2024

West Nile Virus Year End Report

Entomogen Inc.

12/5/2024

CONTENTS

1 Overview

The adult mosquito surveillance program was a key component of the overall mosquito surveillance program for Grey Bruce Public Health in the 2024 season. There were no West Nile Virus (WNV) positive humans, horses or mosquitoes, however three (3) WNV-positive birds were reported in Grey Bruce Public Health in 2024.

In Ontario, a total of sixty-one (61) confirmed and/or probable human cases, one hundred and eight (108) WNV-positive birds, ten (10) WNV-positive horses and two hundred and fourteen (214) WNV-positive mosquito pools were reported (Public Health Ontario, 2024). One (1) Eastern Equine Encephalitis (EEEV) human case (PHAC, 2024), twenty-four (24) EEEV-positive horses (CAHSS, 2024) and two (2) EEEV-positive mosquito pools were reported in 2024 (PHO, 2024).

2 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 2024 Ontario reported sixty-one (61) confirmed and/or probable human cases, one hundred and eight (108) WNV-positive birds, ten (10) WNV-positive horses and two hundred and fourteen (214) WNV-positive mosquito pools. (PHO, 2024)

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.

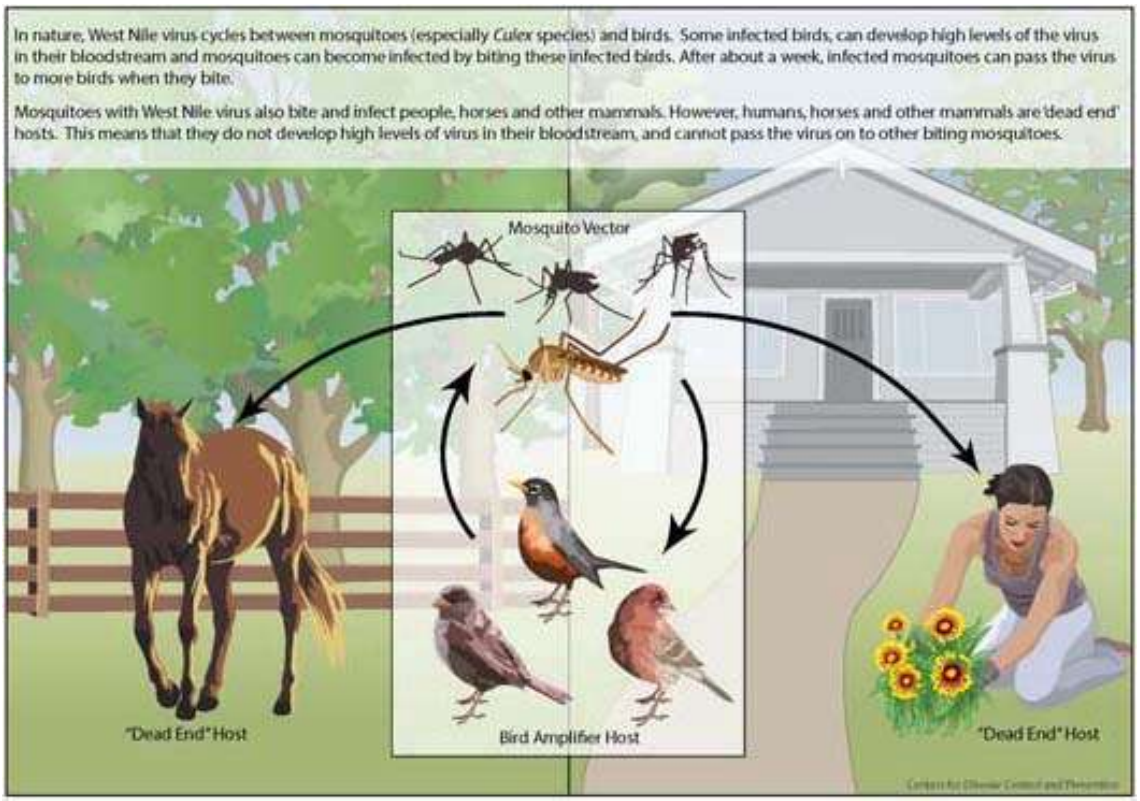

Figure 1. West Nile Virus Transmission Cycle (Centers for Disease Control and Prevention, 2024).

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.

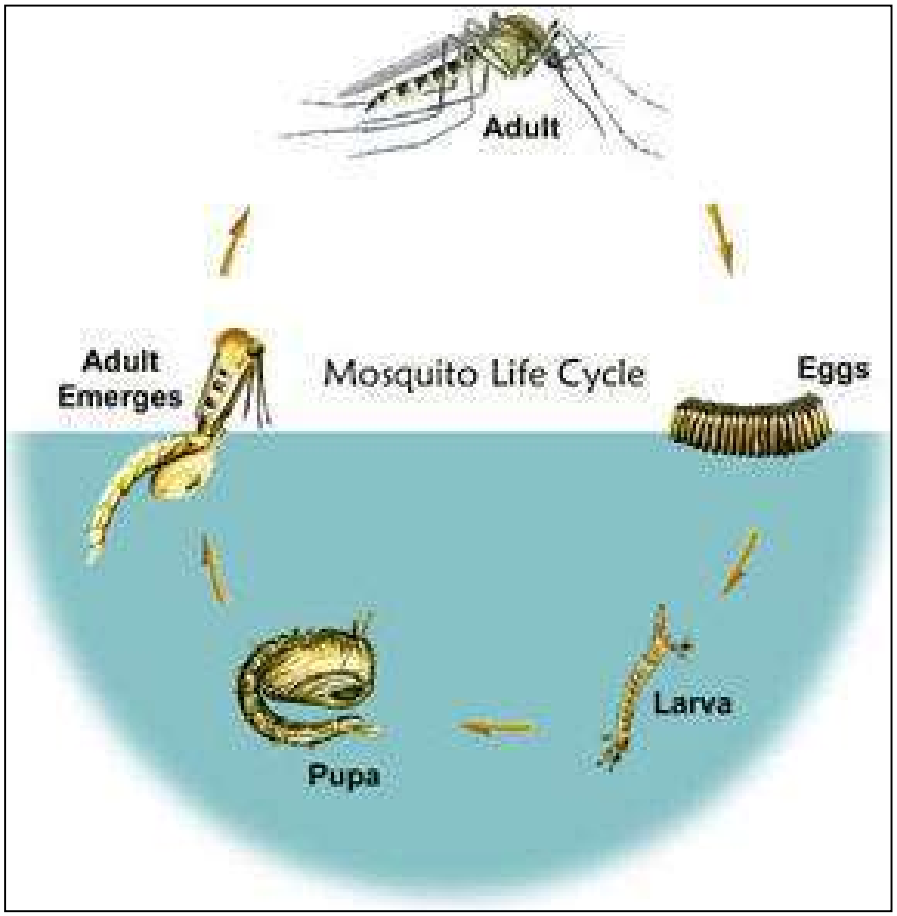

Figure 2. Mosquito Life Cycle (United States Environmental Protection Agency, 2024, recreated from a publication by D.M. Wood).

3 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.

Clinical symptoms of equine encephalitis were first discovered in Massachusetts, USA in 1831, but the name Eastern Equine Encephalitis was not used until 1933 (Armstrong, 2022). 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.

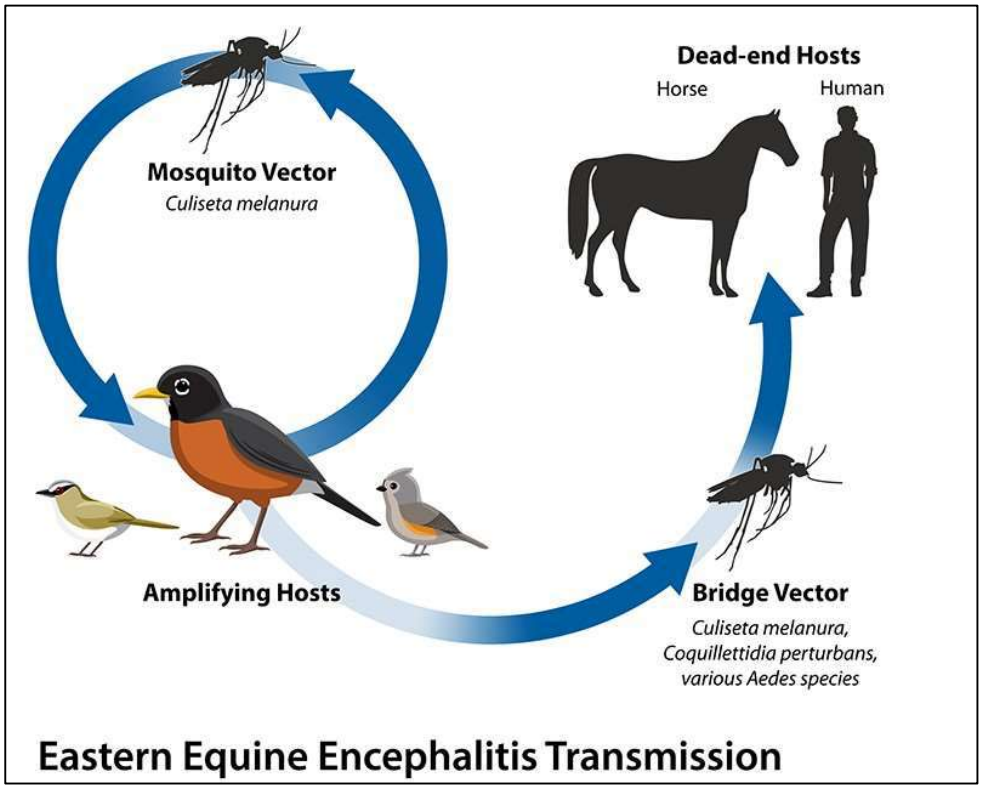

Figure 3. Eastern Equine Encephalitis Virus Transmission Cycle (CDC, 2024).

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, 2024). Reports of human infection are rare, with the United States reporting an average of eleven (11) human cases per year. (CDC, 2024). One (1) EEEV-positive human case, two (2) positive mosquito pools, and twenty-four (24) EEEV-positive horses were reported in Ontario as of October 11, 2024 (PHO, 2024).

4 West Nile Virus Activity Summary for Canada, 2024

The numbers presented below are an amalgamation of data provided by the Public Health Agency of Canada (PHAC), Public Health Ontario (PHO), Canadian Wildlife Health Cooperative (CWHC) and the Canadian Animal Health Surveillance System (CAHSS) as of October 11, 2024.

4.1 West Nile Virus Human Cases in Canada, 2024

A total of one hundred and thirty-two (132) West Nile virus (WNV) confirmed or probable cases have been reported in Canada in 2024 (Table 1). The human cases were reported from Ontario, Quebec, Manitoba, Saskatchewan and British Columbia. There have been no deaths associated with WNV reported to PHAC in 2024. Of the reported human cases 65 were classified as neurological, 30 were classified as non neurological, 12 were unspecified clinical cases, 17 were or currently pending classification and 8 of the cases were asymptomatic (PHAC, 2024).

4.2 West Nile Virus Positive Bird Cases in Canada, 2024

Dead birds were collected and submitted to CWHC as part of the 2024 surveillance season. In total, 322[1] birds tested positive for WNV. The birds that tested positive were submitted from Quebec, Ontario, Manitoba, and Saskatchewan (PHAC, 2024).

4.3 West Nile Virus Positive Equine Cases in Canada, 2024

A total of twenty-seven (28) West Nile virus (WNV) confirmed or probable equine cases have been reported in Canada in 2024 (Table 1). The cases were reported in Saskatchewan, Alberta, Ontario, Quebec and Manitoba (CAHSS, 2024).

4.4 West Nile Virus Positive Mosquito Cases in Canada, 2024

A total of 343 mosquito pools tested positive for West Nile virus in Canada in 2024 (Table 1). Most positive mosquito pools were reported from Ontario with the remaining cases being reported in Manitoba and Saskatchewan (PHAC, 2024). All remaining provinces and territories do not conduct mosquito surveillance.

[1] The total number of avian cases combines data from PHAC and CHWC to provide the most current numbers available for the province of Ontario.

Table 1. Total West Nile Virus cases in Canada, 2024. (Reported by PHAC as of October 11, 2024).

Province	WNV Human Cases	WNV Avian Cases	WNV Equine Cases	WNV Mosquito Pools
Ontario	61[2]	108[3]	10	214
Quebec	64	120	6	N/A
Manitoba	3	16	1	125
Saskatchewan	3	86	7	4
British Columbia	1	0	0	N/A
Newfoundland and Labrador	0	0	0	N/A
Prince Edward Island	0	0	0	N/A
Nova Scotia	0	0	0	N/A
New Brunswick	0	0	0	N/A
Alberta	0	0	7	N/A
Yukon	0	0	0	N/A
Northwest Territories	0	0	0	N/A
Nunavut	0	0	0	N/A
Total	**132**	**330**	**31[4]**	**343**

5 West Nile Virus Activity in the United States, 2024

As of October 2, 2024, a total of 976 cases of West Nile virus disease in people have been reported from 46 states. Of these, 684 (70%) were classified as neuroinvasive disease. Exact numbers of mosquito, bird and equine cases were not readily available.

[2] Human case numbers for Ontario Provided by PHO as of October 11, 2024

[3] Avian cases for Ontario provided by Leonard Shirose of CWHC on September 30, 2024

[4] Equine cases provided by CAHSS as of October 11, 2024

6 West Nile Virus Activity Summary for Ontario, 2024

6.1 West Nile Virus Human Cases in Ontario, 2024

As of October 11, 2024, 61 human WNV cases have been reported from 20 different health units (Table 2). Positive cases were reported by Toronto (9), Ottawa (7), Windsor-Essex (6), Halton (5), Peel (5), Middlesex-London (4), Niagara (3), Waterloo (3), York (3), Algoma (2), Durham (2), Eastern Ontario (2), Kingston-Frontenac and Lennox and Addington (2), Simcoe Muskoka (2), and one (1) human case in each of the following health units; Hamilton, Haldimand-Norfolk, Haliburton-Kawartha-Pine Ridge, Lambton, Peterborough and Timiskaming.

6.2 West Nile Virus Positive Bird Cases in Ontario, 2024

Thirty (30) of the thirty-four (34) provincial health units reported positive avian cases in 2024. As of September 30, 2024, one hundred and eight (108) WNV-positive birds were reported from Health Units in Ontario. The majority of the WNV-positive birds were American Crow (*Corvus brachyrhynchos*), followed by Blue Jay (*Cyanocitta cristata*), Red-Tailed Hawk (*Buteo jamaicensis*), Merlin (*Falco columbarius*), Coopers Hawk (*Accipiter cooperii),* Great Horned Owl (*Bubo virginianus*), Loggerhead Shrike (*Lanius ludovicianus*), American Kestrel (*Falco sparverius*), Bald Eagle (*Haliaeetus leucocephalus*), Broad-Winged Hawk (*Buteo platypterus*) and Sharp-Shinned Hawk (*Accipiter striatus*) (Table 3).

6.3 West Nile Virus Positive Equine Cases in Ontario, 2024

According to the Canadian Animal Health Surveillance System (CAHSS), there were ten (10) WNV-positive cases in the equine population in 2024. The positive cases were reported from Thunder Bay (2), Eastern Ontario (1), Haldimand Norfolk (1), Hastings Prince Edward County (1), Lambton County (1), Middlesex-London (1), Simcoe (1), Sudbury (1) and Wellington, Dufferin, Guelph (1).

6.4 West Nile Virus Positive Mosquito Cases in Ontario, 2024

From mosquito surveillance conducted by provincial health units, 214 WNV-positive mosquito pools were confirmed from 24 separate Ontario Health Units in 2024 (Figure 10). The positive pools were from Toronto (42), Peel (35), Ottawa (20), York (18), Durham (13), Niagara (12), Chatham Kent (11), Hamilton (10), Halton (9), Middlesex-London (9), Windsor-Essex (8), Simcoe-Muskoka (5), Southwestern (4), Haldimand-Norfolk (3), Huron Perth (3), Brant (2), Hastings Prince Edward (2), Waterloo (2), and one (1) WNV-positive pool reported from each of the following Eastern Ontario, Haliburton, Kawartha, Pine Ridge, Kingston-Frontenac and Lennox and Addington, Lambton, Peterborough, and Renfrew (PHO, 2024).

Table 2. West Nile Virus Activity in Ontario, 2024. (Reported by PHO, CWHC and CAHSS as of October 8, 2024).

Health Unit	WNV Human Cases	WNV Avian Cases	WNV Equine Cases	WNV Mosquito Pools
Algoma District	2	2		
Brant County				2
Chatham-Kent		3		11
City of Hamilton	1	4		10
City of Ottawa	7	8		20
City of Toronto	9	6		42
Durham Regional	2	2		13
Eastern Ontario	2	4	1	1
Grey Bruce		3		
Haldimand-Norfolk	1	1	1	3
Haliburton-Kawartha-Pine Ridge District	1	3		1
Halton Regional	5	4		9
Hastings and Prince Edward Counties			1	2
Huron Perth Health Unit		2		3
Kingston-Frontenac and Lennox and Addington	2	4		1
Lambton	1	1	1	1
Leeds-Grenville and Lanark District		10		
Middlesex-London	4	6	1	9
Niagara Regional Area	3	6		12
North Bay Parry Sound District		3		
Northwestern		1		
Peel Regional	5	3		35
Peterborough County-City	1	5		1
Porcupine		1		
Renfrew County and District				1
Simcoe Muskoka District	2	5	1	5
Southwestern Public Health		2		4
Sudbury and District		1	1	
Thunder Bay District		5	2	
Timiskaming	1	5		
Waterloo	3	3		2
Wellington-Dufferin-Guelph		3	1	
Windsor-Essex County	6			8
York Regional	3	2		18
Total	**61**	**108**	**10**	**214**

Table 3. Avian Species Tested Positive for WNV in Ontario, 2024. (CWHC as of October 8, 2024).

Common Name	Species	Number of Positives
American Crow	*Corvus brachyrhynchos*	64
American Kestrel	*Falco sparverius*	1
Bald Eagle	*Haliaeetus leucocephalus*	1
Blue Jay	*Cyanocitta cristata*	18
Broad-Winged Hawk	*Buteo platypterus*	1
Cooper's Hawk	*Accipiter cooperii*	3
Great Horned Owl	*Bubo virginianus*	2
Loggerhead Shrike	*Lanius ludovicianus*	2
Merlin	*Falco columbarius*	4
Red-Tailed Hawk	*Buteo jamaicensis*	11
Sharp-Shinned Hawk	*Accipiter striatus*	1
Total		**108**

7 Eastern Equine Encephalitis Virus Activity Summary for Ontario, 2024

There was one (1) Eastern Equine Encephalitis Virus (EEEV) human case reported in 2024. The case was reported in the City of Ottawa and resulted in a man's death. Additionally, there were twenty-four (24) equine cases, and two (2) EEEV-positive mosquito pools reported for Ontario in 2024. The equine cases were reported from Eastern Ontario, Hastings, Prince Edward Island, Lambton, Leeds, Niagara, North Bay Parry Sound, Ottawa (Table 4). EEEV-positive mosquito pools were reported by the City of Ottawa.

Table 4. Equine Cases of EEEV in Ontario, 2024. (CAHSS as of October 11, 2024).

Date Reported	Region	Health Unit	Virus
30-Jul-24	Lanark County	Leeds, Grenville and Lanark	EEEV
12-Aug-24	City of Ottawa	Ottawa	EEEV
12-Aug-24	Leeds and Grenville	Leeds, Grenville and Lanark	EEEV
14-Aug-24	Lanark County	Leeds, Grenville and Lanark	EEEV
15-Aug-24	Leeds and Grenville	Leeds, Grenville and Lanark	EEEV
15-Aug-24	Prescott and Russell	Eastern Ontario	EEEV
17-Aug-24	Leeds and Grenville	Leeds, Grenville and Lanark	EEEV
17-Aug-24	Lanark County	Leeds, Grenville and Lanark	EEEV
19-Aug-24	Leeds and Grenville	Leeds, Grenville and Lanark	EEEV
26-Aug-24	Lanark County	Leeds, Grenville and Lanark	EEEV
30-Aug-24	City of Ottawa	Ottawa	EEEV
30-Aug-24	Leeds and Grenville	Leeds, Grenville and Lanark	EEEV
3-Sep-24	Parry Sound	North Bay Parry Sound	EEEV
3-Sep-24	Leeds and Grenville	Leeds, Grenville and Lanark	EEEV
11-Sep-24	Niagara Region	Niagara Region	EEEV
12-Sep-24	Hastings County	Hastings Prince Edward County	EEEV
19-Sep-24	Lambton County	Lambton County Public Health	EEEV
23-Sep-24	Hastings County	Hastings Prince Edward County	EEEV
30-Sep-24	Stormont, Dundas and Glengarry	Eastern Ontario	EEEV
2-Oct-24	Leeds and Grenville	Leeds, Grenville and Lanark	EEEV
7-Oct-24	Middlesex County	Middlesex-London	EEEV
7-Oct-24	Prescott and Russell	Eastern Ontario	EEEV
9-Oct-24	Lanark County	Leeds, Grenville and Lanark	EEEV
21-Oct-24	Haldimand County	Haldimand Norfolk	EEEV

8 TIMING OF WNV-POSITIVE *CULEX PIPIENS/RESTUANS* IN GREY BRUCE PUBLIC HEALTH BASED ON 2024 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 4a shows the total accumulated degree-days that occurred during the 2024 season in Ontario, highlighting Grey Bruce Public Health (red arrow).

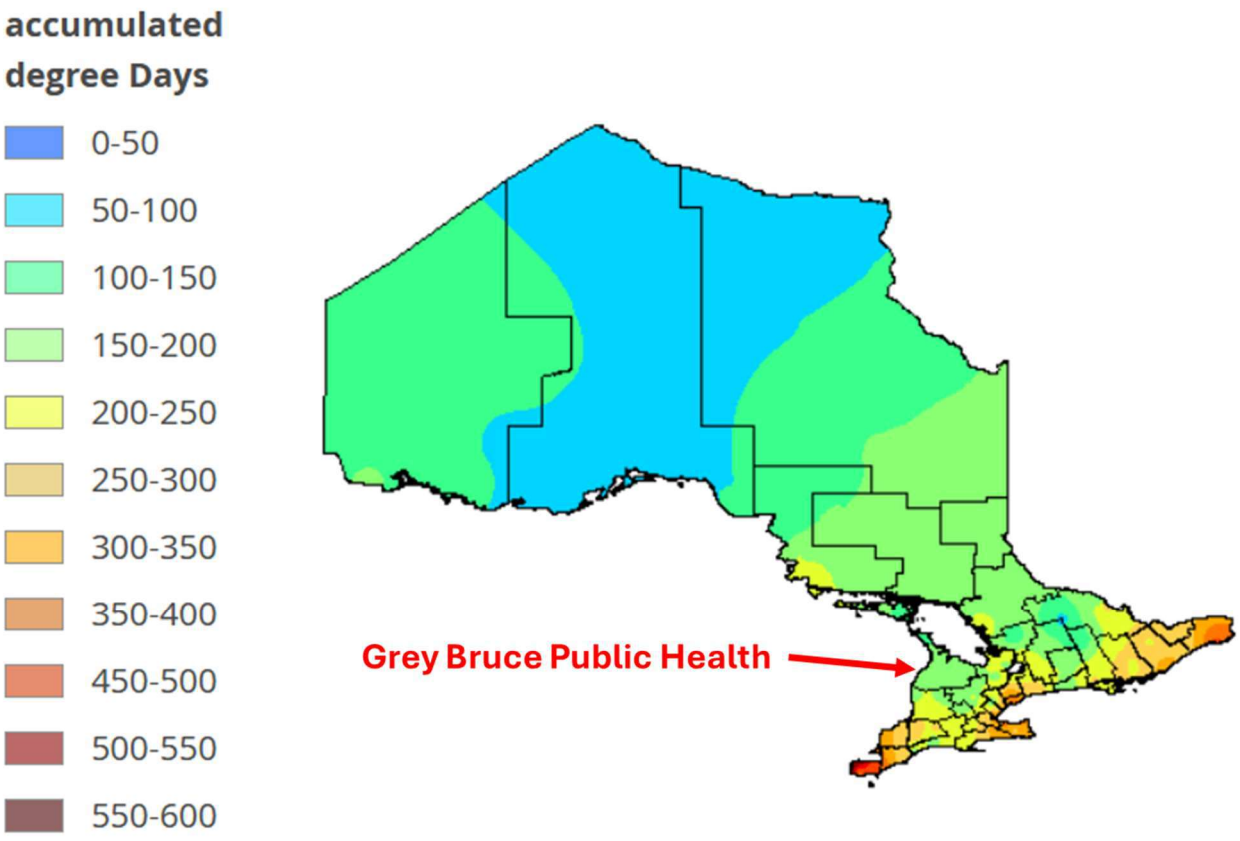

Figure 4a. Accumulated Degree-Day Graph for Ontario, highlighting Grey Bruce Public Health, 2024 (PHO, 2024)

Figure 4b shows the gradual increase in Accumulated degree-days (grey shaded area) that occurred during the 2024 season in Grey Bruce Public Health. In total, there were 129.8 accumulated degree days, based on temperature readings taken from the Wiarton A Station. Please note that although there is a discrepancy in the numbers of accumulated degree days between figures 4a and 4b, both models indicate that there are insufficient heat units in 2024 for significant amplification of the virus in *Culex* spp. mosquitoes.

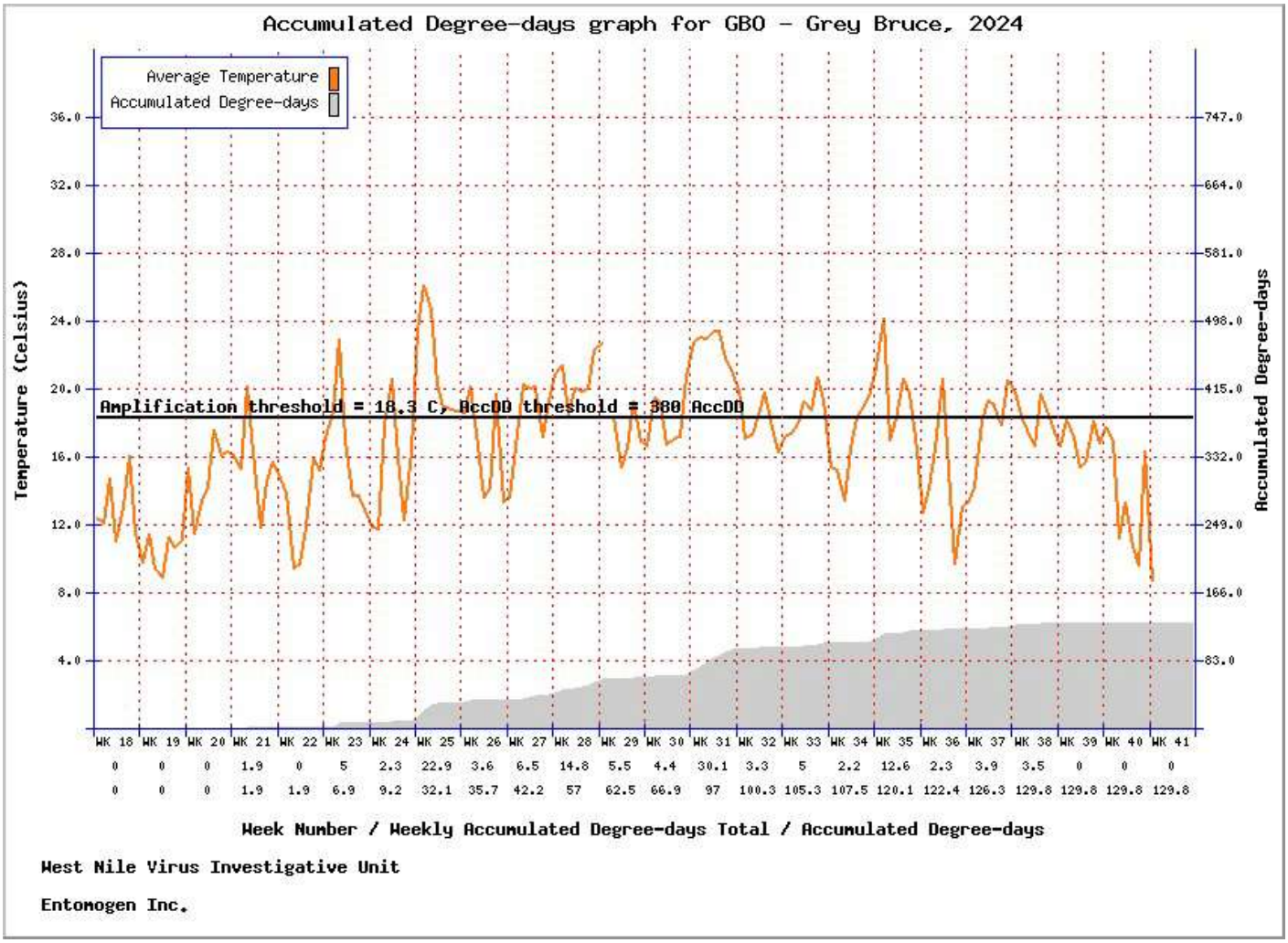

Figure 4b. Accumulated Degree-Day Graph for Grey Bruce Public Health, 2024.

9 Grey Bruce Public Health Adult Mosquito Surveillance Data, 2024

A total of eighty-seven (87) traps were submitted during the 2024 trapping season. Alternating traps were set weekly, with and average of 17 traps set per month beginning the week of May 13, 2024 (epi week 20) and ending the week of September 23, 2024 (epi week 39). In total, traps were set at 19 different sites in 2024, however traps were only set once at sites ESDD and ESJD. Table 5 provides a breakdown of the trapping numbers for each of the 19 sites within Grey Bruce Public Health.

Traps were sorted to a maximum subsample of up to 150 mosquitoes. Any additional mosquitoes were labeled stored as extras. Sorted mosquitoes were identified to the species level and individuals of the same species were pooled for subsequent viral testing. In total, there were 5,299 mosquitoes collected, of which 48 were unidentified males and 1 unidentifiable female. A subsample of 2,830 mosquitoes was examined under a dissecting microscope.

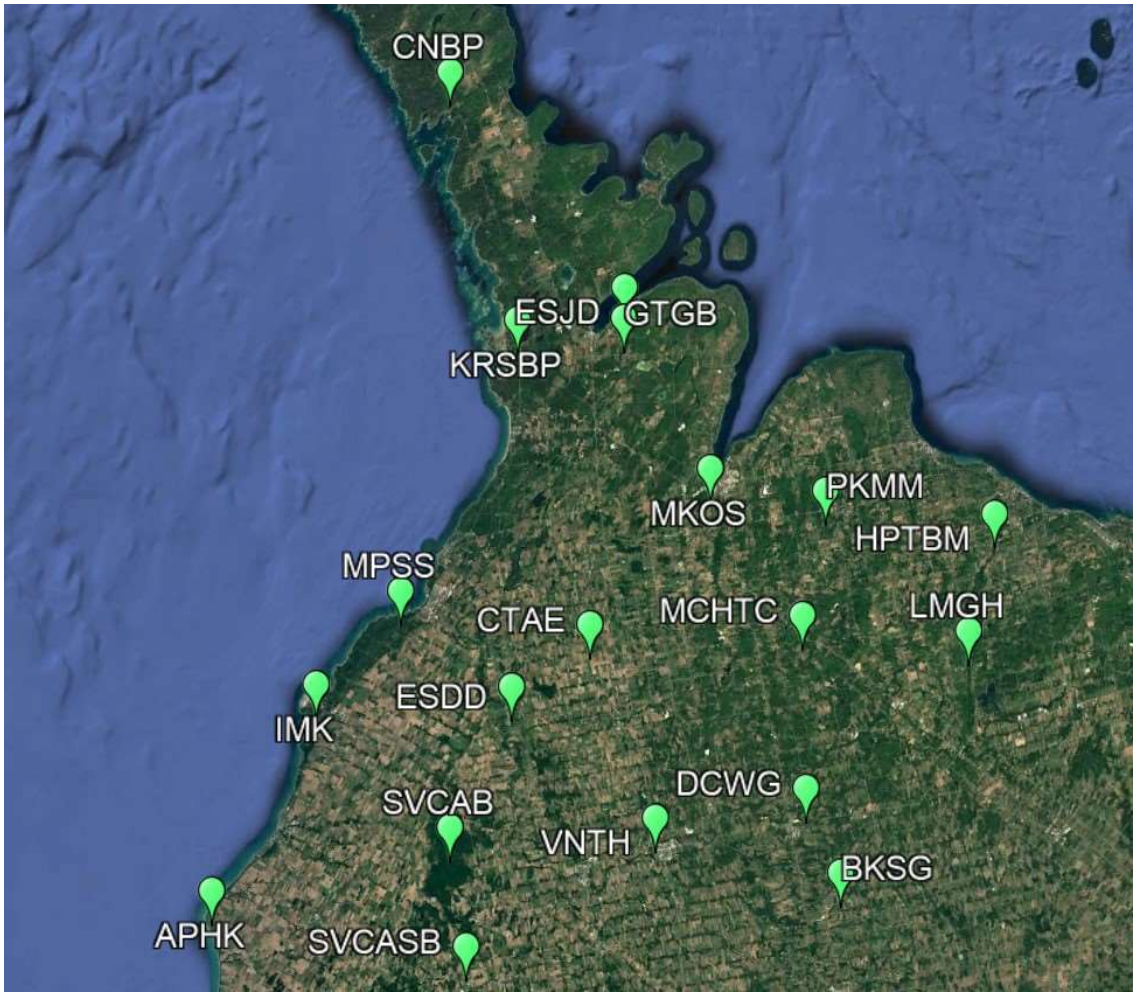

Figure 5. Adult mosquito trap sites for Grey Bruce Public Health, 2024

9.1 Mosquito Species Collected in Grey Bruce Public Health, 2024

Figure 6 shows the species found in Grey Bruce Public Health broken down by epidemiological week. Yellow bars represent WNV enzootic vector species, the pink bars represent the WNV bridge vector species and the green bars indicate non vector species. The blue bar represents the EEEV enzootic vector species *Culiseta melanura*, although it should be noted that a number of other species from each of the other categories may potentially play a role as bridge vector species for EEEV. Historically in Ontario the numbers of EEEV positive mosquito pools are quite low, therefore the focus of testing is geared towards WNV.

Figure 7 represents the percentage of identified mosquitoes belonging to each of the groups mentioned above. Enzootic vectors, or bird-biting mosquitoes, composed primarily of *Culex pipiens/restuans*, made up 2.1% 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*.

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 made up 47.8% of the species identified from traps collected in 2024; thus, humans living within Grey Bruce Public Health may have come in contact with blood feeding *Aedes/Ochlerotatus* mosquitoes as well. Non vector species, which are of no significant concern with regards to WNV, comprised 49% of the identified specimens and EEEV vector species *Culiseta melanura* accounted for the remaining 1% of the total mosquito population.

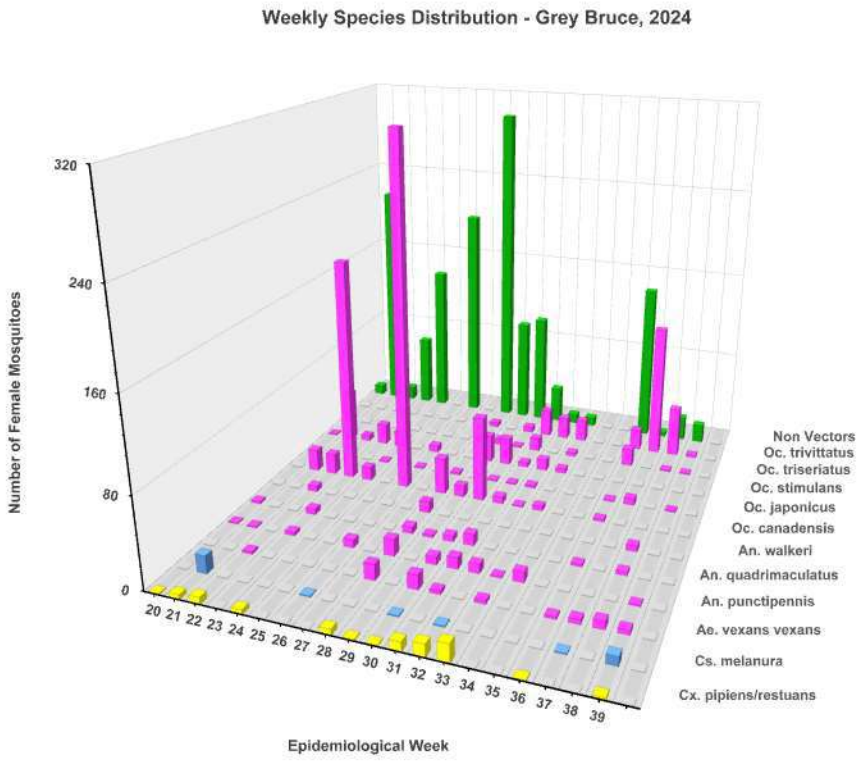

Figure 6. Species Distribution for Grey Bruce Public Health, 2024. Yellow bars represent bird-biting (WNV enzootic vector) species, pink bars represent WNV bridge vectors, blue bars represent EEEV vectors and green bars represent non-vector species.

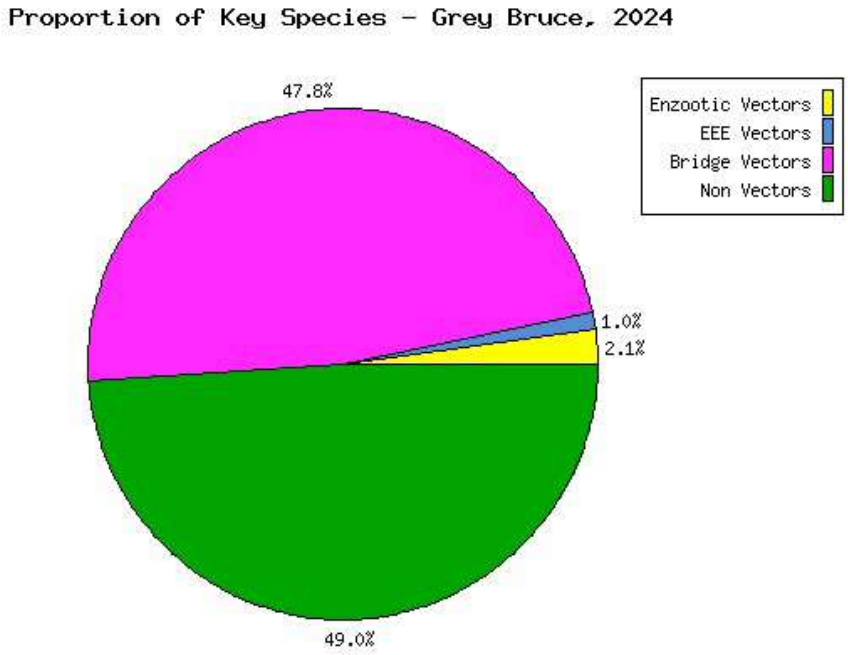

Figure 7. Proportion of Key Species Groups in Grey Bruce Public Health in 2024

Figure 8 provides a full breakdown of all species - including a breakdown of all specimens classified as non-vector species. In total, there were twenty-three (23) different species, or species complexes identified from Grey Bruce Public Health in 2024.

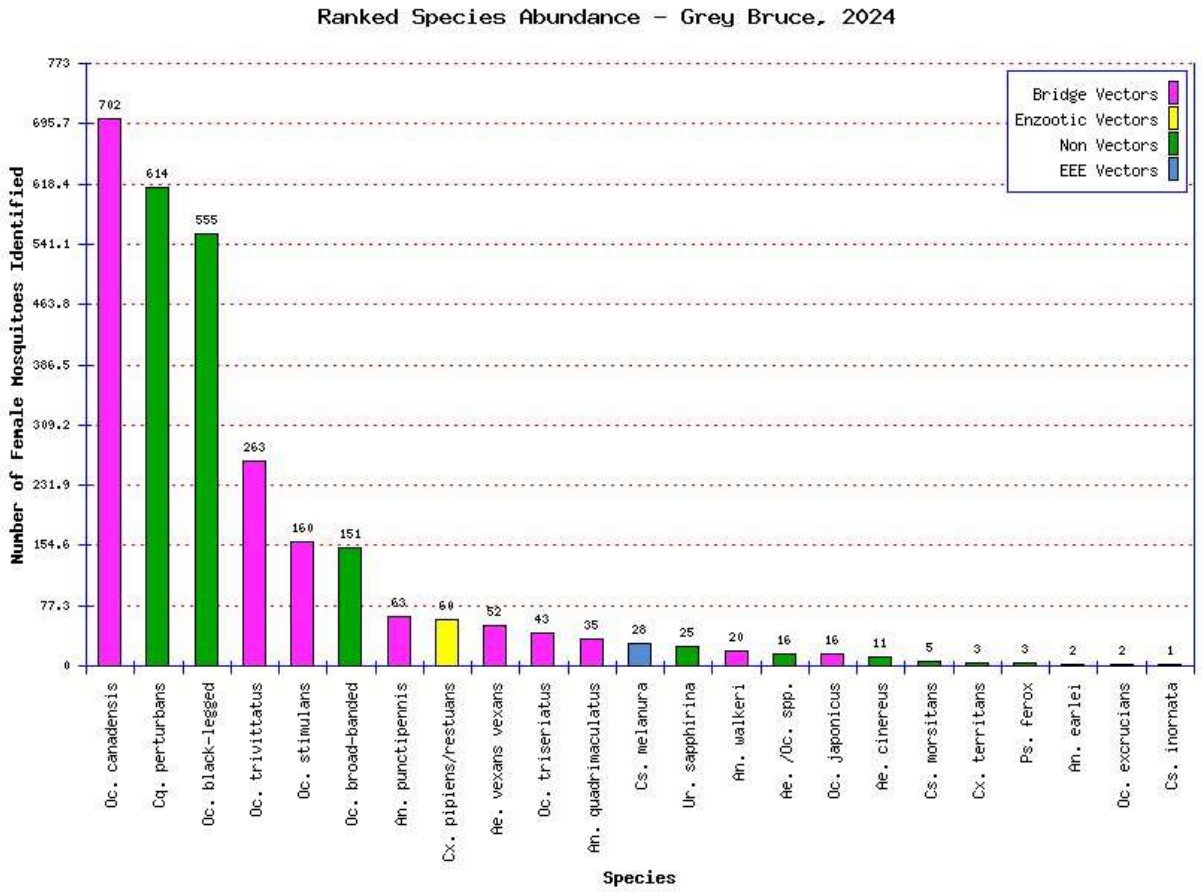

Figure 8. Ranked Species Abundance in Grey Bruce Public Health in 2024

9.2 TRAP INDEX OF GROUPS BY WEEK IN GREY BRUCE PUBLIC HEALTH, 2024

The **trap index (TI)** is a useful tool for summarizing trap data and for comparing different time periods and locations (Figure 9). 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 high bridge vector population throughout most of the season, which peaked during week 26 with a TI value of 178. At the same time, a low enzootic population, consisting entirely of *Cx. pipiens/restuans*, peaked at week 33 with a TI value of 2.7.

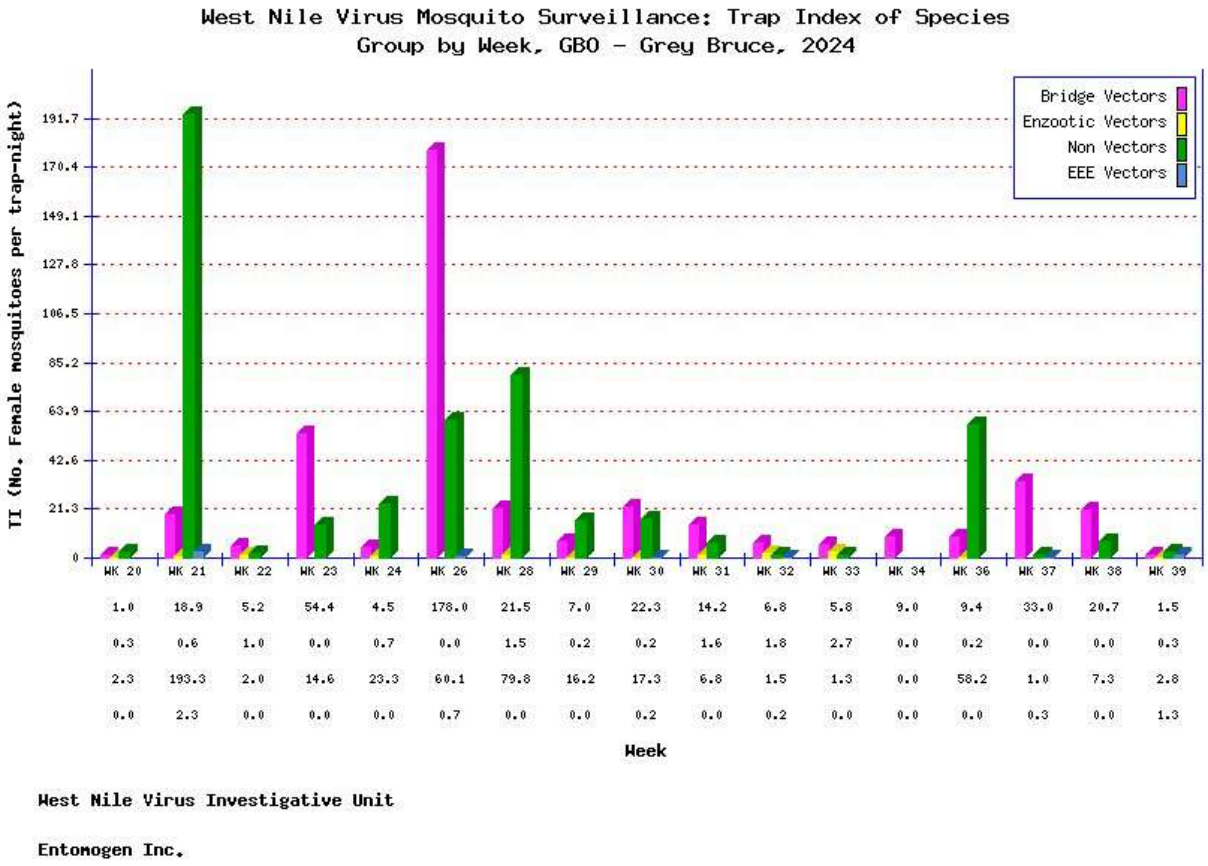

Figure 9. Trap Indices of WNV Enzootic Vectors (yellow), WNV Bridge Vectors (pink), non-Vectors (green), and EEEV Vectors (blue) in Grey Bruce Public Health, 2024

9.3 MOSQUITO SPECIES DISTRIBUTION AMONG SITES IN GREY BRUCE PUBLIC HEALTH, 2024

Total numbers and percentages broken down by groups are provided in Table 5. Site VNTH produced the highest percentage of WNV enzootic vectors (34.5%). Site MKOS and MPSS produced the highest percentage of WNV bridge vectors[5] at 87.5% and 89.5%, respectively. EEEV vector *Culiseta melanura* was collected from three sites DCWG, MKOS and SVCAB representing 6.7%, 0.6% and 7.3% of the overall species collected at those sites, respectively. Overall, site HPTBM had the highest number of identified mosquitoes while site ESDD had the lowest number.

[5] 100% of the specimens collected from sites ESDD and ESJD were WNV bridge vector species, however only one trap was set at each location all season.

Table 5. Grey Bruce Public Health Key Species Distribution Report, 2024

Site Code	WNV Enzootic Vector	WNV Bridge Vector	EEEV Vectors	Non Vectors	Total Mosquitoes Identified	Number of Extras	Number of Traps
APHK	3 (11.1%)	17 (63.0%)	0 (0.0%)	7 (25.9%)	27	0	5
BKSG	0 (0.0%)	37 (69.8%)	0 (0.0%)	16 (30.2%)	53	0	5
CNBP	8 (3.4%)	123 (52.8%)	0 (0.0%)	102 (43.8%)	233	0	5
CTAE	5 (10.0%)	39 (78.0%)	0 (0.0%)	6 (12.0%)	50	0	5
DCWG	2 (6.7%)	15 (50.0%)	2 (6.7%)	11 (36.7%)	30	0	5
ESDD	0 (0.0%)	6 (100.0%)	0 (0.0%)	0 (0.0%)	6	0	1
ESJD	0 (0.0%)	9 (100.0%)	0 (0.0%)	0 (0.0%)	9	0	1
GTGB	2 (0.6%)	200 (57.5%)	0 (0.0%)	146 (42.0%)	348	70	5
HPTBM	0 (0.0%)	46 (9.7%)	0 (0.0%)	429 (90.3%)	475	1350	5
IMK	0 (0.0%)	11 (40.7%)	0 (0.0%)	16 (59.3%)	27	0	5
KRSBP	0 (0.0%)	54 (33.3%)	0 (0.0%)	108 (66.7%)	162	0	5
LMGH	1 (0.9%)	23 (20.0%)	0 (0.0%)	91 (79.1%)	115	0	5
MCHTC	0 (0.0%)	19 (50.0%)	0 (0.0%)	19 (50.0%)	38	0	5
MKOS	1 (0.6%)	154 (87.5%)	1 (0.6%)	20 (11.4%)	176	0	5
MPSS	5 (2.2%)	190 (81.9%)	0 (0.0%)	37 (15.9%)	232	300	5
PKMM	6 (2.3%)	84 (32.2%)	0 (0.0%)	171 (65.5%)	261	100	5
SVCAB	16 (4.7%)	246 (71.5%)	25 (7.3%)	57 (16.6%)	344	500	5
SVCASB	1 (0.5%)	66 (30.7%)	0 (0.0%)	148 (68.8%)	215	100	5
VNTH	10 (34.5%)	15 (51.7%)	0 (0.0%)	4 (13.8%)	29	0	5

10 West Nile Virus and Eastern Equine Encephalitis Virus Analysis, 2024

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, 2024

Entomogen staff tested a maximum of three pools per trap submitted, based on recommendations provided by Public Health Ontario (PHO, 2023). From the 87 traps submitted, a total of 164 pools were sent for testing. Table 6 presents the species breakdown and number of pools of each species tested separated into their respective categories. Ten (10) different species or species complexes were tested for the presence of WNV**.** Bridge vector species *Ochlerotatus canadensis* (N=30, ~19%) and Ocherotatus stimulans (N=26, ~17%) accounted for the greatest percentage of the pools that were tested followed by WNV Enzootic vector *Culex pipiens/restuans* (N=20, ~13%). There were no confirmed WNV-positive pools reported in Grey Bruce Public Health in 2024.

10.2 Eastern Equine Encephalitis Virus Analysis, 2024

A total of seven (7) *Culiseta melanura* pools were tested from traps submitted by Grey Bruce Public Health in 2024. All seven pools tested negative for the presence of EEEV.

Table. 6 Grey Bruce Public Health, Number of Pools Tested for WNV or EEEV by Species in 2024

Species	Total Number of Mosquitoes	Number of Pools	Positive Pools	Virus
Ae. vexans vexans	52	15	0	WNV
An. punctipennis	47	15	0	WNV
An. quadrimaculatus	20	9	0	WNV
An. walkeri	10	2	0	WNV
Cs. melanura	28	7	0	EEEV
Cx. pipiens/restuans	60	20	0	WNV
Oc. canadensis	515	30	0	WNV
Oc. japonicus	16	10	0	WNV
Oc. stimulans	158	26	0	WNV
Oc. triseriatus	42	11	0	WNV
Oc. trivittatus	120	19	0	WNV
Total	**1068**	**164**	**0**	

11 Summary

Of the 2,830 mosquitoes identified in 2024, 60 of those were *Culex pipiens/restuans*, the main enzootic mosquito complex. This number made up 2.1% of the total identified mosquito population in the 2024 season, which is a decrease from the 2023 numbers (116 mosquitoes, 2.6% of the population). In total, 157 mosquito pools consisting of ten (10) different species or species complexes were tested for WNV in 2024. There were no WNV-positive humans, horses, or mosquito pools reported in Grey Bruce Public Health; however, three (3) avian cases were reported in 2024.

The threshold value of 380 accumulated degree-days was not crossed indicating there were insufficient heat units for significant amplification of the virus in the enzootic mosquito population. Provincially, mosquito cases of WNV decreased in 2024, however avian and human cases increased from 2023.

12 Recommendations

Provincially, two (2) EEEV-positive mosquito pools, and twenty-four (24) EEEV-positive horses were reported in Ontario. There was one (1) EEEV-positive human case resulting in death reported in Ottawa region in 2024. 1% of all mosquitoes collected in Grey Bruce Public Health were EEEV enzootic vector *Culiseta melanura*. In addition, EEEV bridge vector species - *Ochlerotatus canadensis*, *Aedes vexans vexans* and *Coquillettidia perturbans* – accounted for a further 48% (N=1,368) of all mosquito species collected in in 2024. While human cases of EEEV are extremely rare in Canada, EEEV infection in horses has increased over the past two years. Grey Bruce Public Health should continue to monitor these numbers and may want to consider targeted trapping or expanding testing to include potential EEEV bridge vector species in the future.

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.

13 Acknowledgments

Entomogen Inc. would like to thank Stephanie Nickels, Madeline Torrie, Zach Meikle and the staff at Grey Bruce Public Health for their contributions to the West Nile Virus and Eastern Equine Encephalitis program in 2024.

14 APPENDICES

14.1 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

15 REFERENCES

Armstrong, Philip M., Andreadis, Theodore G. 2022. Ecology and Epidemiology of Eastern Equine Encephalitis Virus in the Northeastern United States: An Historical Perspective, *Journal of Medical Entomology*, 59(1): 1–13

Bingham, Andrea M., Burkett-Cadena, Nathan D., Hassan, Hassan K. and Unnasch, Thomas R. 2015. Vector Competence and Capacity of *Culex erraticus* (Diptera: Culicidae) for Eastern Equine Encephalitis Virus in the Southeastern United States. *Journal of Medical Entomology* 0(0): 1-4.

Centers for Disease Control and Prevention. 2024. Citing Online Sources: Eastern Equine Encephalitis Virus [online] Available from https://www.cdc.gov/easternequineencephalitis/transmission/index.html [accessed 15 October 2024].

Centers for Disease Control and Prevention. 2024. Citing Online Sources: Current Year Data (2024) [online] Available from https://www.cdc.gov/westnile/statsmaps/current-season-data.html [accessed 15 October 2024].

Kilpatrick, A. M., L. D. Kramer, S. R. Campbell, E. O. Alleyne, A. P. Dobson, and P. Daszak. 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerging Infect. Dis*. 11(3): 425-429.

Canadian Animal Health Surveillance System, 2024: Equine Diseases Dashboard Canada [online]. Available from https://app.powerbi.com/view?r=eyJrIjoiOGFiMTAwYjAtZDkzZC00ZmZhLTk4ZTgtNGJjYmNjZjU3MmE4IiwidCI6IjE4YjVhNWVkLTFkODYtNDFkMy05NGEwLWJjMjdkYWUzMmFiMiJ9 [accessed 15 October 2024].

Ministry of Health and Long-Term Care. 2023. Citing online sources: West Nile Virus Preparedness and Prevention Plan, Ministry of Health, April 2023 [online]. Available from https://www.health.gov.on.ca/en/pro/programs/publichealth/oph_standards/docs/reference/WNV_plan_en.pdf [accessed 15 October 2024].

Government of Canada. 2024. Vector-borne disease surveillance in Canada: Mosquito-borne disease surveillance: Seasonal update (Last updated: 2023-10-23) [online]. Available from https://health-infobase.canada.ca/zoonoses/mosquito/ [accessed 15 October 2024].

Public Health Ontario. 2024. Citing online sources: West Nile Virus Surveillance Reports [online]. Available from https://www.publichealthontario.ca/en/data-and-analysis/infectious-disease/west-nile-virus [accessed 31 October 2024].

Russell, C. B. 2008. Analysis of the feeding behaviour of the mosquito *Culex pipiens* L. (Diptera: Culicidae) in relation to West Nile virus. PhD. Thesis, Brock University, St. Catharines, ON.

Shirose, Leonard. 7 October 2024, Personal Communication.

United States Environmental Protection Agency. Citing online sources: Mosquito Control: Mosquito Life Cycle [online]. Available from http://www2.epa.gov/mosquitocontrol/mosquito-life-cycle [accessed 15 October 2024].