

Eastern Equine Encephalitis Virus

History and Enhanced Surveillance
in Ontario

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Introduction

Eastern equine encephalitis virus (EEEV) is a mosquito-borne virus (*Togaviridae: Alphavirus*) found throughout the Western Hemisphere. In North America, EEEV is restricted to areas east of the Mississippi River in the US and southeastern Canada. The first human case of EEEV infection (eastern equine encephalitis; EEE) occurred in Massachusetts in 1938, yet EEE was initially recognized about a century earlier in equines.¹ Eastern equine encephalitis is considered the most severe of the mosquito-borne diseases in North America, given its high case fatality ratio (estimated 30–75%) and the high incidence of permanent neurological sequelae (e.g., paralysis, brain dysfunction, seizures) in EEE survivors.²⁻⁴ Infections are characterized by fever, headache, nausea and vomiting, malaise and weakness, confusion, myalgia, arthralgia, and neck stiffness.⁴ While EEEV activity has been noted in Canada, no human EEE cases have been reported. In the US, 41 human EEE cases occurred from 2008 through 2013 in 14 states, with the majority of cases occurring in Massachusetts (n = 11), Florida (9), North Carolina (4), Michigan (3) and New York (3).⁵

The earliest evidence of EEEV activity in Canada was in 1938 when equine cases were reported in the Ontario cities of St. George (present day Brant County Health Unit; BRN) and St. Catharines (Niagara Regional Health Unit; NIA).^{6,7} In 1972, equine cases occurred in Eastern Townships, Quebec, and in 2008, over 15 equines and a flock of emus were positive for EEEV in Quebec's Estrie, Centre-du-Québec, Lanaudière and Montérégie regions.^{8,9} In 2009, equine cases were reported in Nova Scotia for the first time in that province.¹⁰

The first detection of EEEV in Canadian mosquitoes occurred in September 2009, where an EEEV-positive pool of *Culiseta melanura* was detected in the First Nations Community of Whata Mohawk (located within Simcoe Muskoka District Health Unit; SMD).¹¹ While limited EEEV testing of mosquitoes has occurred in Ontario since 2004, it was not until 2011 that EEEV testing became a part of the province's vector surveillance program.

Current situation

In 2010, there was increased EEEV activity across the eastern portion of North America, with equine epizootics occurring in neighbouring Michigan and New York states. In late 2010, the Ontario Ministry of Health and Long-term Care made recommendations to public health units (PHUs) that initiated a three year enhanced EEEV vector surveillance program. PHUs were advised to include EEEV-vector testing within the larger framework of their West Nile virus (WNV) vector surveillance programs. In 2011, PHUs could include pools of potential EEEV vectors within the allotted three pools originally available for WNV testing for a given light trap. The period from 2011 through 2013 was used as an enhanced surveillance period to determine baseline EEEV activity in Ontario's mosquito vector populations. However, this report also includes Ontario data from a broader period from 2008 through 2013.

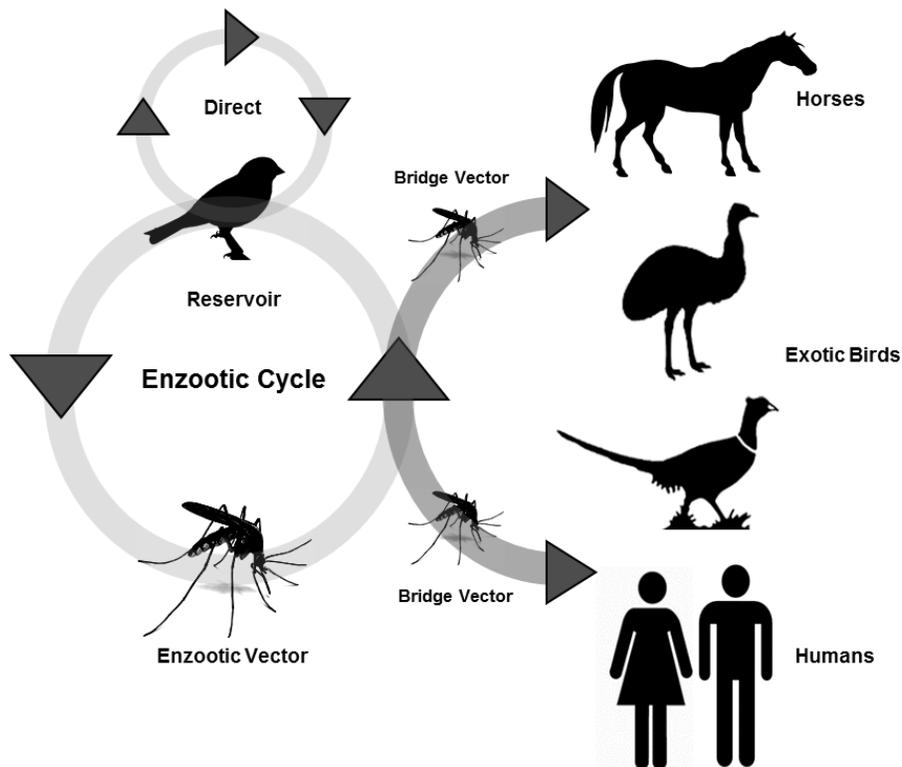
Purpose of this report

The purpose of this report is to share results from the enhanced surveillance work with PHUs and to interpret recent EEEV activity in Ontario in order to guide future evidence-informed decision-making for EEEV surveillance in Ontario. Specifically, we report on EEEV testing results in potential vector populations and positive equine cases from 2008 through 2013. The report provides the historical context of EEEV in Ontario, the ecological factors that contribute to EEEV transmission and aspects of EEEV monitoring in Ontario.

Transmission

Transmission of EEEV is primarily through the bite of an infective mosquito. EEEV is maintained within a bird–mosquito–bird enzootic cycle, whereby passerine birds are the primary reservoirs and the mosquito *Cs. melanura* the primary enzootic vector (Figure 1). While the WNV-transmission cycle is associated with urban landscapes and with urban mosquito species (i.e., *Culex pipiens* and *Culex restuans*), the EEEV-transmission cycle is associated with rural landscapes with appropriate habitat for the development of *Cs. melanura*. Areas with enzootic EEEV cycles are typified by low-lying areas and swamps or bogs dominated by hardwoods such as red maple and hornbeam.

Figure 1. Transmission of EEEV with passerine birds as enzootic hosts or reservoirs; exotic birds, humans and equines are dead-end hosts. *Culiseta melanura* is the primary enzootic/bridge vector with *Aedes vexans*, *Coquilleltidia perturbans* and other *Ochlerotatus* species as other bridge vectors.



Culiseta melanura is considered a highly ornithophilic mosquito, meaning it is effective at maintaining the EEEV enzootic cycle in birds. In New York State, *Cs. melanura* feed mostly on avian sources ($\approx 94\%$ of the blooded mosquitoes assayed), followed by mammalian sources (1%).¹² *Culiseta melanura* can be considered a bridge vector, potentially transmitting EEEV to equines and humans; however, this is considered a rare transmission route due to the vector's overwhelming preference for avian blood. Birds are the primary means by which EEEV disperses across the landscape; the specific bird species involved will vary among foci based on bird populations present.¹³ The avian blood-meal hosts for *Cs. melanura* in

a study conducted in New York State were the wood thrush, American robin and song sparrow.¹² The mammalian hosts of *Cs. melanura* in the New York study were comprised mostly of white-tailed deer and equines; however, the composition of the vertebrate hosts in a particular focus will likely mirror the population densities of available hosts. Recent work in Massachusetts revealed the most common avian blood meal hosts for *Cs. melanura* were the American robin, Tufted Titmouse and black-capped chickadee.¹⁴

Bridge vectors are mosquito species that transmit EEEV from within the bird enzootic cycle to other susceptible hosts such as humans and equines. Normally, bridge vectors include species that will feed on reservoir species (i.e., birds) and then dead-end hosts (i.e., humans, equines, exotic bird species) and are abundant during peak EEEV activity in late summer and early fall (Figure 1). In Ontario, potential bridge vectors include *Aedes vexans*, *Coquillettidia perturbans*, *Ochlerotatus canadensis* and other *Ochlerotatus* species.¹⁰ In Southern Quebec, *Cs. melanura* and *Oc. canadensis* were positive for EEEV during 2009 and 2010 epizootics in equines (L. Robbin Lindsay personal communication).

Alternate modes of enzootic transmission

Once infected, birds can transmit EEEV to other birds through pecking one another in flocks or via cannibalism, especially when birds are in a captive environment and there are high viral loads in nasal and anal secretions (Figure 1). In the 1970s, an epizootic occurred in South Carolina in which over 90,000 Coturnix quail in a commercial facility died of EEEV infections acquired through bird-to-bird transmission; however, initial bird infection was likely mosquito-borne.¹⁵ Susceptible birds that become infected will develop symptoms that include ataxia, constant circular movements, depression, drowsiness, leg paralysis and vocalization changes. In some cases, birds will not develop clinical signs and will die suddenly.

Captive exotic birds (e.g., chukar partridge, emu, ring-necked pheasant) on farms or in zoos are immunologically naïve and susceptible to EEEV infection, making them a passive surveillance indicator for local activity. Native bird species have developed immunity to EEEV, thus typically do not show clinical signs of infection, rather they act as reservoirs for the virus.

Mosquitoes are the primary means for transmission of EEEV; however, the arbovirus has been associated with other arthropods. Black flies and biting midges, along with other biting flies, have been implicated as vectors of EEEV.^{16,17} Further, EEEV has been isolated from ectoparasites such as chicken mites and chicken lice.¹⁸ It is unknown whether these arthropods play a significant role in the maintenance and transmission of EEEV. EEEV transmission to chickens from infective chicken mites has been demonstrated in a laboratory setting, a finding that indicates other possible vectors may exist in nature.¹⁹

Ecological considerations

Female *Cs. melanura* will lay eggs (as rafts) within the root passages of hardwood trees within a swamp, particularly in areas with low pH and high organic content. In Southern Quebec, larvae inhabited deep watery depressions of sphagnum bogs.²⁰ In Ontario, the distribution of *Cs. melanura* roughly coincides with the northern distribution limits of red maple and hornbeam.

In Michigan, New York and Ohio (USA), EEEV vectors and reservoir bird populations have been associated with low-lying, dry land between lakes, ponds, swamps and bogs interconnected by streams. Equine epizootics have occurred in agricultural areas neighboring these types of hydrographic regions.²¹ An important risk factor for humans contracting EEEV is their proximity (within approximately 8 km) to swamps that support populations of *Cs. Melanura*; often this means residence in a rural or suburban area.²² In Southern Ontario, the majority of equine cases (12/14) occur in the Simcoe-Lake Rideau Ecoregion, a region typified by a higher proportion of bogs (13% of land cover) and wetlands (3%) compared to the lower number of cases (2/14) in the Lake Erie-Lake Ontario Ecoregion with lower proportions of bogs (5%) and wetlands (1%).²³ In Oxford County Health Unit (OXF) in 1995, two equine cases were pastured on land with access to a hardwood swamp.⁷

Climate and ecological factors play important roles in predicting if a particular year will have an increase in equine and human EEEV cases. In Massachusetts, Michigan and New Jersey (USA), equine epizootics have occurred when there is relatively higher rainfall in the late summer and early fall of the previous year and during the current summer of an outbreak.^{22,24,25} Historically, increased rainfall, flooding and hurricanes preceded major epizootics and epidemics, e.g., outbreaks in 1955 in Massachusetts and in 1959 in New Jersey.^{26,27} Rainfall is an important part of the development of *Cs. melanura* and other bridge vectors, as it increases their population size, increasing their likelihood of acquiring EEEV.

Epizootics can potentially occur at any time of the mosquito season, but usually coincide with peaks in vector populations (*Cs. melanura*). In Ontario, *Cs. melanura* populations increase in the last week of August through mid-September, followed by equine cases after this period. Large epizootics in equines often foretell human epidemics.^{27,28,29} In Michigan, the majority of equine cases have occurred in September.²² In the 2008 equine epizootic in Southern Quebec, cases began in early September and ended in mid-October.⁹

Ontario surveillance

Historically, EEEV surveillance in Ontario has been undertaken primarily through the reporting of positive equine and bird cases and, more recently, vector surveillance. Although birds can act as useful surveillance indicators, there is no systematic collection of EEEV-positive bird data; therefore, mosquito and equine data will be presented here.

Equine

Equine cases usually occur before the onset of human cases, making equine surveillance a valuable tool in assessing risk to humans. In 1947, an epizootic of EEE involved more than 14,000 equines in Louisiana and Texas; human cases did not appear until after the peak of equine cases had begun to decrease.²⁸ A 1959 outbreak in New Jersey saw a peak of equine cases followed by the onset of human cases (32 cases), with human EEE cases lasting until the first frost in October.²⁷ The initial signs of EEEV infection in equines include anorexia, depression and fever. Infected equines usually show signs of disruption of the central nervous system, with ataxia, erratic behavior, paresis, paralysis, seizures, and vision impairment. The onset of symptoms is relatively quick after the infective mosquito bite; once symptoms begin, death can occur in 24–48 hours (case fatality ratio 70–90%).¹

Table 1: Mosquito and equine indicators of eastern equine encephalitis virus activity in Ontario (2008–13).*

Year	Public Health Unit (no. positive pools)			Equine cases‡
	<i>Culiseta melanura</i>	<i>Aedes vexans</i>	<i>Coquillettidia perturbans</i>	
2008	0	0	0	LGL (2), DUR (1), GBO (1)
2009	SMD (10)†	SMD (2)†	0	HDN (2)
2010	SMD (2)†, NPS (1)	0	0	SMD (2), GBO (1)
2011	0	0	0	EOH (2), LGL (1), SMD (1)
2012	0	0	0	0
2013	0	0	EOH (1)	SMD (1)

*Public Health Unit abbreviations: DUR, Durham Regional; EOH, Eastern Ontario; GBO, Grey-Bruce; HDN, Haldimand-Norfolk; LGL, Leeds-Grenville and Lanark District; NPS, North Bay Parry Sound District; SMD, Simcoe Muskoka District;

†First Nation Community, Wahta Mohawk, data from First Nations Inuit Health Branch (Health Canada) other mosquito pool data from PHO Mosquito Database

‡Data from OMAF (<http://www.omafra.gov.on.ca/english/livestock/horses/westnile.htm>)

From 2008 through 2013, there were 14 equine cases reported in Ontario within 6 public health units: Durham Regional Health Unit (DUR), Eastern Ontario Health Unit (EOH), Grey Bruce Health Unit (GBO), Haldimand-Norfolk Health Unit (HDN), Leeds-Grenville and Lanark District (LGL), and Simcoe Muskoka District (SMD) (Table 1; Figure 2).³¹ From 2008 through 2013, the highest number of equine cases was reported in SMD (4) and LGL (3). During the enhanced surveillance period (2011–2013), five equine cases occurred in Ontario from EOH (2), SMD (2) and LGL (1) (Table 1).³⁰

Equine exposure to EEEV continues to occur in Ontario, primarily in specific foci located in the Central region (GBO, SMD), Southern region (HDN) and in the Eastern region (EOH, LGL) (Table 2; Figure 2). The public health units with the majority of EEE equine cases coincide with where equine farms are more numerous (e.g., GBO, LGL, SMD) (Figure 2).²⁹

Figure 2A. Equine and equine farm census in Ontario (2011).

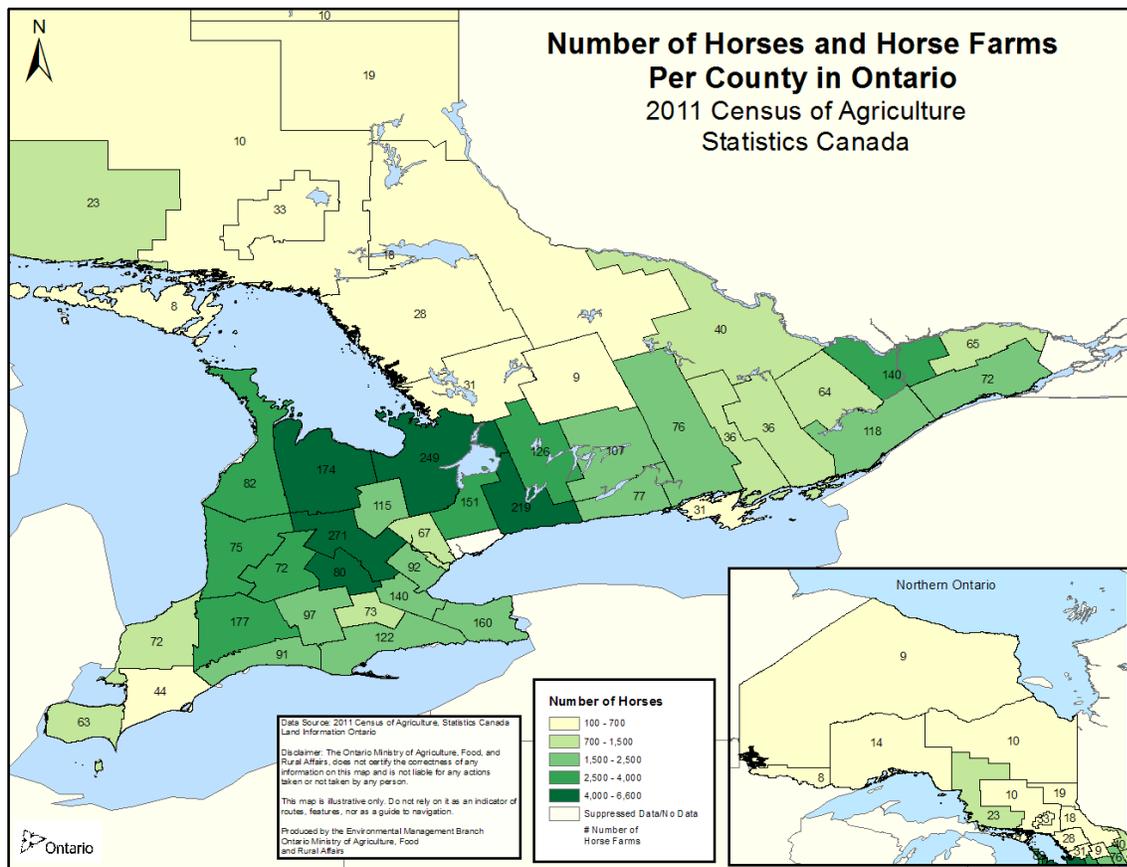
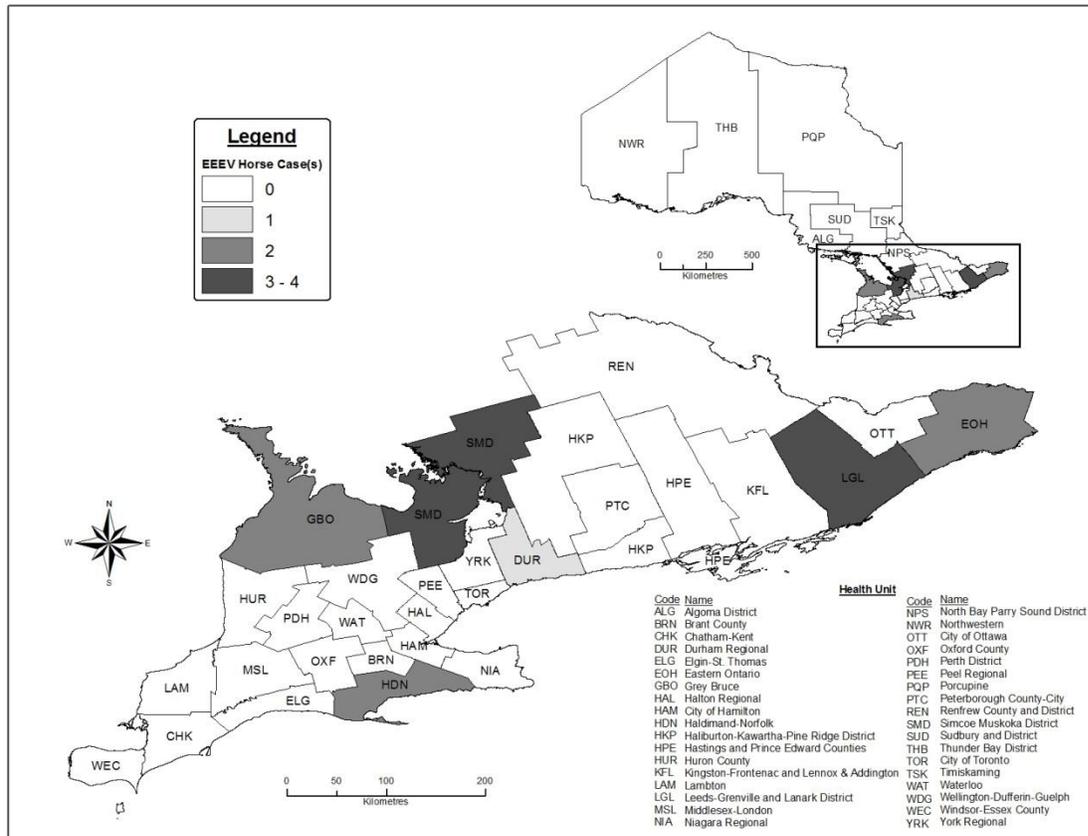


Figure from Ontario Ministry of Agriculture and Food (used by permission).³⁰

Figure 2B. Eastern equine encephalitis equine cases in Ontario (2008–13).



Vector

For Ontario’s WNV mosquito surveillance program, health units place CDC miniature light traps throughout their region and send the captured mosquitoes to their contracted service providers to be identified to species and tested for the WNV. From 2008 through 2013, during Ontario’s WNV mosquito surveillance program, service providers were directed to test the primary enzootic vector, *Cs. melanura*, for EEEV. In 2011, the health units were asked to modify their mosquito viral testing order of preference and test for the main WNV vector, followed by specific EEEV vectors:

1. *Culex pipiens/restuans* – WNV
2. *Culiseta melanura* – EEEV
3. *Coquillettidia perturbans* – EEEV
4. *Aedes vexans* – EEEV
5. Remaining order of WNV vectors³²

In September 2009, the first EEEV-positive pool of *Cs. melanura* was detected in the Wahta Mohawk First Nation; which is located within SMD (Table 1). During the fall of 2009, nine more EEEV-positive

pools of *Cs. melanura* were detected in the Wahta Mohawk First Nation, along with two positive pools of *Aedes vexans* (data from First Nations Inuit Health Branch, Health Canada). In 2010, two positive pools of *Cs. melanura* were again detected in Wahta Mohawk First Nation along with a positive pool of *Cs. melanura* in North Bay Parry Sound District Health Unit (NPS).

From 2008 through 2013, the 16 positive pools were detected involving *Cs. melanura* (13), *Ae. vexans* (2) and *Cq. perturbans* (1) (Table 1). The highest numbers of *Cs. melanura* per trap night (2008–13) were collected in NPS (0.8 per trap night), HKP (0.4) and SMD (0.2) (Figure 3). Despite the testing of over 245,000 mosquitoes in over 18,000 pools during the enhanced surveillance period (2011–13), one EEEV-positive pool was detected (*Cq. perturbans*, 2013 in EOH) (Table 2).

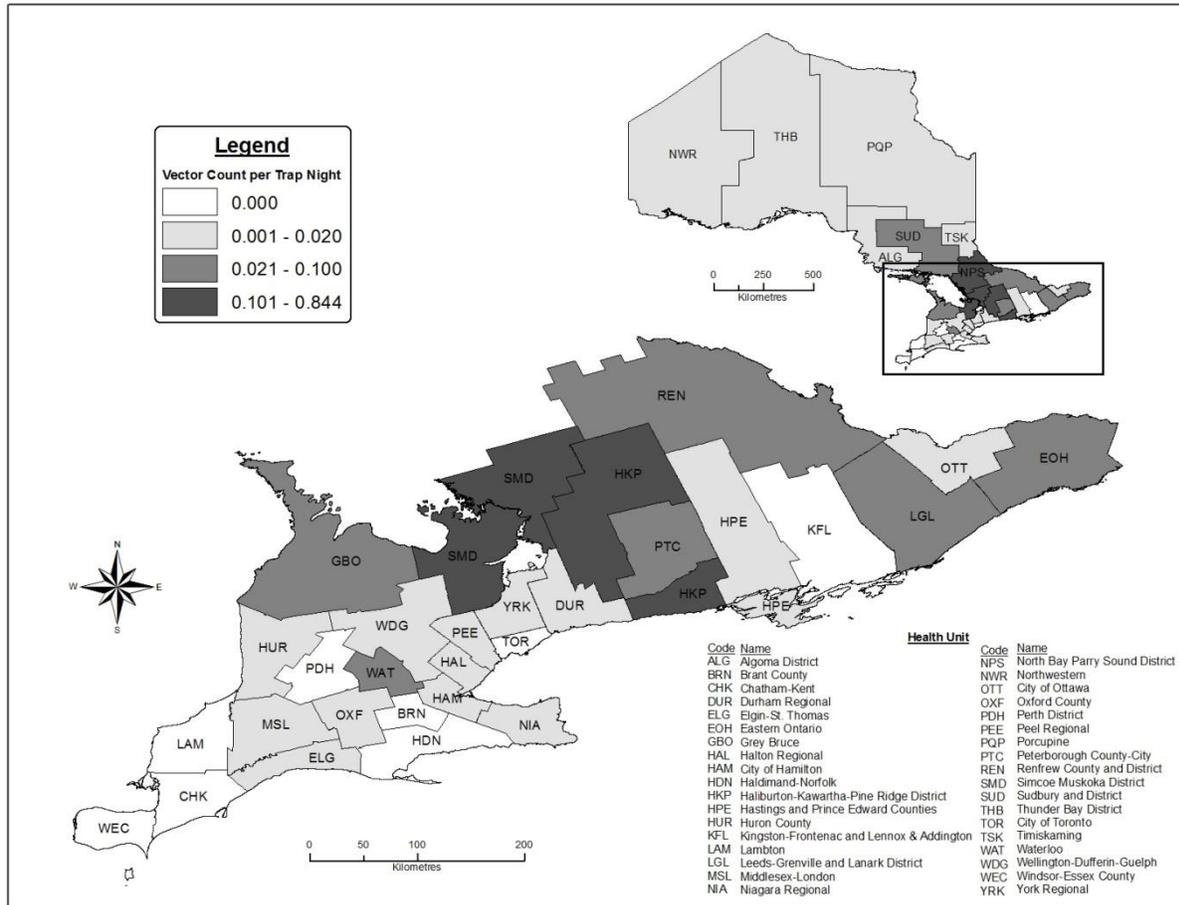
Table 2: Mosquito species tested for eastern equine encephalitis virus in Ontario, noting volume of pools and mosquitoes tested (2011–13).

Mosquito species	Total pools tested (total mosquitoes tested)*			Total
	2011	2012	2013	
<i>Coquillettidia perturbans</i>	3,385 (59,892)	2,649 (43,986)	2,107 (31,529)†	8,141 (135,407)
<i>Aedes vexans</i>	3,392 (46,656)	2,355 (21,188)	1,755 (29,635)	7,502 (97,479)
<i>Ochlerotatus canadensis</i>	893 (5,820)	295 (1,041)	1,054 (9,120)	2,242 (15,981)
<i>Culiseta melanura</i>	84 (218)	28 (65)	69 (243)	181 (526)
<i>Culex erraticus</i>	0	35 (69)	0	35 (69)
<i>Ochlerotatus japonicus</i>	3 (4)	9 (17)	20 (72)	32 (93)
<i>Culex pipiens/restuans</i>	15 (69)	3 (38)	0	18 (107)
<i>Anopheles punctipennis</i>	8 (9)	0	1 (1)	9 (10)
<i>Anopheles quadrimaculatis</i>	4 (15)	0	0	4 (15)
<i>Ochlerotatus trivittatus</i>	4 (21)	0	0	4 (21)
<i>Ochlerotatus stimulans</i>	2 (4)	0	1 (1)	3 (5)
<i>Ochlerotatus triseriatus</i>	3 (10)	0	0	3 (10)
<i>Anopheles walkeri</i>	1 (2)	1 (50)	0	2 (52)
Total	7,794 (112,720)	5,375 (66,454)	5,007 (70,601)	18,176 (249,775)

*Data from PHO Mosquito Database

†Includes a EEEV-positive pool from EOH

Figure 3. *Culiseta melanura* per trap night in Ontario (2008–13).



Discussion

During the enhanced surveillance period, 18,176 mosquito pools (249,775 mosquitoes) were assayed for EEEV. One pool of *Coquillettidia perturbans* (in 2013 in EOH) was positive. In addition, the Ontario Ministry of Agriculture and Food reported five equine cases during the enhanced surveillance period located in the public health units of EOH (2), SMD (2) and LGL (1).

Culiseta melanura are not readily collected in CDC light traps, as they are not particularly attracted to the traps. The traps (as a part of the WNV program) are typically located in urban/suburban areas where *Cs. melanura* populations are negligible. Furthermore, the *Cs. melanura* trapped in a CDC light trap are host seeking and less likely to have previously fed upon an EEEV-viremic bird; therefore, the probability of detecting a positive pool is considerably reduced. While equine cases occur sporadically in specific regions of the province, EEEV activity in vector mosquitoes is limited. The discordance between equine cases, vector population sizes and positive mosquito pools may indicate that current vector surveillance methods are not optimal for detection of the enzootic EEEV vector *Cs. melanura*. Regardless of the lack of *Cs. melanura* positive pools, EEEV activity, if present, should be detectable in bridge vector species, which are more readily captured in CDC light traps (such as *Ae. vexans* and *Cq. perturbans*).

Although *Cs. melanura* is most commonly found in traps in North Bay Parry Sound District Health Unit (NPS), the health unit has lacked equine EEE cases. One explanation may be that this health unit is further north and temperatures may not allow for maintenance of EEEV within mosquitoes. However, NPS has relatively fewer equines and equine farms, with the result that EEE equine cases may not be detected even if EEEV activity is present.

Summary and options

In summary:

1. EEEV detection and avian/equine infections have been documented in Ontario, dating back to the 1930s
2. No human case of EEEV infection has been reported in Ontario
3. Equine cases appear sporadically in Ontario
4. Enhanced mosquito surveillance from 2011 through 2013 demonstrated negligible EEEV in activity in the vector species tested.

Given the results of EEEV testing during the enhanced surveillance period, province-wide EEEV testing of potential vectors is not indicated. However, where risk assessments and animal surveillance indicate EEEV activity in an area or where historical data indicates a likelihood of EEEV activity, a public health unit may still wish to continue or initiate EEEV mosquito testing. Equine cases will remain the primary surveillance tool for determining EEEV activity. If a public health unit detects EEEV-positive animals, it may still want to conduct temporary and enhanced vector surveillance (additional CDC light traps or “hot-spot trapping”) around the area of the infected equine case. Enhanced surveillance may include putting additional CDC light traps in the area with excess dry ice as bait (to make the trap more attractive to *Cs. melanura*). Enhanced surveillance around EEEV-positive cases may only be required for a few weeks after detection of an infected animal. If active EEEV foci emerge in the future, then targeted vector surveillance can be employed at the discretion of the responsible public health unit.

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