sonnesyn 2012; Sung et al. 2013; El Khoury et al. 2013a; Piantadosi et al. 2015; Centers for Disease Control and Prevention 2017). Serological surveys revealed anti-POWV antibodies in the following additional states: Alaska, California, Colorado, Connecticut, New Mexico and Rhode Island (Thomas et al. 1960; Main et al. 1979; Johnson 1987; Ebel et al. 2000; Deardorff et al. 2013). Thus, there is widespread evidence of POWV across much of North America, and human (and possibly equine; *Equus caballus*) cases are likely under-diagnosed due to lack of widespread recognition of POWV as a cause of encephalitis in these species (Little et al. 1985; El Khoury et al. 2013a).

Although POWV has been detected across a wide geographic range, there is still a lack of knowledge regarding the extent of endemic regions in North America and Russia. Surveys have been widespread but are inconsistent in terms of long-term or continuous data sets. For instance, there have been no serological surveys conducted in Ontario, Canada since 1989, even though it is the location of the first POWV case as well as numerous studies that followed soon after (McLean and Donohue 1959; McLean et al. 1967; Artsob et al. 1986; Farkas 1989). Due to its apparent rarity, the detection and surveillance of POWV may be challenging and is often limited to the locations and time immediately following diagnosis of clinical cases in humans.
(Vasconcelos and Calisher 2016). However, the apparent high POWV-associated case fatality rate in humans (20%-35%), coupled with its suspected increased incidence and detection in new geographic regions (e.g., Connecticut; Tutolo et al. 2017), emphasize the importance of ongoing surveillance and investigations into the ecoepidemiology of this virus (Hinten et al. 2008; El Khoury et al. 2013a).

In 1997, molecular genetics work led to the delineation of two separate lineages of POWV: Powassan prototype (POWV-p) and deer tick virus (DTV) (Telford et al. 1997). These two lineages are believed to be maintained in separate enzootic cycles. The prototype virus (POWV-p) is likely maintained between I. cookei and I. marxi ticks and wildlife reservoir hosts, such as groundhogs, striped skunks and other mammals. Deer tick virus is likely maintained between I. scapularis ticks and white-footed mice (Ko 1971; Ebel et al. 2001; Hinten et al. 2008). Deer tick virus strains (lineage I) are antigenically indistinguishable from the POWV-p strain (lineage II), although they may be segregated by genetic analysis (Kuno et al. 2001). Furthermore, POWV-p and DTV appear to have separate, albeit overlapping, geographic ranges. The Powassan prototype virus circulates in New York and Ontario, Canada, while DTV strains have been isolated along the Atlantic coast of the United States and in Wisconsin and New York (Ebel 2010; Dupuis II et al. 2013). These geographic ranges are based on a relatively small number of isolates and further studies are required to better define the geographic distributions and enzootic transmission cycles of POWV-p and DTV.

1.3 CLINICAL MANIFESTATIONS OF POWV IN HUMANS AND OTHER ANIMALS
1.3.1 HUMAN

Clinical disease

Since the initial isolation of POWV in 1958 from a human patient, there have been ~90 human cases reported between 1958 and 2016; 55 of these have been published (see Table 1.1). The remainder is unpublished (El Khoury et al. 2013a; Centers for Disease Control and Prevention 2017). Minnesota and Wisconsin account for the majority of cases, with 22 and 20 cases, respectively, identified since 2006 (Centers for Disease Control and Prevention 2017). Of the total number of published cases reviewed (n=55), the highest number of cases were diagnosed in New York (23/55; 41.8%) and Massachusetts (7/55; 12.7%), United States and Ontario, Canada (6/55; 10.9%; Table 1.1). Powassan virus is currently reportable in the United
States (MedlinePlus 2017), but not Canada (Canadian Food Inspection Agency 2017), which may affect access to knowledge on human cases.

Some infections with POWV may be asymptomatic or are limited to mild, transient clinical disease in humans (Tavakoli et al. 2009). However, diagnosed clinical cases are often characterized by signs of encephalitis or meningoencephalitis. Clinical signs were reviewed among 14 hospitalized, human patients with POWV in New York between 2004 and 2012, and most commonly included fever (100%), generalized weakness (86%) and lethargy (72%) (El Khoury et al. 2013a). More severe symptoms may also include vomiting, respiratory distress with periods of apnea, prolonged sustained fever that persists until the acute neurological changes abate, and coma (Smith et al. 1974). Powassan virus encephalitis presents itself similarly both antigenically and symptomatically to some other arboviral diseases (e.g., California encephalitis, Eastern equine encephalitis, St. Louis encephalitis, Western equine encephalitis and West Nile fever/neuroinvasive disease) and central nervous system diseases (e.g., herpes simplex encephalitis) that may require specific treatment; therefore, etiologic diagnosis is necessary (Hicar et al. 2011; El Khoury et al. 2013a; Centers for Disease Control and Prevention 2017). Preliminary diagnosis is often based on patient symptoms and history; laboratory diagnosis is generally accomplished by testing of serum or cerebrospinal fluid (CSF) to detect virus-specific IgM and neutralizing antibodies (Centers for Disease Control and Prevention 2017).

The incubation period for POWV is between 8 and 34 days (Gholam et al. 1999; El Khoury et al. 2013a; Piantadosi et al. 2015). The fatality rate of POWV is generally reported as between 15% and 20% (Deibel et al. 1979; Gholam et al. 1999; Hinten et al. 2008). El Koury et al. (2013a) reported a higher fatality rate of 36%, but hypothesized that a longer follow-up period may have accounted for this higher rate, because the 30-day fatality rate among the same patients was <20%. Thus, lower long-term survival may also be a factor. Further, the majority of known survivors of clinical POWV disease have suffered long-term neurologic sequelae and impaired ability to perform routine daily activities (Hinten et al. 2008). Of the 55 cases reviewed (Table 1), the male to female ratio was 3:1 (27:12), which is consistent with other studies (El Khoury et al. 2013a). Children (≤18) and seniors (≥60) were most commonly diagnosed with POWV-associated disease, with 31.4% (16/51) of cases aged ≤18, 23.5% (12/51) aged between 21 and 59, and 45.1% (23/51) aged ≥60 years (Table 1.1; four cases had no information on age).

Annually, POWV transmission rates increase in the early spring and late summer, and
although human infections have been detected throughout the year, infection risk is greatest between June and September (McLean et al. 1967; Gholam et al. 1999; Hinten et al. 2008). Of the published human case reports reviewed, 78.4% (40/51) were diagnosed between May and September, and 21.6% (11/51) between October and April (Table 1.1; four cases had no information on month). This pattern is likely due to the increased activity of ticks, the vector of POWV, as well as wildlife hosts, both of which may contribute to potential exposure of humans to infected ticks (Hinten et al. 2008).

The differentiation between POWV-p and DTV first occurred in 1997, owing to advanced molecular genetic techniques (Telford et al. 1997). Subsequently, there have been three published cases of DTV encephalitis in humans: two fatal and one non-fatal case (Gholam et al. 1999; Tavakoli et al. 2009; El Khoury et al. 2013b). One of the fatal DTV cases involved a patient with a known tick bite 32 days prior to clinical disease. Based on timing (October 2010) and the geographic location of this patient in the Lower Hudson Valley of New York State (also an endemic area for Lyme disease, for which I. scapularis is the main vector), it is likely that the tick was an adult I. scapularis (El Khoury et al. 2013b). In addition, among the 14 cases of POWV encephalitis reviewed by El Khoury et al. (2013a) in New York State from 2004 through 2012, ten patients (72%) were from counties located in the Lower Hudson Valley. These cases further support the notion that DTV is likely transmitted to humans via I. scapularis and could be attributed to the changing epidemiology of POWV (Hinten et al. 2008). Further, some cases of POWV-p in humans may have been previously misclassified, and thus, the recent recognition of DTV and future studies will help reveal the prevalence, distribution, and potential differences in clinical manifestation of DTV-associated disease in humans as compared to POWV-p.

**Prevention and Treatment**

Similar to other arboviral encephalitides, there is no vaccine or specific therapy for POWV-p or DTV-associated encephalitis. Current guidelines recommend supportive therapy (El Khoury et al. 2013a; Sung et al. 2013; Piantadosi et al. 2015). In some cases, patients treated with high-dose corticosteroids have survived (El Khoury et al. 2013a; Sung et al. 2013). However, no studies have confirmed the relationship between corticosteroids and outcome of POWV encephalitis (Piantadosi et al. 2015). Currently, prevention of tick bites and promoting awareness of POWV disease among clinicians, laboratory diagnosticians, public health
professionals, and the general public are the best approaches for reducing the risk of POWV encephalitis (El Khoury et al. 2013a).

1.3.2 ANIMAL

Clinical Disease and Subclinical Infections

Powassan virus can cause both clinical and subclinical disease in domestic and wild animals. Domestic animals that have undergone clinical disease and corresponding neurologic lesions include horses and dogs (*Canis lupus familiaris*) (Furumoto 1969; Little et al. 1985; Keane et al. 1988). In horses, POWV may produce non-suppurative, focal necrotizing meningoencephalomyelitis (Little et al. 1985). The clinical progression of POWV in these horses mimicked cases of undiagnosed equine viral encephalitides observed in Ontario (Little et al. 1985; Keane et al. 1988). Furthermore, dogs have been shown to develop clinical signs after inoculation with POWV. All dogs (*n*=6) intravenously injected with POWV had resulting viremia; 4/6 were febrile (up to 40°C) and 1/6 dogs had mild listlessness and hyperemia of mucous membranes (Furumoto 1969). Advancements through clinical studies of POWV in domestic animals may lead to improved recognition of POWV as a differential diagnosis and diagnostic capabilities for detecting this pathogen in these animals.

In contrast, other domestic animals, including rabbits (*Oryctolagus*), cats (*Felis catus*), goats (*Capra aegagrus hircus*), chickens (*Gallus gallus domesticus*), pigs (*Sus scrofa domesticus*) and sheep (*Ovis aries*) have undergone subclinical infections after inoculation with POWV (McLean et al. 1960; Kokernot et al. 1969; Woodall and Roz 1977; Little et al. 1985; Keane et al. 1987). For instance, intracerebrally inoculated rabbits (*n*=4) appeared clinically normal but had extensive widespread encephalitis characterized by lymphocytic perivascular cuffs, lymphocytic meningitis and prominent lymphocytic choroiditis in the brain (Little et al. 1985). McLean et al. (1960) also observed a lack of clinical signs in two POWV-inoculated rabbits, but the rabbits were viremic and had detectable neutralizing antibodies 14 days after inoculation. In addition, histologic lesions of nonsuppurative encephalitis and encephalomyelitis were observed in intracerebrally- and intravenously-inoculated cats, but no clinical signs were observed (Keane et al. 1987). Laboratory studies have shown that POWV is notoriously difficult to re-isolate, revealing one of the likely difficulties in identifying POWV as a cause of encephalitis in naturally acquired cases (Little et al. 1985; Keane et al. 1987, 1988).
Similar to domestic animals, POWV has also led to subclinical infections in many wildlife species that are considered potential POWV reservoir hosts (Kokernot et al. 1969; Zarnke and Yuill 1981). For example, no signs of illness were observed in groundhogs (n=4), Virginia opossums (Didelphis virginiana; n=4), a grey fox (Urocyon cinereoargenteus; n=1), red foxes (Vulpes vulpes; n=2), striped skunks (n=4) or raccoons (Procyon lotor; n=6) inoculated with the POWV (Kokernot et al. 1969). However, viremia was detected in the grey fox, red foxes and groundhogs (Kokernot et al. 1969). Furthermore, no clinical signs were observed following POWV-inoculation in nine snowshoe hares (Lepus americanus); however, between days 1 and 3 post-inoculation, all hares had viremia titers sufficient to infect Dermacentor andersoni ticks (Zarnke and Yuill 1981). The detectable viremia titers support these wildlife species as potential amplifying hosts.

**Aspects of POWV Pathogenesis**

Laboratory studies are useful for studying the etiology and pathogenesis of disease and may lead to improved surveillance, diagnosis and treatment for both humans and animals affected by POWV. Powassan virus was observed to be highly pathogenic in rhesus monkeys (Macaca rhesus) and laboratory mice, with both species demonstrating severe neurological signs preceding death (Sobolev and Shustopalova 1977; Frolova et al. 1985; Hermance and Thangamani 2015; Hermance et al. 2016). Pathogenesis studies of rhesus monkeys detected POWV in neurons, glial cells and intercellular spaces of the central nervous tissue of these animals, indicating that POWV can spread throughout the central nervous system (Frolova et al. 1985). Similar results were detected in adult mice in which POWV particles were detected within all parts of the neurons (perikarya, dendrites and axon) and the intercellular spaces of the brain (Sobolev and Shustopalova 1977). Furthermore, mouse skin biopsies were used to identify early cell targets of infection at the POWV-infected tick feeding site. Neutrophils and mononuclear cells (e.g., macrophages) infiltrates were recruited earlier to the feeding site of a POWV-infected tick versus an uninfected tick, suggesting that macrophages may subsequently contain POWV antigen or virus, which may help disseminate the virus systemically (Hermance et al. 2016). Few pathogenesis studies have been conducted on POWV; however, these studies are vital in defining the interaction between POWV infection and host response.

Pathogen establishment in the host following vector-borne transmission from the salivary
glands of the vector is a complex process (Ebel and Kramer 2004; Hermance and Thangamani 2015). For POWV, tick saliva from *I. scapularis* has been demonstrated to enhance virus transmission to the host, influence dissemination within the host, and affect the course of disease (Hermance and Thangamani 2015). When low doses of POWV were inoculated into mice in the absence of salivary gland extract (SGE), there were no clinical signs of infection and no mice succumbed to disease. However, inoculation with low doses of POWV in the presence of SGE resulted in all infected mice experiencing viral neuroinvasion, paralysis, and death (Hermance and Thangamani 2015). This experiment demonstrated virus dose-dependent saliva-activated transmission of POWV. However, further investigations into the pathogenesis, including the specific salivary factors responsible for enhancing POWV transmission, are required.

1.4 PHYLOGENY OF POWASSAN PROTOTYPE VIRUS AND DEER TICK VIRUS

Viruses can only be classified as distinct if they can be segregated serologically and/or phylogenetically. The two POWV lineages, POWV-p and DTV are antigenically indistinguishable and can both may cause a central nervous syndrome in humans, making differentiation based on clinical presentation impossible (Kuno et al. 2001). Phylogenetically, the DTV genome is approximately 10,800 kilobases and is 1 base shorter at the 3' untranslated region (3'-UTR) than the corresponding sequence of POWV-p virus (Kuno et al. 2001). The nucleotide and amino acid sequences of DTV were determined to differ by 16% and 6% from POWV-p, respectively (Thomas et al. 1960; Kuno et al. 2001).

Although the POWV-p and DTV lineages share the same origin, they are distinct and co-exist independently in separate enzootic transmission cycles with overlapping geographic distribution (Ebel et al. 2001; Kuno et al. 2001). Powassan virus prototype is mainly associated with a groundhog–*I. cookei* transmission cycle, but is also associated with a red squirrel–*I. marxi* transmission cycle (McLean and Larke 1963; Ebel et al. 2001). Deer tick virus is associated with a white-footed mouse–*I. scapularis* transmission cycle (Ebel et al. 2001). Both lineages have been isolated from ticks or human/animal samples in Ontario, Canada and likely co-exist independently (Kuno et al. 2001). In addition, the POWV-p lineage is composed of isolates from the northeastern United States and far East Russia where the DTV lineage is currently comprised of isolates from Colorado, Wisconsin and the Atlantic coast of the United States (Ebel et al. 2001; Kuno et al. 2001; Leonova et al. 2009; Anderson and Armstrong 2012; Subbotina and
Currently, phylogenetic studies of POWV are limited by the relatively small number of sample strains available for analysis and by the relatively small genome fragments that have been analyzed to date.

1.5 TRANSMISSION ECOLOGY - VECTOR AND HOST DYNAMICS

1.5.1 VECTOR

There are two well established families of ticks known to transmit pathogens in humans and animals: Ixodidae (hard ticks) and Argasidae (soft ticks). The main vectors of POWV in Ontario are *I. scapularis, I. cookei* and *I. marxi*, which are classified as hard ticks and are defined by the “plate” on their back called a scutum, visible mouth parts and three distinct life stages: larvae, nymph and adult. All three tick species are currently found across eastern Canada and northeastern United States (Bishopp and Trembley 1945; Nelder et al. 2014; Lindquist et al. 2016). Climate change and migrating passerine birds are suspected to be causing or contributing to the northward range expansion of some tick species, including *I. scapularis* (Ogden et al. 2006; Ogden 2015). No data are currently available on the potential range expansion of *I. cookei* and *I. marxi*.

Complex laboratory studies have been used to characterize the vector competence of ticks for various zoonotic pathogens, including POWV. For example, all life stages of *I. scapularis* (i.e., larvae, nymph and adult) have been confirmed to become infected with POWV, and oral transmission to hamsters or rabbits by ticks increased with each stage of development (larvae 10%; nymph: 40%; adult females 57%). In addition, POWV transstadial transmission (i.e., from one life stage to the next) was confirmed in *I. scapularis* ticks at rates of 9.5% (for nymphs exposed as larvae), 10% (for adults exposed as larvae), and 54% (for adults exposed as nymphs). Transovarial transmission (i.e., from parent to offspring) was observed at a rate of 16.6% (Costero and Grayson 1996). Ebel and Kramer (2004) observed similar results and reported a transstadial transmission efficiency of 22% for *I. scapularis* nymphs that fed as larvae on mice infected with DTV. Furthermore, *I. scapularis* nymphs were demonstrated to transmit DTV to mice in as few as 15 minutes (Ebel and Kramer 2004). These results suggest that *I. scapularis* is a highly competent vector of POWV.

In contrast to the laboratory data available for *I. scapularis* and POWV vector competence, there is currently a lack of knowledge of the proficiency of *I. cookei* and *I. marxi* as
POWV vectors. *Ixodes cookei* ticks are believed to reside primarily within groundhog burrows since tick dragging (e.g., sampling in open environments, such as fields and forest floor) has not been productive as a means of detecting this species. Further, even though groundhogs were observed to come out of their burrows for only 1-2 hours/day, *I. cookei* ticks were often found on them with prevalence of tick detection ranging between 24% and 40%, (Grizzell 1955; Ko 1971; Farkas and Surgeoner 1990). In one study, 91% of adult *I. cookei* ticks (n=98) recovered from groundhogs were female, and adult males placed on groundhogs were never observed to feed, suggesting that female *I. cookei* ticks are the only sex to transmit POWV (Farkas and Surgeoner 1990). In addition, *I. cookei* were able to transmit POWV to their eggs after taking a blood meal from a viremic groundhog (Farkas 1989). No published studies specifically address POWV transmission via *I. marxi*, but POWV was isolated from *I. marxi* collected from a red squirrel in 1962 (McLean and Larke 1963). This tick species is most commonly known as the “squirrel tick”, but it has also been less commonly collected from red foxes, eastern chipmunks and eastern cottontails (*Sylvilagus floridanus*) (Tugwell and Lancaster 1962).

The notion that ticks are the major vector of POWV is supported by virus isolation from ticks, the detection of ticks on likely reservoir vertebrate host species (e.g., groundhogs, striped skunks and red squirrels), and the lack of detection of POWV in other arthropods, including mosquitoes (McLean et al. 1960). For instance, POWV was detected from pooled *I. scapularis* ticks collected between 1997 and 1999 in Massachusetts and Rhode Island (Ebel et al. 2000). Powassan virus was also isolated from pooled *I. cookei* ticks that were removed from groundhogs in northern Ontario (1964), a groundhog in New York (1964), 18/273 pools of *I. cookei* collected in northern Ontario (1964-1966), and 2/583 pools of *I. cookei* collected in New England (1964-1979) (McLean et al. 1964a, 1967; Whitney and Jamnback 1965; Main et al. 1979). In addition, POWV was isolated from a pool of 35 *I. marxi* collected from a red squirrel in northern Ontario in 1962 (McLean and Larke 1963). Deer tick virus was detected via reverse transcriptase (RT)-PCR and subsequently isolated from 17 *I. scapularis* ticks collected in northern Wisconsin between 2007 and 2008 and from 58 tick pools collected from the Hudson Valley, New York between 2007 and 2011 (Ebel et al. 2000; Brackney et al. 2008; Dupuis II et al. 2013). In far eastern Russia, POWV was first isolated in 1972 from a *Haemaphysalis longicornis* (*neumanni*) tick, which is now thought to be the most common vector in that region (Leonova et al. 2009). Collectively, these results suggest that *I. scapularis, I. cookei* and *I. marxi*
are the main vectors of POWV in North America, and that a different tick vector(s) may predominate in Russia.

1.5.2 POTENTIAL VEREBRATE RESERVOIR HOSTS

**Domestic Animals**

Evidence of natural POWV infections has been found in four species of domestic animals. Serological surveys have revealed a seroprevalence of 12.9% (13/101) in horses in Ontario, 1.8% (9/499) in goats in New York, and 12.3% (9/73) and 10.1% (90/889) in dogs in Illinois and Ontario, respectively (Furumoto 1969; Woodall and Roz 1977; Artsob et al. 1984; Little et al. 1985). In search of local candidate host species following a detection of a human POWV case in Powassan, Ontario, researchers documented a cat with antibodies to POWV (Wilson et al. 1979). However, a larger serological survey conducted on cats in southern Ontario revealed that 0/175 had POWV-neutralizing antibodies (Keane et al. 1987). No anti-POWV antibodies were detected in small groups of cattle, fowl or sheep in Ontario (McLean et al. 1960; McLean and Larke 1963). Laboratory-based studies would provide information on POWV viremia profiles and seroconversion rates in domestic animal species to help further assess their potential role as virus-amplifying hosts.

**Wildlife**

Defining the geographic range and susceptibility of candidate amplifying host(s) of POWV-p and DTV in nature is important to help assess the epidemiologic patterns and potential risk to humans in a given region. Evidence thus far has implicated several small mammal and rodent species as likely candidates (McLean et al. 1967; Artsob et al. 1986; Ebel et al. 2000). Infections within the natural or reservoir hosts are often subclinical, and thus, despite their limitations, serological studies provide information on surviving animals that have been previously infected in nature. Anti-POWV antibodies have been detected across a broad variety of wildlife taxa, including birds, reptiles and mammals. Compared to mammals, in which prevalence can be as high as 83.3% (10/12; striped skunk), the prevalence of anti-POWV antibodies detected in birds and reptiles is often much lower (i.e., 0.5-4.5% for birds, and 3.6-25.0% for reptiles) (Whitney et al. 1968; Main et al. 1979; Dupuis II et al. 2013). Small- to medium-sized mammals have historically been the main target for serological surveys for
POWV in wildlife, and antibodies have been detected in Columbian ground squirrels (*Urocitellus columbianus*), deer mice (*Peromyscus leucopus*), eastern chipmunks, eastern grey squirrel (*Sciurus carolinensis*), groundhogs, house mice (*Mus musculus*), long-tailed weasels (*Mustela frenata*), meadow jumping mice (*Zapus hudsonius*), meadow voles (*Microtus pennsylvanicus*), Norway rats (*Rattus norvegicus*), porcupines (*Erethizon dorsatum*), raccoons, red squirrels, short-tailed weasels (*Mustela erminea*), snowshoe hares, striped skunks, Virginia opossums, woodland jumping mice (*Napaeozapus insignis*), yellow-bellied marmots (*Marmota flaviventris*) and yellow-pine chipmunks (*Tamias amoenus*) (McLean et al. 1960, 1964a, 1964b, 1967, 1968, 1970, 1971; McLean and Larke 1963; Whitney et al. 1968; Main et al. 1979; Zarnke and Yuill 1981; Artsob et al. 1984, 1986; Ebel et al. 2000; Deardorff et al. 2013; Dupuis II et al. 2013). Fewer surveillance studies have been conducted on the prevalence of POWV in large mammals. Anti-POWV antibodies have also been detected in white-tailed deer (*Odocoileus virginianus*) and coyotes (*Canis latrans*) (Artsob et al. 1986; Nofchissey et al. 2013). Although small samples size warrants caution when analyzing these results, no antibodies were detected in sera from the following eastern North America mammal species: eastern cottontail (*n*=3), muskrat (*Ondatra zibethicus*; *n*=15), North American beaver (*Castor canadensis*; *n*=1), northern flying squirrel (*Glaucomys sabrinus*; *n*=1) and shot-tailed shrew (*Blarina brevicauda*; *n*=21) (McLean and Larke 1963; McLean et al. 1964b, 1967; Whitney et al. 1968; Artsob et al. 1984).

In eastern Canada and the northeastern United States, groundhogs, striped skunks and red squirrels are considered to be important amplifying hosts for POWV (McLean et al. 1967; Main et al. 1979; Artsob et al. 1984). Vector species of interest, including *I. cookei* and *I. marxi*, have been removed from these animals and groundhogs, striped skunks and red squirrels have consistently demonstrated a high seroprevalence to POWV (between 34%-67%, 16%-83% and 7%-59%, respectively) (McLean et al. 1960, 1964a, 1964b, 1967, 1970, 1971; McLean and Larke 1963; Main et al. 1979; Artsob et al. 1984, 1986; Dupuis II et al. 2013). These medium-sized mammal species are ideal POWV-amplifying hosts because of their population structure; groundhogs, striped skunks and squirrels have short life-spans and high-reproductive rates, which ensures that a large number of “susceptibles” (i.e., immunologically naive) are available every year to maintain virus transmission in nature (Grizzell 1955; Bailey 1971; Main et al. 1979) Furthermore, POWV seroprevalence in groundhogs may vary by age. For example, 51% (119/234) of adult groundhogs were seropositive compared to 15% (8/52) of juveniles collected
in the same year (McLean et al. 1967). Therefore, while serologic surveys reveal animals have been previously infected, likely via infectious ticks, it fails to reveal the hosts’ ability to amplify virus within the blood, and thus help perpetuate natural transmission cycles.

To further evaluate potentially competent, vertebrate virus-amplifying hosts, as well as to identify endemic regions of POWV, virus isolation is necessary as it provides proof of active infection (i.e., viremic stage) in a host within a geographic region. Powassan virus has been isolated from the blood (i.e., serum) and brain of a variety of wildlife species, including a red squirrel in 1962 (designated POWV strain no. 1828), two groundhogs and a grey fox in New York in 1964, a striped skunk trapped in California in 1969 and two groundhogs in Ontario in May 1964 (McLean and Larke 1963; McLean et al. 1964a; Whitney and Jamnback 1965; Johnson 1987). Although it often requires extensive field efforts including sampling of high numbers of animals and ticks, the acquisition of virus isolates is essential to attain isolates for further phylogenetic characterization and to better understand virus properties and host relationships through both in vitro and in vivo studies.

Sample collection from wildlife is increasingly incorporated into surveillance strategies for zoonotic pathogens (Smiley et al. 2015). Although groundhogs, striped skunks, and red squirrels are thought to be amplifying hosts of POWV in North America, further research is needed to identify the spectrum of species that may be infected and involved in transmission in nature. In addition, the major amplifying host for DTV is thought to be the white-footed mouse, but the DTV seroprevalence was relatively low in this species, ranging between 3.0% and 3.9% (Ebel et al. 2000). Other mammalian species, such as white-tailed deer, which is a major host for blood-feeding adult *I. scapularis* ticks (Ogden et al. 2006a), may be a potential amplifying host for DTV, since neutralizing antibodies were recently detected in deer in Connecticut (37.2%; 84/266), Maine (4.0%; 13/326) and Vermont (2.3%; 11/487) (Nofchissey et al. 2013). However, further research should be conducted on DTV in white-tailed deer, including determination of viremia profiles and assessment of whether transstadial (i.e., transmission between tick life stages) and transovarial transmission of DTV occurs in *I. scapularis*. Determining which vertebrate host species may serve as amplifying hosts for DTV is essential to understanding the epidemiology of this disease. This information is also important for better defining the geographic distributions of POWV-p and DTV lineages of POWV (Ebel 2010).
1.6 CONCLUSIONS

Powassan virus encephalitis causes high morbidity and mortality in humans with a reported case fatality rate of 15%–20% and residual neurologic deficits in 50% of survivors (Hinten et al. 2008; Ebel 2010; El Khoury et al. 2013a). The number of human cases of POWV appears to be increasing, in addition to recent POWV diagnoses in previously unrecorded regions, such as Maine, Michigan, Vermont and Wisconsin (Hinten et al. 2008; Piantadosi et al. 2015). Whether this observed increase in detections is due to increased awareness and corresponding rise in diagnostic testing or reflects an actual emergence of the POWV-associated disease is unknown. The recent finding of white-tailed deer that were seropositive for DTV and the expansion of *I. scapularis* in the United States and Canada suggest that it is important to continue efforts to monitor *I. scapularis* activity, and to include testing for DTV among these ticks as well as potential reservoir host species (Ogden et al. 2006a; Nofchissey et al. 2013; Nelder et al. 2014).

Knowledge of the enzootic cycles of POWV has grown considerably since it was first isolated and identified in 1958; however, information on the current status of POWV geographical distribution, including a better delineation of endemic regions, as well as the range and involvement of various wild mammal species as POWV-amplifying hosts is needed. For instance, although POWV was first isolated in Ontario, Canada and was subsequently shown to circulate in ticks and wildlife in the region (Artsob et al. 1984), no surveillance-focused studies, either in wildlife, domestic animals or humans, have been conducted in Ontario since 1989 (McLean and Donohue 1959; Farkas 1989). This lack of information is problematic since POWV was detected from a human in 2015 in Kingston, Ontario and from an *I. scapularis* tick collected from a dog in 2016 in Barrie, Ontario (R. Lindsay, personal communication). In addition, *I. scapularis, I. cookei* and *I. marxi* have all been collected through passive surveillance from humans in Ontario from 2008 to 2012 (Nelder et al. 2014). The importance of identifying the current range of POWV in Ontario is further underscored by predictions of continued *I. scapularis* range expansion in Ontario (Ogden et al. 2006a). Determining the roles of tick vectors and wildlife host species and their current and projected geographic distribution in Ontario will contribute to an improved understanding of the POWV epidemiology in south-central Ontario as well as aid in assessing the potential risks of POWV infection to humans and other animals.
1.7 STUDY RATIONALE AND OBJECTIVES

Powassan virus has been known to circulate in some areas of southern Ontario since 1958, with human cases diagnosed as recently as 2016 (McLean et al. 1959; Tutolo et al. 2017). Powassan virus is maintained in nature between wild mammals and Ixodid tick species; rarely, the virus has caused fatal neurologic disease in humans. However, reported human cases have been on the rise in recent years in the northeastern United States. The geographic distribution and diversity of POWV vectors (i.e., ticks), and potential vertebrate hosts within Ontario are poorly understood. Thus, the specific objectives of the research proposed herein are to:

a) Investigate the geographic distribution and tick burden on medium-sized mammalian wildlife and dogs (Chapter 2);

b) Test collected ticks for evidence of zoonotic diseases, namely Anaplasma phagocytophilum, Babesia microti, Borrelia miyamotoi, B. burgdorferi, Ehrlichia chaffeensis and POWV (Chapter 2);

c) Determine the seroprevalence of POWV among a variety of local, medium-sized, wild mammalian species in Ontario, Canada to help delineate the range of species that are naturally infected and the relative prevalence among these species (Chapter 3);

d) Assess the potential utility of various medium-sized, wild mammalian species for use in surveillance and determine potential candidate reservoir hosts for experimental trials through the identification of ticks on wildlife, and testing of ticks and tissues collected from wildlife (Chapter 3);

e) Provide current documentation of geographic areas of POWV detection through testing of wildlife-derived samples (Chapter 3).
1.8 REFERENCES


Subbotina EL, Loktev VB. 2012. Molecular evolution of the tick-borne encephalitis and


Table 1.1 Published human cases of Powassan virus from 1958 to present with location and demographic data, clinical signs, and references.

<table>
<thead>
<tr>
<th>Date</th>
<th>Location</th>
<th>Sex</th>
<th>Age</th>
<th>Fatal</th>
<th>Acute Clinical Syndromes</th>
<th>Chronic Effects</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Sept. 1958</td>
<td>Ontario</td>
<td>M</td>
<td>5</td>
<td>Yes</td>
<td>Drowsy, fever, neck stiffness, ataxia, spastic hemiplegia, encephalitis</td>
<td></td>
<td>McLean and Donohue 1959</td>
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<tr>
<td>Jul. 1970</td>
<td>New Jersey</td>
<td>F</td>
<td>57</td>
<td>No</td>
<td>Headache, hearing loss, dizziness, lethargy, fever, random twitching in muscles, stupor, muscular rigidity, encephalitis</td>
<td>Frequent headaches</td>
<td>Goldfield et al. 1973</td>
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<tr>
<td>Jun. 1972</td>
<td>New York</td>
<td>M</td>
<td>1</td>
<td>No</td>
<td>Upper respiratory tract symptoms, sore throat, fever, status epilepticus, lethargic, left hemiparesis, encephalitis</td>
<td>Spasticity of the left leg</td>
<td>Smith et al. 1974</td>
</tr>
<tr>
<td>Oct. 1972</td>
<td>Pennsylvania</td>
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<td>8</td>
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<td>Headache, vomiting, fever, malaise, anorexia, neck stiffness, seizures, comatose, left facial palsy, bilateral pyramidal tract signs, meningoencephalitis</td>
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<td>Rossier et al. 1974</td>
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25
<table>
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<tr>
<th>Date</th>
<th>Location</th>
<th>Gender</th>
<th>Age</th>
<th>Hospitalized</th>
<th>Symptoms</th>
<th>Coma</th>
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<tr>
<td>Oct. 1975</td>
<td>Québec</td>
<td>M</td>
<td>3</td>
<td>No</td>
<td>No information</td>
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<td>1</td>
<td>No</td>
<td>Anorexia, lethargy, fever, rash, lethargic, neck stiffness, erythematous morbilliform rash over the legs and arms, seizure, comatose</td>
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<td>1977</td>
<td>Ontario</td>
<td>F</td>
<td>18</td>
<td>No</td>
<td>Encephalitis</td>
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<td>Wilson et al. 1979</td>
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<td>1978</td>
<td>Russia</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td>No information</td>
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<td>Leonova et al. 2009</td>
</tr>
<tr>
<td>Aug. 1978</td>
<td>New York</td>
<td>M</td>
<td>8</td>
<td>No</td>
<td>Malaise, seizures, comatose, fever, drowsy, headache, mild left hemiparesis, encephalitis</td>
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<td>Dec. 1979</td>
<td>Ontario</td>
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<td>7</td>
<td>No</td>
<td>Fever, vomiting, lethargy, headache, coma, weakness of left arm and leg, erythematous rash on upper body and face, comatose</td>
<td></td>
<td>Partington et al. 1980</td>
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<td>1979</td>
<td>Russia</td>
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<td>-</td>
<td>Yes</td>
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</tr>
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<td>New Brunswick</td>
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<td>76</td>
<td>No</td>
<td>Fever, malaise, headache, vomiting, neck stiffness, left-side weakness, viral encephalitis, obtunded</td>
<td></td>
<td>Fitch and Artsob 1990</td>
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<td>Year</td>
<td>Location</td>
<td>Sex</td>
<td>Age</td>
<td>Case Status</td>
<td>Symptoms</td>
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<td>1991</td>
<td>Russia</td>
<td>-</td>
<td>-</td>
<td>No</td>
<td>No information</td>
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<td>1994</td>
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<td>Child</td>
<td>-</td>
<td>No</td>
<td>Fever, seizure</td>
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<td>64</td>
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<td>Headache, fever drowsiness, slurred speech, mild right facial weakness, respiratory failure, comatose</td>
<td></td>
<td>Gholam et al. 1999</td>
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<td>Jul. 2000</td>
<td>Maine</td>
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<td>25</td>
<td>No</td>
<td>Encephalopathy</td>
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<td>53</td>
<td>No</td>
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<td>Doll's eye movements</td>
<td>Lessell and Collins 2003</td>
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<td>Age</td>
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<td>Outcome</td>
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<tr>
<td>May 2002</td>
<td>Michigan</td>
<td>F</td>
<td>60</td>
<td>muscle weakness of all limbs progressing to total paralysis, respiratory failure, diplopia</td>
<td>No</td>
<td>Upper extremity function did not recover, required assistance feeding and dressing</td>
<td>Hinten et al. 2008</td>
</tr>
<tr>
<td>Jun. 2003</td>
<td>Wisconsin</td>
<td>M</td>
<td>69</td>
<td>febrile illness, no noted neurologic abnormality; cerebrospinal fluid laboratory findings consistent with central nervous system infection</td>
<td>No</td>
<td>Febrile illness, no noted neurologic abnormality; cerebrospinal fluid laboratory findings consistent with central nervous system infection</td>
<td>Hinten et al. 2008</td>
</tr>
<tr>
<td>Jul. 2004</td>
<td>Maine</td>
<td>F</td>
<td>74</td>
<td>encephalopathy, aphasia, tremor</td>
<td>No</td>
<td>Encephalopathy, aphasia, tremor</td>
<td>Hinten et al. 2008</td>
</tr>
<tr>
<td>2006</td>
<td>Russia</td>
<td>-</td>
<td>-</td>
<td>no information</td>
<td>No</td>
<td>No information</td>
<td>Leonova et al. 2009</td>
</tr>
<tr>
<td>Jul. 2006</td>
<td>Wisconsin</td>
<td>M</td>
<td>49</td>
<td>fever, chills, headaches, myalgia, fatigue, nausea, stiff neck, muscle aches, viral encephalitis</td>
<td>No</td>
<td>Fever, chills, headaches, myalgia, fatigue, nausea, stiff neck, muscle aches, viral encephalitis</td>
<td>Johnson et al. 2010</td>
</tr>
<tr>
<td>Aug. 2007</td>
<td>New York</td>
<td>-</td>
<td>5</td>
<td>no information</td>
<td>No</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
</tr>
<tr>
<td>Jul. 2007</td>
<td>New York</td>
<td>-</td>
<td>81</td>
<td>no information</td>
<td>Yes</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
</tr>
<tr>
<td>Jun. 2007</td>
<td>New York</td>
<td>-</td>
<td>70</td>
<td>no information</td>
<td>Yes</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
</tr>
<tr>
<td>Date</td>
<td>Location</td>
<td>Gender</td>
<td>Age</td>
<td>Outcome</td>
<td>Symptoms</td>
<td>Reference</td>
<td></td>
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<tr>
<td>Nov. 2007</td>
<td>New York</td>
<td>-</td>
<td>77</td>
<td>Yes</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
</tr>
<tr>
<td>Nov. 2007</td>
<td>New York</td>
<td>-</td>
<td>81</td>
<td>No</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
</tr>
<tr>
<td>Jun. 2007</td>
<td>Wisconsin</td>
<td>F</td>
<td>47</td>
<td>No</td>
<td>Aches, nausea, light-headiness, rash, headaches</td>
<td>Johnson et al. 2010</td>
<td></td>
</tr>
<tr>
<td>Jul. 2008</td>
<td>New York</td>
<td>-</td>
<td>9</td>
<td>No</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
</tr>
<tr>
<td>Jul. 2008</td>
<td>New York</td>
<td>F</td>
<td>9</td>
<td>No</td>
<td>Fever, headache, abdominal pain, emesis, nuchal rigidity, rigidity in upper extremities, acute disseminated encephalomyelitis</td>
<td>Hicar et al. 2011</td>
<td></td>
</tr>
<tr>
<td>Apr. 2009</td>
<td>New York</td>
<td>-</td>
<td>4</td>
<td>No</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
</tr>
<tr>
<td>Jan. 2009</td>
<td>New York</td>
<td>-</td>
<td>76</td>
<td>No</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
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<tr>
<td>Jun. 2009</td>
<td>New York</td>
<td>-</td>
<td>73</td>
<td>No</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
</tr>
<tr>
<td>Feb. 2009</td>
<td>New York</td>
<td>M</td>
<td>22</td>
<td>No</td>
<td>Fever, eye pain, influenza-like symptoms, eye pain, lateral gaze palsy, ataxia, dysarthria, stomach pain and neck stiffness, decreased alertness, dysarthria and ataxia, viral encephalitis</td>
<td>Sung et al. 2013</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>New York</td>
<td>M</td>
<td>62</td>
<td>Yes</td>
<td>Fatigue, fever, bilateral maculopapular palmar rash, onset of diplopia, dysarthria and weakness in the right arm and leg, hydrocephalus, meningoencephalitis</td>
<td>Tavakoli et al. 2009a</td>
<td></td>
</tr>
<tr>
<td>Dec. 2010</td>
<td>New York</td>
<td>-</td>
<td>77</td>
<td>Yes</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Location</td>
<td>Gender</td>
<td>Age</td>
<td>Status</td>
<td>Symptoms and Findings</td>
<td>Reference</td>
<td></td>
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<tr>
<td>Dec. 2010</td>
<td>New York</td>
<td>M</td>
<td>77</td>
<td>Yes</td>
<td>Fever, enlarged prostate, confused, comatose, myoclonic jerking of right upper extremity, neck stiffness, epileptic activity in the left frontal central area, viral encephalitis</td>
<td>El Khoury et al. 2013b</td>
<td></td>
</tr>
<tr>
<td>May 2011</td>
<td>Minnesota</td>
<td>F</td>
<td>67</td>
<td>Yes</td>
<td>Fever, dizziness, chills, malaise, nausea, occasional slurred speech, neck stiffness, encephalopathy, apneic, ocular deviation, absent deep tendon reflexes, positive Babinski response, bilateral flaccid paralysis of extremities and flaccid paralysis</td>
<td>Brige and Sonnesyn 2012</td>
<td></td>
</tr>
<tr>
<td>Apr. 2012</td>
<td>New York</td>
<td>-</td>
<td>32</td>
<td>No</td>
<td>No information</td>
<td>El Khoury et al. 2013a</td>
<td></td>
</tr>
<tr>
<td>May 2012</td>
<td>New York</td>
<td>M</td>
<td>34</td>
<td>No</td>
<td>History of genital herpesvirus infection admitted to hospital with lower extremity weakness and altered mental status, headache, fever, chills, bilateral ankle pain, bilateral leg weakness, confusion, diplopia, decreased alertness, bilateral proximal leg weakness, absence of neck rigidity, 2 transient rashes (history, 1 each on trunk and arm)</td>
<td>Sung et al. 2013</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Relapsed 1 month after release. Returned to work after 10 months despite residual leg weakness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Location</td>
<td>Gender</td>
<td>Age</td>
<td>Note</td>
<td>Symptoms</td>
<td>References</td>
<td></td>
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</tr>
<tr>
<td>May 2013</td>
<td>New Hampshire</td>
<td>M</td>
<td>44</td>
<td>No</td>
<td>Headache, fatigue, diplopia, diffuse rash over trunk and extremities, word-finding difficulty, generalized tonic-colonic seizure</td>
<td>Piantadosi et al. 2015</td>
<td></td>
</tr>
<tr>
<td>Jun. 2014</td>
<td>Massachusetts</td>
<td>F</td>
<td>65</td>
<td>No</td>
<td>Fever, headache, confusion, vomiting, mild right nasolabial fold flattening, mild postural and action tremor in the upper extremities</td>
<td>Piantadosi et al. 2015</td>
<td></td>
</tr>
<tr>
<td>May 2014</td>
<td>Massachusetts</td>
<td>M</td>
<td>49</td>
<td>Yes</td>
<td>Fever, headache, cerebellar swelling, acute herniation symptoms</td>
<td>Piantadosi et al. 2015</td>
<td></td>
</tr>
<tr>
<td>May 2014</td>
<td>Massachusetts</td>
<td>M</td>
<td>52</td>
<td>No</td>
<td>Fever, myalgia, developed: inattention, somnolence, left upper extremity dysmetria</td>
<td>Fifteen months later still had persistent headaches, cerebellar dysarthria, delayed motor function and incoordination</td>
<td>Piantadosi et al. 2015</td>
</tr>
<tr>
<td>Sep. 2014</td>
<td>Massachusetts</td>
<td>M</td>
<td>21</td>
<td>No</td>
<td>Vomiting, confusion, fever, maculopapular rash on his trunk, became obtund and required intubation</td>
<td>Piantadosi et al. 2015</td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Location</td>
<td>Gender</td>
<td>Age</td>
<td>Lived</td>
<td>Symptoms</td>
<td>Outcome</td>
<td>Reference</td>
</tr>
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<td>---------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>Sep. 2014</td>
<td>Massachusetts</td>
<td>M</td>
<td>67</td>
<td>No</td>
<td>Confusion, vomiting, diarrhea, fever, maculopapular rash over chest and upper back, encephalopathy, fore-head sparing right facial droop, diffusely hyperactive reflexes, extensor plantar responses</td>
<td></td>
<td>Piantadosi et al. 2015</td>
</tr>
<tr>
<td>Jun. 2015</td>
<td>Massachusetts</td>
<td>M</td>
<td>74</td>
<td>No</td>
<td>History of psoriatic arthritis on methotrexate, upper respiratory symptoms, right eye pain, and visual blurring, fever, required intubation</td>
<td>Residue left-side weakness, memory impairment</td>
<td>Piantadosi et al. 2015</td>
</tr>
<tr>
<td>Sep. 2015</td>
<td>Massachusetts</td>
<td>M</td>
<td>82</td>
<td>Yes</td>
<td>Fever, vomiting, upward gaze deviation, direction-changing nystagmus, severe axial rigidity, and bilateral Babinski signs, required intubation for airway protection</td>
<td>Death with a comorbidity of coronary artery disease. Died after 18 days</td>
<td>Piantadosi et al. 2015</td>
</tr>
</tbody>
</table>
CHAPTER 2: TICKS AND TICK-BORNE PATHOGENS INVOLVING WILDLIFE AND COMPANION ANIMALS IN SOUTHERN ONTARIO, CANADA

2.1 ABSTRACT

Due to the risk of tick-borne pathogen transmission to humans in Ontario, Canada, a better understanding of the current regional tick distribution and tick burdens of various local wildlife species, as well as prevalence for select zoonotic, tick-borne pathogens in ticks is warranted. The objectives of this study were to investigate the geographic distribution and magnitude of infestation of medium-sized mammalian wildlife and companion animals (i.e., dogs) with ticks and to test these ticks for evidence of zoonotic tick-borne pathogens, including *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia miyamotoi*, *B. burgdorferi* and *Ehrlichia chaffeensis* and in southern Ontario. Ticks were collected from carcasses donated to the Canadian Wildlife Health Cooperative (CWHC), from live wildlife and companion animals, and by tick dragging in southern Ontario during 2015-2016, as well as archived ticks from CWHC wildlife cases from 2011-2013. Testing for evidence of select zoonotic, tick-borne pathogens was done by real-time PCR; *Ixodes scapularis* was tested for *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia miyamotoi* and *B. burgdorferi* and *Amblyomma americanum* were tested for *Ehrlichia chaffeensis*. A total of 1,687 ticks of six species from 334 tick-infested animals were collected, including 1,577 adults, 107 nymphs and 3 larvae. Ticks were removed from 224 raccoons (*n=1,381* ticks) and 50 dogs (*n=67* ticks), among others. Of the parasitized raccoons, the most common ticks collected were *I. texanus* (*n=666* ticks) and *D. variabilis* (*n=600* ticks), which were removed from 58.5% (median: 2 ticks; range: 1-36 ticks) and 49.1% (median: 2 ticks; range: 1-64 ticks) of these raccoons, respectively. Of *I. scapularis* tested, 9.3% (*n=4/43; 95% CI: 2.6—22.1%) were positive for *B. burgdorferi* (the causative agent of Lyme disease) and 2.3% (*n=1/43; 95% CI: 0.1—12.3%) for *A. phagocytophilum* (the causative agent of human granulocytic anaplasmosis). *Borrelia burgdorferi*-positive ticks were removed from a coyote (*Canis latrans*) in the Clifford area and dogs in the Guelph and Lyndhurst area. The *A. phagocytophilum*-positive tick was from a red fox (*Vulpes vulpes*) in Marysville, Ontario. All *I. scapularis* ticks tested negative for *B. miyamotoi* and *B. microti* and one *Amblyomma americanum* tested negative for *E. chaffeensis*. These results reveal that various tick species are found on common wildlife species, and that numerous zoonotic, tick-borne pathogens circulate...
in southern Ontario. However, due to ongoing climate and landscape changes, continued research is needed to monitor and understand changes in the geographic distribution and prevalence of zoonotic tick-borne pathogens among ticks removed from companion and wild animals, to better assess public health risks.

Key words: *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, dog, *Ixodes scapularis*, Ontario, raccoon, tick, tick-borne pathogen, wildlife

2.2 INTRODUCTION

Ticks (Acari: Ixodidae) are important vectors of zoonotic pathogens (e.g., viruses, bacteria and protozoa), many of which also infect and sometimes include wildlife and companion animals in transmission (De la Fuente et al. 2008). Transmission cycles of tick-borne pathogens often involve complex vector-host interactions in which ticks may feed on numerous vertebrate hosts and simultaneously act as vectors for multiple pathogens (Dantas-Torres et al. 2012; Lindquist et al. 2016). Ticks and tick-borne pathogens are of global importance due to their associated economic impacts, which are largely a result of the burden they place on public health (e.g., high morbidity and mortality rates), as well as livestock and companion animal health (Jongehan and Uilenberg 2009). The increase in incidence and geographic range of tick-borne pathogens in North America over the past few decades has led to mounting concern that they will pose an even greater threat to public health in the future (Ogden et al. 2006a; Estrada-Peña and De La Fuente 2014).

Global climate change has made northern latitudes (such as Ontario, Canada) vulnerable to the influx and potential establishment of ticks that may carry zoonotic pathogens (Estrada-Peña and De La Fuente 2014). For example, the northward expansion of *Ixodes scapularis* has led to an increased incidence of Lyme disease (causal agent: *Borrelia burgdorferi*) among humans in Ontario (Ogden et al. 2006a, 2015). Additionally, *I. scapularis* may carry a variety of other zoonotic pathogens, including *Anaplasma phagocytophilum*, *Babesia microti* and *B. miyamotoi* (Lindquist et al. 2016). However, relatively little is known about the latter three potentially zoonotic pathogens in Ontario, including the geographic distribution of tick-borne pathogens, their vectors (i.e., ticks), and potential vertebrate hosts in Ontario (Clow et al. 2016).

The present study was carried out to examine medium-sized mammalian wildlife and to a lesser extent, dogs, with ticks in southern Ontario. The objectives included: 1) to investigate the
geographic distribution and tick burdens of small mammalian wildlife and dogs with ticks, and 2) to test these ticks for evidence of zoonotic tick-borne pathogens, including *A. phagocytophilum, Ehrlichia chaffeensis, B. microti, B. miyamotoi* and *B. burgdorferi*. Ongoing passive and active surveillance of ticks and the pathogens they carry is vital for monitoring the incidence in human cases and potential risk factors for infection, and for evaluating the associated threat to public health (Clow et al. 2016).

2.3 MATERIALS AND METHODS

**Tick Collection**

Ticks were collected opportunistically from May 2015 to December 2016 through a variety of methods. Ticks were removed from wildlife carcasses donated to the Canadian Wildlife Health Cooperative (CWHC) (May-December 2015-2016) and were archived from previous CWHC diagnostic cases (2011-2013). Ticks were also removed from wildlife that were live-trapped during the summer of 2016 in the regions of Guelph, Hamilton and Peterborough, Ontario, Canada. Tomahawk live traps (sizes 106 and 108; Tomahawk Live Trap Co. Tomahawk, Wisconsin, USA) were set in the evenings and checked in the mornings. Raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) were anesthetized with ketamine (Bioniche Animal Health, Belleville, ON, Canada) at a dose of 6.5-13 mg/kg. Groundhogs (*Marmota monax*) and eastern grey squirrels (*Sciurus carolinensis*) were anesthetized within an induction chamber filled with isoflurane gas (Baxter Corporation, Mississauga, Ontario, Canada) at concentrations from 1-3%. Anesthetized animals were weighed with a pesola (Pesole AG, Chaltenbodenstrasse, Schindellegi, Switzerland), groomed for ticks for approximately 2 minutes and individually identified with ear tags (raccoons and striped skunks; National Band and Tag Co. Newport, Kentucky, USA) or subcutaneously injected pit tags (groundhogs and grey squirrels; Biomark, Idaho, USA) before release. For each wild animal sampled, species, sex, age (i.e., immature <1 year and adult ≥1 year; determined by weight), geographic coordinates and number of ticks were recorded. In addition, ticks were opportunistically collected from dogs at seven veterinary clinics within a seven-kilometer radius of Guelph, Ontario. All tick collections occurred from May 2015 until May 2017 and similar to wildlife sampling, data for ticks collected from dogs included date, source species and latitude and longitude of the town where the veterinary clinic was located, as well as any known travel history outside of Ontario. Ticks were either stored in cryovials at -
80°C (from wildlife) or placed into 70% ethanol and maintained at ambient temperature (for ticks collected veterinary clinic animals and archived CWHC diagnostic cases) for later identification. All ticks were identified to species, sex, and life stage (i.e., adult, nymph or larvae) using a dissecting microscope.

All wildlife trapping and handling was done under institutional animal care and use committee approval (University of Guelph; AUP#3471).

**Laboratory Analyses**

Adult and nymphal ticks of the species *Amblyomma americanum*, and *I. scapularis*, were submitted to the National Microbiology Laboratory (Public Health Agency of Canada, Winnipeg, Manitoba, Canada) for testing by real-time polymerase chain reaction (RT-PCR) as previously described (Ogden et al. 2006b; Dibernardo et al. 2014). Ticks from the same source animal were sorted into pools of 1-10 adults or up to 25 nymphs (Dupuis II et al. 2013). Total DNA from ticks was extracted by using QIAGEN DNeasy 96 tissue kits (Qiagen Inc., Mississauga, Canada). Pathogen testing was vector species-dependent, with *A. americanum* ticks tested for *E. chaffeensis* using real-time PCR targeting the 16S ribosomal RNA (rRNA) gene as outlined in the methods described by Loftis et al. (2003). *Ixodes scapularis* ticks were initially screened for *Borrelia* spp. and *A. phagocytophilum* using a duplex real-time PCR assay that targeted the 23S rRNA and *msp2* genes respectively (Courtney et al. 2004). Positive *Borrelia* spp. samples were subsequently tested using a confirmatory *ospA* real-time PCR assay for *B. burgdorferi* and a glpQ real-time PCR assay for *B. miyamotoi* (Ullmann et al. 2005). In addition, DNA extracts from *I. scapularis* ticks were tested for the presence of *B. microti* by a real-time PCR assay described by Nakajima et al. (2008) targeting the *CCTƞ* gene. *Ixodes scapularis* were also tested for Powassan virus (POWV) for a companion study (Chapter 3 of this thesis) targeting the NS5 region of the POWV genome using an in-house method (TIB molbiol, Adelphia, NJ).

**Statistics**

The prevalence and exact 95% confidence intervals of tick-borne pathogens were estimated using STATA14® Intercooled (StataCorp, College Station, Texas USA).
2.4 RESULTS

A total of 1,687 ticks of six species were collected from wildlife and companion animals in southern Ontario between May 2015 and May 2017 (Table 2.1). Of these ticks, 107 were from archived CWHC wildlife diagnostic cases from 2011-2013. The most common tick species identified among all animals was *I. texanus* (*n*=686/1,687; 40.7%; 95% CI: 38.3—43.1%) followed by *Dermacentor variabilis* (*n*=666/1,687; 39.5%; 95% CI: 37.1—41.9%; Table 2.1). The majority of ticks collected were adult females (*n*=1,083/1,687; 64.2%; 95% CI: 61.9—66.5) followed by adult males (*n*=368/1,687; 21.8%; 95% CI: 19.9—23.9), unknown adults (sex undetermined; *n*=126/1,687; 7.5%; 95% CI: 6.3—8.8%), nymphs (*n*=107/1,687; 6.3%; 95% CI: 5.2—7.6%) and larvae (*n*=3/1,687; 0.2%; 95% CI: 0.0—0.5%; Table 2.1). Raccoons (*n*=224) and dogs (*Canis lupus familiaris*; *n*=50) were the most commonly examined tick-source animals (Table 2.2). The vast majority of ticks collected from tick-infested raccoons was *I. texanus* (*n*=666 ticks) and *D. variabilis* (*n*=600), which were removed from 58.5% (median: 2 ticks; range: 1-36 ticks) and 49.1% (median: 2 ticks; range: 1-64 ticks) of raccoons, respectively (Table 2.2).

Of *I. scapularis* tested, 9.3% (*n*=4/43; 95% CI: 2.6—22.1%) were positive for *B. burgdorferi* and 2.3% (*n*=1/43; 95% CI: 0.1—12.3%) for *A. phagocytophilum* (Figure 2.1). The *B. burgdorferi*-positive ticks were collected from a coyote and three different dogs with no history of travel outside of Ontario. The coyote was collected from the city of Clifford, Wellington County, southwestern Ontario. One dog was from Lyndhurst, United Counties of Leeds and Grenville, eastern Ontario, and two were from Guelph, Wellington County, southwestern Ontario. The *A. phagocytophilum*-positive tick was collected from a red fox (*Vulpes vulpes*) located in Marysville, Frontenac County, eastern Ontario. All *I. scapularis* tested negative for *B. miyamotoi* and *B. microti* (*n*=0/43; 0.0%; 95% CI: 0.0—8.2%). An *A. americanum* tick collected from a dog in Rockwood, Ontario tested negative for *E. chaffeensis* (*n*=1/1; 100%; 95% CI: 2.5—100.0%).

2.5 DISCUSSION

Monitoring for tick-borne pathogens is important in light of complex tick-host-pathogen interactions overlaid by dynamic, diverse environments that are continuously subjected to climatic and landscape changes. Such surveillance strategies are varied and are dependent upon
knowledge of the complex pathogen transmission cycles that may involve numerous vertebrate hosts and tick life stages (Dantas-Torres et al. 2012). Further, understanding these host-vector interactions is essential to predicting changes in geographic distribution of ticks and assessing for the potential emergence or re-emergence of zoonotic pathogens. Tick surveys both on wildlife and in the environment may provide information on the geographic locations of, the prevalence of zoonotic pathogens in ticks, and thus, contribute to risk assessments pertaining to public health. The present study focused on ticks and wildlife in southern Ontario Canada, a location where there is a heightened awareness of the ongoing effects and imminent threat of climate change (Ogden et al. 2006a).

Raccoons were the most common animal observed with tick burdens in the present study, followed by dogs, striped skunks and groundhogs. *Ixodes texanus* and *D. variabilis* were the most common tick species collected from these raccoons (Table 2.2). Knowledge of *I. texanus* life history and distribution in Canada is scarce, and the role of *I. texanus* as a potential vector of tick-borne pathogens is unclear (Lindquist et al. 2016). For example, *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever and a disease uncommonly detected in Ontario (Gary et al. 2006), was isolated from a nymphal *I. texanus* in Connecticut (Anderson et al. 1986) and studies have suggested that *I. texanus* may be a possible vector for *Babesia lotori* and *Ehrlichia* spp. in raccoons (Anderson et al. 1981; Dugan et al. 2005). However, the vector potential of this species has not been examined experimentally (Lindquist et al. 2016). In addition, *I. texanus* was recently found on approximately 70% of examined raccoons in the Ontario-neighbor state of Michigan (Hamer et al. 2010). The potential of *I. texanus* as a vector of tick-borne pathogens and the significance of the apparently high frequency of these tick detections on raccoons in nearby regions warrants further investigation. In addition to raccoons, *D. variabilis* ticks were also removed from dogs, striped skunks, coyotes and a Virginia opossum (*Didelphis virginiana*) in the present study (Table 2.2). These results support the previous observation that *D. variabilis* ticks have a broad range of host species (Lindquist et al. 2016). The observation of *I. cookei*, *I. marxi*, and *I. texanus* on striped skunks and *I. cookei* from groundhogs is also consistent with previous reports (Bishopp and Trembley 1945; Ko 1971; Lindquist et al. 2016). Collectively, the occurrence of ticks on numerous host species highlights the importance of conducting surveys of multiple wild and domestic animal hosts to monitor changing geographic distributions of both ticks and emerging zoonotic pathogens, as well as
possible adaptations to new hosts.

In the present study, adult females were the most common life stage collected for all tick species (Table 2.1). This result is not surprising since adult male ixodid ticks (i.e., hard ticks) blood feed little or not at all, whereas adult female ticks require a blood meal to lay eggs (Lindquist et al. 2016). Adult female ticks accounted for 96% of *I. scapularis* ticks submitted by the public in a sweeping passive surveillance study conducted in Canada from 1990-2003 (Ogden et al. 2006b), suggesting that adult female ticks are more easily found or feed more often on humans. Smaller tick life stages (i.e., larvae and nymphs) are more difficult to locate than adults, especially on heavily haired areas such as on many wildlife species, and thus the smaller proportion of these life stages collected in the present study may be falsely low due to decreased detection abilities. All stages of ticks (i.e., adult, nymph and larva) are capable of transmitting some pathogens and are thus are important in tick-borne pathogen surveillance; the increased difficulty in their detection may hinder such efforts (Piesman et al. 1987; Costero and Grayson 1996; Lindquist et al. 2016). Therefore, surveillance efforts and field studies should aim to include all tick life stages by being aware of the challenges their detection presents and by ensuring sampling during periods when targeted life stages are active (Clow et al. 2016).

Passive surveillance has been a vital component of monitoring the changing geographic distribution of ticks and tick-borne pathogens in Ontario, Canada. Analysis of samples submitted by members of the Ontario public between 2008 and 2012 illustrate the range expansion of *I. scapularis* and highlights the increasing prevalence and non-uniform distribution of *B. burgdorferi*, the causative agent of Lyme disease (Nelder et al. 2014). In the present study, testing of *I. scapularis* ticks collected from wildlife and companion animals revealed evidence of zoonotic pathogens, including *B. burgdorferi* and less commonly, *A. phagocytophilum*. Similarly, drag sampling of *I. scapularis* in Michigan revealed and expanding population within this Ontario-neighbor state and detected both *B. burgdorferi* and *A. phagocytophilum* (Hamer et al. 2007). Lyme disease represents an increasing threat to public health in Ontario and parts of the eastern U.S., and can cause lifelong clinical sequelae including joint, cardiovascular, neurologic, and ocular manifestations as well as systemic pain and fatigue (Parola and Raoult 2001). Human granulocytic anaplasmosis can cause severe, febrile illness with headache, myalgia, and malaise (Dumler et al. 2005). The detection of *B. burgdorferi* in ticks in Ontario is consistent with recent studies conducted in southern and eastern Ontario that focused on *I.*
*scapularis* collection from the environment via tick dragging in 2014 and 2016 (Clow et al. 2016, Clow et al. *in review*) and between 2008-2012, when *B. burgdorferi* and less commonly, *A. phagocytophilum*, were removed from humans and identified in a public health survey (Nelder et al. 2014).

Currently, *I. scapularis* is found throughout Ontario, although more commonly in the southern and eastern regions (Bouchard et al. 2015; Clow et al. 2017). However, climate change models have predicted increased habitat suitability for these ticks in Ontario, Canada, supporting the continued northward range expansion and establishment of this species (Brownstein et al. 2005; Ogden et al. 2006a). A recent study detected *I. scapularis* in areas outside of known endemic regions of southern, eastern and central Ontario and identified a ‘hotspot’ of tick activity in eastern Ontario (Clow et al. 2016). One of the *B. burgdorferi*-positive cases in the present study originated in Lyndhurst, Ontario, and the *A. phagocytophilum*-positive tick was removed from a dog in Garden Island, Marysville, which are both within the recently described hotspot region of eastern Ontario. The other two *B. burgdorferi*-positive *I. scapularis* ticks were removed from dogs in Guelph, Ontario, an area of southern Ontario where *B. burgdorferi* has previously been recorded (Nelder et al. 2014); however, the establishment of *I. scapularis* in this highly populated region remains in question. The small sample sizes and advantageous collection method used in the present study make such determinations impossible. In addition, *E. chaffeensis*-negative *A. americanum* tick was collected from a dog in Rockwood, Ontario with no reported travel history outside of Ontario. Low submission rates of *A. americanum* across multiple Ontario studies suggest that this species has not yet established in Ontario (Nelder et al. 2014; Scott et al. 2016). However, it has been detected in regions bordering Ontario, including Michigan, where it was the fifth most common species submitted in a 12-year (1985-1996) passive surveillance study, and in New York State, where the range of *A. americanum* has expanded into northern and western regions (1998-1996) (Means and White 1997; Walker et al. 1998). Powassan virus was an additional zoonotic tick-borne pathogen detected from an *I. cookei* tick removed from a free-ranging Canada Goose (*Branta canadensis*) included in the present study but reported elsewhere (Chapter 3 of this thesis). The geographic expansion of *I. scapularis* and other tick species in parts of the northern U.S. and southern Canada support likely secondary effects of global climate change on tick-borne, zoonotic diseases (Ogden et al. 2006a; Nelder et al. 2014).
Passive surveillance is a valuable tool for monitoring tick activity both temporally and spatially over large geographic regions (Ogden et al. 2006b; Nelder et al. 2014). Wildlife have become increasingly used in passive surveillance for zoonotic and vector-borne pathogens, such as West Nile virus (Nemeth et al. 2007), as this represents an opportunity to expand knowledge about host range and their respective geographic distribution. In the present study, opportunistic tick collections from wildlife were used to detect tick-borne pathogens in southern Ontario and provide insight into the relationships between ticks and their respective wildlife and domestic animal hosts. Further, many of the wildlife species included in this study are adaptive species that thrive in variety of environments, including urban and peri-domestic (e.g., raccoons, skunks), and thus, may come into contact with humans and companion animals. Additionally, some tick vector species, such as *I. scapularis*, are promiscuous feeders and quest for hosts in habitats where humans and companion animals may frequent (Bouchard et al. 2015). Despite its utility, however, opportunistic sampling has inherent limitations, such as lack of systematic data collection over time and space.

The results from the present study provide further evidence of the presence of tick-borne pathogens in southern Ontario and outline the common tick species found on various wildlife hosts. The detection of *B. burgdorferi* and *A. phagocytophilum* also highlights the importance of continued tick surveillance and the promotion of public health awareness within Ontario, Canada and other regions of similar latitudes that may be subject to changing systems. A more thorough understanding of vector-host relationships, especially involving wildlife, will help in future assessments of the geographic distribution of a variety of potential tick vector species that will, in turn, allow for improved, targeted strategies to monitor and control tick-borne diseases.
REFERENCES


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Piesman J, Mather TN, Sinsky RJ, Spielman A. 1987. Duration of tick attachment and *Borrelia*


Table 2.1. Tick species and number opportunistically collected from free-ranging wildlife and companion animals in southern Ontario between May 2015 and May 2017 and in archived tick samples collected from 2011-2013.

<table>
<thead>
<tr>
<th>Tick Species</th>
<th>Total (% ticks)a</th>
<th>Adults (% ticks)b</th>
<th>Nymphs (% ticks)b</th>
<th>Larvae (% ticks)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>Male</td>
<td>Unknown</td>
</tr>
<tr>
<td>Ixodes texanus</td>
<td>686 (40.7)</td>
<td>593 (86.4)</td>
<td>13 (1.9)</td>
<td>63 (9.2)</td>
</tr>
<tr>
<td>Dermacentor variabilis</td>
<td>666 (39.5)</td>
<td>319 (47.9)</td>
<td>347 (52.1)</td>
<td>-</td>
</tr>
<tr>
<td>I. cookei</td>
<td>275 (16.3)</td>
<td>133 (48.4)</td>
<td>-</td>
<td>56 (20.4)</td>
</tr>
<tr>
<td>I. scapularis</td>
<td>43 (2.5)</td>
<td>32 (74.4)</td>
<td>8 (18.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>I. marxi</td>
<td>15 (0.9)</td>
<td>5 (33.3)</td>
<td>-</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>Amblyomma americanum</td>
<td>1 (0.1)</td>
<td>1 (100.0)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (0.1)</td>
<td>-</td>
<td>-</td>
<td>1 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>1,687</td>
<td>1,083 (64.2)</td>
<td>368 (21.8)</td>
<td>126 (7.5)</td>
</tr>
</tbody>
</table>

a Percentage of all ticks.

b Percentage of ticks (per tick species).
Table 2.2. Tick species and number collected from 334 parasitized wildlife and companion animals by species between May 2015 and December 2016 and in archived tick samples collected from 2011-2013.

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Total (%)</th>
<th>Number of Hosts (%; median; range)</th>
<th>Adults (%)</th>
<th>Nymphs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>with Tick Species</td>
<td></td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Raccoon (Procyon lotor; n=224)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ixodes texanus</em></td>
<td>224</td>
<td>666 (48.2)</td>
<td>131 (58.5; 2; 1-36)</td>
<td>590 (88.6)</td>
<td>13 (2.0)</td>
</tr>
<tr>
<td><em>Dermacentor variabilis</em></td>
<td>224</td>
<td>600 (43.4)</td>
<td>110 (49.1; 2; 1-64)</td>
<td>278 (46.3)</td>
<td>322 (53.7)</td>
</tr>
<tr>
<td><em>I. cookei</em></td>
<td>224</td>
<td>100 (7.2)</td>
<td>20 (8.9; 1; 1-44)</td>
<td>40 (40.0)</td>
<td>-</td>
</tr>
<tr>
<td><em>I. marxi</em></td>
<td>224</td>
<td>13 (0.9)</td>
<td>3 (1.3; 5; 1-7)</td>
<td>4 (30.8)</td>
<td>-</td>
</tr>
<tr>
<td><em>I. scapularis</em></td>
<td>224</td>
<td>2 (0.1)</td>
<td>1 (0.4; 2; NA)</td>
<td>2 (1.00)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>224</td>
<td>1,381</td>
<td>914 (66.2)</td>
<td>335 (24.3)</td>
<td>97 (7.0)</td>
</tr>
</tbody>
</table>

| Dog (Canis lupus familiaris; n=50) | | | | | |
| *D. variabilis* | 50 | 37 (59.7) | 24 (48.0; 1; 1-4) | 22 (59.5) | 15 (40.5) | - | - |
| *I. scapularis* | 50 | 27 (43.5) | 25 (50.0; 1; 1-3) | 23 (85.2) | 3 (11.1) | - | 1 (3.7) |
| *I. cookei* | 50 | 2 (3.2) | 2 (4.0; 1; 1-1) | 1 (50.0) | 0 (0.0) | - | 1 (50.0) |
| *Amblyomma americanum* | 50 | 1 (1.6) | 1 (2.0; 1; NA) | 1 (100.0) | 0 (0.0) | - | - |
| Total | 50 | 67 | 47 (70.1) | 18 (26.9) | - | 2 (3.0) |

| Striped Skunk (Mephitis mephitis; n=32) | | | | | |
| *I. cookei* | 32 | 92 (78.0) | 26 (81.3; 1; 1-16) | 46 (50.0) | - | 5 (5.4) | 41 (44.6) |
| *I. texanus* | 32 | 20 (16.9) | 2 (6.3; NA; 3-17) | 3 (15.0) | - | 17 (85.0) | - |
| *D. variabilis* | 32 | 5 (4.2) | 3 (9.4; 1; 1-3) | 3 (60.0) | 2 (40.0) | - | - |
| *I. marxi* | 32 | 1 (0.8) | 1 (3.1; NA; NA) | 1 (100.0) | - | - | - |
| Total | 32 | 118 | 53 (44.9) | 2 (1.7) | 22 (18.6) | 41 (34.7) |

| Groundhog (Marmota monax; n=12) | | | | | |

| | | | | | |
| | | | | | |
| | | | | | |

47
<table>
<thead>
<tr>
<th>Species</th>
<th>Count (Percentage)</th>
<th>Count (Percentage; Median)</th>
<th>Count (Percentage)</th>
<th>Count (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. cookei</td>
<td>37 (100.0)</td>
<td>12 (100.0; 2; 1-11)</td>
<td>17 (45.9)</td>
<td>-</td>
</tr>
</tbody>
</table>
| All other wildlife (n=16)
I. cookei        | 41 (50.6)          | 8 (50.0; 4; 1-20)         | 27 (65.9)          | 2 (4.9)            |
| D. variabilis    | 24 (29.6)          | 2 (12.5; NA; 1-23)        | 16 (66.7)          | 5 (35.7)           |
| I. scapularis    | 14 (17.3)          | 6 (37.5; 1; 1-8)          | 7 (50.0)           | 8 (33.3)           |
| I. marxi         | 1 (1.2)            | 1 (6.3; 1; NA)            | -                  | -                  |
| Unknown          | 1 (1.2)            | 1 (6.3; 1; NA)            | -                  | -                  |
| Total            | 81                 | 50 (61.7)                 | 15 (18.5)          | 1 (1.2)            |
| Total            | 1,687              | 1,081 (64.4)              | 370 (21.9)         | 126 (7.5)          | 107 (6.3)

a Percentage of all ticks (per host species).
b Percentage of ticks (per tick species).
c Median number of ticks per parasitized animal.
d NA=not applicable.
e Species with <5 parasitized animals submitted included: fisher (Martes pennanti; n=3), red fox (Vulpes vulpes; n=3), porcupine (Erethizon dorsatum; n=2), beaver (Castor canadensis; n=1), black bear (Ursus americanus; n=1), Canada goose (Branta canadensis; n=1), domestic cat (Felis catus; n=1), red squirrel (Sciurus vulgaris; n=1) and Virginia opossum (Didelphis virginiana; n=1).
f Three I. cookei larvae collected from a groundhog were included in the total number of ticks.
Figure 2.1. The distribution of tick samples opportunistically collected between May 2015 until May 2017 from free-ranging wildlife and dogs in southern Ontario, Canada.
CHAPTER 3: SURVEY OF POWASSAN VIRUS AND OTHER ARBOVIRUSES FROM WILDLIFE IN SOUTHERN ONTARIO

3.1 ABSTRACT

Powassan virus (POWV) is a tick-borne virus capable of causing fatal encephalitis in horses and humans. It is maintained in a sylvatic transmission cycle between ixodid ticks and small- and medium-sized wild mammals. However, the current geographic distribution of POWV within Ontario and the involvement of numerous candidate vertebrate host species in transmission are poorly understood. The present study assessed for evidence of Powassan virus infection among potential wildlife host species and ticks collected from animals in southern Ontario. Tissues and blood from mammalian wildlife and ticks removed from wild animals and dogs were collected in the summers of 2015-2016; ticks removed from wildlife diagnostic cases in 2011-13 were also tested. Tissue and tick (Ixodes spp.) homogenates were tested by reverse transcriptase (RT)-PCR for POWV, and sera from wildlife were screened by hemagglutination inhibition (HI) test. For the latter, reactive serum samples were subjected to plaque reduction neutralization test (PRNT) for POWV and competitive-enzyme linked immune sorbent assay (cELISA) and PRNT for West Nile virus (WNV). In addition, sera were tested for antibodies to Heartland virus (HRTV) by PRNT. All 724 (0.0%; 95% CI: 0.0—0.5%) tissue samples were negative for POWV by RT-PCR. One pool of I. cookei collected from a Canada goose (Branta canadensis) tested positive via RT-PCR for POWV (n=1/98; 1.0%; 95% CI: 0.0—5.6%). Sera were collected from 266 individuals of seven medium-sized wild mammal species. Anti-flavivirus antibodies were detected in 13.5% (n=36/266; 95% CI: 9.7—18.2%) of samples. Powassan virus and WNV neutralizing antibodies were detected in 0.8% (n=2/265; 95% CI: 0.1—2.7%) and 8.0% (n=21/264; 95% CI: 5.0—11.9%) of samples, respectively. All 219 serum samples tested negative for antibodies to HRTV (n=0/219; 0.0%; 95% CI: 0.0—1.7%). Although evidence of POWV infection was rarely detected in wildlife and tick-derived samples in the present study, recent human cases in the northeastern United States and historic and current evidence in southern Ontario highlights the importance of continued POWV surveillance in the region.
Key words: arbovirus, Heartland virus, Ontario, Powassan virus, tick-borne, wildlife, West Nile virus

3.2 INTRODUCTION

Powassan virus (POWV) is a tick-borne virus (family Flaviviridae; genus Flavivirus) that was first isolated in 1958 from a fatal human case of encephalitis in the village of Powassan, Ontario (McLean and Donohue 1959). It has since been documented across numerous regions of North America, including eastern Canada, northeastern United States, north-central United States and occasionally in western North America (Hermance and Thangamani 2017). The virus is composed of two genetically distinct but serologically indistinguishable lineages: lineage I, prototype Powassan virus (POWV-p), and lineage II, deer tick virus (DTV) (Ebel 2010). Both lineages circulate within North America and may cause severe neurologic disease in humans, with a high case fatality rate and long-term neurologic sequelae in survivors ( Hinten et al. 2008; Ebel 2010; Piantadosi et al. 2015). Powassan virus is maintained in enzootic cycles that involve small- and medium-sized mammalian wildlife species and ticks (Ebel 2010).

Powassan virus is a growing public health concern due to its increasing incidence in North America, specifically in the northeastern United States of America. Further, northward-expanding tick populations, aided by global climatic change, have led to predictions of an increased incidence of tick-borne pathogens, including POWV (Ogden et al. 2006a).

Approximately 100 human cases have been reported in North America since 1958, and more recently, the average number of POWV human cases reported per year has increased from 0.7 (1958-1998) to 1.3 cases per year (1999-2005) ( Hinten et al. 2008; Piantadosi et al. 2015). Further, within the most recent seven years, human POWV encephalitis cases have been detected in new geographic locations, including several eastern states such as Connecticut, Minnesota, New Hampshire, and Virginia ( Tutolo et al. 2017).

Historically within Ontario, POWV has been detected both by human cases, and by host-vector studies conducted in the 1960s-1980s (McLean and Donohue 1959; Artsob et al. 1986). More recently, a fatal human case of POWV encephalitis was diagnosed in 2015 in Kingston, Ontario and POWV RNA was detected in an Ixodes scapularis tick from Barrie, Ontario in 2016 (R. Lindsay, pers. comm.). Furthermore, the public health risks of other North American arboviruses, including West Nile virus (WNV; family Flaviviridae; genus Flavivirus) and
Heartland virus (HRTV; family *Bunyaviridae*; genus *Phlebovirus*) are dynamic and warrant continued awareness and monitoring. For instance, the overall risk of WNV in Ontario is changing from year to year and the risk of HRTV in the mid-western United States has been increasing annually (Vasconcelos and Calisher 2016; Thompson and Berke 2017). Despite this, the current geographic distribution of these arboviruses and the potential vertebrate reservoir hosts within Ontario remain poorly understood. The objectives of the present study were to: 1) compare the prevalence of likely recent [by real-time, reverse transcriptase (RT)-PCR] and prior POWV infections (by serology) among candidate wildlife reservoir host species; 2) estimate the prevalence of POWV among ticks removed from wildlife and companion animals in southern Ontario; and 3) investigate the geographic distribution of POWV, WNV and HRTV among local wildlife in southern Ontario.

### 3.3 MATERIALS AND METHODS

**Sample Collection**

Ticks and tissues (including blood) were collected from wildlife carcasses of small- to medium-sized mammals submitted or donated to the Canadian Wildlife Heath Cooperative (CWHC) from mid-May to mid-October in 2015 and 2016 (when seasonal transmission of POWV is expected to occur in southern Ontario; Farkas 1989). Additional ticks were collected from previous CWHC diagnostic cases, which included a variety of wild mammal and bird species (2011-2013). In most cases, carcasses were frozen to -20°C prior to arrival. Blood collection from carcasses via cardiac puncture was performed when possible. Heart, kidney, spleen, and brain samples were also collected and approximately 0.5-cm³ pieces of each tissue were pooled into cryovials and frozen at -80°C in 2015, or were placed in RNA-later (to decrease RNA degradation) and stored at -20°C in 2016. Tissues were selected based on previous studies of *Flavivirus* infection (e.g., West Nile virus) in wild mammals (Root et al. 2006).

Opportunistic live mammal trapping was performed during summer 2016. Raccoons (*Procyon lotor*) and striped skunks (*Mephitis mephitis*) were trapped in the Hamilton (Bronte Creek Provincial Park and Stony Creek) and Peterborough regions in June. In addition, during September, groundhogs (*Marmota monax*) and eastern grey squirrels (*Sciurus carolinensis*) were trapped in the Guelph region at Guelph Lake, University of Guelph campus and University of Guelph Arboretum. Tomahawk live traps (sizes 106 and 108; Tomahawk Live Trap Co.
Tomahawk, Wisconsin, USA) were set in the evening, baited with apple and canteloupe for groundhogs and oat-peanut butter for squirrels, and checked the following morning. Animals were anesthetized with ketamine (raccoons and striped skunks; Bioniche Animal Health, Belleville, ON, Canada) at a dose of 6.5-13 mg/kg or isoflurane gas (groundhogs and grey squirrels; Baxter Corporation, Mississauga, Ontario, Canada) at concentrations from 1-3% within an induction chamber, followed by blood collection (via jugular or saphenous vein) and tick grooming. For each trapping session, species, sex, age (i.e., immature <1 year and adult ≥1 year; determined by weight), geographic coordinates and number of ticks were recorded for each animal, and animals were individually tagged with ear tags (raccoons and striped skunks; National Band and Tag Co. Newport, Kentucky, USA) or pit tags (groundhogs and grey squirrels; Biomark, Idaho, USA) before release. Blood samples collected from each animal were less than 5% of the animal’s body weight. All wildlife trapping and handling was done under institutional animal care and use committee approval (University of Guelph; AUP#3471).

Carcasses and anesthetized live wildlife were groomed for ticks in the hair and skin for approximately 5 minutes per animal. In addition to ticks removed from wildlife, ticks from companion animals were opportunistically collected from seven veterinary clinics located within a seven-kilometer radius of Guelph, Ontario from May to November, 2015-2016. For every submitted tick, veterinary clinics were asked to provide data on source species, date of collection, and general location (e.g., town; location was considered the town-center), as well as any known history of travel outside of Ontario.

Test results from additional tick-borne pathogens for *I. scapularis* ticks collected and tested for POWV in the present study are reported in Chapter 2 of this thesis.

**Sample Processing and Storage**

Blood samples were immediately refrigerated at 4°C after collection, and centrifuged within 12 hours for 10 minutes at 2,500 x g. Sera were separated from packed cells, placed into cryovials, and stored at -20°C.

Ticks collected from wildlife were refrigerated at 4°C for up to 72 hours prior to identification via stereomicroscope and then stored at -80°C (Lindquist et al. 2016). Ticks from companion animals were immediately placed into 70% ethanol and stored at ambient temperature.
Serologic Assays

Serum samples were screened at a 1:10 dilution for anti-Flavivirus antibody reactivity by hemagglutination inhibition test (HI) (Clarke and Casals 1958). Reactive or borderline reactive samples were then titrated to a dilution of 1:160. Positive HI samples (dilutions ≥1:10) were assessed for POWV-neutralizing antibodies by plaque reduction neutralization test (PRNT) and considered positive at a dilution of ≥1:20 (Dupuis II et al. 2013). Positive HI samples (≥1:10 dilution) were also tested for anti-WNV antibodies by competitive enzyme-linked immunosorbent assay (cELISA), in which positive samples exhibited >30% inhibition (Hirota et al. 2012). West Nile virus cELISA-positive samples were confirmed for WNV-neutralizing antibodies using PRNT (Beaty et al. 1995) considered positive at a dilution of ≥1:20. In addition, all samples with sufficient remaining volume were screened for antibodies to HRTV via PRNT, with positive samples having at least 70% neutralization at a 1:10 dilution (Bosco-Lauth et al. 2015).

All serologic assays were completed in biosafety level-3 facilities at the National Microbiology Laboratory in Winnipeg, Manitoba, Canada (POWV, WNV) or at the Animal Disease Laboratory at Colorado State University, Fort Collins, Colorado, U.S. (HRTV).

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Tissues (50-100 mg each of brain, heart, kidney and spleen) and ticks (1-10 adult ticks/animal and ≥25 nymphs) from the same animal were pooled for testing (Dupuis II et al. 2013). Ticks were cut in half with a sterile scalpel, and one-half was stored at -80°C for potential future virus isolation attempts. The remaining half-ticks and pooled tissue samples were homogenized for RNA extraction using a TissueLyser II (Qiagen Inc., Valencia, CA). For ticks, RNA was extracted according to the manufacturer’s protocols using the RNeasy mini kit (Qiagen Inc., Valencia, CA). For tissues, RNA was extracted via the TRizol (TRI reagent) according to the manufacturer’s protocol (Thermo Fisher Scientific, MA). Samples were tested by RT-PCR targeting the NS5 region of the POWV genome. RT-PCR assays were performed on ABI Prism 7500 or 7900 Sequence Detection System using TaqMan One-Step RT-PCR master mix (Thermo Fisher Scientific, MA). One primer and probe set was used for the detection of Lineage I and II POWV (FWD- TGGATGACAACAGAAGACATGCT; REV-
GGCAGATAGGGATGTCTCT; probe FAM-BBQ-AAGTCTGGATTGATGTCGC; REV-AGTCCTCAAACCAGTCACGAT; probe FAM-BBQ-TGATGTCACTCGGCAGCAGCAA). The reaction was as follows: initial denaturation at 94°C for 1 minute, followed by 35 cycles of 94°C for 45 seconds, 40°C for 45 seconds, and 72°C for 1 minute. A final extension step of 72°C for 6 minutes was also performed (Ebel et al. 1999).

Statistics

The prevalence data and 95% exact confidence intervals of each pathogen were estimated using STATA14® Intercooled (StataCorp, College Station, Texas USA).

3.4 RESULTS

Tissues, blood and ticks were collected from wildlife and a smaller number of domestic dogs (Canis lupus familiaris) across southern Ontario, Canada. Tissues were collected from 724 individuals of 15 species (Table 3.1). A total of 238 ticks of three Ixodid species, I. cookei (n=131), I. scapularis (n=43) and I. marxi (n=15), were collected from wildlife and companion animals (n=82) between May—November in 2015 and 2016 and I. cookei (n=49) were collected from wildlife between 2011 and 2013. Source species and numbers detected varied among tick species: I. cookei were removed from striped skunks (n=20), raccoons (n=12), groundhogs (n=5), red foxes (Vulpes vulpes; n=2), dogs (n=2), a beaver (Castor canadensis; n=1), a Canada goose (Branta canadensis; n=1), a cat (Felis catus; n=1) and a porcupine (Erethizon dorsatum; n=1); I. scapularis were removed from dogs (n=25), a black bear (Ursus americanus; n=1), a coyote (Canis latrans; n=1), a fisher (Pekania pennanti; n=1), a porcupine (n=1), a raccoon (n=1), a red fox (n=1) and a red squirrel (Sciurus vulgaris; n=1); and, I. marxi were removed from raccoons (n=2) and a striped skunk (n=1). Some source animals had multiple tick species on them, including a red fox that had both I. scapularis and I. cookei, a fisher (n=1) with I. cookei and I. marxi, and a raccoon (n=1) with I. scapularis and I. marxi.

All pooled tissue samples tested via RT-PCR were negative for POWV RNA (n=0/724; 0.0%; 95% CI: 0.0—0.5%; Table 3.1). Based on source species, tick homogenates of I. cookei, I. marxi and I. scapularis comprised 98 pools. One pool of I. cookei, which was comprised of two
adult females and two nymphs, tested positive via RT-PCR for POWV \( (n=1/98; 1.0\%; 95\% \text{ CI: } 0.0—5.6\%) \). The ticks in this pool were removed from a Canada goose at the Toronto Zoo in 2012.

Sera were collected from 266 wild animals of seven species. Samples were collected from southern Ontario (Figure 3.1). The majority of samples were collected from raccoons followed by striped skunks (Table 3.2). Initial screening by HI detected anti-flavivirus antibodies in 13.5\% \( (n=36/266; 95\% \text{ CI: } 9.7—18.2\%) \) of samples; of these, 50.0\% \( (n=18/36; 95\% \text{ CI: } 33.0—67.1\%) \) had a titer of \( \geq 1:10 \) and 50.0\% \( (n=18/36; 95\% \text{ CI: } 33.0—67.1\%) \) were indeterminate. Powassan virus-neutralizing antibodies were detected 0.8\% \( (n=2/265; 95\% \text{ CI: } 0.1—2.7\%) \) of samples, including a groundhog \( (n=1/17; 5.9\%; 95\% \text{ CI: } 0.1—28.7\%) \) and a striped skunk \( (n=1/36; 2.8\%; 95\% \text{ CI: } 0.1—14.5\%; \text{ Table 3.2}) \).

Anti-WNV antibodies were detected by cELISA in 8.0\% \( (n=21/264; 95\% \text{ CI: } 5.0—11.9\%) \) of samples. West Nile virus-neutralizing antibodies were detected via PRNT in 5.7\% of samples \( (n=15/264; 95\% \text{ CI: } 3.2—9.2\%) \). West Nile virus neutralizing antibodies were detected in 17.6\% of groundhogs \( (n=3/17; 95\% \text{ CI } 3.8—43.4\%) \), 14.3\% of striped skunks \( (n=5/35; 95\% \text{ CI } 4.8—30.3\%) \) and 4.0\% of raccoons \( (n=7/176; 95\% \text{ CI } 1.6—8.0\%) \). All 219 serum samples tested negative for HRTV by PRNT \( (n=0/219; 0.0\%; 95\% \text{ CI: } 0.0—1.7\%; \text{ Table 3.2}) \).

3.5 DISCUSSION

An alarming trend of an increased incidence in clinical (and sometimes fatal) human POWV cases as well as continued recently reported cases in the northeastern U.S. (Hinten et al. 2008; Piantadosi et al. 2015; Tutolo et al. 2017) and southern Ontario (R. Lindsay, pers. comm.) suggest an ongoing but poorly understood public health threat. Powassan virus surveillance in the short-term will likely continue to rely on human case reporting, and thus may underestimate POWV activity and distribution. Thus, additional strategies for future surveillance may help in early seasonal detections, better define the geographic range and temporal patterns, and enhance public awareness. Based on the involvement of wildlife in the enzootic POWV maintenance cycles for both currently recognized lineages, the use of wildlife and ticks may be useful. However, enzootic cycles differ for each POWV lineage, and targeted wildlife sampling should accommodate both lineages. Powassan virus-prototype (i.e., lineage I) is thought to be maintained primarily by \textit{I. cookei} (i.e., groundhog ticks) and groundhogs. However, evidence
from serological surveys and virus isolation studies suggests that additional species, such as striped skunks and eastern grey (Sciurus carolinensis) and red squirrels, may also be involved in natural transmission cycles (McLean et al. 1964a, 1964b, 1967; Artsob et al. 1986). Deer tick virus circulates between I. scapularis ticks and white-footed mice (Peromyscus leucopus), and its relatively recent discovery as a separate POWV lineage (i.e., lineage II) that can also cause fatal encephalitis in humans (Telford et al. 1997; Ebel et al. 2001; Tavakoli et al. 2009) warrants further investigation.

Results from early POWV serosurveillance studies in northern and southern Ontario indicated that groundhogs and striped skunks were infected more often than other species tested (McLean et al. 1967; Artsob et al. 1986), and that groundhogs were commonly infested with I. cookei ticks (Farkas 1989). The present study serves as a follow up to this early work to further assess the potential involvement of a variety of wildlife and tick species in POWV transmission and to gain insight into current POWV circulation in the region. The present results reaffirm past findings that groundhogs, striped skunks, and I. cookei ticks are infected with POWV in Ontario, albeit at a low prevalence, supporting low levels of virus circulation. These data were not collected systematically across species or spatiotemporally and therefore, the accuracy with which these results reflect natural conditions is unknown. An additional challenge to this opportunistic data collection among wildlife include the likely transient, short-lived period of POWV circulation in blood and other tissues of vertebrate hosts (Kokernot et al. 1969; Telford et al. 1997; Mlera et al. 2017), thereby limiting the ability to detect viral RNA in tissues. Further, POWV experimental trials in groundhogs, Virginia opossums (Didelphis virginiana), grey foxes (Urocyon cinereoargenteus), striped skunks, snowshoe hares (Lepus americanus), white-footed mice and raccoons suggested low virulence in these hosts, evidenced by lack of clinical signs and low-titered, transient viremia (Kokernot et al. 1969; Zarnke and Yuill 1981; Mlera et al. 2017). Small numbers of a few species have shown clinical signs of illness following experimental POWV infection, including eastern grey squirrels, horses (Equus caballus) and a rhesus monkey (Macaca mulatta) (Artsob 1989; Little et al. 1985; Keane et al. 1988).

The lack of detection of recent POWV infections through tissue-PCR testing in mammalian wildlife species suggests that this strategy is not worthwhile in defining POWV activity. However, the detection of neutralizing antibodies in about 6% of groundhogs and 3% of striped skunks, both wild mammal species with relatively limited (i.e., local) home ranges
(Grizzell 1955; Bailey 1971) suggests that these wildlife species may be helpful in defining POWV-active regions, and they play a potential role in transmission. During the 1960s and 1980s in Ontario, antibodies to POWV (detected via HI test) were consistently detected in groundhogs and striped skunks as well as other species, such as eastern grey squirrels, red squirrels, red foxes and occasionally raccoons (McLean and Larke 1963; McLean et al. 1964a, 1964b, 1967; Whitney et al. 1968; Artsob et al. 1984, 1986). Although the timing of POWV infections in these seropositive animals is unknown, many of these species are relatively short-lived in the wild (e.g., groundhogs have an average lifespan of five to six years; striped skunks four to six years) indicating the likelihood that seropositive animals may have been infected in the recent year or two, and especially for animals deemed <1 year of age (Grizzell 1955; Casey and Webster 1975). Additional experimental trials should be conducted on groundhogs and striped skunks to further define their role as likely reservoir hosts. The detection of POWV RNA from a pool of *I. cookei*, which is known to feed primarily on groundhogs in some regions (Farkas and Surgeoner 1990), provides further evidence of current POWV circulation in southern Ontario. The observation of *I. cookei* on a variety of different hosts in the present study suggests that it may opportunistically feed upon other animals that enter groundhog burrows (e.g., skunks, dogs, foxes), which may facilitate POWV spread and maintenance among numerous hosts.

West Nile virus is an endemic, mosquito-borne virus in much of North America that was first detected in Ontario from a WNV-infected bird in 2001 and has since been annually reported in southern Ontario (Drebot et al. 2003; Public Health Ontario 2017; Thompson and Berke 2017). West Nile virus-neutralizing antibodies were detected in approximately 18% of groundhogs, 14% of striped skunks and 4% of raccoons in the present study. These findings are consistent with past studies in the United States Colorado, in which multiple mammalian species including fox squirrels (*Sciurus niger*), house mice (*Mus musculus*), raccoons, striped skunks, and Virginia opossums were seropositive for WNV (Root et al. 2013). These mammalian species are generally considered dead-end hosts (i.e., play little to no role in transmission), although naturally infected fox squirrels were diagnosed with WNV-associated neurological disease (Kuipel et al. 2003; Root et al. 2006). West Nile virus-positive mosquito pools and human cases were reported within Ontario in 2015 and 2016 (Public Health Ontario, 2017), and our study results suggest that the mammalian wildlife species examined were infected with WNV during a similar time frame in southern Ontario.
Heartland virus is a tick-borne virus that infects a large variety of mammalian hosts, including raccoons, white-tailed deer (*Odocoileus virginianus*), and Virginia opossums. The recognized tick vector is the lone star tick, *Amblyomma americanum* (Bosco-Lauth et al. 2015). This virus has not yet been detected in Ontario or other regions of Canada, although it has been detected in the midwestern and eastern U.S., with a fatal human case diagnosed in Oklahoma in 2014 (Vasconcelos and Calisher 2016). Despite the lack of antibodies to HRTV detected in the present study, the expanding geographic distribution of its candidate vector species, *A. americanum*, and the local abundance of candidate host species (e.g., white-tailed deer and raccoons), support preemptive surveillance efforts in Ontario (Nelder et al. 2014; Bosco-Lauth et al. 2015).

In North America, climate change has been associated with the northward incursion and expansion of various tick species, including *I. scapularis* (Ogden et al. 2006a; Nelder et al. 2014). In addition to regionally important tick-borne pathogens, such as *Borrelia burgdorferi*, *I. scapularis* is considered the primary vector of DTV (Telford et al. 1997; Ebel 2010). Deer tick virus has also been found to be widespread in *I. scapularis* in states near Ontario, including New York and Wisconsin and can cause fatal encephalitis in humans (Brackney et al. 2008; Tavakoli et al. 2009; Dupuis II et al. 2013). The present study included a small sample of *I. scapularis*, from which there was no evidence of DTV or POWV. *Ixodes scapularis* ticks are an important target for future POWV research, as this species has been increasing in spatial distribution in the northeastern U.S. states as well as southern Ontario, and is projected to continue their northern expansion and establish in more northern regions (Brownstein et al. 2005; Ogden et al. 2006a). Further, *I. scapularis* are known to feed on a much wider variety of vertebrate hosts than *I. cookei*, including white-footed mice, white-tailed deer and, more importantly, humans (Hermance and Thangamani 2017). Passive surveillance shows that these ticks feed on humans more often than other known POWV tick vectors (i.e., *I. cookei* and *I. marxi*) (Nelder et al. 2014). Therefore, from a zoonotic disease standpoint, monitoring of *I. scapularis* for DTV in endemic regions, such as Ontario should be considered to characterize the geographic range of POWV and concurrent human health risks.

The present study results of rare detections of anti-POWV antibodies in wildlife and viral RNA in ticks and the lack of human case reports from Ontario suggest that POWV currently circulates at low levels in parts of Ontario. However, the expanding geographic range of *I.*
*scapularis* as well as some other tick species and the recent diagnosis of POWV in humans in neighboring U.S. states as well as in Ontario merit increased recognition and monitoring of this virus (Ogden et al. 2006a; Nelder et al. 2014; Piantadosi et al. 2015). The clinical and laboratory symptoms of POWV resemble those of other arboviruses, and, consequently, POWV may be under-diagnosed and should be considered in seasonal neurologic cases in regions with known tick vector populations (Piantadosi et al. 2015). Powassan virus infection is especially concerning due to the short time required for transmission via tick bite (e.g., as low as 15 minutes; Ebel and Kramer 2004), a 50% case fatality rate, and potential for long term-neurologic sequelae in survivors (Ebel and Kramer 2004; Hinten et al. 2008; Hermance and Thangamani 2017). In conclusion, continued surveillance and investigation into host-vector dynamics of POWV in Ontario and other regions is essential to more fully understand the role wildlife plays in pathogen transmission and to document any alterations in POWV epidemiology associated with changes in the environment, with the goal of reducing public health impacts.
3.6 REFERENCES


Table 3.1. Species of origin and number of wildlife tissue samples tested for Powassan virus RNA by reverse transcriptase polymerase chain reaction from May to October, 2015-2016.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue Samples (% positive)</th>
<th>95% CI^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raccoon (Procyon lotor)</td>
<td>225 (0.0)</td>
<td>0.0—1.6</td>
</tr>
<tr>
<td>Eastern Grey Squirrel (Sciurus carolinensis)</td>
<td>217 (0.0)</td>
<td>0.0—1.7</td>
</tr>
<tr>
<td>Striped Skunk (Mephitis mephitis)</td>
<td>96 (0.0)</td>
<td>0.0—3.8</td>
</tr>
<tr>
<td>Groundhog (Marmota monax)</td>
<td>56 (0.0)</td>
<td>0.0—6.4</td>
</tr>
<tr>
<td>Eastern Chipmunk (Tamias striatus)</td>
<td>44 (0.0)</td>
<td>0.0—8.0</td>
</tr>
<tr>
<td>Beaver (Castor canadensis)</td>
<td>30 (0.0)</td>
<td>0.0—11.6</td>
</tr>
<tr>
<td>Red Squirrel (Sciurus vulgaris)</td>
<td>19 (0.0)</td>
<td>0.0—17.6</td>
</tr>
<tr>
<td>Other^b</td>
<td>37 (0.0)</td>
<td>0.0—9.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>724 (0.0)</strong></td>
<td><strong>0.0—0.5</strong></td>
</tr>
</tbody>
</table>

^a Exact 95% confidence interval or one-sided 97.5% confidence intervals if prevalence was equal to zero.

^b Species with <10 individuals sampled: deer mice (Peromyscus maniculatus; n=0/9; 0.0%; 95% CI: 0.0—33.6), Virginia opossum (Didelphis virginiana; n=0/6; 0.0%; 95% CI: 0.0—45.9), eastern cottontail (Sylvilagus floridanus; n=0/5; 0.0%; 95% CI: 0.0—52.3), muskrat (Ondatra zibethicus; n=0/5; 0.0%; 95% CI: 0.0—52.3), fisher (Pekania pennanti; n=0/4; 0.0%; 95% CI: 0.0—60.2), North American river otter (Lontra canadensis; n=0/3; 0.0%; 95% CI: 0.0—70.8), red fox (Vulpes vulpes; n=0/3; 0.0%; 95% CI: 0.0—70.8) and porcupine (Erethizon dorsatum; n=0/1; 0.0%; 95% CI: 0.0—97.5).
**Table 3.2.** Number of wildlife serum samples tested by species from May—October in 2015 and 2016 and tested for Powassan virus (POWV), West Nile virus (WNV) and Heartland virus (HRTV).

<table>
<thead>
<tr>
<th>Source Species</th>
<th>HI test (1:10)</th>
<th>POWV PRNT</th>
<th>WNV (cELISA)</th>
<th>WNV PRNT</th>
<th>HRTV PRNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Raccoon (Procyon lotor)</td>
<td>20/177 (11.3)</td>
<td>0/176 (0)</td>
<td>11/176 (6.3)</td>
<td>7/176 (4.0)</td>
<td>0/155 (0)</td>
</tr>
<tr>
<td>Striped Skunk (Mephitis mephitis)</td>
<td>9/36 (25.0)</td>
<td>1/36 (2.8)</td>
<td>7/35 (14.3)</td>
<td>5/35 (14.3)</td>
<td>0/40 (0)</td>
</tr>
<tr>
<td>Groundhog (Marmota monax)</td>
<td>7/17 (41.2)</td>
<td>1/17 (5.9)</td>
<td>3/17 (17.6)</td>
<td>3/17 (17.6)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>Eastern Grey Squirrel (Sciurus carolinensis)</td>
<td>0/22 (0)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>Beaver (Castor canadensis)</td>
<td>0/12 (0)</td>
<td>0.0-26.5</td>
<td>-</td>
<td>-</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>Virginia Opossum (Didelphis virginiana)</td>
<td>0/1 (0)</td>
<td>0.0-97.5</td>
<td>-</td>
<td>-</td>
<td>0/1 (0)</td>
</tr>
<tr>
<td>Red Squirrel (Sciurus vulgaris)</td>
<td>0/1 (0)</td>
<td>0.0-97.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>36/266 (13.5)</td>
<td>2/265 (0.8)</td>
<td>21/264 (8.0)</td>
<td>15/264 (5.7)</td>
<td>0/219 (0)</td>
</tr>
</tbody>
</table>

*a* Serum samples were first screened by Hemagglutination inhibition (HI) test for POWV and WNV and competitive enzyme-linked immunosorbent assay (cELISA) for WNV. Positive screened samples were confirmed by plaque reduction neutralization test (PRNT) for both POWV and WNV. A subset of samples were tested for HRTV by PRNT.

*b* One sample did not have enough serum to test for POWV via PRNT or for WNV via cELISA or PRNT.

*c* One sample did not have enough serum to test for WNV via cELISA or PRNT.
Figure 3.1. The distribution of Powassan virus- and West Nile virus-positive samples by plaque reduction neutralization test in southern Ontario, Canada. Serum samples were collected from carcasses and live-trapped mammals from May-September in 2015 and 2016.
Powassan virus (POWV; family *Flaviviridae*; genus *Flavivirus*) was first isolated in 1958 from the brain of a child in Powassan, Ontario (McLean and Donohue 1959), and although its eco-epidemiology and geographic distribution were subsequently documented in northern Ontario via serological surveys of both wildlife and humans (McLean et al. 1960, 1962), the present research represents the first POWV survey conducted in Ontario since the 1980s (Artsob et al. 1986). Recent evidence of POWV activity in Ontario, including a fatal human case of POWV encephalitis diagnosed in 2015 in Kingston, Ontario and the detection of POWV genetic material (RNA) from an *Ixodes scapularis* tick in Barrie, Ontario in 2016 (R. Lindsay, personal communication), emphasizes the importance of conducting surveys to determine prevalence and geographic distribution of POWV among wildlife hosts and tick vectors. In the present study, sample collections and laboratory testing were designed to assess for both recent (i.e., tissues tested by RT-PCR) and previous (i.e., for anti-POWV antibodies) POWV infections. We did not detect evidence of recent infections among likely wildlife reservoir species, as all tissues tested were negative; however, POWV-neutralizing antibodies were detected in a striped skunk (*Mephitis mephitis*) and a groundhog (*Marmota monax*) in Guelph, Ontario. Both of these species would be expected to live locally where found, and are not generally long-lived species (e.g., groundhogs have an average lifespan of five to six years; striped skunks four to six years) (Grizzell 1955; Casey and Webster 1975). We also detected POWV RNA from a pooled sample of *I. cookei* ticks, which were collected in 2012 from a Canada goose (*Branta canadensis*) in Toronto, Ontario. These findings suggest recent circulation of POWV in wildlife and ticks in southern Ontario and support the notion that testing of ticks collected from wildlife, as well as serum samples are a more effective strategy to assess for POWV than tissue testing.

West Nile virus (WNV; family *Flaviviridae*; genus *Flavivirus*) is a mosquito-borne zoonosis that has been circulating in Ontario, Canada, with public risk varying annually and regionally (Drebot et al. 2003; Thompson and Berke 2017). Neutralizing antibodies to WNV were detected among raccoons (*Procyon lotor*), striped skunks and groundhogs in the present study. Birds are the primary amplifying hosts of WNV, whereas mammalian vertebrates, including those with neutralizing antibodies detected in the present study, are considered dead-end hosts (Root et al. 2003). These results indicate WNV continues to infect wild mammals in
southern Ontario, and supports detection of cases in mosquitoes and birds in recent years (Canadian Wildlife Health Cooperative, unpublished data; Public Health Ontario 2017).

The present study is novel in that it included testing of serum samples collected from various wild mammals in southern Ontario for Heartland virus (HRTV; family Bunyaviridae; genus *Phlebovirus*). Heartland virus is a tick-borne, zoonotic virus that currently circulates in the midwestern and eastern United States. In humans, HRTV may cause a flu-like illness, including fever, headache, muscle aches, diarrhea, appetite loss, and lethargy (Bosco-Lauth et al. 2015; Vasconcelos and Calisher 2016). This virus has not yet been detected in Ontario or elsewhere in Canada; however, the recognized tick vector species, *Amblyomma americanum*, is thought to be biologically capable of establishing itself in Ontario (Nelder et al. 2014). Although evidence of past HRTV infections was not revealed in the present study, the abundance of suspect candidate host species, including white-tailed deer (*Odocoileus virginianus*) and raccoons, and the changing distribution (i.e., establishing in new areas) of other tick species (e.g., *I. scapularis* and *A. americanum*) in southern Ontario support the need for pre-emptive surveillance studies in Ontario (Ogden et al. 2006a; Bosco-Lauth et al. 2015).

The present research also included assessment of ticks collected from wildlife and companion animals for other zoonotic, tick-borne pathogens. Knowledge of the wildlife host species that most commonly harbor known tick vectors of zoonotic pathogens may contribute to understanding eco-epidemiological aspects such as geographic distribution of hosts, ticks and pathogens, and potential pathogen transmission, maintenance and spread. *Ixodes scapularis* ticks may transmit *Borrelia burgdorferi* (the causative agent of Lyme disease), *Anaplasma phagocytophilum* (the causative agent of human granulocytic anaplasmosis), and several other pathogens (e.g., *B. miyamotoi* and *Babesia microti*). Both *B. burgdorferi* and *A. phagocytophilum* were previously detected in Ontario in a provincial-wide survey of ticks submitted by the public from 2008-2012, with a detection prevalence of 15.5% and 0.3%, respectively (Nelder et al. 2014). Both pathogens were detected, albeit rarely (2-10%), among ticks removed from canid species, including, dogs (*Canis lupus familiaris*), red foxes (*Vulpes Vulpes*) and coyotes (*Canis latrans*) in the present study. As global climate change continues to affect and alter the geographic distributions of both ticks and wildlife host species (Ogden et al. 2006a), continued surveillance in Ontario for both tick vectors as well as the pathogens they carry is essential from a public health standpoint.
In addition to assessing for evidence of tick-borne pathogens, the present research also attempted to characterize relationships between tick species and their wildlife hosts in southern Ontario. Consideration of this relationship is vital when attempting to understand eco-epidemiologic aspects of tick-borne pathogen transmission cycles (Dantas-Torres et al. 2012). Some tick species, such as *I. scapularis*, are known to feed from a wide variety of hosts whereas others, such as *I. texanus*, are more host-specific (Lindquist et al. 2016). In the present study, *I. scapularis* ticks were removed from eight source species, most commonly dogs, and less commonly black bears (*Ursus americanus*), coyotes, fishers (*Pekania pennanti*), porcupines (*Erethizon dorsatum*), raccoons, red foxes and red squirrels (*Sciurus vulgaris*). This host diversity contrasts with *I. texanus* ticks, which were collected from only two species, raccoons and striped skunks, possibly reflecting differences in tick-feeding strategies, seasonal tick-feeding activity, or tick density and distribution within the trapping area. Furthermore, in the present study, *I. texanus* were removed from more than 50% of parasitized raccoons. Little is known about the vector potential of *I. texanus* for zoonotic pathogens; however, indirect evidence has implicated it as a possible vector of *Rickettsia rickettsii* (the causative agent of Rocky Mountain spotted fever), *Babesia lotor* and *Ehrlichia* spp. infection in raccoons (Anderson et al. 1981, 1986; Dugan et al. 2005). In addition, *I. texanus* was found on approximately 70% of raccoons examined in the neighboring state of Michigan (Hamer et al. 2010). The potential of these ticks to act as a vector for zoonotic pathogens and the apparent abundance of these ticks on raccoons in nearby regions warrants further investigation. These results outline the importance of wildlife-based surveillance studies to better understand tick-host relationships and to build a more accurate understanding of the diversity of tick species in Ontario. Overall, these studies may be used to further build upon the understanding of complex and evolving tick-host-pathogen relationships in changing environments.

4.1 STUDY LIMITATIONS

The overall goal of the present research was to conduct a survey of wildlife, ticks and companion animals from May—October in 2015 and 2016 and to use these samples to acquire data on geographic locations, ticks found on different wildlife species, and tick-borne pathogen prevalence among ticks removed from numerous wild and domestic animals. The logistical challenges to collecting wildlife-derived samples, including ticks, are many. First, collecting
high numbers of wildlife carcasses or trapping high numbers of live wild animals often relies on opportunistic sampling. This affects the timing, location, numbers, and species of animals received. Thus, it impedes the ability to ensure that animals included are an accurate representation of the population. For instance, POWV seroprevalence in groundhogs in the present study was 5.9%, which is considerably lower than previous studies (44—67%) (McLean et al. 1964a, 1964b, 1967; Whitney et al. 1968; Dupuis II et al. 2013). It is possible the low number of groundhogs collected in this study resulted in a falsely low POWV seroprevalence that may not accurately reflect that of the regional population. In addition, the opportunistic nature of data collection often resulted in a lack of data concerning tick-negative animals (i.e., an accurate denominator) so the prevalence of parasitism with ticks could not be estimated. However, opportunistic sampling was essential for covering a wide geographic area and for sampling a wide array of species at the highest numbers as possible within southern Ontario. The resulting tick and host species collected enabled a wide diversity of sample sources for zoonotic pathogen testing. The information gathered from this study will be useful for designing targeted studies of POWV and other tick-borne viruses in wildlife within southern Ontario.

Maintaining the integrity of tissue, blood and tick samples over long periods of time, in varying temperatures and conditions is an additional challenge involved in the field collection of wildlife-derived samples. Serum samples are derived from blood samples collected from live or recently dead animals that were immediately spun down and separated. However, field conditions are often less than ideal. In the beginning of this study, carcasses were regularly submitted frozen or with the animal having been dead for more than a few hours; both scenarios result in an inability to separate serum from the packed blood cells, preventing serologic testing of these samples. The second year of the study, the protocol was adapted so that wildlife trappers collected blood in the field. In addition, avoiding freeze-thaw cycles is essential for maintaining high quality RNA in tick and tissues samples. The lack of a Biosafety Level 3 Laboratory at the University of Guelph required that the collected samples be shipped to a collaborating laboratory, which put these sensitive samples at risk of prolonged shipment times and temperature changes. In an effort to minimize these risks, during the second year of the study, RNAlater was added to pooled tissue samples. This additional step allowed the samples to maintain high quality RNA both prior to and while in transit. Although often a considerable challenge, maintaining the integrity of samples until measurements are performed is essential, as
wildlife-derived samples are vital for studies involving many zoonotic pathogens.

The duration for which POWV is in the blood (i.e., viremia) and other tissues of wild mammalian hosts is likely transient; consequently, the period when the virus or viral RNA may be detected is likely short-lived (Kokernot et al. 1969; Telford et al. 1997; Mlera et al. 2017). It is possible that wildlife experience transient infections with POWV that are likely cleared within less than one week after infection, creating a small window within which limited amounts of virus are present within tissues (Kokernot et al. 1969). Based on past studies with WNV in mammals (Root et al. 2006; Platt et al. 2008), four of the most likely tissues (i.e., brain, heart, kidney and spleen) were chosen in this study to target for POWV RT-PCR. In addition, tissues were pooled for testing to maximize the chances of detection. However, no evidence of viral RNA was found in any of the tissue samples (n=724). Thus, while great efforts and resources were invested in this aspect of the study, these results suggest that testing for POWV through tissue collection of wild mammals would likely not be a useful component of passive POWV surveillance in wildlife.

The majority of samples in this study were collected at one time point, limiting the potential for investigating seasonal or annual patterns. Powassan virus prevalence in groundhogs has been observed to change over the course of a transmission season (i.e., May—August) and vary between juveniles and adults (15.4% vs 50.9%) (McLean et al. 1967). In the present study, groundhogs were only live-trapped in September 2016; therefore, differences among seasons or age groups could not be examined. Tick activity is also seasonal (e.g., I. scapularis nymphs are most active in Ontario from June-August; Ogden et al. 2005), and the present study did not target the collection of ticks during periods of high tick activity. Instead, ticks were collected throughout the summers (May-October) of 2015 and 2016, which could lead to an underestimation of tick abundance as well as prevalence of tick-borne pathogens in southern Ontario. Despite this limitation, the results from the present study include a variety of sample sources and species that are useful for hypothesis generation.

The small numbers of medium-sized wild mammals collected, other than raccoons, represented additional limitations to this study. Due to the opportunistic sampling, as mentioned above, we received more non-target species (such as raccoons) versus target species (such as groundhogs). In addition, the majority of samples in the first year of the study were from carcasses, from which ticks often do not remain attached, thus limiting numbers of ticks.
collected. Thus, we adapted the study protocol to include live-trapped animals and companion animals at veterinary clinics. Small sample sizes of ticks and targeted wildlife species reduced the ability to make statistical inferences, including the clear distinction of likely important candidate reservoir host species of POWV in southern Ontario. However, the data gathered in this study can be used as a baseline for some regions and to select geographic areas that could be more systematically sampled for POWV in the future.

4.2 FUTURE STUDIES

Recent increased numbers of reports of human POWV cases in the northeastern United States (Hinten et al. 2008; Piantadosi et al. 2015; Tutolo et al. 2017) as well as several recent cases in southern Ontario (R. Lindsay, personal communication) highlight the importance of regional POWV monitoring. In the present study, evidence of past POWV infections was detected in striped skunks and groundhogs, which is consistent with previous studies (McLean et al. 1960, 1964a, 1964b; Main et al. 1979; Artso et al. 1986). These results may contribute to the design of future surveillance studies in southern Ontario, for which these species may be targeted and serosurveillance may maximize the chances of detecting evidence of past infection (versus tissue PCR). In addition, two POWV lineages are currently recognized: Lineage I (Powassan-prototype; POWV-p), which is maintained primarily between I. cookei ticks and groundhogs, and Lineage II (Deer Tick virus; DTV), which is maintained between I. scapularis ticks and white-footed mice (Peromyscus leucopus) (Telford et al. 1997; Ebel 2010). Deer tick virus was not detected in the present study; however, the ongoing northward expansion of I. scapularis (Brownstein et al. 2005; Ogden et al. 2006a; Clow et al. 2016) and the tick’s aggressive feeding behaviour (Hermance and Thangamani 2017) warrant further research on the potential circulation of the DTV in southern Ontario. Furthermore, in the present study, a large number of I. texanus ticks were collected from raccoons; this finding is consistent with previous studies and suggests a strong vector-host relationship between these two species (Lindquist et al. 2016). Currently, the role of I. texanus in pathogen transmission is still being explored; however, the high numbers observed on raccoons in various geographic regions (Hamer et al. 2010; Lindquist et al. 2016) and the capability of Ixodes spp. to act as vectors for a variety of zoonotic pathogens warrants further investigation.
4.3 CONCLUSIONS

The present research suggests that POWV circulation is currently at low levels among various wildlife species in southern Ontario. However, the northward expansion of some vector species, such as *I. scapularis* (Ogden et al. 2006), recent detection of human cases (Piantadosi et al. 2015), and the high human case fatality rate associated with POWV infection (Hinten et al. 2008) underscores the importance of continued surveillance for this tick-borne pathogen. In addition, our research detected *B. burgdorferi* and *A. phagocytophilum* in ticks collected in southern Ontario. Continued surveillance efforts and investigations into host-vector dynamics of POWV in Ontario and other regions is essential to mitigate public health risk, and to more fully understand the role wildlife plays in pathogen transmission.
4.4 REFERENCES


