

Emergence of Eastern Equine Encephalitis Virus in Northeastern Massachusetts: Improving surveillance of infected mosquitoes.

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Overall Introduction

The Northeast Massachusetts Mosquito Control and Wetlands Management District is the state governmental agency responsible for monitoring mosquitoes, and the pathogens they transmit, in Essex County. A wealth of mosquito-breeding habitats abounds throughout the District, from expansive coastal salt marshes to abundant hardwood freshwater swamps and associated wetlands, as well as urbanized regions with an "artificial" assemblage of water-filled artificial containers.

Up to 2000, the District's mandate focused primarily on control of aggressive mosquitoes that have impacted tourism, local economy, and overall quality of life. Control practices included traditional ground-based abatement, but also in the previous decade were implemented novel larviciding and open-water management methodologies. However, the District was challenged with the arrival of West Nile Virus (WNV) in Essex County in 2000 and the bulk of its operations was redirected towards vector control. The District instituted a "Vector Management Plan" (VMP), which directed limited assets more efficiently and effectively by adhering to targeted, measured, and pre-emptive responses to specific risk. This program has successfully reduced WNV isolations in mosquitoes and hence, WNV spread has been kept to a relative minimum. And now, the District is being challenged again, not by a novel exotic arthropod, but by an old Massachusetts nemesis, Eastern Equine Encephalitis virus (EEEV).

EEEV has historically been limited to southern Massachusetts, an area dominated by expansive 6,000 acre white-oak Hackmatack Swamps. This is prime habitat for the vector of EEEV, *Culiseta melanura*, which is found in great abundance. Although mosquito isolations are found almost yearly, horse and human infections are far less common, and are more often than not are clustered in roughly three-year epidemic cycles occurring every 15 to 20 years. No such expansive wetlands exist in northern MA and as a consequence, *C. melanura* is nowhere as abundant. This may be the primary reason for the historical scarcity of EEEV isolations in northern MA.

Increases in EEEV-infected mosquito pools were recorded in 2004 in southern MA signaling the possible start of another EEEV outbreak (Table 1). However, EEEV isolations now began to appear in northern MA in well. And furthermore, EEEV was now being isolated in southeastern New Hampshire (see Figure 1), primarily Rockingham County bordering Essex County, for the first time since CDC began recording EEE infections in the US in 1964 (Stull *et al.*, 2006).

With concern that EEEV may "spillover" further into Essex County, the District increased EEEV surveillance and developed preemptive control strategies for 2005. And while isolations of EEEV were recorded in 2005, there was even greater EEEV activity in southern New Hampshire during county residents (see Figure 1). With EEEV isolations in Rockingham counties, but also with human infection and mortalities, aerial abatement was considered in early September 2005 over District communities along the NH border. But with prevailing cooler temperatures and declining mosquito populations, the operation was not recommended to the State. However, had an aerial spray been mandated, there would have been unavoidable bureaucratic and fiscal, as well as operational delays in its execution, which could have increased the risk to the population. It became evident that more effective planning was needed to insure that aerial abatement could be implemented quickly if necessary in 2006.

Concluding that EEEV had become "an emerging problem" in Northeastern MA, the District's 2006 VMP included both increased surveillance along the border with New Hampshire and the development of a comprehensive "Emergency Response Aerial Abatement Plan" (ERAAP). This poster presents how our vector surveillance program was enhanced.

Enhanced Surveillance using "Fiber-Pot" Resting Boxes

Introduction & Materials/Methods

The primary objective was to detect the presence of EEEV, then recommend the appropriate actions. Prior to 2006, *C. melanura* comprised less than 1% of all mosquitoes collected in our surveillance traps, traps employed at each District historical collection site west of CO₂-baited NJ and Resting-Boxes ground traps. However, most of the District's trapping sites are locations that favor the collection of other species of concern; these include *Aedes triseriatus*, *Culiseta inornata*, *Cx. sollicitans*, *Copitomyia perturbans*, *Culex pipiens*. Additionally, political & fiscal realities have impeded our ability to perform countywide comprehensive data on *C. melanura*. Only in 2006 have all Essex County communities bordering southeastern New Hampshire were members of the District, so effective sampling for *C. melanura* could finally proceed along the entire border.

To improve surveillance, resting boxes were selected as the preferred trap for *C. melanura* and for reasons of economy and ease, boxes consisting of recycle pulp fiber was the preferred type of resting box (Kumar *et al.*, 1995). The boxes, first used in 2005, were modified in 2006 (made painted black, drainage holes added with insulation foam, and circular holes drilled then plugged with rubber stoppers). It was decided to concentrate all Resting Boxes along the NH border. Ten locations, no farther than one-quarter mile of the NH border, were selected based on presence of high tree canopy and low vegetative cover (see Figure 2). At each site, six boxes were deployed; each site was visited twice weekly, between 9 am and 7 pm, from the mid-June through early September (see Figures 7 & 8). The anesthetizing agent, Triethylamine or TEA, was administered and mosquitoes aspirated and chilled; they were frozen upon arrival in the lab, identified, and sent to the State Labs in Jamaica Plain (Boston) to test for presence of EEEV.

Results & Conclusions:

C. melanura and *Cx. varians* were not collected in District's traps with any frequency until the weeks of 10 and 17 July (CDC Epidemiological Week 28 & 29). And while these two species comprised a small percentage of all species collected with CO₂-baited traps, they were a consistent and increasing presence in the Resting Boxes, in some instances, the majority of the species collected (see Table 2). Direct comparisons between CO₂-baited traps and Resting Boxes to collect *C. melanura* was only made at one location, where each trap was separated from one another by nearly 1000 feet. As seen in Figures 7 and 8, the Resting Boxes were superior in attracting both *C. melanura* and *Cx. varians*, and in decreased below, the boxes were superior in attracting EEEV-infected females as well.

As for EEEV isolations from Resting Box-captured mosquitoes, five of the ten Box sites yielded positive EEEV isolations (in Amesbury at both sites, and one site each in Merrimack, Haverhill, and Methuen). For the 2006 season, a total of eleven EEEV positive pools were identified in the District, nine collected from Resting Boxes and two from CO₂-baited traps. The first positive pools collected from Resting Boxes occurred four weeks earlier than positive pools isolated from CO₂-baited traps. This demonstrates the effectiveness of resting box surveillance to detect EEEV-infected mosquitoes earlier in the transmission cycle than with baited traps in areas where *C. melanura* population are not high.

In southern NH, EEEV was isolated in mosquitoes from twenty-one towns (Figure 1), all within twenty miles from Essex County, primarily in *C. melanura*, but also in *Cx. varians*. Mosquitoes in NH are collected exclusively via CO₂-baited CDC traps. No infected mosquitoes were recovered in the 27 miles between the northeastern MA community with an infected isolate (Brookline) and Methuen, the closest Essex county city with a positive EEEV pool. Due to the greater proximity to NH communities rather than those in southeastern MA, we conclude that EEEV transmission in Essex County is a "consequence" of the transmission cycle-focus occurring in New Hampshire.

References:

- Kumar, Nicholas; Pollack, Richard, and Spielman, Andrew. 1995. A reusable fiber pot for sampling resting mosquitoes. *Journal of the American Mosquito Control Association*. 11(4): 463-467.
- Stull, J.W. *et al.* 2006. Eastern Equine Encephalitis- New Hampshire and Massachusetts, August-September 2005. *Morbidity and Mortality Weekly Report*. 55(25): 697-700 (June 30, 2006).

Table 1. EEEV Isolations in Massachusetts & New Hampshire: 2001 to 2006.

Year	Southeastern MA			Northeastern MA			Southeastern NH		
	# collected	% EEEV	# EEEV	# collected	% EEEV	# EEEV	# collected	% EEEV	# EEEV
2001	12	0	1 // 0	0	0	0	0	0	0
2002	1	0	0	0	0	0	0	0	0
2003	9	3*	0	0	0	0	0	0	0
2004	37	4	4 // 2	2**	3***	0	19	2	0
2005	40	1***	4 // 2	2	2	0	15	6	7 // 2
2006	157	6*	5 // 2	11	0	0	40	1	0

* - one other horse infection in Hampton Co.
 ** - from towns west of Essex Co. (Billerica & Wilmington)
 *** - 2 from towns west of Essex Co. (Billerica & Wilmington)
 **** - one other horse infection in MA (Billerica & Wilmington)
 † - from community along border; Essex from Plum Island Co. & Haverhill from Bristol Co.

Table 2. Percentage of trap collections consisting of *Culiseta melanura* & *Cx. varians* in 2006 at District locations along border with New Hampshire.

Town	CO ₂ traps			Ground			Resting Boxes					
	# collected	% EEEV	# EEEV	# collected	% EEEV	# EEEV	# collected	% EEEV	# EEEV			
Salisbury	0	0.0	0	3	1.6	0	135	75.4	4	2.2		
Amesbury	5	0.03	0	0	0.0	0	172	23.7	9	1.2		
Merrimack	281	3.7	16	0.2	11	1.7	1	0.7	283	40.7	186	26.5
Haverhill	31	1.6	0	0.0	8	0.4	0	0.0	179	52.4	37	10.9
Methuen	18	0.1	0	0.0	0	0.0	0	0.0	345	34	8	0.8

Figure 3. Modified recycled pulp fiber nursery pot as "Resting Box" on locations.

Figure 2. "Resting Box Habitat" with high canopy and low ground vegetation.



Figure 4. Prior to applying TEA, Plexiglas plate is placed over resting box to contain resting mosquitoes.



Figure 5. Application of TEA into resting box.

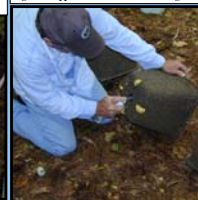
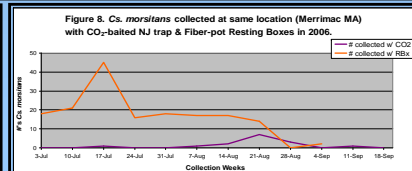
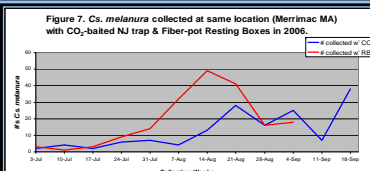
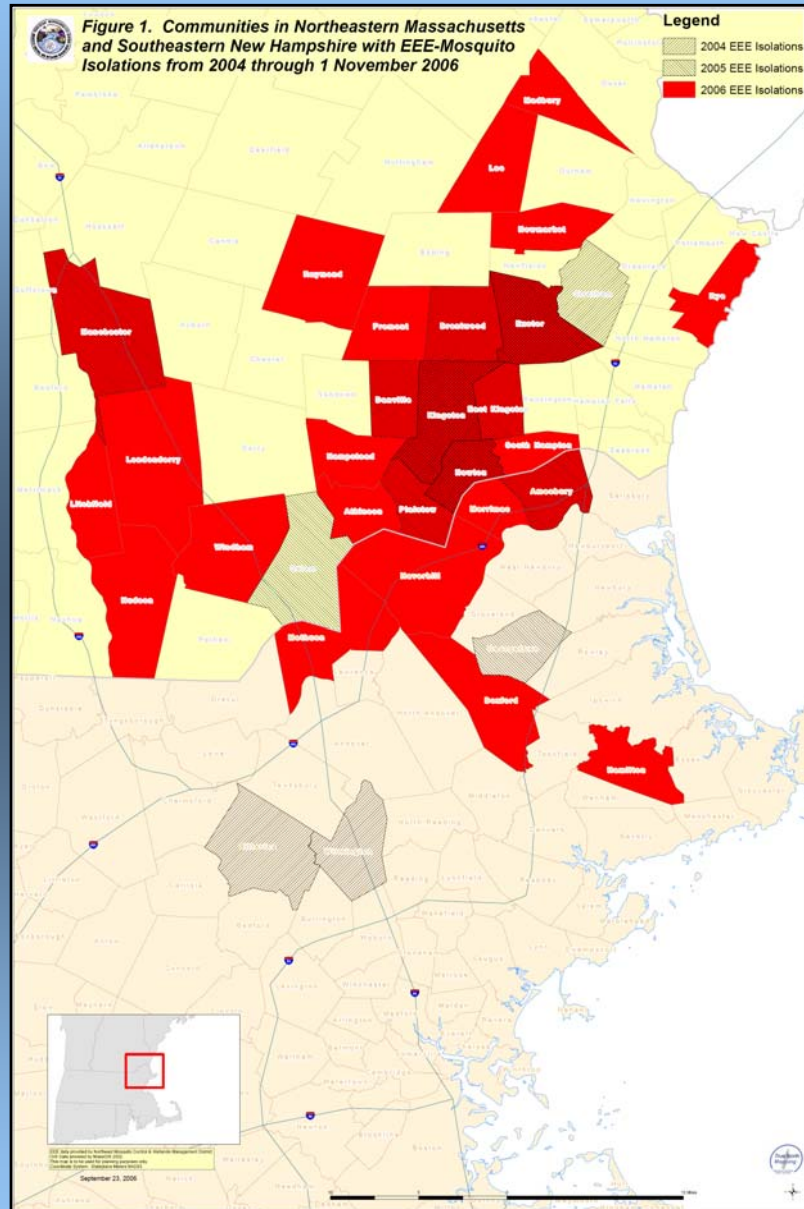


Figure 6. Anesthetized resting mosquitoes being collected.



Acknowledgments

- Suzanne Lathis, True North Mapping, Inc. for design of maps;
- Eric Swanson, Field Technician & "Associate Entomologist" for expertise in trap improvements & identifications;
- Emily W.D. Sullivan, Wetlands Project Coordinator & Anthony Corvelli, Field Technician for assistance in trap collections & maintenance;
- Deena Gallant, Field Technician for Resting Box Collections (along with Director Montgomery, pictured above);
- Matthew Osborne, Field Coordinator (Mosquito Lab - MA DPH State Lab Building) for supervision of mosquito pool processing & arbovirus testing;
- John Smith (Norfolk Co. Mosquito Control), Wayne Andrews & Phyllis Collins (Bristol Co. Mosquito Control), & Dr. Richard Pollack (Harvard School of Public Health) for their valuable suggestions regarding *C. melanura* surveillance.