2025

West Nile Virus Year End Report

Entomogen Inc.

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1 Overview

Adult mosquito surveillance is a key component of the overall WNV program for Grey Bruce Public Health. In 2025, 86 traps were submitted, 4,516 mosquitoes were examined under a dissecting microscope, one hundred and sixty (160) mosquito pools were tested for WNV and seven (7) mosquito pools were tested for EEEV. No mosquito pools tested positive for WNV however, three (3) human cases, six (6) avian cases and three (3) equine cases of WNV were reported in Grey Bruce Public Health in 2025. There was no EEEV activity reported in Grey Bruce Public Health in 2025.

In Ontario, a total of one hundred and seventy-six (176) confirmed and/or probable human cases, one hundred and eight (108) WNV-positive birds, nine (9) WNV-positive horses and two hundred and sixty-seven (267) WNV-positive mosquito pools were reported (Public Health Ontario, 2025). One (1) Eastern Equine Encephalitis (EEEV) human case (PHAC, 2025) and eight (8) EEEV-positive horses (CAHSS, 2025) were reported in 2025.

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 2025, Ontario reported one hundred and seventy-six (176) confirmed and/or probable human cases, one hundred and eight (108) WNV-positive birds, nine (9) WNV-positive horses and two hundred and sixty-seven (267) WNV-positive mosquito pools. (PHO, 2025)

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.

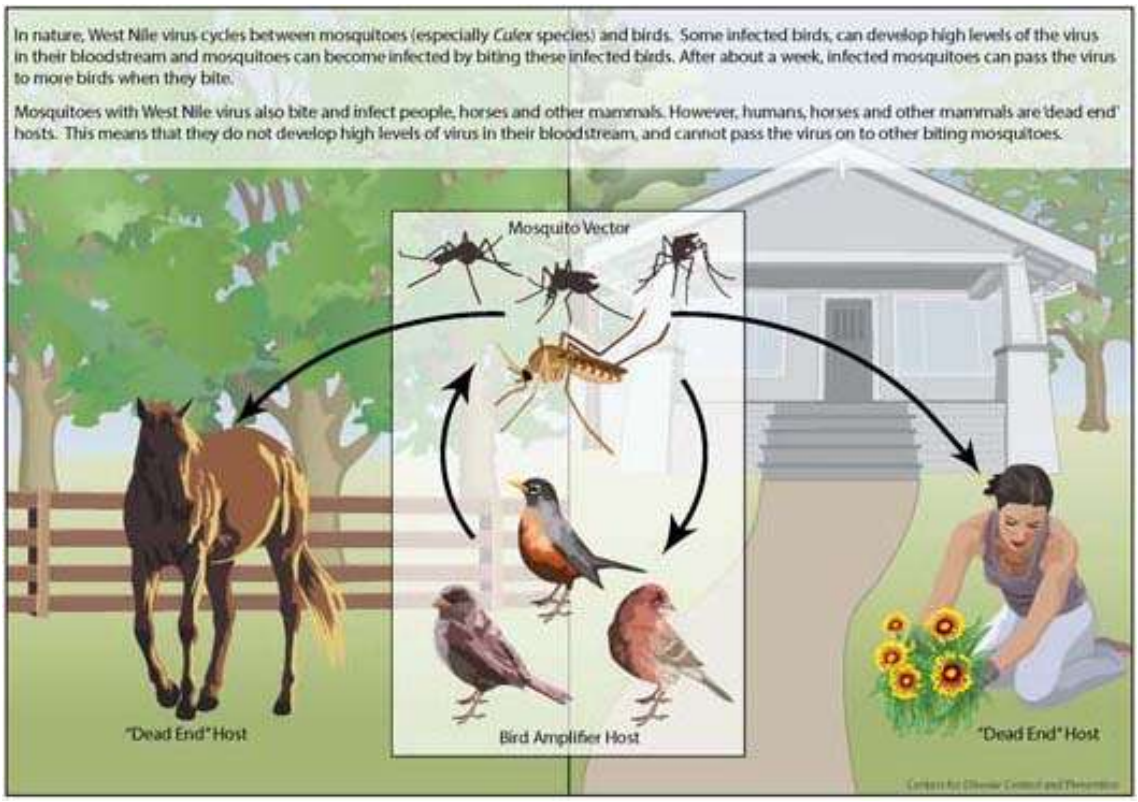

Figure 1. West Nile Virus Transmission Cycle (Centers for Disease Control and Prevention, 2025).

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.

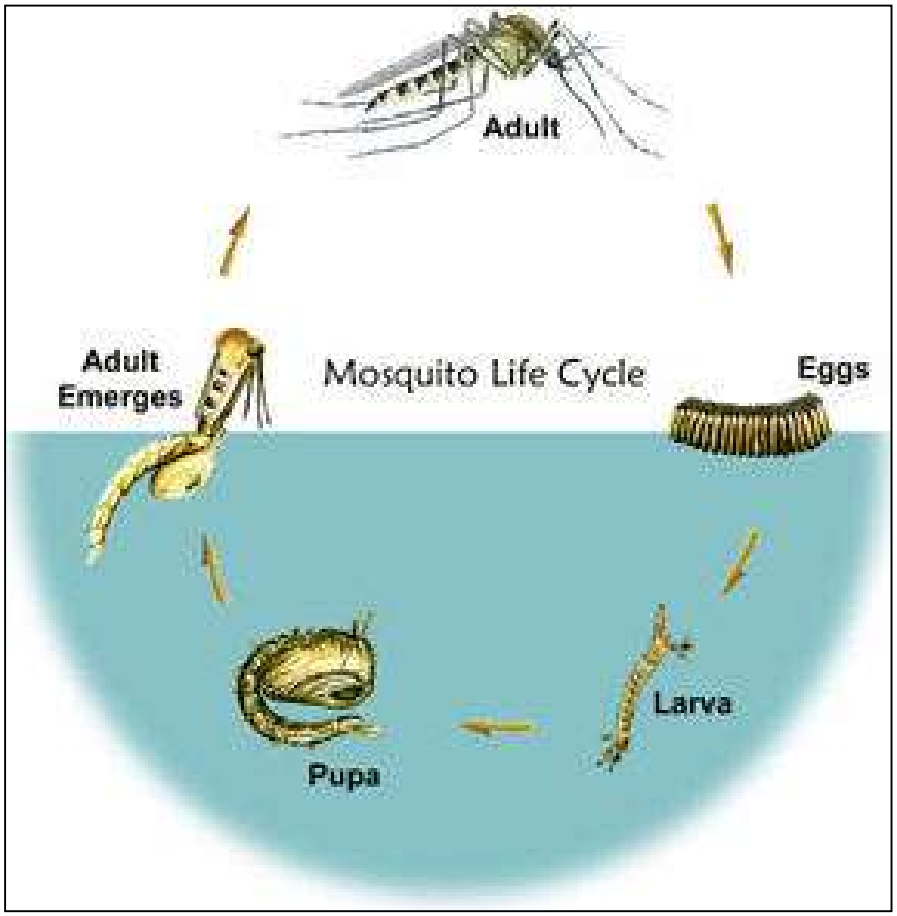

Figure 2. Mosquito Life Cycle (United States Environmental Protection Agency, 2025, recreated from a publication by D.M. Wood).

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.

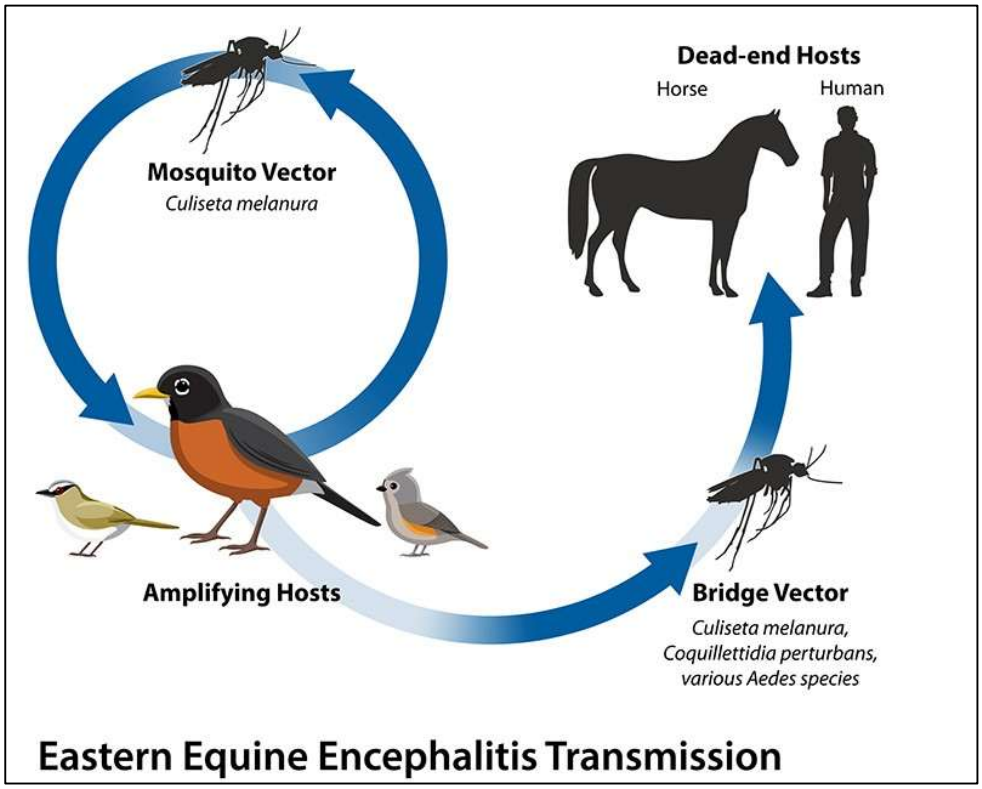

Figure 3. Eastern Equine Encephalitis Virus Transmission Cycle (CDC, 2025).

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, 2025). Reports of human infection are rare, with the United States reporting an average of 11 human cases per year. (CDC, 2025). One (1) EEEV-positive human case, and eight (8) EEEV-positive horses were reported in Ontario as of October 14, 2025 (PHO, 2025).

4 West Nile Virus Activity Summary for Canada, 2025

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 15, 2025.

4.1 West Nile Virus Human Cases in Canada, 2025

A total of three hundred and two (302) West Nile virus (WNV) confirmed or probable human cases have been reported in Canada in 2025 (Table 1). The human cases were reported from Ontario, Quebec, Manitoba, Alberta, British Columbia and Saskatchewan. Nine (9) deaths associated with WNV were reported by PHAC in 2025. Of the reported human cases 175 were classified as neurological, 74 were classified as non neurological, 32 were unspecified clinical cases and 14 cases were asymptomatic (PHAC, 2025) with 7 cases currently unclassified.

4.2 West Nile Virus Positive Bird Cases in Canada, 2025

Dead birds were collected and submitted to CWHC as part of the 2025 surveillance season. In total, three hundred and eighteen (318)[1] birds tested positive for WNV. The birds that tested positive were submitted from Quebec, Ontario, Manitoba and Saskatchewan, (PHAC, 2025).

4.3 West Nile Virus Positive Equine Cases in Canada, 2025

A total of forty-nine (49) West Nile virus (WNV) confirmed or probable equine cases have been reported in Canada in 2025 (Table 1). The cases were reported in Ontario, Quebec, Manitoba, Saskatchewan, Alberta and British Columbia (PHCA, 2025).

4.4 West Nile Virus Positive Mosquito Cases in Canada, 2025

A total of 415 mosquito pools tested positive for West Nile virus in Canada in 2025 (Table 1). Most positive mosquito pools were reported from Ontario with the remaining cases being reported in Manitoba (PHAC, 2025). All remaining provinces and territories do not conduct mosquito surveillance.

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

Table 1. Total West Nile Virus cases in Canada, 2025. (Reported by PHAC as of November 28, 2025).

Province	WNV Human Cases	WNV Avian Cases	WNV Equine Cases	WNV Mosquito Pools
Ontario	176	108	9	267
Quebec	114	67	6	N/A
Manitoba	3	28	2	148
Alberta	5		12	N/A
British Columbia	1		1	N/A
Saskatchewan	3	115	19	N/A
Total	**302**	**318**	**49**	**415**

5 West Nile Virus Activity in the United States, 2025

As of November 28, 2025, a total of 1,941 cases of West Nile virus disease in people have been reported from 46 states. Of these, 1,336 (~69%) were classified as neuroinvasive disease. Exact numbers of mosquito, bird and equine cases were not readily available.

6 West Nile Virus Activity Summary for Ontario, 2025

6.1 West Nile Virus Human Cases in Ontario, 2025

As of November 28, 2025, 176 human WNV cases have been reported from 22 different health units (Table 2). Positive cases were reported by Toronto (75), York (18), Ottawa (10), Halton (9), Niagara (8), Peel (8), Windsor-Essex (8), Hamilton (7), Durham (6), Southeast (5), Grey Bruce (3), Middlesex-London (3), Waterloo (3), Chatham Kent (2), Grand Erie (2), Simcoe Muskoka (2), Wellington-Dufferin Guelph (2), and one (1) human case in each of the following health units; Eastern Ontario, Huron, Lakelands, North Bay Parry Sound, Northeastern.

6.2 West Nile Virus Positive Bird Cases in Ontario, 2025

All twenty-nine provincial health units reported positive avian cases in 2025. As of November 6, 2025, 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 Red-Tailed Hawk (*Buteo jamaicensis*), Ring-Billed Gull (*Larus delawarensis*), and the Coopers Hawk (*Accipiter cooperii)*. In total, fifteen (15) different species of birds tested positive for WNV in 2025 (Table 3).

6.3 West Nile Virus Positive Equine Cases in Ontario, 2025

According to the Canadian Animal Health Surveillance System (CAHSS), there were nine (9) WNV-positive cases in the equine population in 2025. The positive cases were reported from Grey Bruce (3), Waterloo (2), Algoma (1), North Bay (1), Peel (1) and Sudbury (1).

6.4 West Nile Virus Positive Mosquito Cases in Ontario, 2025

From mosquito surveillance conducted by provincial health units, 267 WNV-positive mosquito pools were confirmed from 20 separate Ontario Health Units in 2024 (Figure 10). The positive pools were from Toronto (55), Peel (38), Durham (28), Halton (28), Hamilton (23), Ottawa (13), York (13), Niagara (12), Windsor-Essex (10), Southwestern (8), Middlesex-London (7), Chatham Kent (6), Grand Erie (5), Waterloo (5), Eastern Ontario (4), Simcoe-Muskoka (4), Southeast (3), Huron Perth (2), Wellington-Dufferin-Guelph (2), and Lambton (1) (PHO, 2025).

Table 2. West Nile Virus Activity in Ontario, 2025. (Reported by PHO, CWHC and CAHSS as of November 28, 2025).

PHU Code	Health Unit	WNV Human Cases	WNV Avian Cases	WNV Equine Cases	WNV Mosquito Pools
ALG	Algoma District		4	1	
CHK	Chatham-Kent	2	2		6
HAM	City of Hamilton Public Services	7	3		23
DUR	Durham Region Health Department	6	4		28
EOH	Eastern Ontario Health Unit	1	1		4
GEH	Grand Erie Public Health	2	1		5
GBO	Grey Bruce Public Health	3	6	3	
HNP	Lakelands Public Health	1	8		
HAL	Halton Region Public Health	9	2		28
HPH	Huron Perth Public Health	1	3		2
LAM	Lambton Public Health		1		1
MSL	Middlesex-London Public Health	3	1		7
NIA	Niagara Region Public Health	8	6		12
NPS	North Bay Parry Sound District Health Unit	1	2	1	
NEH	Northeastern Public Health	1	1		
NWR	Northwestern Health Unit		1		
OTT	Ottawa Public Health	10	3		13
PEL	Peel Public Health	8	2	1	38
SUD	Public Health Sudbury and Districts		3	1	
WAT	Region of Waterloo Public Health and Paramedic Services	3	5	2	5
REN	Renfrew County and District Health Unit		1		
SMD	Simcoe Muskoka District Health Unit	2	3		4
SEH	Southeast Public Health	5	5		3
OXE	Southwestern Public Health		2		8
THB	Thunder Bay District Health Unit		3		
TOR	Toronto Public Health	75	11		55
WDG	Wellington-Dufferin-Guelph Public Health	2	11		2
WEC	Windsor-Essex County Health Unit	8	6		10
YRK	York Region Public Health	18	7		13
Total		**176**	**108**	**9**	**267**

Table 3. Avian Species Tested Positive for WNV in Ontario, 2025. (CWHC as of November 6, 2025).

Common Name	Species	Number of Positives
American Crow	*Corvus brachyrhynchos*	64
American Goshawk	*Astur atricapillus*	1
Bald Eagle	*Haliaeetus leucocephalus*	4
Blue Jay	*Cyanocitta cristata*	2
Broad-Winged Hawk	*Buteo platypterus*	3
Canada Goose	*Branta canadensis*	1
Common Raven	*Corvus corax*	1
Cooper's Hawk	*Accipiter cooperii*	5
Great Horned Owl	*Bubo virginianus*	4
Herring Gull	*Larus smithsonianus*	1
Loggerhead Shrike	*Lanius ludovicianus*	1
Mallard	*Anas platyrhynchos*	1
Red-Tailed Hawk	*Buteo jamaicensis*	9
Ring-Billed Gull	*Larus delawarensis*	7
Sharp-Shinned Hawk	*Accipiter striatus*	4
Total		**108**

7 EASTERN EQUINE ENCEPHALITIS VIRUS ACTIVITY SUMMARY FOR ONTARIO, 2025

There was one (1) human case and eight (8) equine cases of Eastern Equine Encephalitis Virus (EEEV) reported in 2025. The human case was reported in the City of Hamilton and the equine cases were reported from Hamilton (2), Wellington-Dufferin-Guelph (2), Halton (1), Ottawa (1), Renfrew (1), and Simcoe Muskoka (1) (Table 4). No mosquito pools tested positive for EEEV in 2025.

Table 4. Equine Cases of EEEV in Ontario, 2025. (CAHSS as of October 11, 2025).

Date Reported	Region	Health Unit	Virus
29-Jul-25	Wellington County	Wellington Dufferin Guelph	EEEV
14-Aug-25	Wellington County	Wellington Dufferin Guelph	EEEV
18-Aug-25	Hamilton	City of Hamilton	EEEV
26-Aug-25	City of Ottawa	Ottawa	EEEV
26-Aug-25	Hamilton	City of Hamilton	EEEV
3-Sep-25	Halton	Regional Municipality of Halton	EEEV
3-Sep-25	Renfrew County	Renfrew County and District Health Unit	EEEV
6-Oct-25	Simcoe County	Simcoe Muskoka District Health Unit	EEEV

8 TIMING OF WNV-POSITIVE *CULEX PIPIENS/RESTUANS* IN GREY BRUCE PUBLIC HEALTH BASED ON 2025 TEMPERATURES

Accumulated degree-days (AccDD) or growing degree days (GDD) are temperature measurements used to estimate the rate of growth of organisms, particularly plants and insects (Government of Canada, 2025). Degree days are calculated using the mean daily temperature for one 24-hour period and a predetermined threshold level. For mosquitoes and WNv, higher temperatures result in quicker growth and therefore, quicker amplification of the virus (PHO, 2013).

The threshold level for WNV within the adult females of *Culex pipiens/restuans* - the main WNv vector in Ontario - is 18.3˚C therefore, every degree above that threshold is considered one degree day. For example, if the mean daily temperature in 19.3˚C that would be 1 degree day (19.3˚C -18.3˚C), however if the mean daily temperature was 23.3˚C that would equal 5-degree days (23.3˚C -18.3˚C)[2]. AccDD are cumulative and can therefore be used to predict when positive mosquito pools and human cases may appear within the population. Positive mosquito pools can occur as early as 30 AccDD with human cases appearing a little later, around 100 AccDD (PHO, 2013).

Figure 4 shows the gradual increase in Accumulated degree-days (grey shaded area) that occurred during the 2025 season in Grey Bruce Public Health. In total, there were 163.4 accumulated degree days, based on temperature readings taken from the Wiarton A Station.

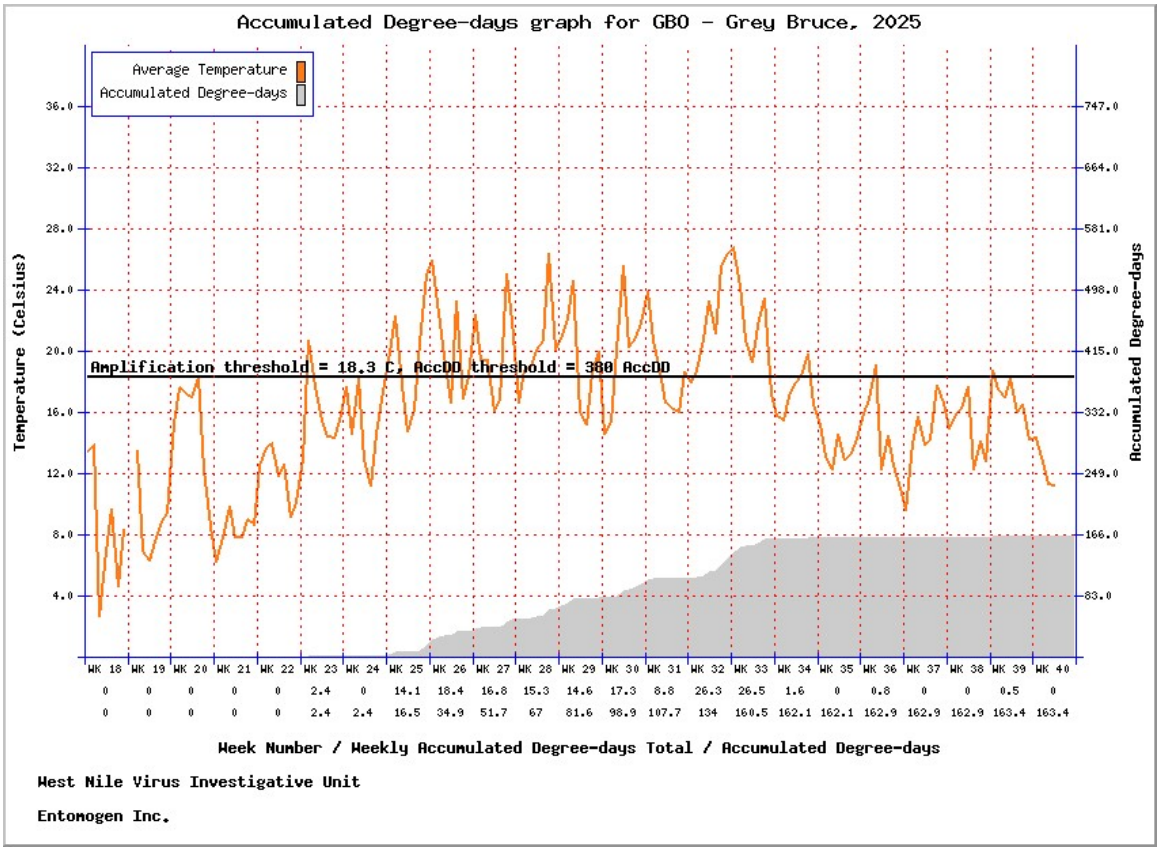

Figure 4. Accumulated Degree-Day Graph - Grey Bruce Public Health, 2025

[2] Negative degree day values are recorded as zero (0) degree days.

9 GREY BRUCE PUBLIC HEALTH ADULT MOSQUITO SURVEILLANCE DATA, 2025

A total of eighty-six (86) traps were submitted from 18 different sites during the 2025 trapping season. Figure 5 shows the location of all 18 sites within Grey Bruce Public Health. Traps were set weekly, beginning the week of May 12, 2025 (epi week 20) and ended the week of September 22, 2025 (epi week 39).

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. Table 5 shows a summary of the number of mosquitoes trapped in each of the 18 sites within Grey Bruce Public Health.

In total, there were 8,253 mosquitoes collected, of which 15 were damaged unidentifiable females and 92 were unidentified males. A subsample of 4,516 mosquitoes was examined under a dissecting microscope.

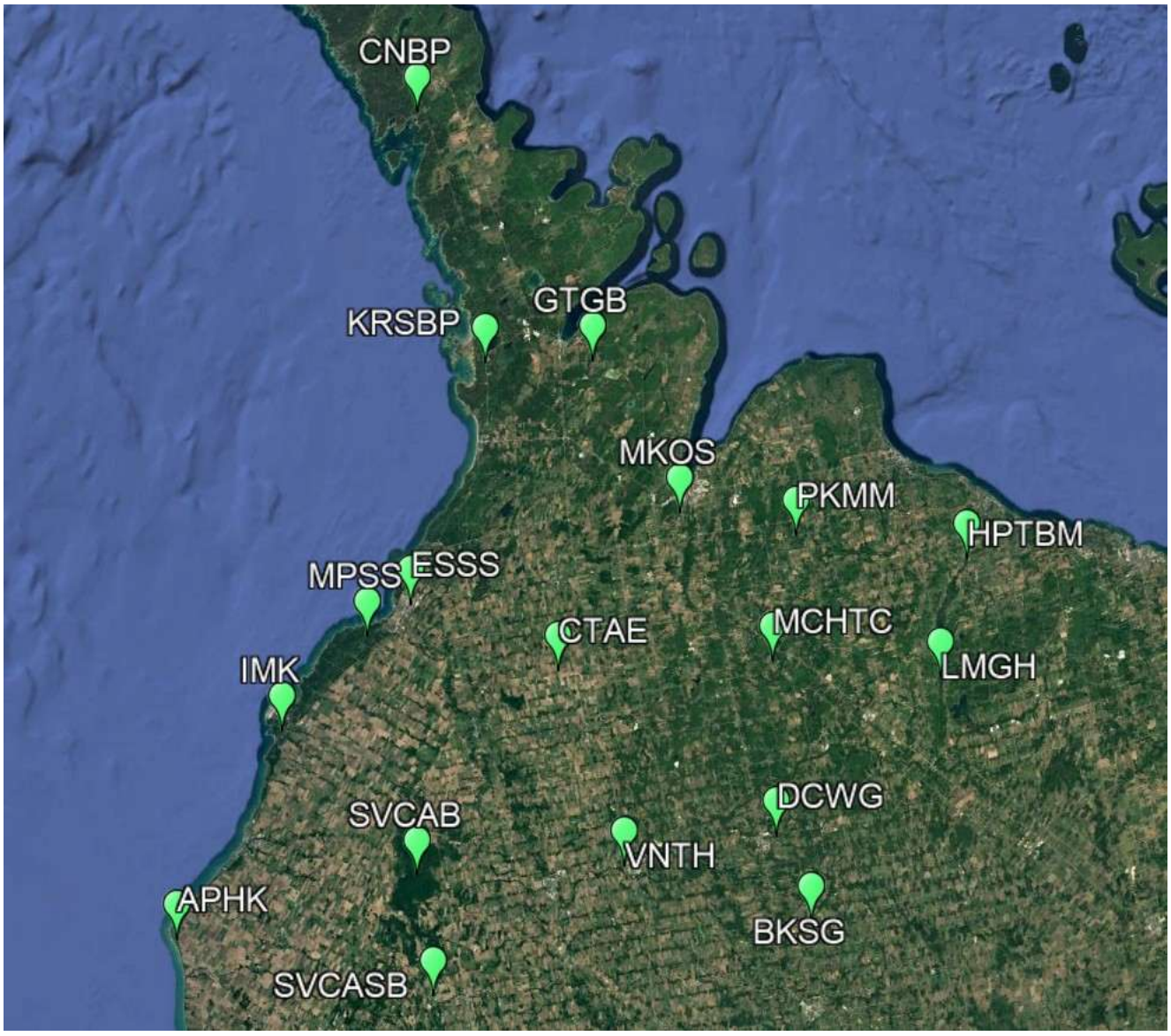

Figure 5. Adult mosquito trap sites for Grey Bruce Public Health, 2025

9.1 Mosquito Species Collected in Grey Bruce Public Health, 2025

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 bars represent the EEEV enzootic vector species *Culiseta melanura*, although it should be noted that a number of other species 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.

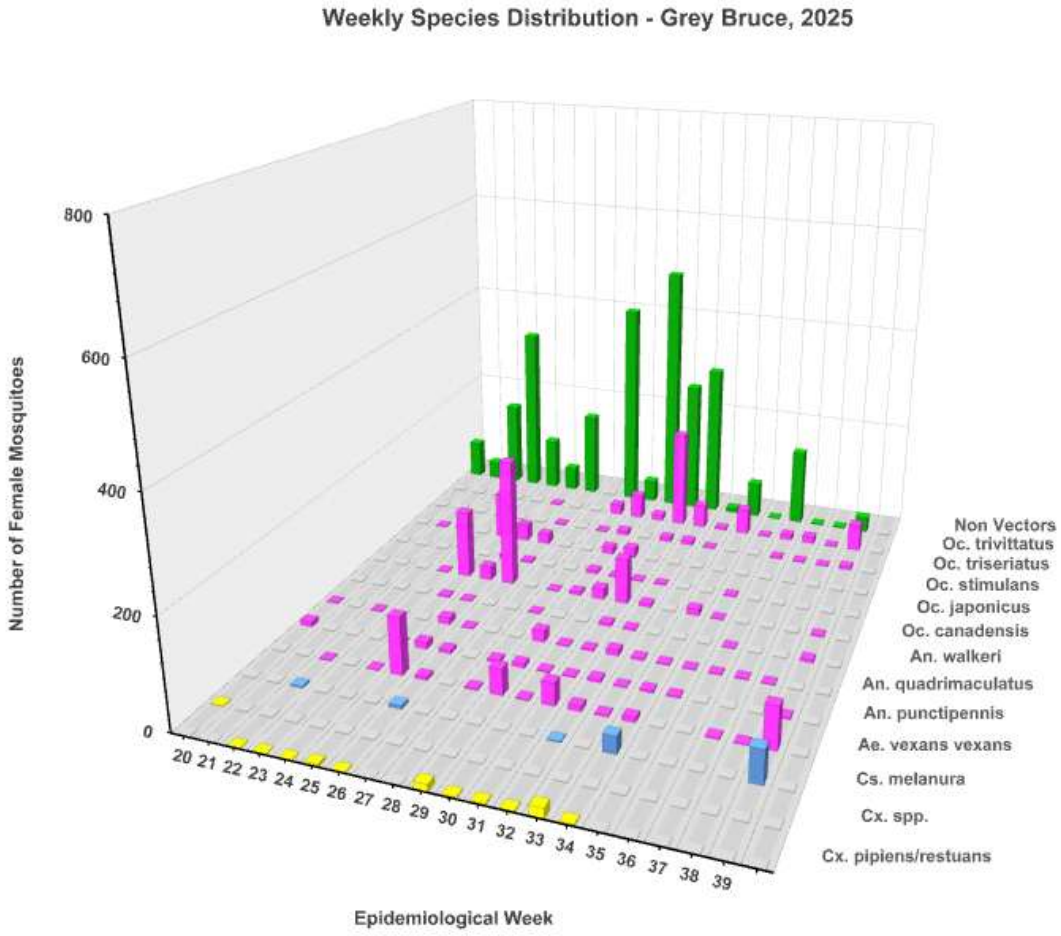

Figure 6. Species Distribution for Grey Bruce Public Health, 2025.

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 1.1% of the species collected. Research indicates that *Culex 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 36.9% of the species identified from traps collected in 2025; thus, humans living within Grey Bruce Public Health may have come in contact with blood feeding *Aedes/Ochlerotatus* mosquitoes as well. The largest group were non vector species, which are of no significant concern with regards to WNV. Non vector species comprised 59.7% of the identified specimens. *Culiseta melanura*, the main EEEV vector species comprised the remaining 2.3% of the mosquito species identified from Grey Bruce Public Health in 2025.

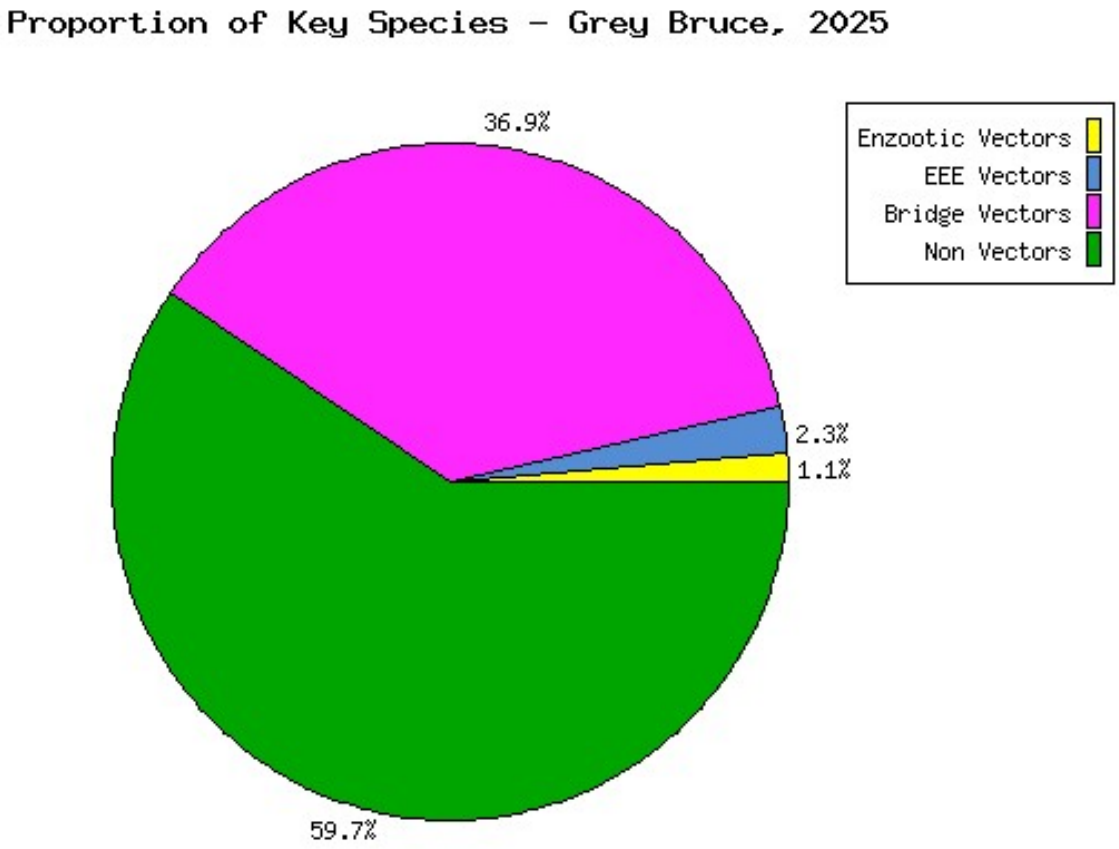

Figure 7. Proportion of Key Species Groups in Grey Bruce Public Health in 2025

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 2025.

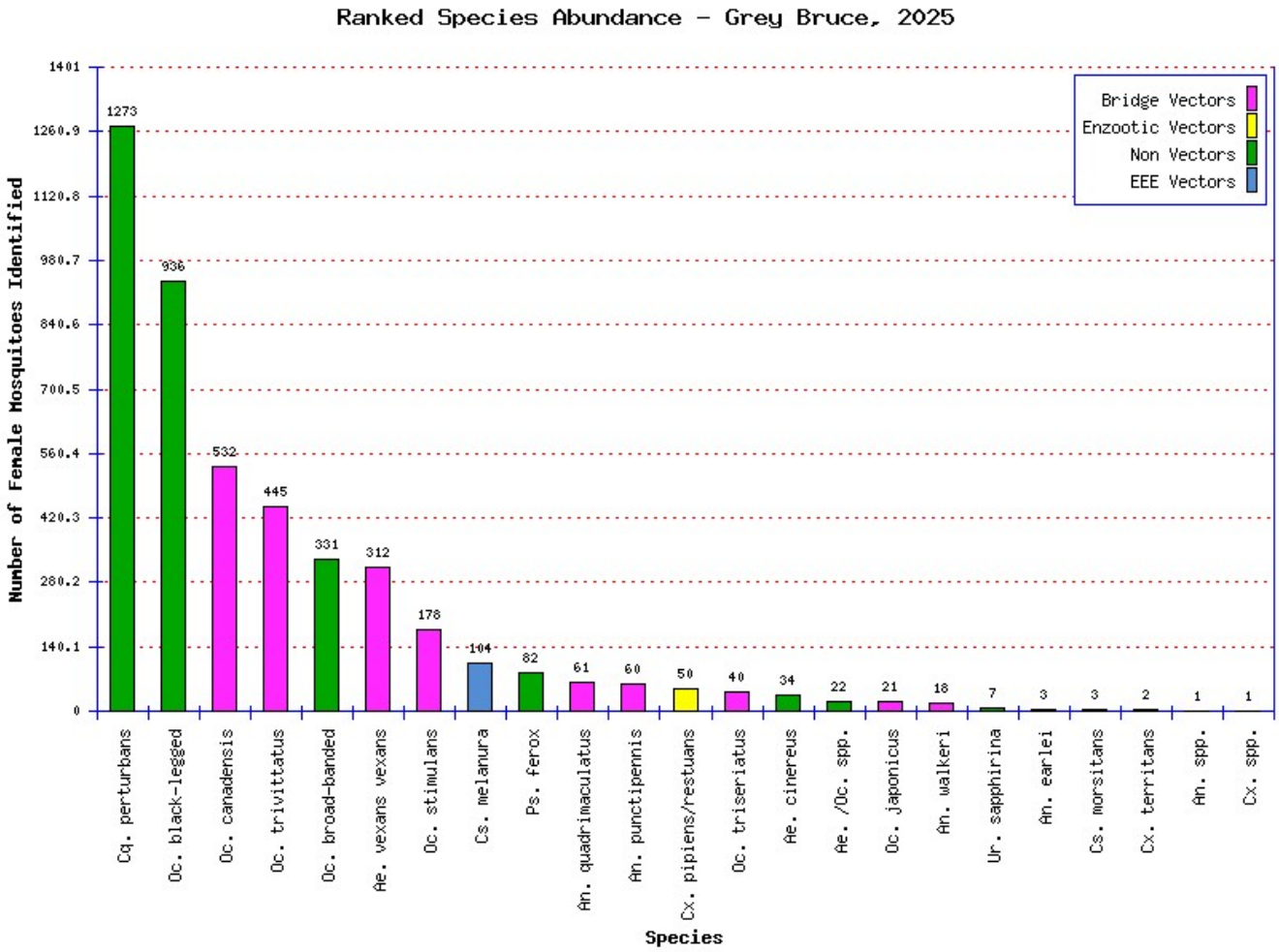

Figure 8. Ranked Species Abundance in Grey Bruce Public Health in 2025

9.2 TRAP INDEX OF GROUPS BY WEEK IN GREY BRUCE PUBLIC HEALTH, 2025

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 significant bridge vector population throughout most of the season, which peaked during week 31 with a TI value of 154.9. At the same time, a low enzootic population, consisting mostly of *Cx. pipiens/restuans*, peaked at week 33 with a TI value of 4.5.

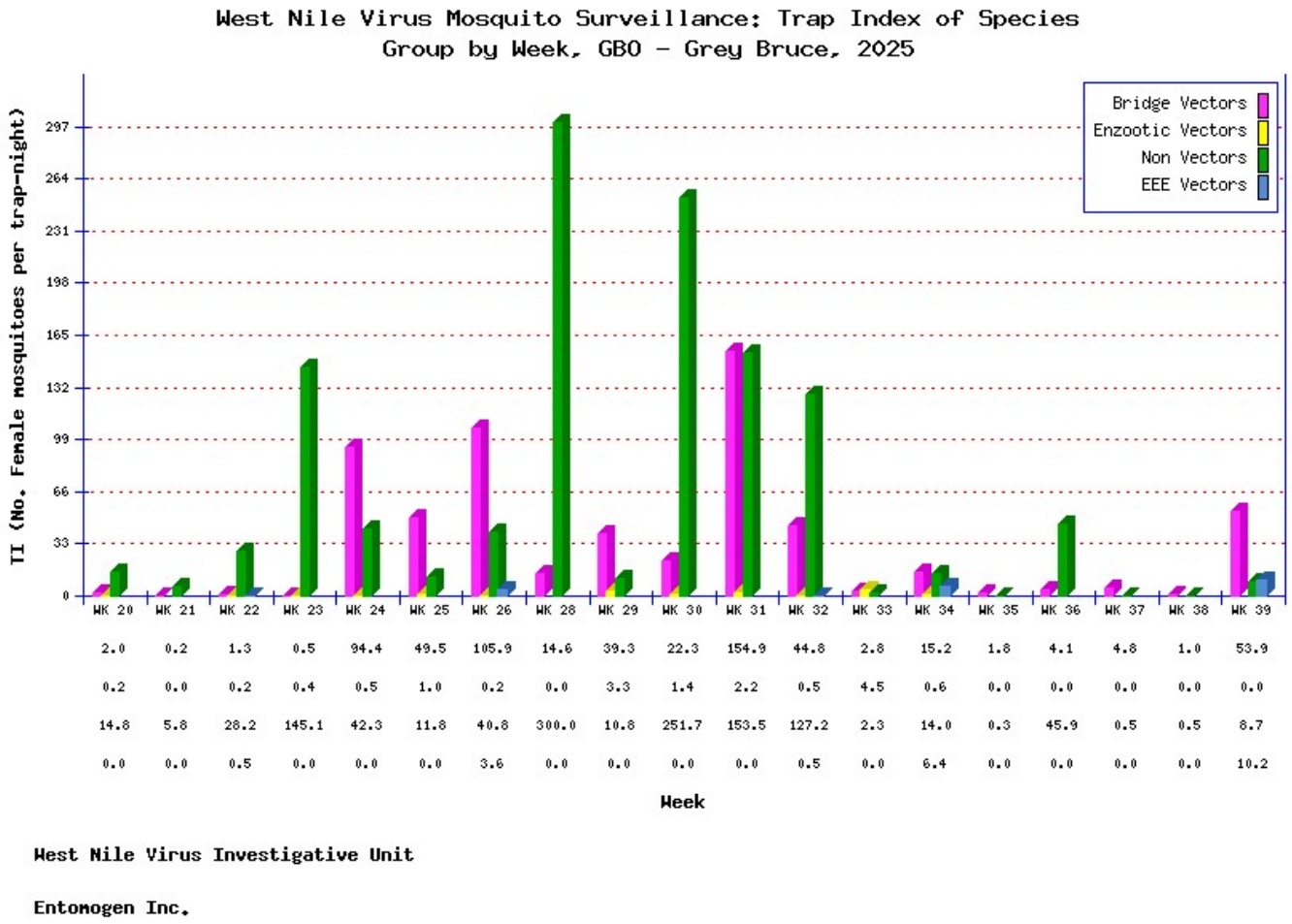

Figure 9. Trap Indices of Species Group in Grey Bruce Public Health, 2025

9.3 Mosquito Species Distribution among sites in Grey Bruce Public Health, 2025

Total numbers and percentages broken down by groups are provided in Table 5. Site CTAE produced the highest percentage of WNV enzootic vectors (13.4%), however the total catch for that site was only 15 mosquitoes over five weeks of trapping. Site VNTH produced the highest percentage of WNV bridge vectors at 88.1%[3]. Almost all of the EEEV vectors species *Culiseta melanura* trapped in 2025 were from site SVCAB. Overall, site SVCAB had the highest number of identified mosquitoes while site APHK had the lowest number.[4]

[3] Site ESSS had a greater percentage of WNV bridge vectors, but only one trap was set all season.

[4] Site ESSS had a lower number, but only one trap was set.

Table 5. Grey Bruce Public Health Key Species Distribution Report, 2025

Site Code	WNV Enzootic Vectors	WNV Bridge Vectors	EEEV Vectors	Non-Vectors	Total Mosquitoes Identified	Number of Extras	Number of Traps
APHK	2 (10.5%)	16 (84.2%)	0 (0.0%)	1 (5.3%)	19	0	5
BKSG	1 (1.0%)	43 (42.2%)	0 (0.0%)	58 (56.9%)	102	0	5
CNBP	0 (0.0%)	29 (15.3%)	0 (0.0%)	161 (84.7%)	190	100	5
CTAE	15 (13.4%)	67 (59.8%)	0 (0.0%)	30 (26.8%)	112	0	5
DCWG	3 (4.4%)	47 (69.1%)	0 (0.0%)	18 (26.5%)	68	0	5
ESSS	0 (0.0%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	3	0	1
GTGB	1 (0.3%)	177 (56.2%)	0 (0.0%)	137 (43.5%)	315	125	5
HPTBM	0 (0.0%)	73 (13.7%)	0 (0.0%)	460 (86.3%)	533	485	5
IMK	2 (0.9%)	106 (47.5%)	0 (0.0%)	115 (51.6%)	223	200	5
KRSBP	2 (0.6%)	91 (28.6%)	0 (0.0%)	225 (70.8%)	318	550	5
LMGH	0 (0.0%)	21 (8.8%)	1 (0.4%)	218 (90.8%)	240	20	5
MCHTC	0 (0.0%)	8 (5.2%)	0 (0.0%)	145 (94.8%)	153	0	5
MKOS	2 (1.6%)	15 (12.0%)	0 (0.0%)	108 (86.4%)	125	0	5
MPSS	1 (0.2%)	330 (71.1%)	0 (0.0%)	133 (28.7%)	464	300	5
PKMM	3 (0.6%)	35 (7.6%)	1 (0.2%)	423 (91.6%)	462	900	5
SVCAB	3 (0.5%)	193 (35.0%)	102 (18.5%)	253 (45.9%)	551	800	5
SVCASB	0 (0.0%)	199 (50.4%)	0 (0.0%)	196 (49.6%)	395	150	5
VNTH	16 (6.6%)	214 (88.1%)	0 (0.0%)	13 (5.3%)	243	0	5

10 West Nile Virus and Eastern Equine Encephalitis Virus Analysis, 2025

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, 2025

Entomogen staff tested a maximum of three pools per trap submitted, based on recommendations provided by Public Health Ontario (PHO, 2025). From the 86 traps submitted a total of 167 pools were sent for testing.

Table 6 presents the species breakdown and number of pools of each species tested separated into their respective categories. Eleven (11) different species or species complexes were tested for the presence of WNV. WNV bridge vector species *Aedes vexans vexans* (N=29) accounted for the greatest percentage (17%) of the pools that were tested followed by WNV bridge vector *Anopheles punctipennis* (N=23, 13%). WNV enzootic vector *Culex pipiens/restuans* (N=20) and WNV bridge vector *Ochlerotatus triseriatus* (N=20) had the third most pools tested in Grey Bruce Public Health, each accounting for approximately 12% of the total pools tested. There were no confirmed WNV-positive pools reported in Grey Bruce Public Health in 2025.

10.2 Eastern Equine Encephalitis Virus Analysis, 2025

Seven (7) *Culiseta melanura* pools were tested for the presence of EEEV. This accounted for 4% of the total number of pools tested in Grey Bruce Public Health in 2025. There were no EEEV positive pools reported.

Table 6. Grey Bruce Public Health, Number of Pools Tested for WNV or EEEV by Species in 2025

Species	Total Number of Mosquitoes	Number of Pools	Positive Pools
Aedes vexans vexans	234	29	0
Anopheles punctipennis	43	23	0
Anopheles quadrimaculatus	8	6	0
Anopheles walkeri	6	5	0
Culiseta melanura	104	7	0
Culex pipiens/restuans	50	20	0
Culex species	1	1	0
Ochlerotatus canadensis	162	10	0
Ochlerotatus japonicus	21	11	0
Ochlerotatus stimulans	102	11	0
Ochlerotatus triseriatus	31	20	0
Ochlerotatus trivittatus	153	24	0
Total	**915**	**167**	**0**

11 SUMMARY

Of the 4,516 mosquitoes identified in 2025, 50 were *Culex pipiens/restuans*, the main enzootic mosquito complex. This number made up 1.1% of the total identified mosquito population in the 2025 season, which is a decrease from the 2024 numbers (60 mosquitoes, 2.1% of the population). In total, 160 mosquito pools consisting of eleven (11) different species or species complexes were tested for WNV in 2025. In addition, seven (7) *Culiseta melanura* pools were tested for EEEV.

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, human and mosquito cases of WNV were higher in 2025, in comparison to 2024.

12 RECOMMENDATIONS

There were no WNV-positive mosquito pools, however three (3) human cases, six (6) avian cases and three (3) equine cases were reported in Grey Bruce Public Health in 2025. Although trapping was conducted weekly, traps were rotated among 17 sites[5] therefore it is possible that positive pools may have been missed during the weeks when trapping was not conducted. It may be more beneficial to submit weekly from sites that produce WNV enzootic vector species *Culex pipiens/restuans* and rotate the remaining traps among the less productive sites.

One hundred and four (104) *Culiseta melanura* were identified in Grey Bruce Public Health in 2025, the majority of which were collected from site SVCAB in Brockton. Additionally, EEEV bridge vector species - *Ochlerotatus canadensis*, *Aedes vexans vexans* and *Coquillettidia perturbans* – made up 47% (N=2,117) of all mosquito species collected in Grey Bruce Public Health in 2025. Historically *Coquillettidia perturbans* and *Aedes vexans vexans* have tested positive for EEEV in Ontario in the past (PHO, 2014). While human cases of EEEV are extremely rare in Canada, one (1) EEEV human case was reported in Hamilton Public Health in 2025, marking the second consecutive year a human case of EEEV has been reported in Ontario. Furthermore, eight (8) EEEV equine cases were reported in 2025. Equine cases have become more common over the past number of years; therefore, Grey Bruce Public Health should continue to monitor these numbers and may want to consider targeted trapping using resting traps at site SVCAB particularly late in the season (end of August through September).

In 2016, *Aedes albopictus* and *Aedes aegypti* – two exotic species that are vectors of many diseases including dengue, Zika and chikungunya - were discovered during routine surveillance in Windsor-Essex County in addition to the discovery of *Psorophora howardii* in 2024 (Benton et. al, 2025). Furthermore, a locally acquired case of chikungunya virus was reported in New York State in 2025 (ABC News, 2025). 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.

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[5] Traps were submitted from a total of 18 sites, however the trap at site ESSS was only set once in response to a reported human case.

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

ABC News. 2025. Citing Online Sources: New York confirms 1st locally acquired case of chikungunya virus in 6 years in US [online] Available from https://abcnews.go.com/Health/new-york-confirms-1st-locally-acquired-case-chikungunya/story?id=126539403 [accessed 21 October 2025]

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

Benton, Nicholas, Krokovsky, Larissa, Gasparotto, Alessio and Hunter, Fiona F. 2025. New record of *Psorophora howardii* (Diptera: Culicidae) in Southern Ontario, Canada. *Journal of Med Entom.* 0(0):1-4.

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. *Jou. l of Med. Ento*. 0(0): 1-4.

Canadian Animal Health Surveillance System, 2025: Equine Diseases Dashboard Canada [online]. Available from https://www.cahss.ca/cahss-tools/disease-alerts [accessed 7 November 2025].

Centers for Disease Control and Prevention. 2025. Citing Online Sources: Eastern Equine Encephalitis Virus [online] Available from https://www.cdc.gov/easternequineencephalitis/transmission/index.html [accessed 3 October 2025].

Centers for Disease Control and Prevention. 2025. Citing Online Sources: Current Year Data (2025) [online] Available from https://www.cdc.gov/west-nile-virus/data-maps/current-year-data.html?CDC_AAref_Val=https://www.cdc.gov/westnile/statsmaps/current-season-data.html [accessed 7 November 2025].

Government of Canada. 2025. Vector-borne disease surveillance in Canada: Mosquito-borne disease surveillance: Seasonal update (Last updated: 2025-11-10) [online]. Available from https://health-infobase.canada.ca/zoonoses/mosquito/ [accessed 28 November 2025].

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.

Ministry of Health and Long-Term Care. 2025. Citing online sources: West Nile Virus Preparedness and Prevention Plan, Ministry of Health, April 2023 [online]. Available from https://files.ontario.ca/moh-ophs-ref-west-nile-virus_plan-2023-en.pdf [accessed 3 October 2025].

Ontario Agency for Health Protection and Promotion (Public Health Ontario). Citing online sources: Eastern equine encephalitis: history and enhanced surveillance in Ontario. Toronto, ON: Queen's Printer for Ontario. 2014. [accessed 30 October 2025].

Public Health Ontario. 2025. Citing online sources: West Nile Virus Surveillance Reports [online]. Available from https://oahpp.maps.arcgis.com/apps/dashboards/bb2f1ae3ae754de5801142e3569f11bb [accessed 28 November 2025].

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. 6 November 2025, 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 3 October 2025.