West Nile Virus Mosquito Surveillance Report and Notes on Eastern Equine Encephalitis Virus Mosquito Testing, 2020

Grey Bruce Public Health

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1.0 Overview

The adult mosquito surveillance program was a critical component of the overall West Nile Virus (WNV) program for Grey Bruce Public Health in the 2020 season.

No WNV human and avian cases, or WNV-positive mosquito pools were reported in Grey Bruce Public Health in 2020; however, in Ontario, a total of seventy-seven (77) human cases were reported (Public Health Ontario, 2020).

There were seven (7) Eastern Equine Encephalitis (EEEV) equine cases in Ontario in 2020. No human cases or positive mosquitoes have been reported (PHO, 2020). One (1) EEEV-positive equine case was reported from Grey Bruce Public Health.

2.0 West Nile Virus Transmission Dynamics

West Nile Virus (WNV) is a member of the viral family Flaviviridae and is considered to be a classic arbovirus (arthropod-borne virus). Arboviruses are a large group of viruses transmitted by blood-feeding insects. WNV is transmitted by mosquitoes, primarily to birds, but it can sometimes spread to mammalian populations as well (Figure 1). There are two types of mosquito vectors involved in the WNV transmission cycle: 1) Enzootic vectors – which feed primarily on birds (and are referred to as bird-biting vectors) and 2) Bridge vectors – which feed on both birds and mammals, but primarily on mammals.

WNV was first isolated in the West Nile district of Uganda in 1937. WNV was initially endemic only in the eastern hemisphere, but spread to the western hemisphere in 1999, where it was first discovered in the greater New York City area. The first positive dead bird was reported in 2001 in Southern Ontario and the virus has since spread throughout Canada and become endemic. In 2020, in Ontario alone, there were 77 WNV-positive humans, 23 WNV-positive dead birds, 2 WNV-positive horses, and 171 WNV-positive mosquito pools (PHO, 2020).

Mammals are considered dead-end hosts of WNV because they do not produce significant viremia to be able to infect any mosquitoes that feed upon them. Mosquitoes from the genus *Culex* are the main enzootic vectors responsible for amplifying WNV in bird populations. Thus, most control programs emphasize the reduction of *Culex* species populations. Without a significant *Culex* population there is inadequate amplification of WNV and therefore limited risk of human infection.

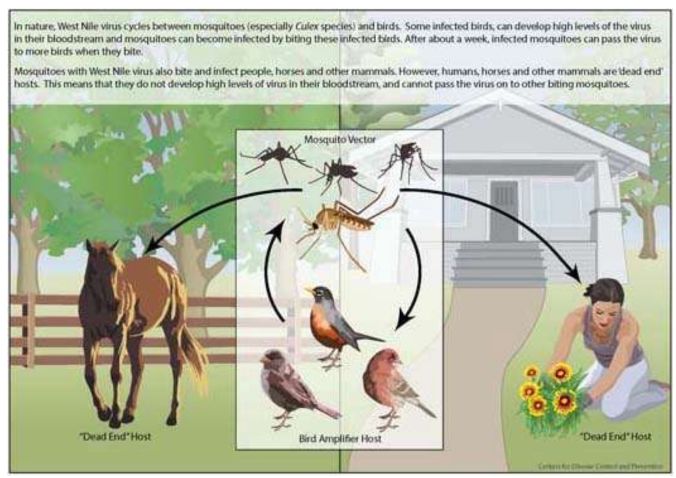

Figure 1. West Nile Virus Transmission Cycle (Centers for Disease Control and Prevention, 2020).

2.1 The Role of *Culex* Species in WNV Transmission

According to most researchers, the major WNV enzootic vectors in Ontario are *Culex pipiens* and *Culex restuans*, which are both very competent vectors. *Cx. restuans* is an early season species and is replaced by *Cx. pipiens* as the season progresses. Research by Dr. Curtis Russell indicates that in certain instances, *Cx. pipiens* may be attracted to humans as well as to birds (Russell, 2008). Thus, *Cx. pipiens* may also serve as a bridge vector of WNV to humans. Other studies have shown that *Cx. pipiens* can transmit WNV to humans, potentially being responsible for up to 80% of human cases (Kilpatrick et al., 2005).

It has been shown that the risk of human disease increases in areas with large numbers of *Culex* mosquitoes throughout the season, whereas areas lacking high numbers of *Culex* mosquitoes have a much lower incidence of human cases. According to Dr. Henry Cuevas (pers. comm.) average daily temperatures must be at least 16.3˚C for amplification of the virus to occur within the mosquito.

Mosquitoes have a complex life cycle, with four discrete stages: egg, larva, pupa and adult (Figure 2). The first three life stages are aquatic and *Culex* mosquitoes thrive in organically enriched standing water.

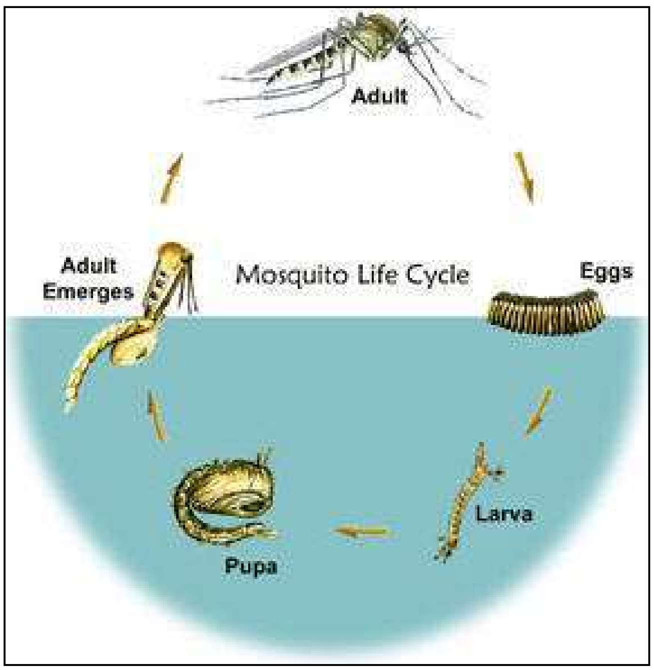

Figure 2. Mosquito Life Cycle (United States Environmental Protection Agency, 2020, recreated from a publication by D.M. Wood).

3.0 Eastern Equine Encephalitis Virus Transmission Dynamics

Eastern Equine Encephalitis virus (EEEV) is a member of the viral family Togaviridae and is considered to be a classic arbovirus (arthropod-borne virus). EEEV is transmitted by mosquitoes, primarily to birds, but it can sometimes spread to mammalian populations as well (Figure 3). There are two types of mosquito vectors involved in the EEEV transmission cycle: 1) Enzootic vectors – which feed primarily on birds (and are referred to as bird-biting vectors) and 2) Bridge vectors – which feed on both birds and mammals, but primarily on mammals.

EEEV was first discovered in Massachusetts, USA in 1831. There are four lineages of EEEV of which Group I is endemic to North America and the Caribbean and is the main cause of human related cases. Groups IIA, IIB and III are primarily responsible for equine illness in Central and South America. The first positive horse was reported in 1938 in Southern Ontario.

Mammals are thought to be dead-end hosts of EEEV because they do not produce significant viremia to be able to infect any mosquitoes that feed upon them. The mosquito *Culiseta melanura* is the main enzootic vector responsible for amplifying EEEV in bird populations. Without a significant *Culiseta melanura* population there is inadequate amplification of EEEV and therefore limited risk of human infection.

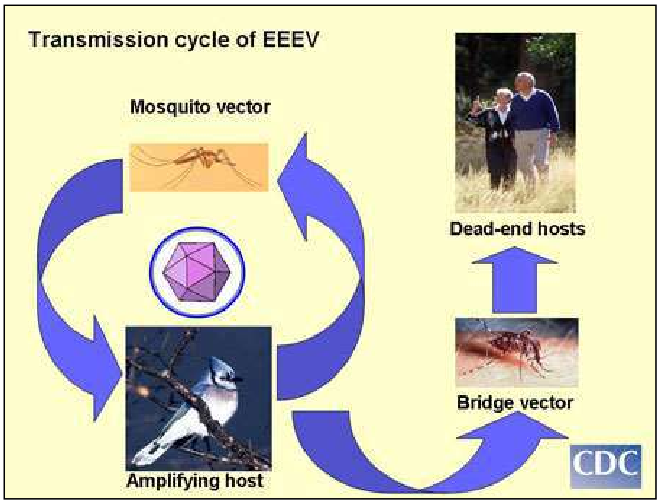

Figure 3. Eastern Equine Encephalitis Virus Transmission Cycle (Atlantic Pest Solutions, 2020).

3.1 The Role of *Culiseta melanura* in EEEV Transmission

Culiseta melanura is the main enzootic vector of EEEV in Ontario. The preferred habitat of this species is freshwater, hardwood swamps where they lay their eggs in the underground crypts in the root mats of trees. While this mosquito will occasionally bite humans, their preference is for an avian host. As a result, contracting EEEV from a bite of *Culiseta melanura* is not considered a significant risk to humans. Transmission to humans is more commonly associated with bridge vectors, such as *Aedes vexans*, *Coquillettidia perturbans*, and some species of the genus *Culex*. In 2020, there were no EEEV-positive mosquito pools reported (PHO, 2020).

Reports of human infection are very rare with the United States reporting an average of 5-10 human cases per year. (CDC, 2020). No human EEEV cases were reported in Canada in 2020.

Horses are susceptible to EEEV infection and some cases can be fatal. However, infected horses are not considered to be of significant risk to humans because, like humans, they are thought to be dead-end hosts (CDC, 2020). In 2020, seven (7) EEEV-positive horses were reported in Ontario (CAHSS, 2020).

4.0 West Nile Virus Activity Summary for Canada, 2020

4.1 West Nile Virus Human Cases in Canada, 2020

A total of ninety-four (94)[1] West Nile virus (WNV) cases have been reported in Canada in 2020 (Figure 4). Eighty-one (81) were clinical cases of which eleven (15) were classified West Nile Virus Neurological syndrome, twenty-seven (27) were classified as West Nile Virus Non-Neurological syndrome and thirty-nine (39) were unclassified. There were five (5) asymptomatic infections reported. There have been no deaths associated with WNV reported to PHAC in 2020.

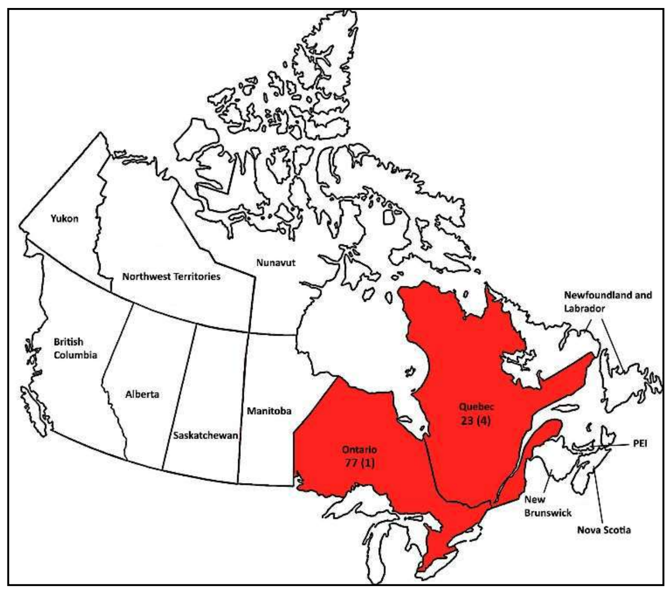

Figure 4. Total Human West Nile Virus cases in Canada, 2020. Red areas indicate WNV-positive cases. Number of reported cases marked within the province with asymptomatic cases in brackets. (Reported by PHAC as of November 13, 2020. Ontario results provided by PHO).

[1] An additional 10 human cases reported by PHO were not included in the PHAC numbers at time of report.

4.2 West Nile Virus Positive Bird Cases in Canada, 2020

Dead birds were collected and submitted to Canadian Wildlife Health Cooperative (CWHC) as part of the 2020 surveillance season. In Canada, samples were submitted from the eastern, western and central parts of the country. In total, 41 birds tested positive for WNV. The birds that tested positive were submitted from Ontario (23), Quebec (18) and Saskatchewan (1). (Figure 5).

Figure 5. West Nile Virus positive bird cases in Canada, 2020. Red areas indicate WNV-positive cases. Number of reported cases in brackets. (Numbers confirmed from CWHC as of December 3, 2020).

4.3 West Nile Virus Positive Mosquito Cases in Canada, 2020

A total of 198 mosquito pools tested positive for West Nile virus in Canada in 2020 (Figure 6). The majority of positive mosquito pools (n=171) were reported from Ontario, followed by Quebec (n=22) and Manitoba (n=5).

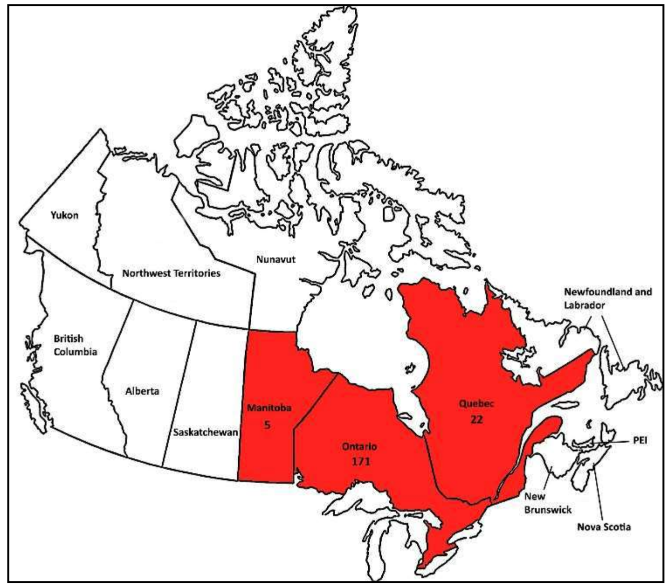

Figure 6. West Nile Virus positive mosquito pools in Canada, 2020. Red areas indicate WNV-positive cases. Number of reported cases in brackets. (Reported by PHAC and PHO as of October 10, 2020).

5.0 West Nile Virus Activity Summary for Ontario, 2020

5.1 West Nile Virus Human Cases in Ontario, 2020

As of November 25, 2020, 77 human WNV cases have been reported in 15 different health units (Figure 7). The positive cases were from Toronto (28), Durham (10), York (7), Halton (6), Ottawa (6) Peel (4), Hamilton (3), Niagara (3), Windsor-Essex (3), Hastings Prince Edward (2), and one each from Brant, Eastern Ontario, Lambton, Middlesex-London and Wellington-Dufferin-Guelph.

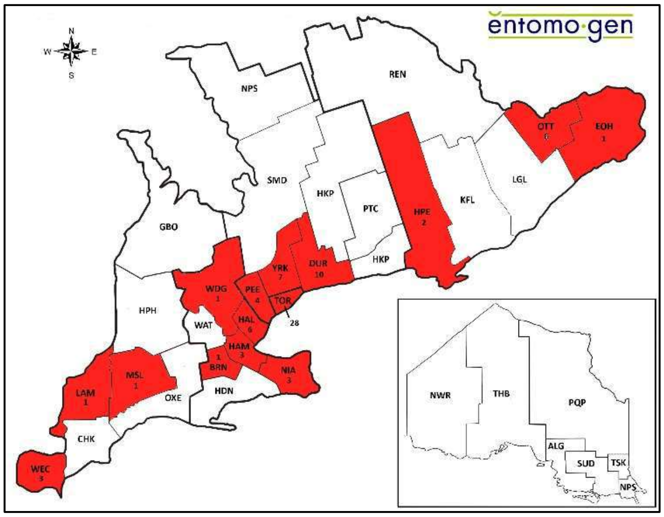

Figure 7. West Nile Virus human cases in Ontario, 2020. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by Public Health Ontario as of November 25, 2020).

5.2 West Nile Virus Positive Bird Cases in Ontario, 2020

Twenty-three (23) WNV-positive birds were reported from nine Health Units in Ontario in 2019 (Figure 8). Seven (7) American Crows (*Corvus brachyrhynchos*), four (4) Red Tailed Hawks (*Buteo jamaicensis*), three (3) Coopers Hawks (*Accipiter cooperii*), three (3) Great Horned Owls (*Bubo virginianus),* two (2) Double-Crested Cormorants (*Phalacrocorax auratus)*, one (1) Bald Eagle (*Haliaeetus leucocephalus*), one (1) Canada Goose (*Branta canadensis*), one (1) Common Raven (*Corvus corax*) and one (1) Sharp Shinned Hawk (*Accipiter striatus*) were positive for WNV.

The WNV-positive birds were reported from Toronto (8), Niagara (4), Durham (2), Northwestern (2), Simcoe Muskoka (2), York (2), and one each from Grey Bruce, Haliburton, Kawartha, Pine Ridge and Wellington-Dufferin-Guelph.

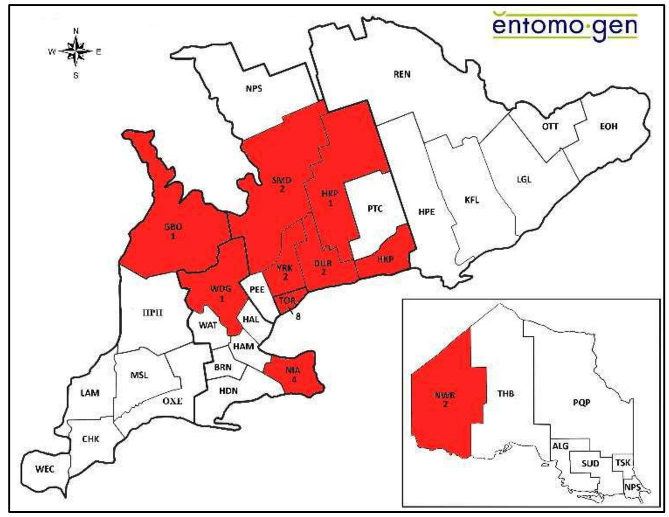

Figure 8. West Nile Virus positive birds in Ontario, 2020. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by CWHC as of December 3, 2020).

5.3 West Nile Virus Positive Equine Cases in Ontario, 2020

Two (2) WNV-positive horses were reported in 2020, one each from Haliburton, Kawartha Pine Ridge and Thunder Bay.

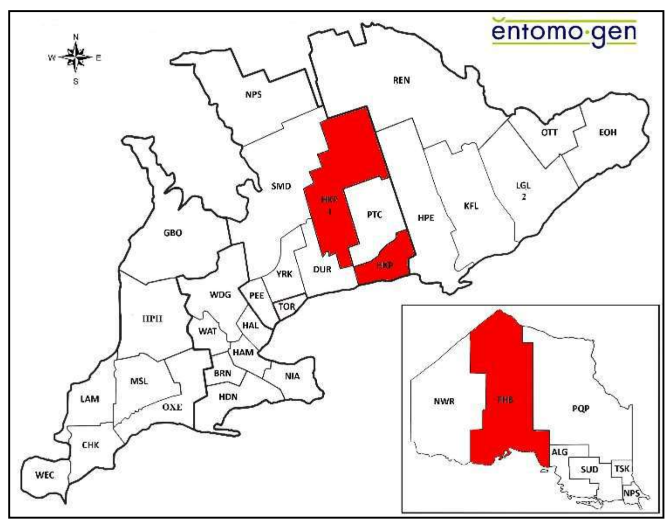

Figure 9. West Nile Virus positive horses in Ontario, 2020. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by CAHSS as of October 22, 2020).

5.4 West Nile Virus Positive Mosquito Cases in Ontario, 2020

From mosquito surveillance conducted by provincial health units, 171 WNV-positive mosquito pools were confirmed from 18 separate Ontario Health Units in 2020 (Figure 10).

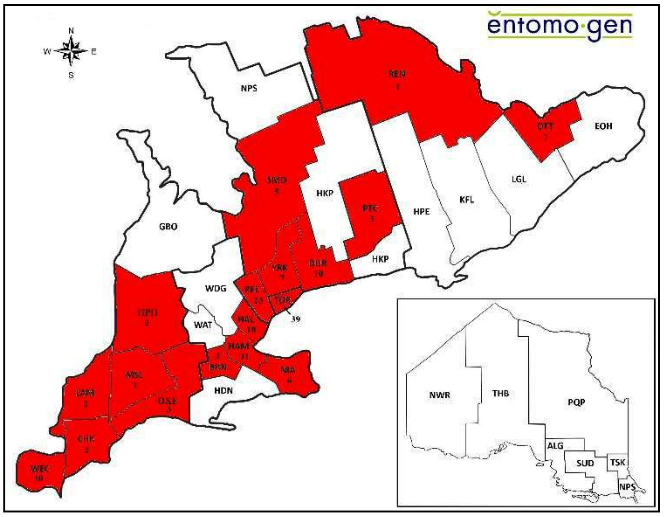

Figure 10. West Nile Virus positive mosquitoes in Ontario, 2020. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by Public Health Ontario as of October 14, 2020).

The positive pools were from Toronto (39), Windsor-Essex (30), Peel (23), Halton (18), Hamilton (11), Durham (10), Ottawa (7), York (7), Niagara (6), Simcoe Muskoka (5), Peterborough (3), Southwestern Public Health (3), Chatham-Kent (2), Huron Perth (2), Lambton (2), Brant (1), Middlesex London (1) and Renfrew (1).

6.0 Eastern Equine Encephalitis Virus Activity Summary for Ontario, 2020

6.1 Eastern Equine Encephalitis Virus Equine Cases in Ontario, 2020

Eight (8) EEEV-positive horses were reported from 4 Health Units in Ontario in 2020. (Figure 11). The cases were reported in Lambton (3), Leeds, Grenville and Lanark (2), Simcoe Muskoka (2) and Grey Bruce (1).

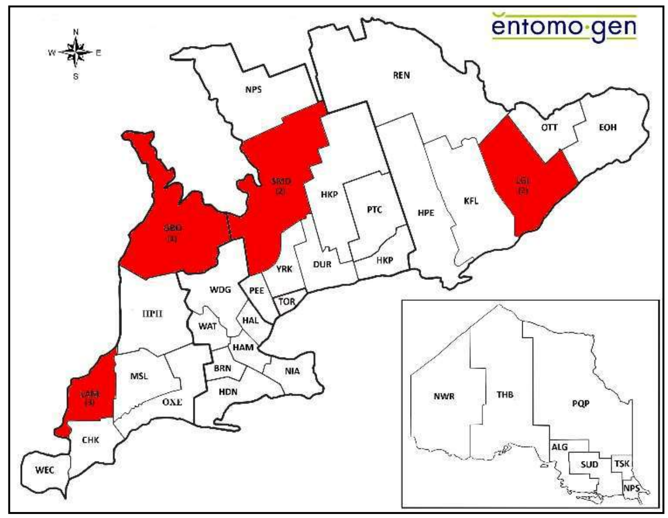

Figure 11. Eastern Equine Encephalitis Virus positive horses in Ontario, 2020. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by CAHSS as of October 22, 2020).

6.2 West Nile Virus Human and Mosquito Cases in Ontario, 2020

There were no EEEV-positive humans or mosquitoes in 2020.

7.0 Timing of WNV-positive *Culex pipiens/restuans* in Grey Bruce Public Health based on 2020 Temperatures

Based on an accumulated degree-day model used by Public Health Ontario, the rate at which WNV replicates within the adult females of *Culex pipiens/restuans* depends on ambient temperatures. Below 18.3˚C (average daily field temperature) there is no extrinsic incubation of WNV but above this temperature threshold the virus will replicate in the mosquito. 380 accumulated degree-days were required for 50% of infected *Culex pipiens/restuans* mosquitoes to test positive for WNV. Figure 12a shows the total accumulated degree-days that occurred during the 2020 season in Ontario, highlighting Grey Bruce Public Health in red. According to this model, there appeared to be insufficient heat units in 2020 for amplification of the virus in *Culex* spp. mosquitoes.

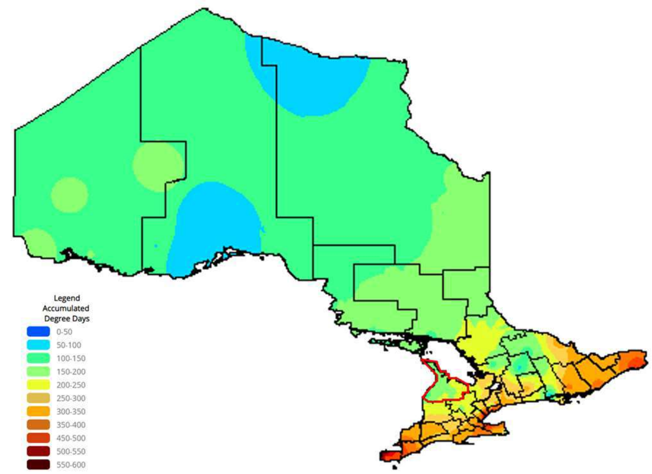

Figure 12a. Accumulated Degree-Day Graph for Ontario, highlighting Grey Bruce Public Health, 2020

Figure 12b shows the gradual increase in Accumulated degree-days (grey shaded area) that occurred during the 2020 season in Grey Bruce Public Health. In total, there were 158.6 accumulated degree days, based on temperature readings taken from the Wiarton A Station.

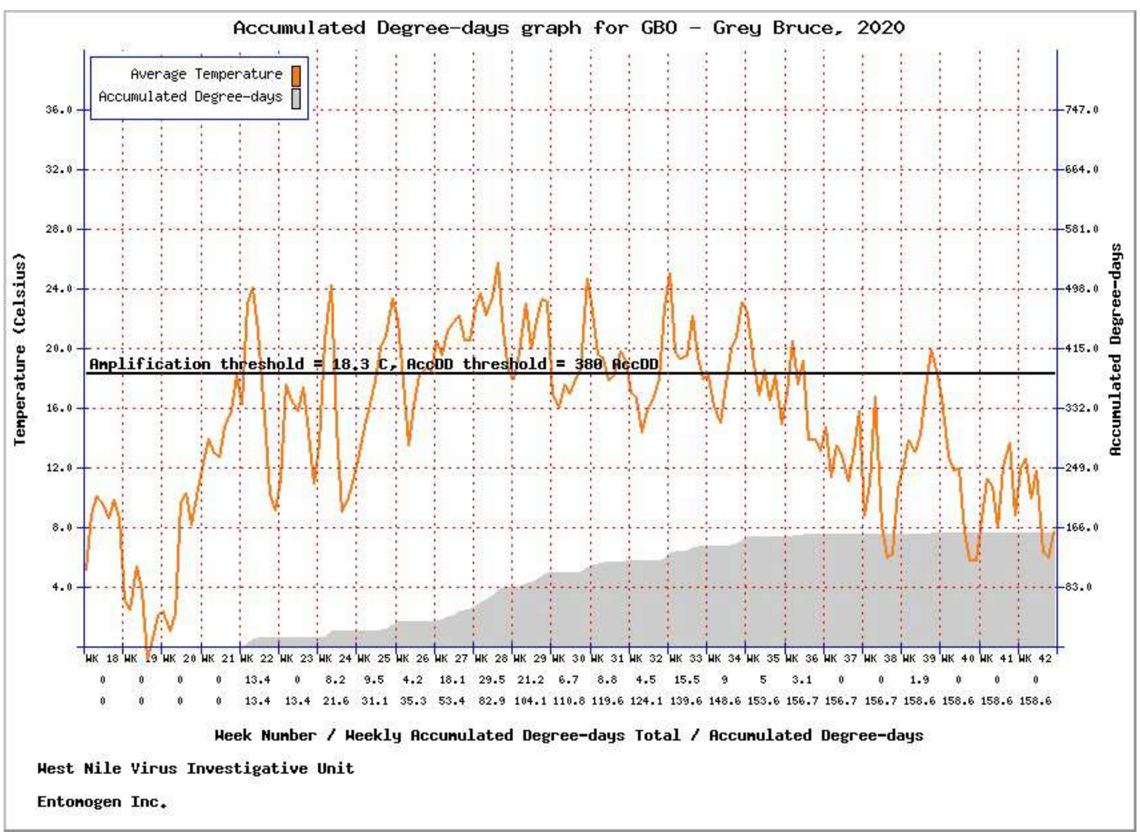

Figure 12b. Accumulated Degree-Day Graph for Grey Bruce Public Health, 2020.

8.0 Grey Bruce Public Health Adult Mosquito Surveillance Data, 2020

A total of eighty-five (85) traps were submitted from 17 different sites during the 2020 trapping season. Figure 13 shows the location of all 17 sites within Grey Bruce Public Health.

An average of 5 traps per week were submitted to Entomogen Inc. for processing, over 17 weeks. Traps were sorted to a maximum subsample of up to 150 mosquitoes. Any additional mosquitoes were labeled and stored as extras. Sorted mosquitoes were identified to the species level and individuals of the same species were pooled for subsequent viral testing. Appendix A shows a summary of the number of mosquitoes trapped in each of the 17 sites within Grey Bruce Public Health.

In total, there were 8,161 mosquitoes collected, of which 10 were unidentifiable (i.e., damaged females) and 94 were unidentified males. A subsample of 3,852 mosquitoes was examined under a dissecting microscope.

Figure 13. Adult mosquito trap sites for Grey Bruce Public Health, 2020 (number of positive pools highlighted in red balloons)

8.1 Mosquito Species Collected in Grey Bruce Public Health, 2020

Figure 14 shows the species found in Grey Bruce Public Health throughout the season. The majority of mosquitoes were non vector species (~64%), which are of no significant concern with regards to WNV. Enzootic vectors, or bird-biting mosquitoes, composed primarily of *Culex pipiens/restuans*, made up approximately 34% of the species collected. Research indicates that *Cx. pipiens* may be attracted to humans as well as to birds (Russell, 2008). Therefore, humans may have come in contact with blood feeding *Culex* as well.

Potential bridge vector species, capable of biting an infected bird and transmitting the virus from the infected bird to a human, horse, or other mammal are highlighted in pink in Figure 14. These species made up 2% of the species identified from traps collected in 2020; thus, humans living within Grey Bruce Public Health may have come in contact with blood feeding *Aedes/Ochlerotatus* mosquitoes.

Table 1 lists the mosquito species identified from sites in Grey Bruce Public Health. The primary WNV enzootic vector was *Culex pipiens/restuans* (1.48% of the population) and the primary WNV bridge vector was *Ochlerotatus stimulans* (15.01% of the population). Three (3) *Culiseta melanura* – the primary EEEV vector – were collected from traps submitted in 2020.

8.2 Trap Index of Groups by Week in Grey Bruce Public Health, 2020

The **trap index (TI)** is a useful tool for summarizing trap data and for comparing different time periods and locations (Figure 15). We use TI to show population fluctuations of a particular group of mosquitoes (enzootic vectors vs. bridge vectors). TI is the average number of females per taxon per trap night.

There was a low bridge vector population throughout most of the season, with a peak TI value of 160.7 in week 29. At the same time, a significant enzootic population, consisting mostly of *Cx. pipiens/restuans*, also peaked at week 32 with a TI value of 4.5.

8.3 Mosquito Species Distribution among sites in Grey Bruce Public Health, 2020

The pie charts in Figure 16 show WNV enzootic species (yellow), WNV bridge species (pink), and non-vectors (green). Site TAH produced the highest percentage of WNV enzootic vectors (28.6%) and site TBM produced the highest percentage of WNV bridge vectors (89.6%). Overall, site SVCAB had the highest number of identified mosquitoes while site TAH had the lowest number. Total numbers and percentages for all sites can be found in Appendix B.

Table 1. Mosquitoes identified from Sites in Grey Bruce Public Health in 2020

	WNV Enzootic Vectors				
57	*Culex pipiens/restuans*	1.48%		**Non Vectors**	
13	*Culex species*	0.34%	1143	*Coquillettidia perturbans*	29.67%
			813	*Ochlerotatus broad-banded*	21.11%
	WNV Bridge Vectors		315	*Ochlerotatus black-legged*	8.18%
578	*Ochlerotatus stimulans*	15.01%	84	*Ochlerotatus provocans*	2.18%
399	*Ochlerotatus canadensis*	10.36%	62	*Aedes/Ochlerotatus species*	1.61%
135	*Aedes vexans*	3.50%	46	*Aedes cinereus*	1.19%
107	*Ochlerotatus trivittatus*	2.78%	6	*Uranotaenia sapphirina*	0.16%
23	*Anopheles punctipennis*	0.60%	2	*Culiseta morsitans*	0.05%
23	*Ochlerotatus triseriatus*	0.60%	2	*Ochlerotatus excrucians*	0.05%
17	*Ochlerotatus japonicus*	0.44%	1	*Anopheles earlei*	0.03%
11	*Anopheles walkeri*	0.29%	1	*Culex territans*	0.03%
9	*Anopheles quadrimaculatus*	0.23%	1	*Ochlerotatus fitchii*	0.03%
			1	*Psorophora ferox*	0.03%
	EEEV Vectors				
3	*Culiseta melanura*	0.08%			

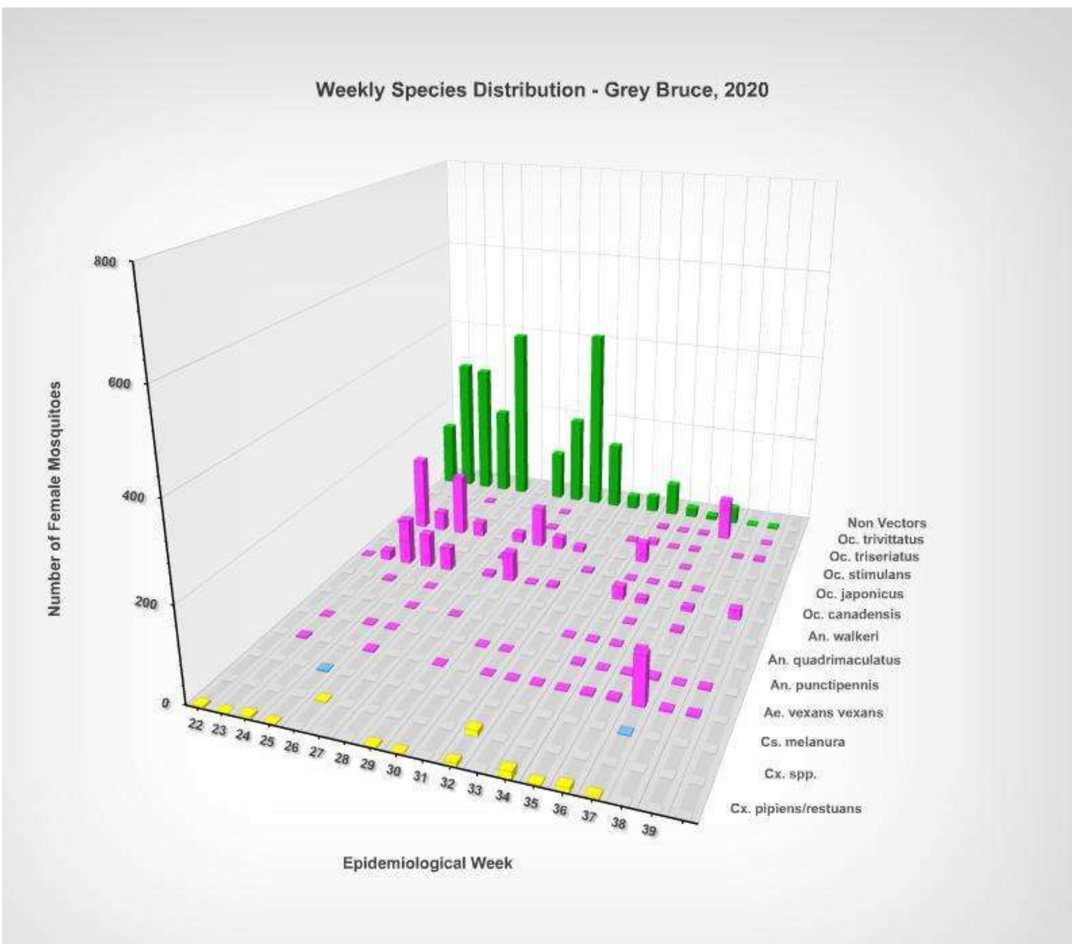

Figure 14. Species Distribution for Grey Bruce Public Health, 2020. Yellow bars represent bird-biting (WNV enzootic vector) species, pink bars represent WNV bridge vectors, and green bars represent non-vector species.

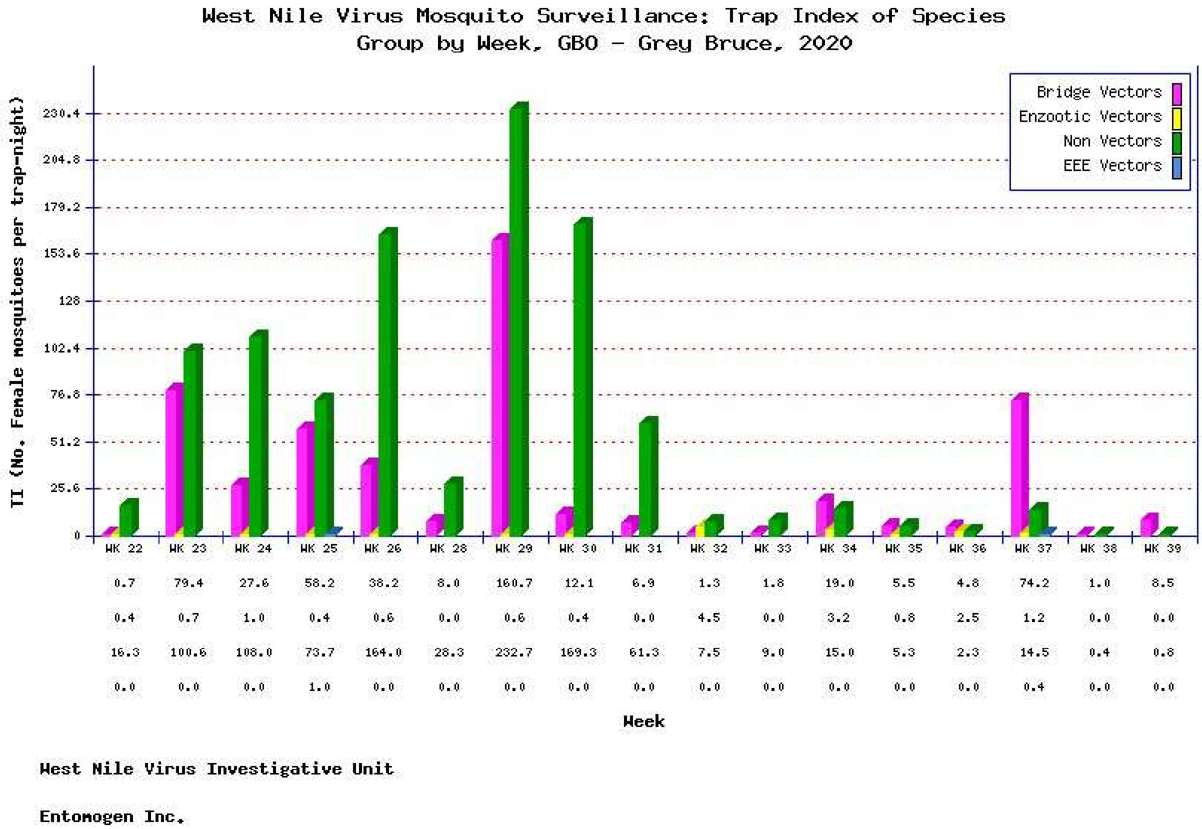

Figure 15. Trap Indices of WNV Enzootic Vectors (yellow), WNV Bridge Vectors (pink) and Non-Vectors (green), Grey Bruce Public Health, 2020

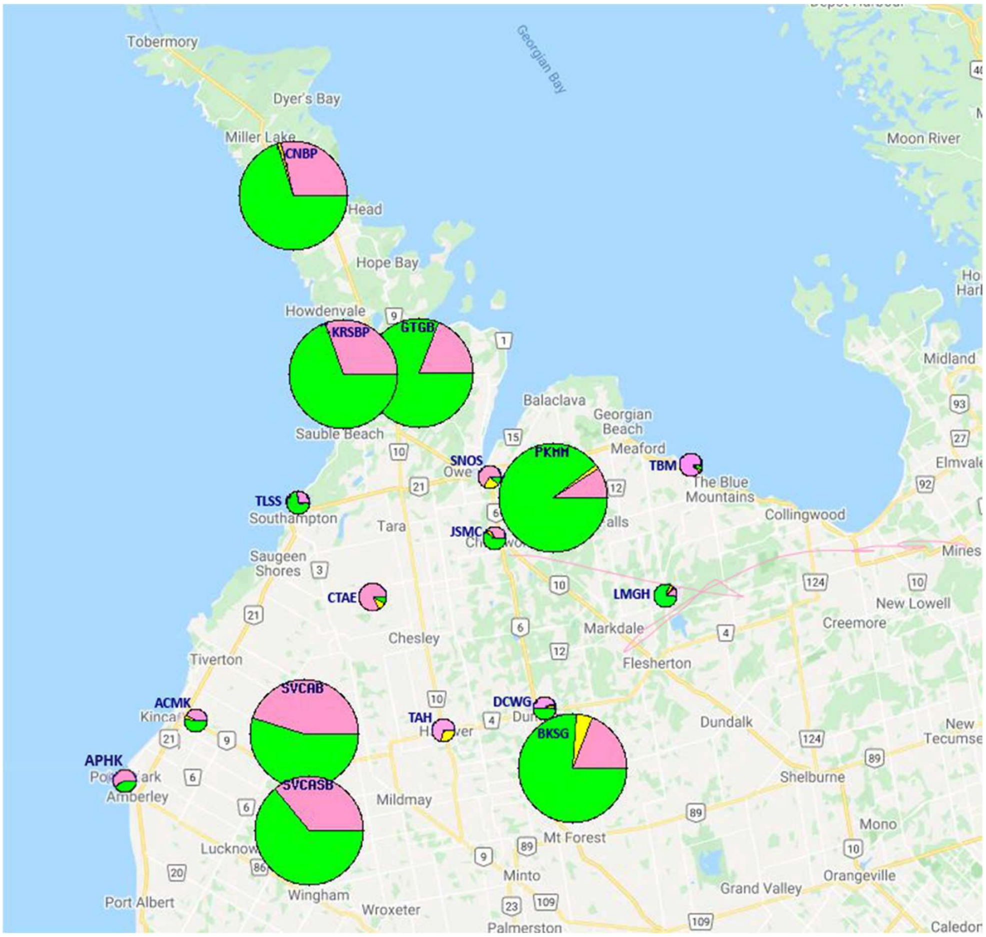

Figure 16. Grey Bruce Public Health Key Species Distribution Map, 2020.

9.0 West Nile Virus and Eastern Equine Encephalitis Virus Analysis, 2020

Appendix C outlines the order of preference for viral testing. A maximum of three pools per trap were sent for WNV and/or EEEV viral testing.

9.1 West Nile Virus Viral Testing Results

Of the 145 pools sent for testing, personnel from the Microbiology Laboratory of Entomogen’s West Nile virus Investigative Unit did not confirm any WNV-positive pools from Grey Bruce Public Health (Table 2).

9.2 Eastern Equine Encephalitis Virus Analysis, 2020

There were two (2) pools of *Culiseta melanura* – the primary vector for Eastern Equine Encephalitis – tested from Grey Bruce Public Health in 2020. Both pools tested negative for EEEV.

10.0 Summary

The percentage of *Culex pipiens/restuans*, the main enzootic mosquito complex, was determined to be 1.48% of the total mosquito population in the 2020 season.

The threshold value of 380 accumulated degree-days was not crossed in 2020 however, WNV human, avian cases or mosquito pools were reported in Grey Bruce Public Health. Not enough accumulated degree-days along with the low WNV activity within its borders would put Grey Bruce Public Health Unit at **low** risk for endemic WNV transmission to humans.

In 2020, there were seven (7) EEEV-positive horses reported in Ontario. Although one (1) of the cases was reported in Grey Bruce Public Health Unit, there was a very low number (n=3) of the main EEEV enzootic vector (*Culiseta melanura*) and therefore, Grey Bruce Public Health Unit would be at a **low** risk for endemic EEEV transmission to humans.

11.0 Recommendations

Temperatures in 2020 were slightly warmer than 2019, however yearly global average temperatures continue to increase so does the risk for potential WNV infections.

In 2016, *Ae. albopictus* and *Ae. aegypti* – two exotic species that are vectors of many diseases including dengue, zika and chikungunya - were discovered during routine surveillance in Windsor-Essex County. These findings demonstrate the importance for continued mosquito surveillance activities, not only for virus detection, but also to monitor the potential introduction of invasive species.

WNV positive humans, birds and mosquitoes were confirmed in Ontario in 2020, therefore the trapping and viral testing program should be continued at the same intensity throughout the 2021 mosquito season.

Since there was one (1) EEEV-positive horse reported in 2020, Grey Bruce Public Health Unit may want to consider more targeted trapping as well as expanding EEEV testing to include bridge vectors such as *Ochlerotatus canadensis*, *Aedes vexans vexans* and *Coquillettidia perturbans*.

12.0 Acknowledgments

Entomogen Inc. would like to thank Stephanie Nickels, Andrew Barton, Jennifer Scott, and Grey Bruce Public Health for their contributions to the West Nile Virus and Eastern Equine Encephalitis program in 2020.

13.0 Appendices

Appendix A – Trap Numbers for the Seventeen Sites in Grey Bruce Public Health

Site Code	Total Mosquitoes Identified	Number of Extras	Number of Traps	Latitude	Longitude	City
ACMK	34	0	5	44.165398	-81.59069	Kincardine
APHK	44	0	5	44.513351	-81.36029	Ripley
BKSG	355	340	5	44.088457	-80.7407	Holstein
CNBP	417	140	5	45.00355	-81.37013	Stoke's Bay
CTAE	172	0	5	44.363441	-81.192861	Arran-Elderslie
DCWG	118	0	5	44.183871	-80.8038	Durham
GTGB	330	175	5	44.721105	-81.088459	Wiarton
JSMC	106	0	5	44.456985	-80.915767	Chatsworth
KRSBP	481	375	5	44.719218	-81.258703	Wiarton
LMGH	142	0	5	44.365889	-80.5326	Flesherton
PKMM	381	300	5	44.521345	-80.785348	Bognor
SNOS	9	0	5	44.554797	-80.929142	Owen Sound
SVCAB	589	2500	5	44.143721	-81.3461	Brockton
SVCASB	469	375	5	43.987853	-81.3354	Teesewater
TAH	7	0	5	44.148964	-81.0316	Hanover
TBM	134	0	5	44.574298	-80.474473	Thornbury
TLSS	64	0	5	44.513351	-81.36029	Southhampton

Appendix B – Number and Percentages of Identified Mosquitoes Separated into Respective Groups in Grey Bruce Public Health

Site Code	WNV Enzootic Vectors	WNV Bridge Vectors	EEEV Vectors	Non Vectors	Total Mosquitoes Identified
ACMK	2 (5.9%)	14 (41.2%)	0 (0.0%)	18 (52.9%)	34
APHK	0 (0.0%)	25 (56.8%)	0 (0.0%)	19 (43.2%)	44
BKSG	16 (4.5%)	67 (18.9%)	0 (0.0%)	272 (76.6%)	355
CNBP	5 (1.2%)	120 (28.8%)	0 (0.0%)	292 (70.0%)	417
CTAE	17 (9.9%)	141 (82.0%)	0 (0.0%)	14 (8.1%)	172
DCWG	2 (1.7%)	57 (48.3%)	0 (0.0%)	59 (50.0%)	118
GTGB	0 (0.0%)	62 (18.8%)	0 (0.0%)	268 (81.2%)	330
JSMC	2 (1.9%)	39 (36.8%)	0 (0.0%)	65 (61.3%)	106
KRSBP	2 (0.4%)	146 (30.4%)	1 (0.2%)	332 (69.0%)	481
LMGH	8 (5.6%)	18 (12.7%)	0 (0.0%)	116 (81.7%)	142
PKMM	5 (1.3%)	34 (8.9%)	0 (0.0%)	342 (89.8%)	381
SNOS	2 (22.2%)	6 (66.7%)	0 (0.0%)	1 (11.1%)	9
SVCAB	2 (0.3%)	263 (44.7%)	2 (0.3%)	322 (54.7%)	589
SVCASB	1 (0.2%)	168 (35.8%)	0 (0.0%)	300 (64.0%)	469
TAH	2 (28.6%)	5 (71.4%)	0 (0.0%)	0 (0.0%)	7
TBM	4 (3.0%)	120 (89.6%)	0 (0.0%)	10 (7.5%)	134
TLSS	0 (0.0%)	17 (26.6%)	0 (0.0%)	47 (73.4%)	64

Appendix C – West Nile Virus and Eastern Equine Encephalitis Virus Viral Testing Order of Preference

Ontario Ministry of Health and Long-Term Care Mosquito Species for Viral Testing	
1	*Culex pipiens/restuans (WNV)*
2	*Culex salinarius (WNV)*
3	*Ochlerotatus japonicus (WNV)*
4	*Culex tarsalis* (WNV)
5	*Aedes vexans vexans/ Aedes vexans nipponi (WNV)*
6	*Ochlerotatus triseriatus (WNV)*
7	*Anopheles punctipennis (WNV)*
8	*Ochlerotatus trivittatus (WNV)*
9	*Anopheles walkeri (WNV)*
10	*Ochlerotatus stimulans (WNV)*
11	*Anopheles quadrimaculatus (WNV)*
12	*Ochlerotatus canadensis (WNV)*
*	*Culiseta melanura (EEEV)*
**	*Aedes albopictus/Stegomyia albopicta (WNV)*

* Since this species is found in low numbers and is the main enzootic vector for EEEV, it is to be tested for EEEV as part of the three-pool limit

** Since this species is found in very low numbers and is a highly competent vector, it is suggested that it be tested for WNV as part of the three-pool limit

14.0 References

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