2022 WEST NILE VIRUS YEAR END REPORT

Grey Bruce Public Health

Decemeber 15, 2022

CONTENTS

1.0 OVERVIEW

The adult mosquito surveillance program was a key component of the overall West Nile Virus (WNV) program for Grey Bruce Public Health in the 2022 season.

There were no WNV-positive humans, horses or mosquitoes in Grey Bruce Public Health in 2022 however; there was one (1) WNV-positive bird reported. In Ontario, a total of twenty-three (23) human cases, forty-six WNV-positive birds (46) and eighty-nine (89) WNV-positive mosquito pools were reported (Public Health Ontario, 2022).

There were no Eastern Equine Encephalitis (EEEV) positive horses or mosquito pools reported in 2022 (PHO, 2022).

2.0 WEST NILE VIRUS TRANSMISSION DYNAMICS

West Nile Virus (WNV) is a member of the viral family Flaviviridae and is 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 2022, in Ontario alone, there were 23 WNV-positive humans, 46 WNV-positive dead birds, and 89 WNV-positive mosquito pools. There were no WNV-positive equine cases reported in 2022. (PHO, 2022).

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.

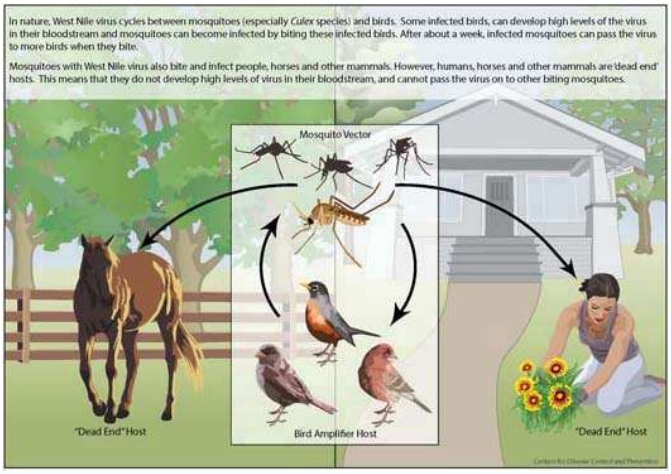

Figure 1. West Nile Virus Transmission Cycle (Centers for Disease Control and Prevention, 2022).

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.

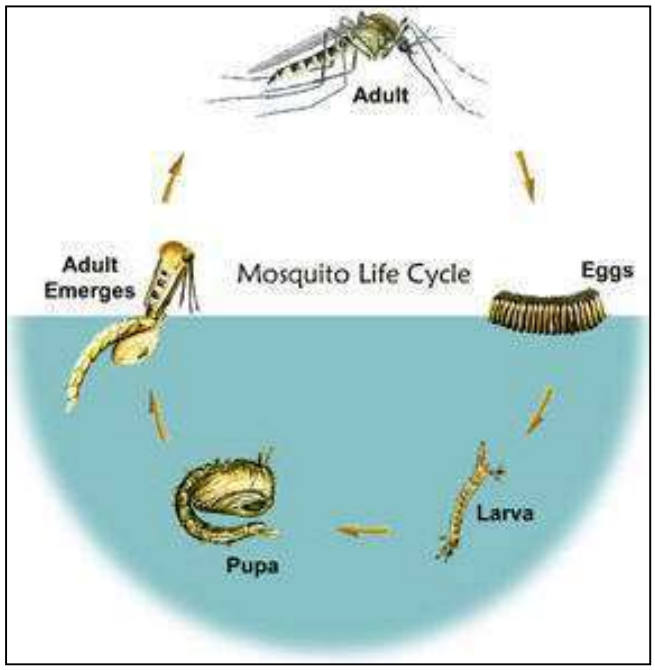

Figure 2. Mosquito Life Cycle (United States Environmental Protection Agency, 2022, recreated from a publication by D.M. Wood).

3.0 EASTERN EQUINE ENCEPHALITIS VIRUS TRANSMISSION DYNAMICS

Eastern Equine Encephalitis virus (EEEV) is a member of the viral family Togaviridae and is 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.

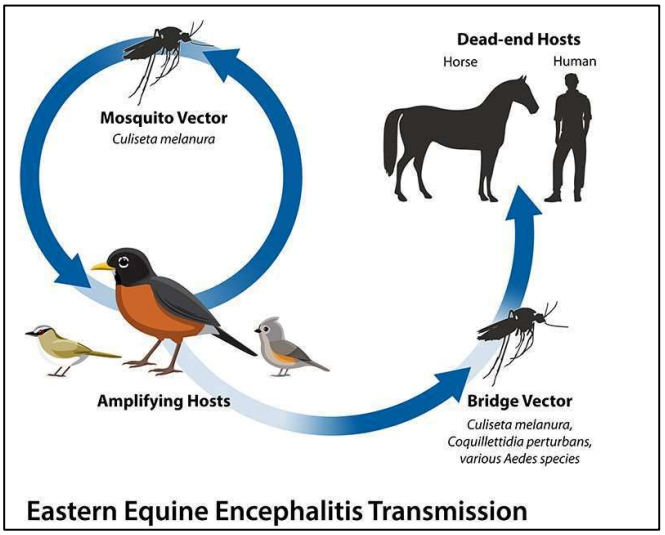

Figure 3. Eastern Equine Encephalitis Virus Transmission Cycle (CDC, 2022).

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*.

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, 2022).

Reports of human infection are rare, with the United States reporting an average of 11 human cases per year. (CDC, 2022). No mosquito, human or equine EEEV cases were reported in Canada in 2022.

4.0 WEST NILE VIRUS ACTIVITY SUMMARY FOR CANADA, 2022

4.1 WEST NILE VIRUS HUMAN CASES IN CANADA, 2022

A total of twenty-five (25) West Nile virus (WNV) confirmed or probable cases have been reported in Canada in 2022 (Figure 4). There have been no deaths associated with WNV reported to PHAC in 2022. Reporting by PHAC is typically three to four weeks behind that of PHO. The numbers presented are an amalgamation of data provided by PHAC and PHO. A full summary report for 2022 has not yet been completed which outline the classification (i.e., West Nile Virus Neurological Syndrome, West Nile Virus Non-Neurological syndrome, Asymptomatic infection) therefore, all 25 WNV-positive human cases are reported as unclassified.

Figure 4. Total Human West Nile Virus cases in Canada, 2022. Red areas indicate WNV-positive cases. Number of reported cases marked within the province (Reported by PHAC and PHO as of October 27, 2022).

4.2 WEST NILE VIRUS POSITIVE BIRD CASES IN CANADA, 2022

Dead birds were collected and submitted to Canadian Wildlife Health Cooperative (CWHC) as part of the 2022 surveillance season. In total, 100 birds tested positive for WNV. The birds that tested positive were submitted from Quebec (54), Ontario (46). PHAC also reports eight (8) WNV-positive birds from Manitoba (Figure 5).

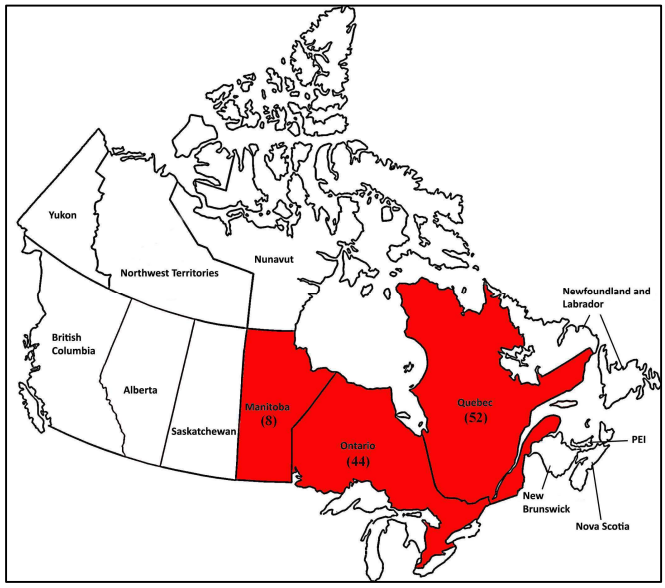

Figure 5. West Nile Virus positive bird cases in Canada, 2022. (Numbers confirmed from PHAC as of October 27, 2022).

4.3 WEST NILE VIRUS POSITIVE EQUINE CASES IN CANADA, 2022

A total of eight (8) West Nile virus (WNV) confirmed or probable equine cases have been reported in Canada in 2022 (Figure 6). The cases were reported in Saskatchewan (n=5), Manitoba (n=2) and Quebec (n=1).

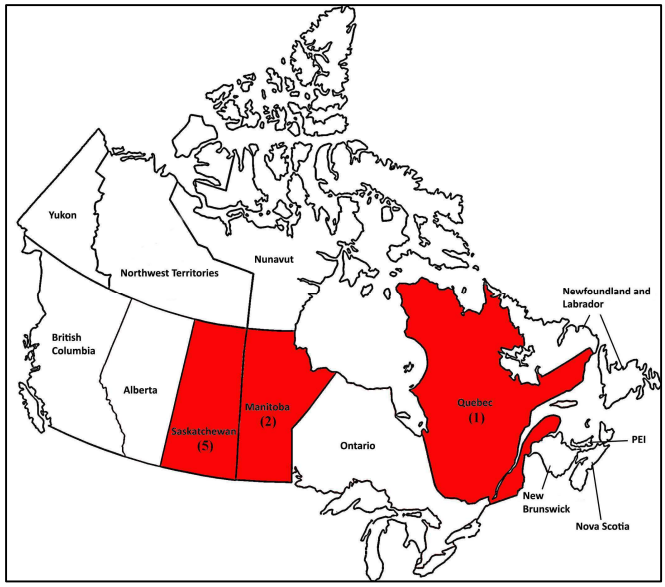

Figure 6. West Nile Virus positive equine cases in Canada, 2022. Red areas indicate WNV-positive cases with numbers indicating the total number of positive horses. (Reported by PHAC as of October 27, 2022).

4.4 WEST NILE VIRUS POSITIVE MOSQUITO CASES IN CANADA, 2022

A total of 128 mosquito pools tested positive for West Nile virus in Canada in 2022 (Figure 7). Most positive mosquito pools were reported from Ontario (n=89) with the remaining cases being reported in Manitoba (n=39).

Figure 7. West Nile Virus positive mosquito pools in Canada, 2022. Red areas indicate WNV-positive cases with numbers indicating the total number of positive pools. (Reported by PHAC as of October 27, 2022).

5.0 WEST NILE VIRUS ACTIVITY IN THE UNITED STATES, 2022

As of October 18, 2022, a total of 766 cases of West Nile virus disease in people have been reported by the CDC. Of these, 528 (69%) were classified as neuroinvasive disease and 238 (31%) were classified as non-neuroinvasive disease. Exact numbers of mosquito, bird and equine cases was not readily available.

6.0 WEST NILE VIRUS ACTIVITY SUMMARY FOR ONTARIO, 2022

6.1 WEST NILE VIRUS HUMAN CASES IN ONTARIO, 2022

As of October 27, 2022, 23 human WNV cases have been reported from 8 different health units (Figure 8). The positive cases were from Toronto (11), Peel (3), Durham (2), Hamilton (2), Windsor-Essex (2), Chatham-Kent (1), Halton (1) and Waterloo (1).

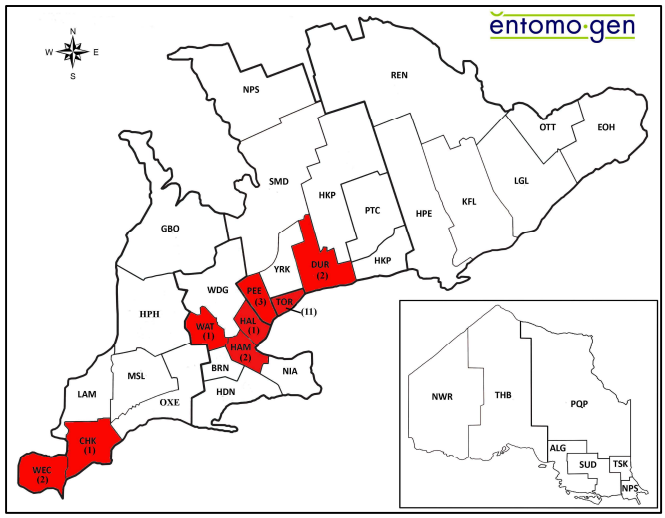

Figure 8. West Nile Virus human cases in Ontario, 2022. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by Public Health Ontario as of October 27, 2022).

6.2 WEST NILE VIRUS POSITIVE BIRD CASES IN ONTARIO, 2022

Forty-six (46) WNV-positive birds were reported from sixteen Health Units in Ontario in 2022. The number of WNV-positive birds presented below and in Figure 9 is based on results provided to Entomogen by Lenny Shirose of CWHC on October 6, 2022. These are the most complete data available at the time of reporting. There is no geographical or species information currently available for two of the forty-six WNV-positive birds.

In total nine different species of bird tested positive for WNV. Twenty-seven (27) American Crows (*Corvus brachyrhynchos*), four (4) Red Tailed Hawks (*Buteo jamaicensis*), four (4) Merlin's (*Falco columbarius*), two (2) Blue Jays (*Cyanocitta cristata*), two (2) Coopers Hawks (*Accipiter cooperii*), two (2) Common Ravens (*Corvus corax*), one (1) Broad-Winged Hawk (*Buteo platypterus*), one (1) Northern Goshawk (*Accipiter gentilis*) and one (1) Ring-Billed Gull (*Larus delawarensis*).

The WNV-positive birds were reported from Toronto (14), Ottawa (5), Waterloo (4), Wellington-Dufferin-Guelph (4), Northwestern (3), Halton (2), Niagara (2), York (2), and one WNV-positive bird from each of the following Health Units; Grey Bruce, Haliburton-Kawartha-Pine Ridge, Hamilton, Middlesex-London, Peel, Renfrew, Sudbury and Thunder Bay.

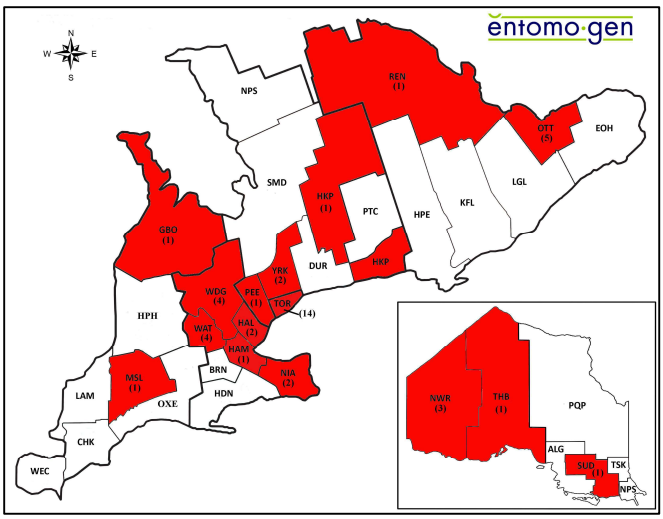

Figure 9. West Nile Virus bird cases in Ontario, 2022. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by CWHC as of October 6, 2022).

6.3 WEST NILE VIRUS POSITIVE EQUINE CASES IN ONTARIO, 2022

According to the Canadian Animal Health Surveillance System (CAHSS), there were no WNV-positive cases in the equine population in 2022.

6.4 WEST NILE VIRUS POSITIVE MOSQUITO CASES IN ONTARIO, 2022

From mosquito surveillance conducted by provincial health units, 89 WNV-positive mosquito pools were confirmed from 12 separate Ontario Health Units in 2022 (Figure 11).

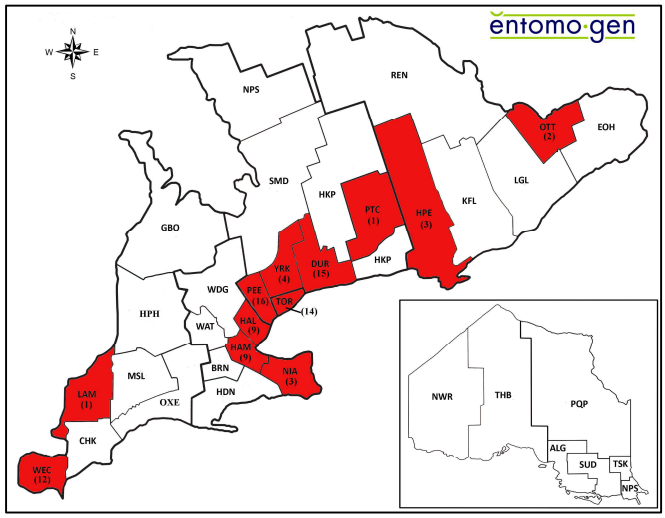

Figure 11. West Nile Virus positive mosquitoes in Ontario, 2022. Red areas indicate WNV-positive cases. Number of reported cases recorded within health unit boundary. (Reported by Public Health Ontario as of October 27, 2022).

The positive pools were from Peel (16), Durham (15), Toronto (14), Windsor-Essex (12), Halton (9), Hamilton (9), York (4), Hastings Prince Edward (3), Niagara (3), Ottawa (2), Lambton (1) and Peterborough (1).

7.0 EASTERN EQUINE ENCEPHALITIS VIRUS ACTIVITY SUMMARY FOR ONTARIO, 2022

There was no Eastern Equine Encephalitis Virus (EEEV) activity reported in the human, equine or mosquito population for Ontario in 2022.

8.0 TIMING OF WNV-POSITIVE *CULEX PIPIENS/RESTUANS* IN GREY BRUCE PUBLIC HEALTH BASED ON 2022 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 are required for 50% of infected *Culex pipiens/restuans* mosquitoes to test positive for WNV. Figure 13a shows the total accumulated degree-days that occurred during the 2022 season in Ontario, highlighting Grey Bruce Public Health in red. According to this model, there appeared to be insufficient heat units in 2022 for amplification of the virus in *Culex* spp. mosquitoes.

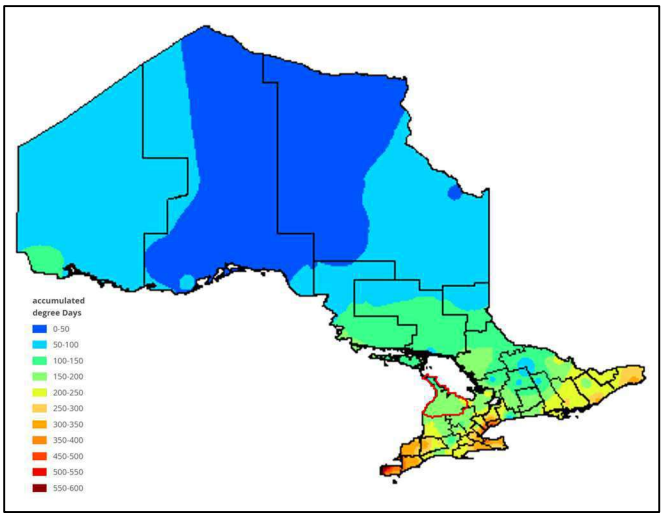

Figure 13a. Accumulated Degree-Day Graph for Ontario, highlighting Grey Bruce Public Health, 2022.

Figure 13b shows the gradual increase in Accumulated degree-days (grey shaded area) that occurred during the 2022 season in Grey Bruce Public Health. In total, there were 128.8 accumulated degree days, based on temperature readings taken from the Wiarton A Station.

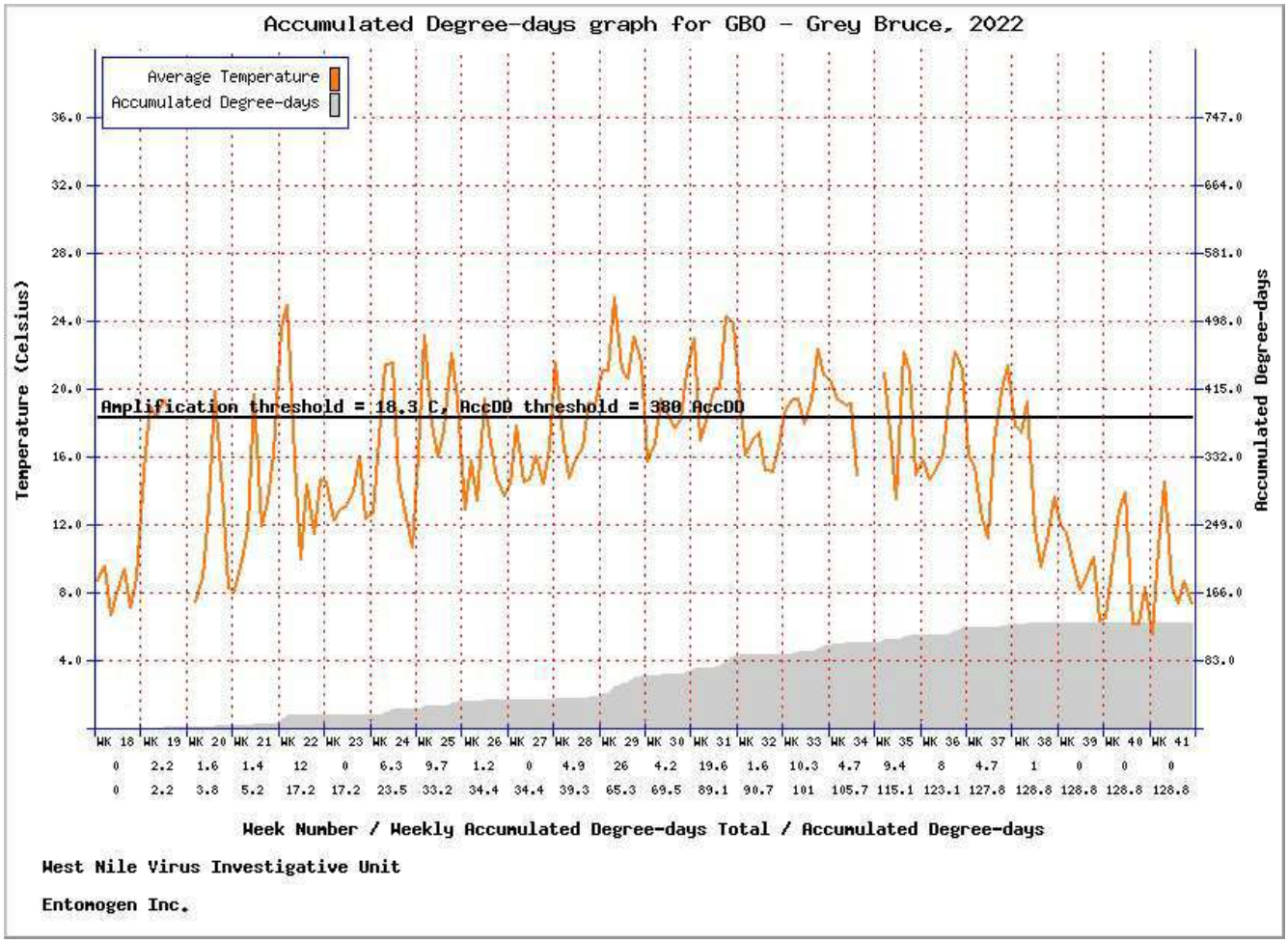

Figure 13b. Accumulated Degree-Day Graph for Grey Bruce Public Health, 2022.

9.0 GREY BRUCE PUBLIC HEALTH ADULT MOSQUITO SURVEILLANCE DATA, 2022

A total of eighty-five (85) traps were submitted from 17 different sites during the 2022 trapping season. Figure 14 shows the location of all 17 sites within Grey Bruce Public Health.

Trapping began the week of May 30th, 2022 (epi week 22) with 10 traps being collected and submitted to Entomogen Inc. for processing. 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.

A subsample of 3,740 mosquitoes was examined under a dissecting microscope, of which 12 were unidentifiable (i.e., damaged females) and 322 were unidentified males.

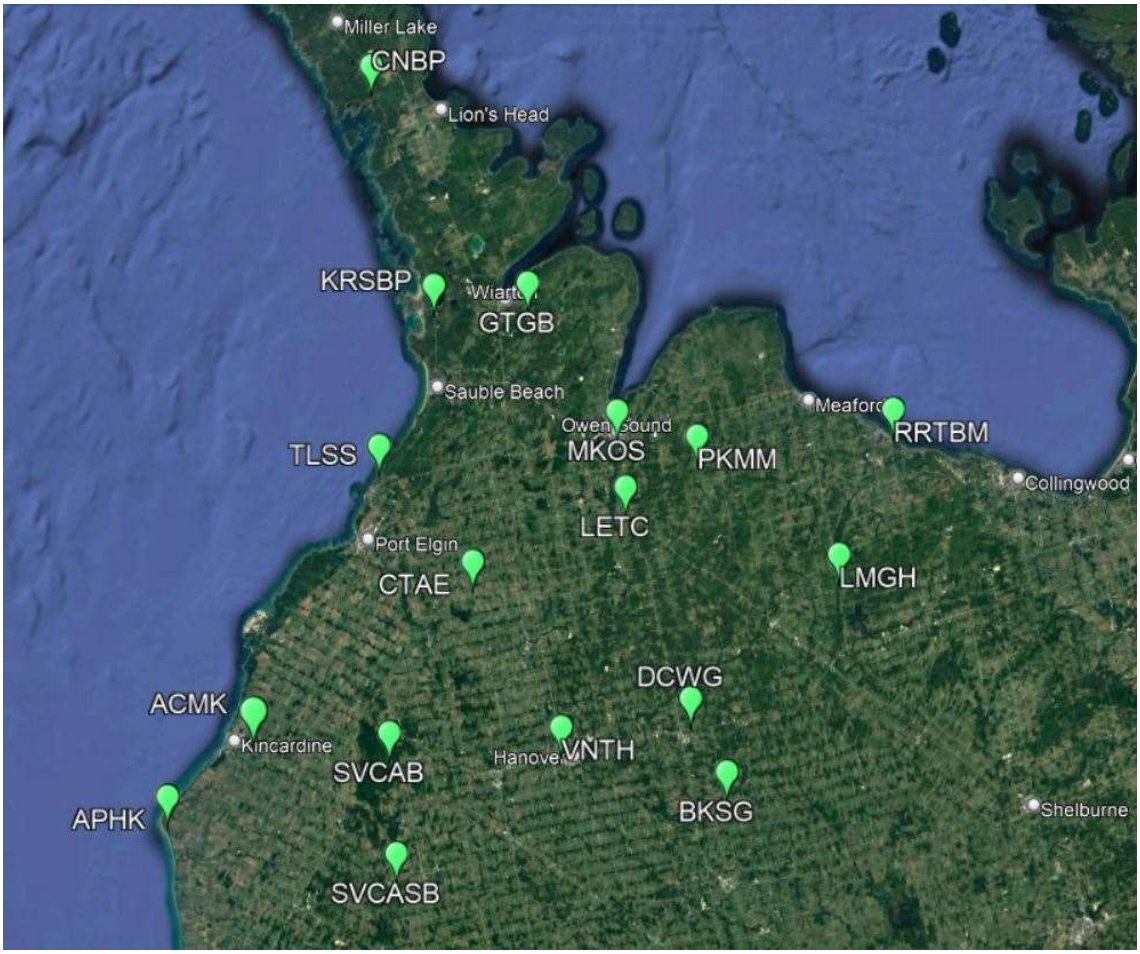

Figure 14. Adult mosquito trap sites for Grey Bruce Public Health, 2022

9.1 MOSQUITO SPECIES COLLECTED IN GREY BRUCE PUBLIC HEALTH, 2022

Figure 15 shows the species found in Grey Bruce Public Health throughout the season. Enzootic vectors, or bird-biting mosquitoes, composed primarily of *Culex pipiens/restuans*, made up approximately 4% 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 15. These species made up the approximately 4% of the species identified from traps collected in 2022; thus, humans living within Grey Bruce Public Health may have come in contact with blood feeding *Aedes/Ochlerotatus* mosquitoes. The majority of species collected (~54%) were non-vector species, which are of no significant concern with regards to WNV.

Table 1 lists the mosquito species identified from sites in Grey Bruce Public Health. The primary WNV enzootic vector was *Culex pipiens/restuans* (3.58% of the population) and the primary WNV bridge vector was *Ochlerotatus canadensis* (20.29% of the population). *Culiseta melanura* – the primary EEEV vector – was collected from traps submitted in 2022, making up 1% of the total.

Table 1. Mosquitoes identified from Sites in Grey Bruce Public Health in 2022

	WNV Enzootic Vectors				
122	*Culex pipiens/restuans*	3.58%			
5	*Culex species*	0.15%		**Non Vectors**	
2	*Culex salinarius*	0.06%	1033	*Coquillettidia perturbans*	30.33%
			323	*Ochlerotatus broad-banded*	9.48%
	WNV Bridge Vectors		203	*Aedes/Ochlerotatus species*	5.96%
691	*Ochlerotatus canadensis*	20.29%	153	*Ochlerotatus black-legged*	4.49%
317	*Aedes vexans vexans*	9.31%	89	*Aedes cinereus*	2.61%
179	*Ochlerotatus stimulans*	5.26%	12	*Psorophora ferox*	0.35%
61	*Ochlerotatus trivittatus*	1.79%	4	*Anopheles earlei*	0.12%
56	*Anopheles punctipennis*	1.64%	3	*Anopheles species*	0.09%
50	*Ochlerotatus japonicus*	1.47%	3	*Culex territans*	0.09%
27	*Anopheles walkeri*	0.79%	2	*Ochlerotatus excrucians*	0.06%
21	*Ochlerotatus triseriatus*	0.62%	2	*Uranotaenia sapphirina*	0.06%
13	*Anopheles quadrimaculatus*	0.38%	1	*Culiseta minnesotae*	0.03%
	EEEV Vectors				
34	*Culiseta melanura*	1.00%			

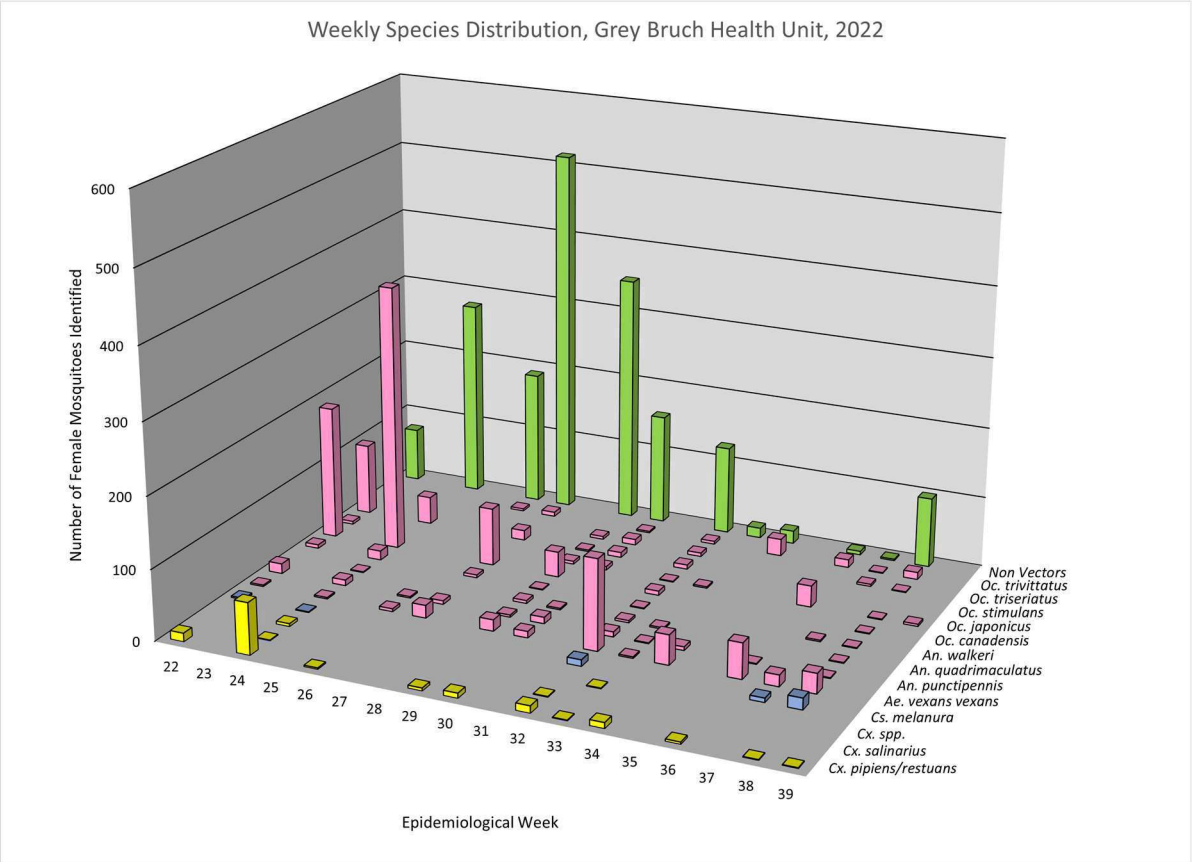

Figure 15. Species Distribution for Grey Bruce Public Health, 2022. Yellow bars (WNV enzootic vector species), pink bars (WNV bridge vector species), blue bars (EEEV vector species) and green bars (non-vector species).

9.2 TRAP INDEX OF GROUPS BY WEEK IN GREY BRUCE PUBLIC HEALTH, 2022

The **trap index (TI)** is a useful tool for summarizing trap data and for comparing different time periods and locations (Figure 16). 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.

A low enzootic population, consisting almost entirely of *Cx. pipiens/restuans* peaked early in the season in week 24, with a TI value of 7.9. The bridge vector population was considerably larger and more consistent throughout the year, but also peaked in week 24 with a TI value of 83.0.

9.3 MOSQUITO SPECIES DISTRIBUTION AMONG SITES IN GREY BRUCE PUBLIC HEALTH, 2022

Total numbers and percentages broken down by groups are provided in Table 2. Overall, site SVCAB had the highest number of identified mosquitoes (N=675) while site ACMK had the lowest number (N=12). Site APHK produced the highest percentage of WNV enzootic vectors (49.0%) while the highest percentage of WNV bridge vectors (69.9%) were collected from site KRSBP. For EEEV vectors site SVCAB produced the highest percentage (3.9%) and majority of the *Culiseta melanura* collected in Grey Bruce. Of the thirty-four (34) specimens identified, twenty-six (26) of them were collected from this SVCAB.

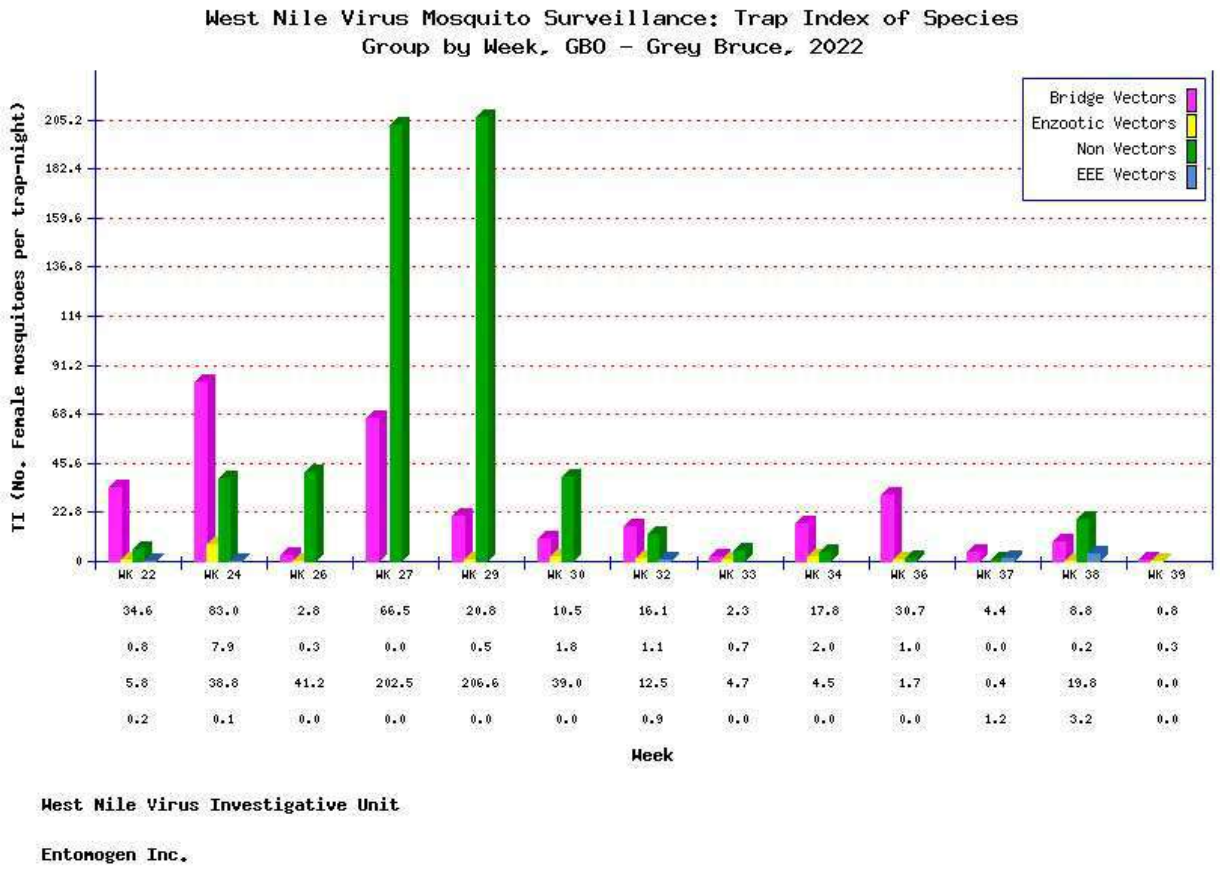

Figure 16. Trap Indices of WNV Enzootic Vectors (yellow), WNV Bridge Vectors (pink) and non-Vectors (green), Grey Bruce Public Health, 2022.

Table 2. Grey Bruce Public Health Key Species Distribution Report, 2022

Site Code	WNV Enzootic Vectors	WNV Bridge Vectors	EEEV Vectors	Non Vectors	Total Mosquitoes Identified	Number of Extras	Number of Traps
ACMK	4 (33.3%)	5 (41.7%)	0 (0.0%)	3 (25.0%)	12	0	5
APHK	58 (69.0%)	11 (13.1%)	0 (0.0%)	15 (17.9%)	84	0	5
BKSG	3 (4.9%)	34 (55.7%)	0 (0.0%)	24 (39.3%)	61	0	5
CNBP	1 (0.2%)	169 (39.3%)	7 (1.6%)	253 (58.8%)	430	100	5
CTAE	28 (44.4%)	25 (39.7%)	0 (0.0%)	10 (15.9%)	63	0	5
DCWG	6 (5.9%)	59 (58.4%)	0 (0.0%)	36 (35.6%)	101	0	5
GTGB	0 (0.0%)	269 (69.9%)	0 (0.0%)	116 (30.1%)	385	600	5
KRSBP	1 (0.3%)	73 (21.9%)	0 (0.0%)	260 (77.8%)	334	300	5
LETC	0 (0.0%)	110 (42.6%)	0 (0.0%)	148 (57.4%)	258	0	5
LMGH	0 (0.0%)	28 (17.4%)	0 (0.0%)	133 (82.6%)	161	0	5
MKOS	12 (10.9%)	56 (50.9%)	1 (0.9%)	41 (37.3%)	110	0	5
PKMM	1 (0.3%)	18 (5.5%)	0 (0.0%)	307 (94.2%)	326	1100	5
RRTBM	2 (1.8%)	77 (67.5%)	0 (0.0%)	35 (30.7%)	114	0	5
SVCAB	1 (0.1%)	349 (51.7%)	26 (3.9%)	299 (44.3%)	675	900	5
SVCASB	0 (0.0%)	92 (63.4%)	0 (0.0%)	53 (36.6%)	145	0	5
TLSS	0 (0.0%)	3 (3.6%)	0 (0.0%)	81 (96.4%)	84	0	5
VNTH	12 (19.0%)	37 (58.7%)	0 (0.0%)	14 (22.2%)	63	0	5

10.0 WEST NILE VIRUS AND EASTERN EQUINE ENCEPHALITIS VIRUS ANALYSIS, 2022

Identified mosquitoes are pooled according to species, location, collection date and number of specimens. Any species of concern (see Appendix A) are sent to our diagnostics laboratory, emDx, for viral analysis using Real Time RT-PCR.

10.1 WEST NILE VIRUS VIRAL TESTING RESULTS, 2022

Entomogen staff tested a maximum of three pools per trap submitted, based on recommendations provided by Public Health Ontario (PHO, 2019). From the 85 traps submitted a total of 154 pools were sent for WNV-testing. Table 3 presents the species breakdown and number of pools of each species tested separated into their respective categories. Twelve (12) different species or species complexes were tested for the presence of WNV**.** Enzootic vector *Culex pipiens/restuans* made up 14% (n=22) of the pools tested in Grey Bruce Public Health. Bridge vectors species *Aedes vexans vexans Anopheles punctipennis* and *Ochlerotatus stimulans* combined to account for another 47% (n=73) of the pools that were tested for WNV. There were no confirmed WNV-positive pools reported in Grey Bruce Public Health in 2022.

10.2 EASTERN EQUINE ENCEPHALITIS VIRUS ANALYSIS, 2022

There were a total of thirty-four (34) specimens of *Culiseta melanura* identified from traps submitted in 2022. These were broken down into six (6) separate pools and tested for the presence of EEEV. None of the six pools tested positive for EEEV.

Table 3. Grey Bruce Public Health, Number of Pools Tested for WNV or EEEV by Species in 2022

WNV Enzootic Vectors	**Number of Pools Tested**	**Total Number of Mosquitoes Tested**
Culex pipiens/restuans	22	122
Culex species	3	5
Culex salinarius	2	2
WNV Bridge Vectors		
Aedes vexans vexans	28	244
Anopheles punctipennis	23	41
Ochlerotatus stimulans	22	150
Ochlerotatus canadensis	16	367
Ochlerotatus japonicus	12	50
Anopheles quadrimaculatus	7	10
Ochlerotatus trivittatus	7	16
Anopheles walkeri	6	24
Ochlerotatus triseriatus	6	19
EEEV Vectors		
Culiseta melanura	6	34

11.0 SUMMARY

The percentage of *Culex pipiens/restuans*, the main enzootic mosquito complex, was determined to be 3.58% of the total mosquito population in the 2022 season.

The threshold value of 380 accumulated degree-days was not crossed indicating there were insufficient heat units for amplification of the virus in the enzootic mosquito population. One (1) WNV avian case was reported in Grey Bruce Public Health however, there were no human, equine or mosquito cases.

The lack of significant amplification of the virus along with the limited WNV-activity within its borders would put Grey Bruce Public Health at **low** risk for endemic WNV transmission to humans.

There were no EEEV-positive horses reported in Ontario; however thirty-four (34) *Culiseta melanura* – the main EEEV enzootic vector - were collected in traps submitted by Grey Bruce Public Health. Due to the absence of EEEV activity in mosquitoes, horses or humans in 2022, Grey Bruce Public Health is at **low** risk for endemic EEEV transmission to humans.

12.0 RECOMMENDATIONS

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 range expansion and potential introduction of invasive species.

The main EEEV enzootic vector *Culiseta melanura* was identified in Grey Bruce Public Health in 2022. In addition, EEEV bridge vector species - *Ochlerotatus canadensis*, *Aedes vexans vexans* and *Coquillettidia perturbans* – made up approximately 60% of all mosquito species collected in Grey Bruce Public Health in 2022. Although no EEEV activity was reported in the province of Ontario in 2022, the presence of the EEEV enzootic vectors and high number of EEEV bridge vector species in Grey Bruce Public Health is something that should continue to be monitored.

There was a decrease in the number of WNV positive humans and mosquitoes in Ontario for 2022. WNV detection in the avian population, however, was more than four times greater in 2022 (n=46) than it was in 2021 (n=11), indicating the virus is still circulating within the amplifying host, therefore the trapping and viral testing program should be continued at the same intensity in 2023.

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14.0 APPENDICES

APPENDIX A – MOSQUITO SPECIES: WNV TESTING ORDER OF PREFERENCE

1	*Culex pipiens/restuans*
2	*Culex salinarius*
3	*Ochlerotatus japonicus*
4	*Culex tarsalis*
5	*Aedes vexans vexans*
6	*Ochlerotatus triseriatus*
7	*Anopheles punctipennis*
8	*Ochlerotatus trivittatus*
9	*Anopheles walkeri*
10	*Ochlerotatus stimulans*
11	*Anopheles quadrimaculatus*
12	*Ochlerotatus canadensis*
*	*Aedes albopictus*
*	*Aedes aegypti*

* Since this species may sporadically occur 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

* Since this species may sporadically occur 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

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