ANNUAL REPORT

TO THE

NEW JERSEY STATE MOSQUITO CONTROL COMMISSION

"THE SURVEILLANCE OF VECTOR-BORNE ARBOVIRUSES IN NEW JERSEY"

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Introduction

The NJ State Mosquito Control Commission (SMCC) has monitored potential vectors of mosquito-borne encephalitis in New Jersey since 1975 with a vector surveillance program designed to keep health related agencies aware of the potential for human involvement. Eastern equine encephalitis (EEE) was an original target for investigation because of its impact on coastal resorts in the southern portion of the state. West Nile virus (WNV) was added to the program in 2000 following an outbreak in New York City the previous year. County mosquito control personnel were recruited to collect and process specimens. This program functions as a cooperative effort that includes the NJ Department of Environmental Protection, the NJ Department of Health, the NJ Agricultural Experiment Station at Rutgers and the 21 county mosquito control agencies in the state. The goal is a disease surveillance effort that provides mosquito control with information to target vector populations for the prevention of human disease. This report documents the results of virus surveillance efforts during the 2008 encephalitis season.

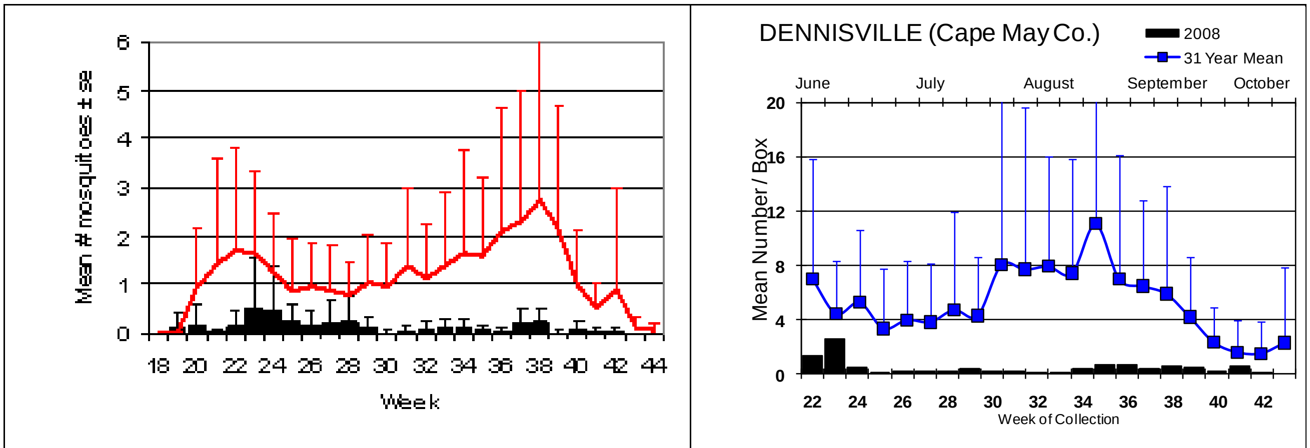
Methodology of EEE Surveillance

The mosquito, *Culiseta melanura*, is monitored from late May to mid-October as the primary indicator of EEE virus in southern New Jersey. This ornithophilic mosquito usually does not bite mammals but can be used to monitor virus levels in local bird populations as the season progresses. Weekly collections of *Cs. melanura* were made from resting boxes at permanent study sites by a team of field staff from Rutgers. The mosquitoes were frozen on dry ice at the collection site and transported to Headlee Research Labs at Rutgers for further processing. The frozen specimens were sorted on a chill table to maintain the cold chain and were identified to species, pooled by stage of blood meal digestion and submitted weekly to the PHEL facility in Trenton for virus testing. Positive pools were detected by Taqman RT-PCR. Information from the investigation was summarized and distributed weekly to mosquito control and public health agencies in New Jersey and the Northeast. The resting box collection sites for 2008 included: Turkey Swamp in Monmouth Co., Green Bank in Burlington Co., Corbin City in Atlantic Co., Dennisville in Cape May Co., Waterford in Camden Co. and Centerton in Salem Co. A new site near Glassboro in Gloucester County was added this year.

Results of EEE Surveillance in 2008

During the previous year, *Culiseta* melanura population levels rose to significant levels prior to and at the beginning of the surveillance season but did not maintain these levels throughout the season. The 2008 mosquito season began with low levels of *Culiseta melanura* in both the Statewide Surveillance light traps of the Pinelands and resting box populations in the Vector Surveillance program (Figure 1). Populations sampled from both types of traps maintained low levels throughout the season with t. Amplification occurring with the second generation should set the stage for horse and human cases, which usually develops during from August to November. In 2006, virus was detected at only one site and appeared to be poorly disseminated in southern New Jersey. EEE was detected later in New Jersey than in neighboring states. This year, virus detection also occurred earlier in some states to the north (by about 6 weeks in Massachusetts and two weeks in New Hampshire). No detection occurred in Pennsylvania and Connecticut first detected EEE the week following New Jersey.

Figure 1. Populations of *Culiseta melanura* in two types of traps in southern New Jersey during 2008.



Light trap (Statewide) surveillance in, Dennisville 2008	Resting box (Vector) surveillance in Dennisville, 2008

Table 1. Total number of *Culiseta melanura* tested for EEE by site in 2008, together with positives and earliest isolation dates.

Site Name	Coastal or Inland	Total Pools	Total Mosquitoes	Positive pools	MFIR	Earliest Date
Corbin City	Coastal	55	161			
Dennisville	Coastal	70	598	1	1.67	15-Oct
Green Bank	Coastal	51	257	1	3.98	11-Oct
Centerton	Inland	69	642	2	3.12	1-Sep
Glassboro	Inland	41	100	1	10.00	12-Oct
Turkey Swamp	Inland	60	270			
Waterford	Inland	15	68	2	29.41	12-Sep
Statewide		**361**	**2096**	**7**	**3.34**	1-Sep

Eastern equine encephalitis virus was first detected at Centerton on September 1st, three weeks later in the season than last year. The second sites for confirmed activity were at Waterford and Glassboro, suggesting that dissemination had occurred. Last infection at a traditional resting box site was detected at Dennisville on 15 October. Cape May also recorded the last positive *Cs. melanura* pool a day later in a Tuckahoe gravid trap. There were no positive EEE pools detected in other mosquito species (Table 2) nor were there other *Cs. melanura* positive pools.

Cape May County Department of Mosquito Control reported that one chicken from two sentinel flocks turned positive for EEE. As with last year, no bridge vectors were found positive with EEE. Table 2 lists all species tested for EEE.

Table 2. Total non-*Cs. melanura* species tested for EEE. No positives occurred among potential bridge vectors.

Species	Total pools	Total mosquitoes
Aedes albopictus	62	583
Aedes canadensis canadensis	21	497
Aedes cantator	3	86
Aedes cinereus	1	3
Aedes communis	1	1
Aedes grossbecki	1	1
Aedes japonicus	24	69
Aedes sollicitans	18	781
Aedes sticticus	2	5
Aedes taeniorhynchus	10	326
Aedes triseriatus	18	43
Aedes trivittatus	2	5
Aedes vexans	63	643
Anopheles bradleyi	5	30
Anopheles crucians	8	10
Anopheles punctipennis	31	71
Anopheles quadrimaculatus	25	81
Coquillettidia perturbans	26	221
Culex erraticus	79	658
Culex pipiens	39	378
Culex restuans	5	11
Culex salinarius	5	5
Culex sp.	84	750
Culex territans	11	22
Culiseta inornata	1	3
Orthopodomyia signifera	4	12
Psorophora ciliata	6	10
Psorophora columbiae	16	50
Psorophora cyanescens	1	1
Psorophora ferox	6	9
Psorophora howardii	1	3
Uranotaenia sapphirina	9	13
Statewide	**588**	**5381**

Horse and Human Involvement with EEE

No horse or human cases occurred.

Methodology of WNV Surveillance

New Jersey's WNV surveillance program in 2008 relies on significant county initiative to conduct meaningful surveillance within their county borders. Counties have various approaches to monitoring West Nile virus activity, ranging from focusing on the enzootic vector, *Culex pipiens* (primarily through the submission of Mixed *Culex* pools) to the submission of a wide range of potential bridge vectors.

The Rutgers program used gravid traps and CO_2 baited traps to collect mosquitoes from areas where human or equine cases required special surveillance investigations. The Rutgers program also conducted WNV surveillance activities for counties that requested assistance.

Results of WNV Surveillance in 2008

During the 2008 mosquito season, a total of 201,483 specimens were tested in 10,385 pools. Results from the surveillance effort produced 644 WNV positive pools, a significant increase of nearly 300 pools from the previous year. All of New Jersey's 21 county mosquito control agencies participated in the state program during 2008. Table 2 indicates species results from county and Rutgers effort in mosquito collection. As with last year, the majority of positive pools came from *Culex* species, either mixed pools or species-identified, with *Culex pipiens*, the enzootic vector of WNV showing the highest degree of infection at 5.564 mosquitoes/1000 of the three mixed species. *Culex restuans* was the second most infected species, with an MFIR value of 1.107. *Culex salinarius* was the least of the infected mosquito species with an MFIR of 0.194. The mixed *Culex* pool had an MFIR value much closer to the value for *Culex pipiens* and it is likely that *Cx. pipiens* contributes proportionally to the overall Mixed *Culex* pools.

Table 3. Mosquitoes tested for West Nile in New Jersey during 2008.

Species	Total pools	Total mosquitoes	Positive pools	MFIR
Aedes abserratus	1	9		
Aedes albopictus	1242	10321	3	0.291
Aedes atlanticus	2	5		
Aedes atropalpus	1	1		
Aedes canadensis canadensis	63	1261		
Aedes cantator	36	417		
Aedes cinereus	3	5		
Aedes communis	1	1		
Aedes grossbecki	3	4		
Aedes japonicus	612	2399	1	0.417
Aedes sollicitans	82	1528		
Aedes sticticus	9	93		
Aedes stimulans	1	1		
Aedes taeniorhynchus	45	836		
Aedes thibaulti	5	13		
Aedes triseriatus	271	729		
Aedes trivittatus	24	172		
Aedes vexans	337	4444		
Anopheles atropos	1	1		

Anopheles barberi	4	16		
Anopheles bradleyi	94	1301		
Anopheles crucians	11	35		
Anopheles earlei	2	2		
Anopheles punctipennis	190	1035		
Anopheles quadrimaculatus	216	2840		
Coquillettidia perturbans	111	963		
Culex erraticus	200	3284		
Culex pipiens	1399	26240	146	5.564
Culex restuans	959	11748	13	1.107
Culex salinarius	282	10308	2	0.194
Culex spp.	3395	117142	476	4.063
Culex territans	92	389		
Culiseta inornata	3	5		
Culiseta melanura	542	2969	3	1.010
Orthopodomyia signifera	12	21		
Psorophora ciliata	9	54		
Psorophora columbiae	36	218		
Psorophora cyanescens	1	1		
Psorophora ferox	39	185		
Psorophora howardii	4	11		
Uranotaenia sapphirina	45	476		
Statewide	**10385**	**201483**	**644**	**3.196**

Table 3 also lists infection rates in potential bridge vectors. In 2007, WNV was detected in *Aedes albopictus, Ae. japonicus, Coquillettidia perturbans* and *Culex salinarius*, representing 4.9% of positive pools. The first two species are highly competent vectors as well as aggressive mammalian biters. (*Coquillettidia perturbans* is a mosquito that is an inefficient vector for WNV) This year, less than 1 percent of the positive pools were in species other than bird biters and the difference between the two proportions was not significantly different (z=0.076). The difference in the proportion of ornithophilic species sampled (*Culex pipiens, Cx. restuans, Culex* Mixed and *Culiseta melanura*) was also not significantly different between the years (2007 = 0.724, 2008 = 0.765, z=0.015, p>0.05). Nor was there a significant difference in the number of species counties sampled from 2007 to 2008 (Paired t=0.09, n=21, p=0.46).

While counties tended to maintain their collection patterns from one year to the next, counties varied on what they collected, likely based upon many factors. The degree of urbanization is a significant feature of West Nile virus activity. The number of pools submitted by counties to detecting WNV continued to play a significant role. Last year, the total number of mosquitoes caught by a county was correlated with the number of positive pools. This year, the trend continued (Spearman’s r = 0.471, n=20, p<0.05), indicating that the greater number of mosquitoes submitted by a county, the more likely the county was to find positive mosquitoes. This effect was reduced considerably from last year (Spearman’s r = 0.84) as counties concentrated on detection in *Culex* species.

Table 4 indicates the cumulative infection rates in each county by the end of the 2008 season.

County	Total pools	Total mosquitoes	Positive pools	MFIR
Atlantic	369	7229	10	1.383
Bergen	668	30941	153	4.945
Burlington	558	4117	5	1.214
Camden	233	3840	18	4.688
Cape May	2125	28464	3	0.105
Cumberland	307	2468	8	3.241
Essex	345	3998	34	8.504
Gloucester	745	13156	56	4.257
Hudson	238	10373	63	6.073
Hunterdon	360	15365	9	0.586
Mercer	727	8701	79	9.079
Middlesex	352	8086	44	5.442
Monmouth	645	5805	26	4.479
Morris	231	6904	31	4.490
Ocean	454	6238	13	2.084
Passaic	121	3859	32	8.292
Salem	514	6055	1	0.165
Somerset	339	4299	16	3.722
Sussex	539	15776	8	0.507
Union	248	4739	35	7.386
Warren	267	11070	0	0.000
Grand Total	**10385**	**201483**	**644**	**3.196**

One sentinel chicken converted in Cape May county. Fifty-three birds sent to PHEL tested positive for the presence of West Nile virus. Infection rates ranged from a high of 56% in American Crows (*Corvus brachyrhynchos*) to a zero for non-corvids (species "Other").

Table 5. Birds tested at PHEL for the presence of WNV and their corresponding infection rates.

Species	Negative	Positive	Tested	IR
American crow	4	5	9	0.56
Blue Jay	18	15	33	0.45
Fish crow	53	27	80	0.34
Hawk	8	2	10	0.20
Other	17		17	0.00
Unidentified crow	7	4	11	0.36
All Birds	107	53	160	0.33

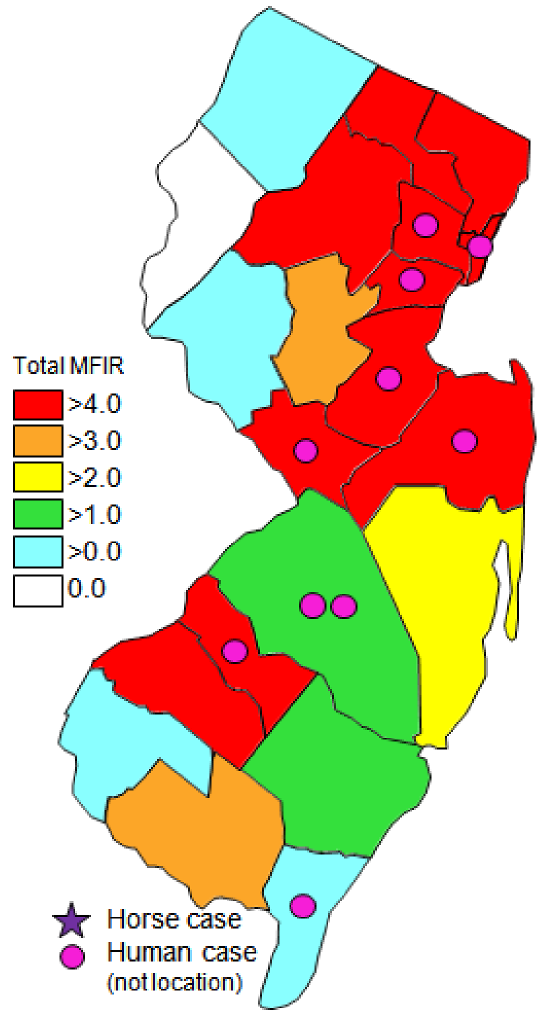
Horse and Human Involvement

During 2008, there were no equine cases reported in 2008. Ten human cases, two of which were fatal occurred in eight counties (Figure 2). This represented a significant rise from the single human case of the previous year. Earliest onset of symptoms occurred on 10 August in a 65 year-old male from Middlesex County. The last human case was reported in December, but onset of symptoms was undoubtedly much earlier.

Conclusions

EEE virus was detected only in *Culiseta melanura* and disseminated patchily throughout southern New Jersey despite low numbers of the primary enzootic vector. WNV was largely limited to bird feeding mosquitoes in 2008 but the number of positive pools increased significantly over 2007. Positive mosquitoes involved beyond the amplification cycle included 3 pools of *Ae. albopictus,* 1 pools of *Ae. japonicus* and 2 pools of *Culex salinarius*.

Figure 2. Cumulative WNV activity by the end of the mosquito season.



West Nile Risk Assessment

In order to produce predictability about how likely disease may be transmitted to humans, we are developing a risk assessment model. This model is used to predict human case occurrence with a variety of factors, including climatic and biotic. Human cases were defined by the NJDHSS. Human cases from 2000-2005 were put into a multiple regression model, predicted by a number of variables. Variables were also tested that occurred two weeks prior to human cases in order to reproduce conditions that would increase the probability of an infected mosquito biting a human and subsequent transmission of West Nile as well as include time for symptoms to appear. Variables included spring rainfall, temperature, precipitation, degree days, mosquito abundance, and MFIR values of various mosquito groups. Variables were calculated as week averages. Variables used in the regression model were chosen to reduce the effects of multicollinearity. The resulting standardized equation was derived: number of human cases = + 0.709 * (*Culex* MFIR two weeks prior) + 0.632 * (Spring Rainfall) - 0.492 * (Cumulative Degree Days) - 0.318 * (MFIR “other” feeders two weeks prior) + 0.328 * (Percent positive dead birds two weeks prior) and the unstandardized equation : number of human cases = -10.925(a constant) + 0.330 * (*Culex* MFIR two weeks prior) + 0.738 * (Spring Rainfall) - 0.002 * (Cumulative Degree Days) - 0.388 * (MFIR “other” feeders two weeks prior) + 1.675 * (Percent positive dead birds two weeks prior) ($F_{14,56}$=9.545, p< 0.000, R^2 = 0.76; significance of all variables p<0.01, except positive dead birds, where p=0.048.).

Other analyses were performed to see if more variability could be explained. For example, a logistics model was derived on the presence or absence of human cases in week using the same variables in the linear regression model, but this model explained less that the above model ($F_{12,88}$=11.622, p<0.000, R^2 = 0.61).

MFIR values of different mosquito species, plus all species combined were also examined in single regression models to predict human cases for possible relationships. Variables were examined both concurrent to weekly cases as well as lagged to account for incubation time before symptoms appeared. Variables that were lagged predicted more variability than concurrent variables (Table 6). Not surprisingly, the MFIR values of *Culex pipiens* predicted the number of human cases best, and this prediction was better when the MFIR value was lagged

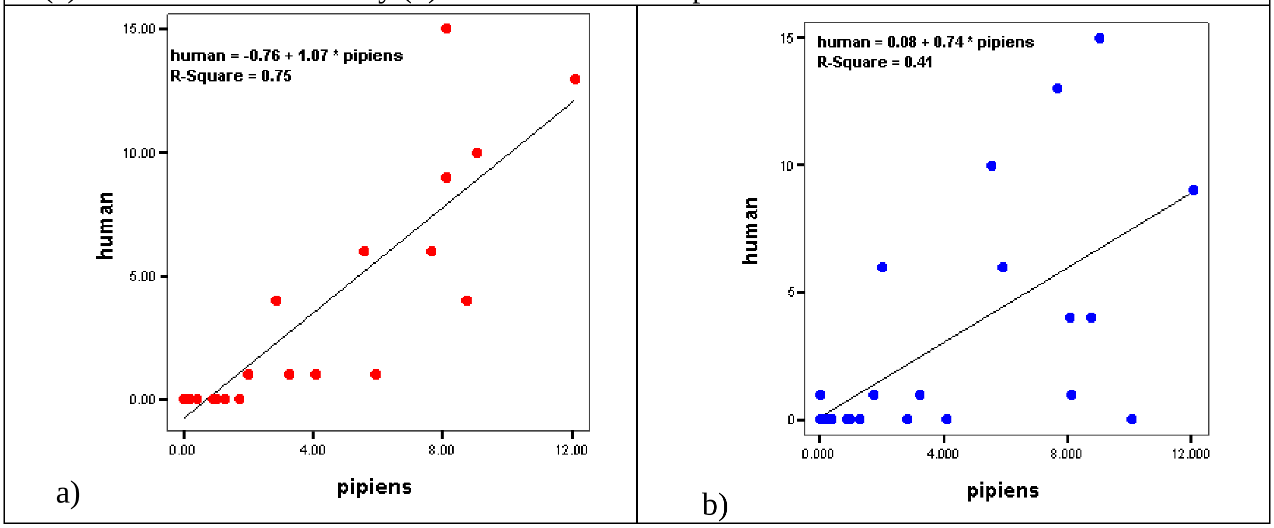
Table 6. Regression lines using MFIR values of mosquito species to predict human cases. Concurrent is when the MFIR value is what is calculated in the week human cases occur while 2 week lag MFIR reflect conditions prior to the occurrence of human cases.

Concurrent	Predicting human cases	
species	equation	R2
sollicitans	2.71 + 0.11*sollicitans	0.00
albopictus	2.72 + 0.01*albopictus	0.00
triseriatus	2.65 + 0.05*triseriatus	0.00
punctipennis	2.63 + 0.06*punctipenn	0.00
trivittatus	2.89 + -0.22*trivittatus	0.02
japonicus	2.35 + 0.20*japonicus	0.03
vexans	1.99 + 1.13*vexans	0.12
allmosquitoes	1.22 + 1.27*allmosquito	0.16
melanura	1.33 + 3.23*melanura	0.17
restuans	0.86 + 0.75*restuans	0.28
pipiens	0.08 + 0.74*pipiens	0.41

2 Week Lag	Predicting human cases	
species	equation	R2
trivittatus	3.03 + -0.09*trivittatus	0.00
albopictus	2.99 + -0.02*albopictus	0.00
sollicitans	2.64 + 1.84*sollicitans	0.06
melanura	1.92 + 2.24*melanura	0.08
triseriatus	2.09 + 0.5*triseriatus	0.09
japonicus	2.16 + 0.40*japonicus	0.13
punctipennis	2.00 + 0.55*punctipenn	0.38
restuans	0.43 + 0.99*restuans	0.49
vexans	1.23 + 2.42*vexans	0.54
allmosquitoes	0.00 + 2.37*allmosquito	0.54
pipiens	"-0.76 + 1.07*pipiens	0.75

(Figure 3). A combination of all mosquito MFIR values also predicted well when lagged as did *Aedes vexans*. This last MFIR is of interest as *Aedes vexans* is often the most abundant mosquito for many counties and it is a moderately competent vector of West Nile virus. However, *Ae. vexans* involvement in WNV in New Jersey has declined in recent years.

Figure 3. Scatterplot of regression lines using MFIR values of *Culex pipiens* to predict weekly human WNV cases. Using MFIR values that occurred two week prior to human cases (a) rather than concurrently (b) resulted in a better predictive value.



The use of single variables to predict human cases may be useful at both a county and statewide level as it is much simpler to implement as well as to understand. However, the use of *Culex pipiens* posed a problem. Currently, in this state, few people are certified to separate the three species of *Culex* that make up mixed pool (*Culex pipiens*, *Cx. restuans* and *Cx. salinarius*). While some counties often do separate these species (hence the MFIR values for the individual species), past experience has shown that people's ability to do so correctly varies widely. We encourage anyone who identifies mosquitoes in New Jersey to contact the Center for Vector Biology for testing *Culex* identification should they wish to separate these species with confidence.

Presentations

2008 Northeastern Mosquito Control Association, Plymouth Massachusetts: *Vector and Mosquito Population Surveillance in New Jersey, 2008.*

2008 New Jersey Mosquito Control Association, Atlantic City, New Jersey: *Vector and Mosquito Population Surveillance in New Jersey, 2008.*

Publications

NJMCA proceedings: New Jersey Vector Surveillance Program, 2008 (*in prep*)

Reed, L. M., Johansson, M. A., Panella, N., McLean, R. Creekmore, T., Puelle, R. and Komar, N. 2009 Declining mortality in American crow (*Corvus brachyrhynchos*) following natural West Nile infection. Avian Diseases (in print).