

ADULT MOSQUITO SURVEILLANCE

Introduction

The West Nile virus survives by circulating between bird and mosquito populations. A female mosquito can acquire the infection by obtaining a blood meal from an infected bird and after a two-to-three week incubation period, can then pass the infection by injecting its saliva into another host (bird, horse, human or other animal) when it takes a blood meal.⁷ Once in the new host, the virus can multiply, causing illness and possibly death.

There are 74 known species of mosquitoes in Canada;³ 57 of these have been identified in Ontario.⁷ According to Health Canada, only 10 species have been found to be infected by WNV in this country.³ The most important “amplification” species in Ontario are thought to be *Culex pipiens* and *Culex restuans*. The most important “bridge vector” mosquitoes are highly dependant on local conditions. *Coquillettidia perturbans* and *Aedes vexans* may be the most important bridge vectors in Peel because of their high numbers. Three other species of mosquitoes (*Culex salinarius*, *Ochlerotatus triseriatus* and *Ochlerotatus trivittatus*) may be more important in the transmission of WNV to humans in Peel, than their small numbers suggest because of their high infection rates and aggressive biting of people.

The purpose of mosquito surveillance programs is to monitor mosquito populations associated with WNV, determine the level of WNV activity among these species and use this information to make decisions regarding the risk for transmission to humans and the need to implement mosquito control plans.

Methods

Two types of specialized traps used to capture adult mosquitoes were deployed in 20 fixed and 10 temporary locations across the Region of Peel from May 27 to October 17, 2002. CDC miniature light traps use carbon dioxide as bait to attract host-seeking adult female mosquitoes looking for a blood meal. Gravid traps are designed to attract adult female mosquitoes searching for a suitable site in which to lay their eggs.¹⁴

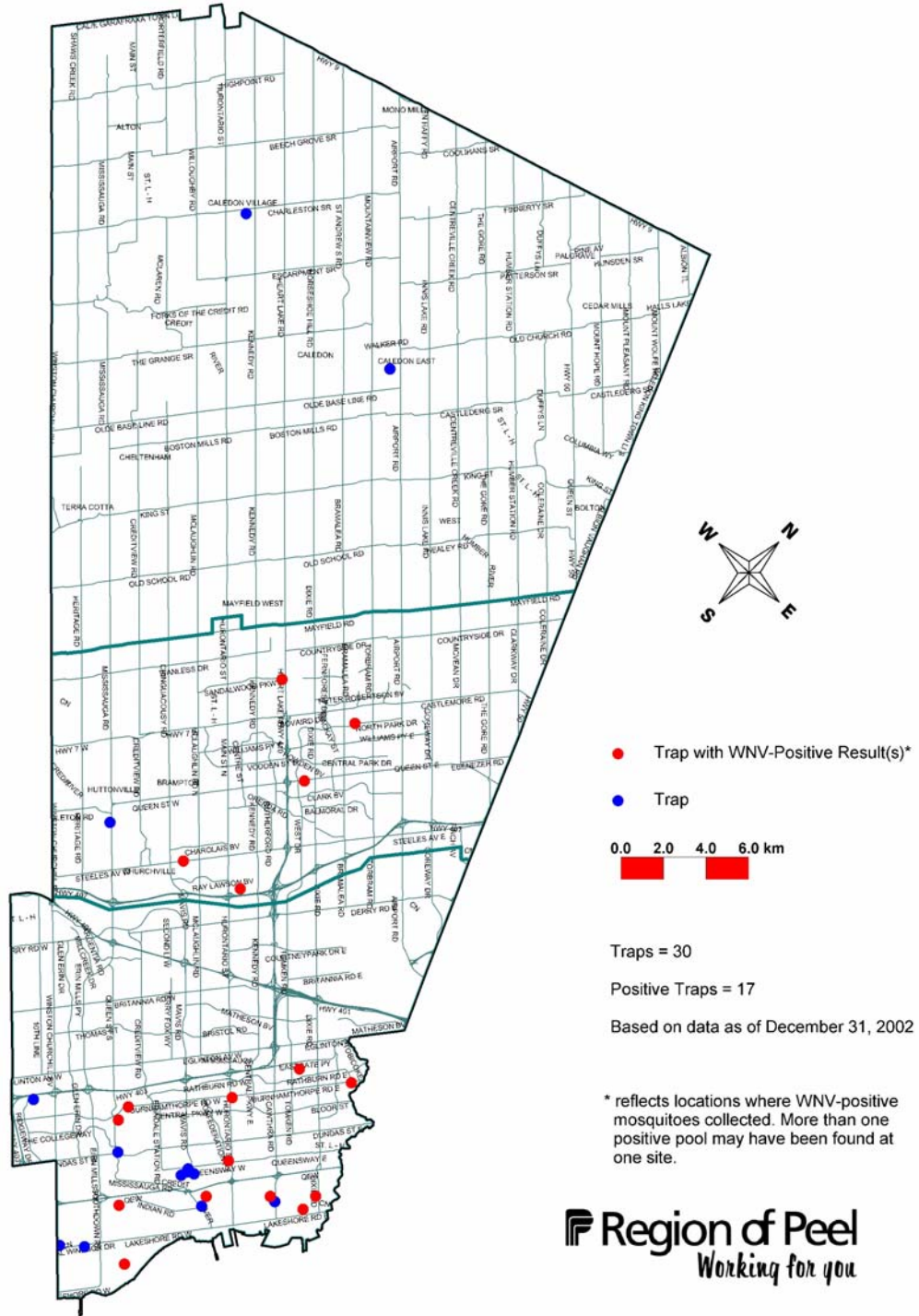
Adult mosquito traps were placed as shown in Figure 4. There were 22 sites in Mississauga, six in Brampton and two in Caledon. In all, 30 sites had light traps deployed and 25 sites had gravid traps set over the course of the mosquito season.



Adult mosquitoes were collected each week from the mosquito traps, refrigerated and transported alive via courier to the Department of Biological Sciences at Brock University in St. Catharines, Ontario. The trap contents were counted and separated into males (which feed on nectar and do not take blood meals) and females. The females were separated into species, recounted and then “pooled” by species, date of collection and location for testing. Pools generally numbered less than or equal to 50 adult female mosquitoes.



Figure 4: Locations of Mosquito Traps and Traps with WNV-Positive Results, Region of Peel, 2002



When species could not be distinguished, they were either classified as a group (e.g. *Culex pipiens/restuans*) or to the level of genus (e.g. *Culex spp.*). Some mosquitoes ended up being classified as unknown or “extras”, likely because the specimens were either unusable due to the natural aging process, or were damaged during collection, shipping or storage, making them difficult to identify.¹⁴

Initially, all testing of female mosquitoes was conducted at Health Canada’s National Microbiology Laboratory in Winnipeg, Manitoba. Later in the year, Brock University developed its own testing capability as part of a pilot program, which allowed some of the viral testing to be done at their facility. The remainder of Peel’s specimens was tested by Health Canada for the presence of WNV, and final results were communicated in April, 2003.

Counts of mosquitoes by species, date collected, trap type and site were entered into an Excel spreadsheet by staff at Brock University; the file was then distributed to the public health units that had submitted specimens. Once they became available, positive pool test results were communicated to public health units via electronic mail.

Upon notification of laboratory evidence of a human case in Peel, the first known human case of WNV encephalitis acquired in Canada, additional light traps were set in the southern part of Mississauga to better monitor the risk of human infection in that area.

Results

In Peel, nearly 42,000 mosquitoes were trapped over the course of the 2002 season; however, only a portion of these was suitable for testing for the presence of WNV. Thus, a total of 24,269 adult female mosquitoes of 41 different species were collected and submitted for testing (Table 1).

The first notification in 2002 of a positive pool of mosquitoes identified in Ontario was a pool that had been collected in Mississauga on June 20, 2002 (Appendix C).¹⁵ As of May 23, 2003, 128 positive mosquito pools from the 2002 season had been identified in the Region of Peel. These included results from two of three area municipalities: 83% (106) of the positive pools were from Mississauga and 17% (22) were from Brampton (Table 2). No positive pools were identified in Caledon. Locations of WNV-positive mosquitoes found in Peel are identified by a red symbol in Figure 4.



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Table 1: Number of Female Adult Mosquitoes Collected by Species and Municipality, Region of Peel, 2002

Species Name	Brampton	Caledon	Mississauga	Peel
<i>Coquillettidia perturbans</i>	340	271	7383	7994
<i>Aedes vexans</i>	1536	27	2765	4328
<i>Culex pipiens/restuans</i>	582	5	2459	3046
<i>Culex spp.</i>	369	5	1445	1819
<i>Culex pipiens</i>	317	2	977	1296
<i>Culex restuans</i>	93	5	863	961
<i>Ochlerotatus excrucians</i>	160	3	533	696
<i>Aedes vexans/cantator</i>	390	2	231	623
<i>Aedes/Ochlerotatus spp.</i>	324	6	263	593
<i>Ochlerotatus triseriatus</i>	24	3	548	575
<i>Ochlerotatus trivittatus</i>	103	3	300	406
<i>Ochlerotatus canadensis</i>	88	6	143	237
<i>Anopheles punctipennis</i>	17	3	125	145
<i>Culex salinarius</i>	10	0	105	115
<i>Anopheles quadrimaculatus</i>	41	10	8	59
<i>Culex pipiens/salinarius</i>	6	0	50	56
<i>Aedes cinereus</i>	47	0	6	53
<i>Culex territans</i>	0	0	34	34
<i>Ochlerotatus triseriatus/hendersonii</i>	0	0	31	31
<i>Ochlerotatus dorsalis</i>	8	0	9	17
<i>Aedes vexans nipponi</i>	4	0	7	11
<i>Ochlerotatus sticticus</i>	1	0	10	11
<i>Culiseta inornata</i>	0	0	10	10
<i>Anopheles spp.</i>	3	2	1	6
<i>Uranotaenia sapphirina</i>	1	0	3	4
<i>Culex quinquefasciatus</i>	1	0	2	3
<i>Ochlerotatus cantator</i>	0	0	3	3
<i>Ochlerotatus hendersonii</i>	0	0	3	3
<i>Ochlerotatus stimulans</i>	0	0	3	3
<i>An. quadrimaculatus/walkeri</i>	1	0	1	2
<i>Culex erythrothorax</i>	0	0	2	2
<i>Culiseta spp.</i>	0	0	2	2
<i>Anopheles barberi</i>	0	0	1	1
<i>Anopheles walkeri</i>	0	1	0	1
<i>Culex reevesi</i>	0	0	1	1
<i>Culex restuans/salinarius</i>	0	0	1	1
<i>Culiseta morsitans</i>	0	0	1	1
<i>Ochlerotatus sollicitans</i>	0	0	1	1
<i>Orthopodomyia alba</i>	0	0	1	1
<i>Psorophora ferox</i>	0	0	1	1
<i>Psorophora spp.</i>	0	0	1	1
Unknown	8	0	1108	1116
Total	4474	354	19441	24269



A comparison of the number of positive pools and their dates of collection by Health Unit in Ontario can be found in Appendix C. Only Toronto had a higher number of positive pools identified (175) than Peel (128). Across Ontario, there was a total of 598 WNV-positive mosquito pools in 2002.¹⁵

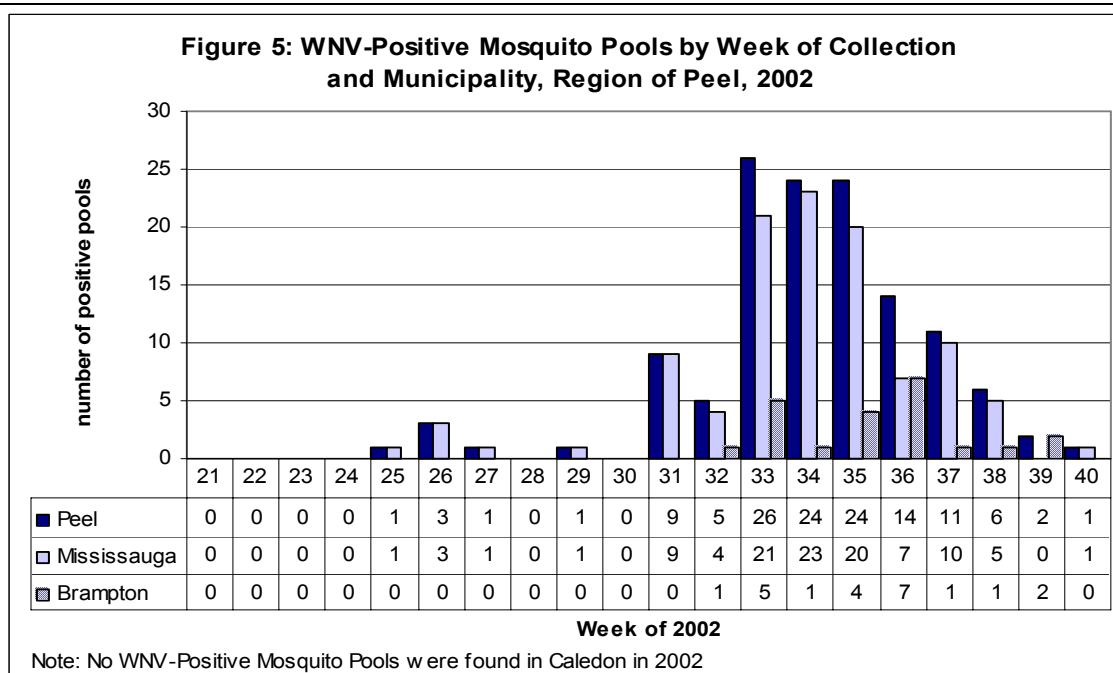
Overall, eight separate species of mosquito were found to be positive in Peel (Table 2). In general, *Culex* mosquitoes were the most important, and accounted for 30% of the mosquitoes that were collected and tested and over three-quarters (77%) of the WNV-positive mosquito samples. However, WNV was not isolated in all species within the genus *Culex*.

Because of their importance in the bird-mosquito amplification cycle, certain *Culex* species were combined for analysis. *Culex pipiens*, *Culex restuans*, *Culex pipiens/restuans*, and *Culex spp.* mosquitoes that could not be separated into species accounted for 29% (7122/24,269) of the mosquitoes collected and 68% (87/128) of the WNV-positive pools in Peel. Meanwhile, the bridge vector species *Coquillettidia perturbans* and *Aedes vexans* combined for 51% (12,322/24,269) of mosquitoes collected and 13% (17/128) of the positive pools.

A temporal analysis of WNV-positive mosquito pools by week of collection was conducted by municipality and is shown in Figure 5. Overall, 20% (26/128) of the positive pools in Peel were collected during week 33 (August 11-17). The highest number of positive pools collected in any one week in Mississauga (23) occurred in week 34 (August 18-24), while the highest number of positive pools collected in Brampton (7) occurred in week 36 (September 1-7).



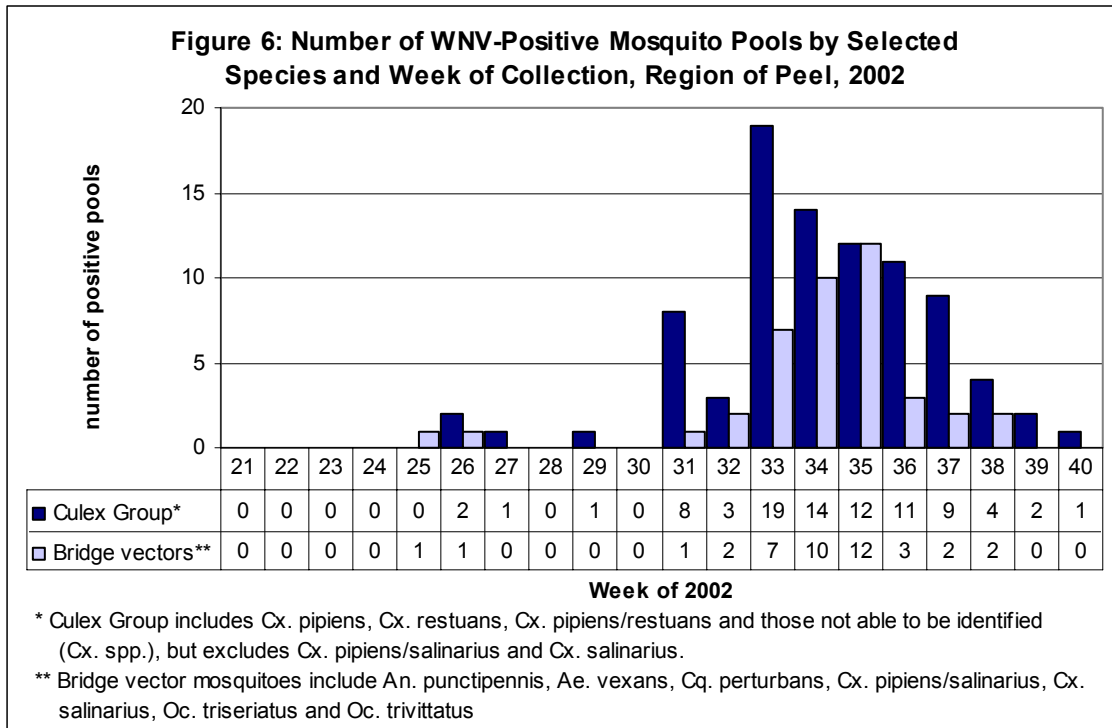
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Another analysis of the timing of WNV-positive mosquito pools can be seen in Figure 6. For this analysis, the *Culex* group included *Culex pipiens*, *restuans*, *pipiens/restuans* and those not able to be identified (spp.), but excluded *Culex pipiens/salinarius*. While the first WNV-positive mosquito pools containing species from the *Culex* group were found within one week of the first positive bridge vector species, a difference was observed between these two groups throughout the remainder of the mosquito season. The number of positive pools for the *Culex* group increased from two during week 26 (June 23-29), peaked at 19 positive pools during week 33 (August 11-17), after which it gradually decreased throughout the remainder of the mosquito season. WNV-positive pools among bridge vector species began to increase after week 32 (August 4-10) and peaked during week 34 (August 18-24).



West Nile Virus in the Region of Peel 2002



The minimum infection rate (MIR) is an indicator of the prevalence of WNV, transmission intensity and thus risk of disease to humans.¹⁴ MIRs of WNV in certain species, expressed as the number infected per 1,000 specimens tested, are shown in Table 2 by municipality and overall. Higher MIRs are usually indicative of greater WNV activity among a given species, but can become unreliable if based on small numbers.¹⁴

Overall, the highest MIRs based on samples of 1,000 or more mosquitoes in Peel, were observed among *Culex spp.* (18.1 per 1,000) followed by *Culex pipiens* (13.1 per 1,000) (Table 2). The third highest MIR occurred in *Culex pipiens/restuans* (9.5 per 1,000). This likely reflects the infection rates found in identified *Culex* species which contribute to these larger categories (i.e. *Culex restuans* < *Culex pipiens* < *Culex salinarius*). While higher MIRs were calculated for other mosquito species, they were based on samples of less than 1,000 mosquitoes and are more likely to be unstable. These include *Culex pipiens/salinarius* (53.6 per 1,000) and *Culex salinarius* (69.6 per 1,000), as well as other mosquito species.



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Table 2: Minimum Infection Rates by Species, Municipality and Overall, Region of Peel, 2002

Municipality	Species	Number Collected and Tested	Positive Pools*	MIR**
Mississauga	<i>Aedes vexans</i>	2765	9	3.25
	<i>Anopheles punctipennis</i>	125	1	8.00 †
	<i>Coquillettidia perturbans</i>	7383	8	1.08
	<i>Culex pipiens</i>	977	11	11.26 †
	<i>Culex pipiens/restuans</i>	2459	24	9.76
	<i>Culex pipiens/salinarius</i>	50	3	60.00 †
	<i>Culex restuans</i>	863	8	9.27 †
	<i>Culex salinarius</i>	105	7	66.67 †
	<i>Culex spp.</i>	1445	25	17.30
	<i>Ochlerotatus triseriatus</i>	548	4	7.30 †
	<i>Ochlerotatus trivittatus</i>	300	6	20.00 †
Brampton	<i>Aedes vexans</i>	1536	2	1.30
	<i>Culex pipiens</i>	317	6	18.93 †
	<i>Culex pipiens/restuans</i>	582	5	8.59 †
	<i>Culex salinarius</i>	10	1	100.00 †
	<i>Culex spp.</i>	369	8	21.68 †
Caledon	(none)	0	0	
Peel	<i>Aedes vexans</i>	4328	11	2.54
	<i>Anopheles punctipennis</i>	145	1	6.90 †
	<i>Coquillettidia perturbans</i>	7994	8	1.00
	<i>Culex pipiens</i>	1296	17	13.12
	<i>Culex pipiens/restuans</i>	3046	29	9.52
	<i>Culex pipiens/salinarius</i>	56	3	53.57 †
	<i>Culex restuans</i>	961	8	8.32 †
	<i>Culex salinarius</i>	115	8	69.57 †
	<i>Culex spp.</i>	1819	33	18.14
	<i>Ochlerotatus triseriatus</i>	575	4	6.96 †
	<i>Ochlerotatus trivittatus</i>	406	6	14.78 †
Total		24269	128	

* Positive pools number 132 in Ontario's report (Appendix C), but 2 pools belonged to Toronto and 2 others could not be reconciled with site or date information to determine the number of mosquitoes tested, and so were excluded.

** The Minimum Infection Rate (MIR) is calculated as the number of positive pools of infected mosquitoes of a given species divided by the total number of mosquitoes of a given species that were tested for the presence of the virus, expressed per 1,000.

† MIRs based on numbers < 1000 are more likely to be unstable than those based on numbers ≥ 1000 (bolded).



MIRs were also calculated for certain species by week of collection. In Figure 7, the *Culex* group of mosquitoes (including *Culex pipiens*, *Culex restuans*, *Culex pipiens/restuans*, and *Culex spp.* mosquitoes that could not be separated into species) had the highest MIRs (31.5 per 1,000 in week 38, September 15-21), and these continued to be above 20.0 per 1,000 in weeks 39 and 40 (ending October 5). This means that their level of infectivity carried well into the fall. Meanwhile, the bridge vector mosquitoes *Aedes vexans* and *Coquillettidia perturbans* had their highest MIRs during week 34 (August 18-24), at 13.6 and 15.6 per 1,000, respectively.

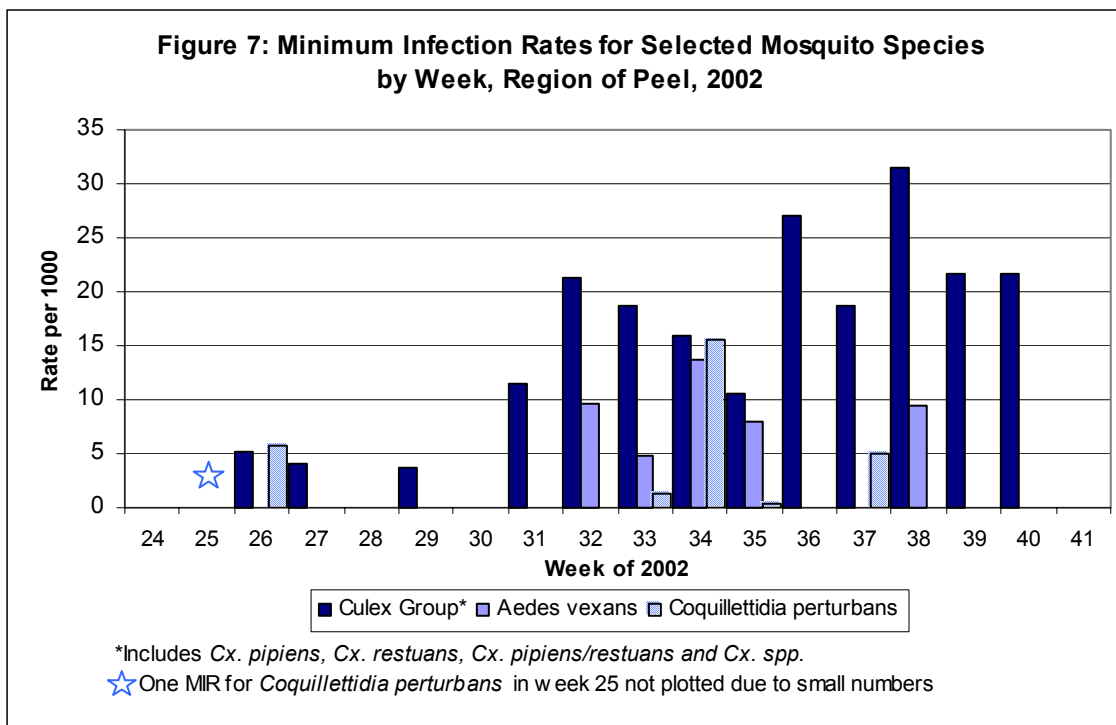


Table 3 shows the exact dates and weeks when the first notification of positive viral test results was made, by species. The first positive pool (*Coquillettidia perturbans*) was found very early in the mosquito season.

Table 3: First Notification of Positive Viral Test Results by Mosquito Species and Date Collected, Region of Peel, 2002

Mosquito Species	Date Collected	Week
<i>Coquillettidia perturbans</i>	20/06/2002	23
<i>Culex restuans</i>	25/06/2002	26
<i>Culex spp.</i>	25/06/2002	26
<i>Culex pipiens/restuans</i>	04/07/2002	27
<i>Culex pipiens</i>	30/07/2002	28
<i>Culex salinarius</i>	30/07/2002	31
<i>Aedes vexans</i>	08/08/2002	32
<i>Ochlerotatus trivittatus</i>	13/08/2002	33
<i>Culex pipiens/salinarius</i>	20/08/2002	34
<i>Ochlerotatus triseriatus</i>	20/08/2002	34
<i>Anopheles punctipennis</i>	29/08/2002	35

The four charts in Appendix D show the level of mosquito activity (# of mosquitoes trapped) by week for the 11 groups or species of mosquitoes for which positive viral results were found. Note that the scales in the charts are different. It is evident from these analyses that certain species appeared to have more than one peak period of activity.

The trapping of mosquito populations can be a function of weather conditions, particularly rain (which increases their numbers due to availability of standing water and thus breeding sites), wind (which tends to decrease numbers collected) and temperature. It is also possible that a generational effect (i.e. a new generation having hatched) is being observed in these charts particularly for *Culex pipiens*. It appears to have 3 increasing peaks or generations which is consistent with the biology of this mosquito.

Summary

Although 41 species of mosquitoes were found in the Region of Peel in 2002, only a small number were found to be important in the transmission of WNV from mosquitoes to humans. While eight separate species of mosquito were found to be positive for WNV, mosquitoes from the genus *Culex* were the most important, accounting for 30% of the mosquitoes tested and 77% of the WNV-positive pools. *Culex* mosquitoes also exhibited some of the highest WNV infection rates in Peel.



In particular, *Culex pipiens* and *Culex restuans* were key “amplification” species, because they prefer to feed on birds, the main reservoir of WNV, and are effective transmitters of the virus. They are more common in urban and suburban settings and use primarily standing or slow-moving water in which to lay their eggs.⁷ They breed quickly, producing as many as four generations such that their numbers greatly increase over the course of the mosquito season.

It is interesting to note that very few *Culex* mosquitoes were trapped in the Caledon area compared to Mississauga or Brampton. No WNV-positive pools of mosquitoes were found in Caledon in 2002.

While other species of mosquito are more likely to bite people, control of *Culex* mosquitoes is one of the most important strategies to reduce the risk of WNV transmission to humans.⁴

