Center for Vector Biology & Zoonotic Diseases

The Center for Vector Biology & Zoonotic Diseases at The Connecticut Agricultural Experiment Station (CAES) brings together the research, surveillance and diagnostic activities of our scientific and technical staff working on arthropods of public health and veterinary importance and the infectious disease organisms they transmit in Connecticut and the northeastern United States. The mission of the Center is to advance knowledge on the ecology and epidemiology of vector-borne disease organisms and to develop novel methods and more effective strategies for their surveillance and control.

The Center is responsible for conducting the state-wide Mosquito and Arbovirus Surveillance Program for eastern equine encephalitis (EEE) and West Nile (WNV) viruses and testing of ticks for the Lyme disease agent. Scientists at the Center are also engaged in full-time laboratory and field research on the biology and control of mosquitoes, ticks and bedbugs, and are investigating the epidemiology and ecology of a variety of mosquito- and tick-associated diseases that occur throughout the region including: eastern equine encephalitis, human babesiosis, ehrlichiosis, granulocytic anaplasmosis, Lyme disease, West Nile virus, Powassan virus and a related deer tick virus.

The Center maintains several microbiology, pathology, immunology, electron microscopy and molecular biology laboratories located at the main campus in New Haven and a Biosafety Level 3 containment facility where a world-wide reference collection of about 475 arboviruses are housed. The laboratory is one of the few in the northeast that is certified by the Centers for Disease Control and Prevention and the US Department of Agriculture to work with “select agents”. Select agents are bio-agents which have been declared by the U.S. Department of Health and Human Services or by the U.S. Department of Agriculture to have the "potential to pose a severe threat to public health and safety”. The Tick Identification, Testing and Information Laboratory and Insect Information Office are also located in New Haven, while insectary facilities for maintaining insect, tick, and vertebrate animal colonies are located at the Station’s 75-acre research farm, Lockwood Farm in Hamden, CT. A 28-acre field station and laboratory for conducting additional studies is located at the Griswold Research Center in Griswold/Voluntown, CT.

Core funding for the Center is provided from the State of Connecticut and federal Hatch funds administered by the U.S. Department of Agriculture. Research and surveillance activities on mosquitoes and mosquito-borne diseases are additionally supported in part by an "Epidemiology and Laboratory Capacity for Infectious Diseases (ELC)" grant from the Centers for Disease Control and Prevention (CDC) administered through the Connecticut Department of Public Health (DPH). The Center has nine lead scientists, one support scientist and seven technicians, currently divided into three Research Groups investigating (1) Mosquitoes, (2) Ticks and (3) Bedbugs. A description of the Mosquito Research and Surveillance Program is described below.

Mosquito Research and Surveillance Group

Scientists:

- Dr. Theodore Andreadis, Director - Medical Entomology
- Dr. Philip Armstrong, Scientist - Virology
- Dr. Douglas Brackney, Assistant Scientist - Virology
- Dr. Goudarz Molaei, Associate Scientist - Insect Physiology and Molecular Biology
- Dr. Charles Vossbrinck, Associate Scientist - Evolution, Ecology and Molecular Biology
- Dr. John Anderson, Distinguished Scientist Emeritus - Medical Entomology

Support Staff

- John Shepard, Assistant Scientist I - Mosquito laboratory, New Haven
- Angela Bransfield, Technician II and Responsible Official - BSL3 laboratory, New Haven
- Michael Thomas, Technician II - Mosquito laboratory, New Haven
- Michael Misencik, Technician I - BSL3 laboratory, New Haven
- Michael Vasil, Technician II - Mosquito and animal colony laboratory, Lockwood Farm
- Heidi Stuber, Technician I - Mosquito and animal colony laboratory, Lockwood Farm

Mosquito Research and Surveillance Programs

Program History

In 1997, Public Act 97-289, *An Act Concerning Mosquito Control and Aerial Application of Pesticides*, (CGS Sec 22a-45b) created a Mosquito Management Program to monitor mosquito breeding populations for the prevalence of infectious agents that can cause disease in humans and to determine when measures to abate a threat are necessary. The original focus of the program was to monitor the threat of EEE virus in southeastern CT and state funding was given to the Experiment Station to create a state-wide mosquito trapping and testing program. The original program, the first of its kind in the State, was established by Dr. Theodore Andreadis and included 36 trapping locations where approximately 50,000 mosquitoes were trapped and tested on an annual basis from June through October. State funding was used to hire one Technician and five seasonal staff to assist in the collection and identification of mosquitoes. Virus isolation work was subcontracted out to Yale University, as no Biosafety Level 3 (BSL3) laboratory for conducting such work existed at the Experiment Station at that time. The following year, a small laboratory at the Experiment Station which had been used for Rocky

Mountain spotted fever and Lyme disease studies, was converted to a BSL3 virus isolation laboratory and certified by the CDC and DPH, as facilities at Yale were closing. Dr. John Anderson, then Experiment Station Director, assumed responsibilities for operation of the virus laboratory and a second laboratory Technician was hired to assist in this work.

The establishment of the mosquito trapping and testing program proved to be most timely, as it allowed Experiment Station staff to respond immediately to the introduction of WNV into the US in 1999. This led Station scientists to obtain the first isolations of WNV from mosquitoes in North America, an achievement that was published in the prestigious journal *Science* (Dec. 17, 1999).

The unprecedented establishment and expansion of WNV in the US necessitated an expansion of the mosquito trapping and testing program to include more densely populated urban and suburban communities in the State where the peridomestic mosquitoes that carried the virus were most abundant and where WNV activity proved to be a greater threat to human health. With grants from the US Department of Agriculture (Congressional award) and CDC (ELC funds) the program was expanded threefold to include trapping at 91 locations throughout the State. These sites were mostly located in southeastern and central CT. Two new Assistant Scientists, 2 Postdoctoral Scientists, 5 Technicians and 8 additional seasonal staff were hired with this funding to sustain the expanded surveillance program and support new research initiatives on WNV. The USDA funding was additionally used in 2003 to equip a new state-of –the-art BSL3 laboratory in the Johnson-Horsefall building with expanded capacity to handle increased numbers of mosquitoes and new mosquito-borne viruses. Permanent cuts in CDC funding ($230,000 to $110,000 annual) which directly supported the surveillance program occurred in 2009, and all USDA funding ($750,000 annual) which supported both surveillance and research programs was eliminated in 2010 with the abolishment of many congressional programs. This resulted in the termination of 2 Postdoctoral Scientists and 2 Technicians and a scaling back of specific research programs. State funding was increased in 2011 from $230,000 to $460,000 to make up for the loss in federal funding and to specifically maintain the surveillance program at its current level thus avoiding elimination of trapping locations in the State.

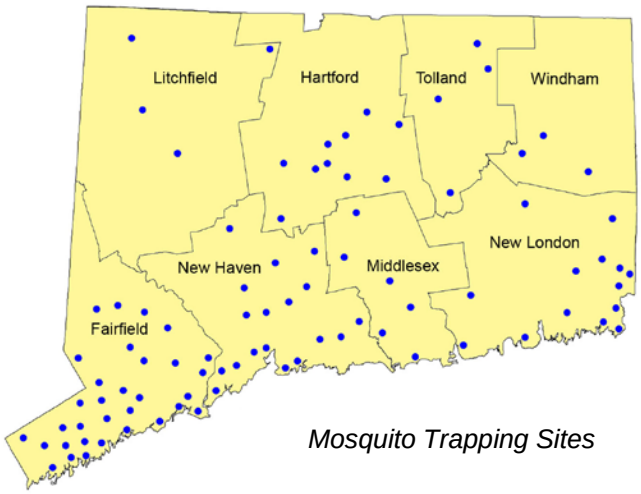
Current Surveillance Program Description

West Nile and EEE viruses constitute ongoing threats to human health in Connecticut causing severe illness and death. Surveillance for EEE and WNV in mosquitoes is integral to the public health response to these mosquito-borne diseases and the Mosquito Trapping and Testing Program conducted by the Experiment Station provides an effective early warning system that directs targeted intervention strategies and prevents disease in humans and domestic animals. The objectives of the surveillance program are to provide:

- Early evidence of local virus activity, including new viruses that may emerge
- Information on the abundance, distribution, identity and infection rates of mosquito vectors
- Information that is used to assess the threat of WNV virus and EEE to the public and guide the implementation of mosquito control measures

The entire state-wide program, including collection and identification of mosquitoes and isolation and identification of viruses is undertaken by staff at the Experiment Station under the direction of Dr. Philip Armstrong who also supervises virus isolation work in the BSL3 laboratory. Support staff include: 4 laboratory technicians and eleven seasonal staff that assist in mosquito collection in the field and identification in the laboratory, virus isolation and identification in the BSL3 laboratory and electronic data management and reporting to the DPH and the National CDC Arbovirus Surveillance System (ArboNet).

The CAES maintains a network of 91 permanent mosquito-trapping stations in 72 municipalities throughout the state. One-third of the sites are located in densely populated areas in southern Fairfield and New Haven Counties where the highest levels of WNV activity in mosquitoes, and humans are detected each year. Another third are located in southeastern CT where EEE activity is most frequently encountered.



Mosquito Trapping Sites

Annual training of seasonal support staff begins in May and formal mosquito trapping and testing begins in June and continues through October. Mosquito trapping is conducted daily and at each site every 10 days on a regular rotation until virus is detected. Thereafter, trapping frequency is increased to 2-3 times per week in an effort to obtain more information on the abundance of specific mosquito vectors and virus activity.

CO_2-baited CDC Light Trap

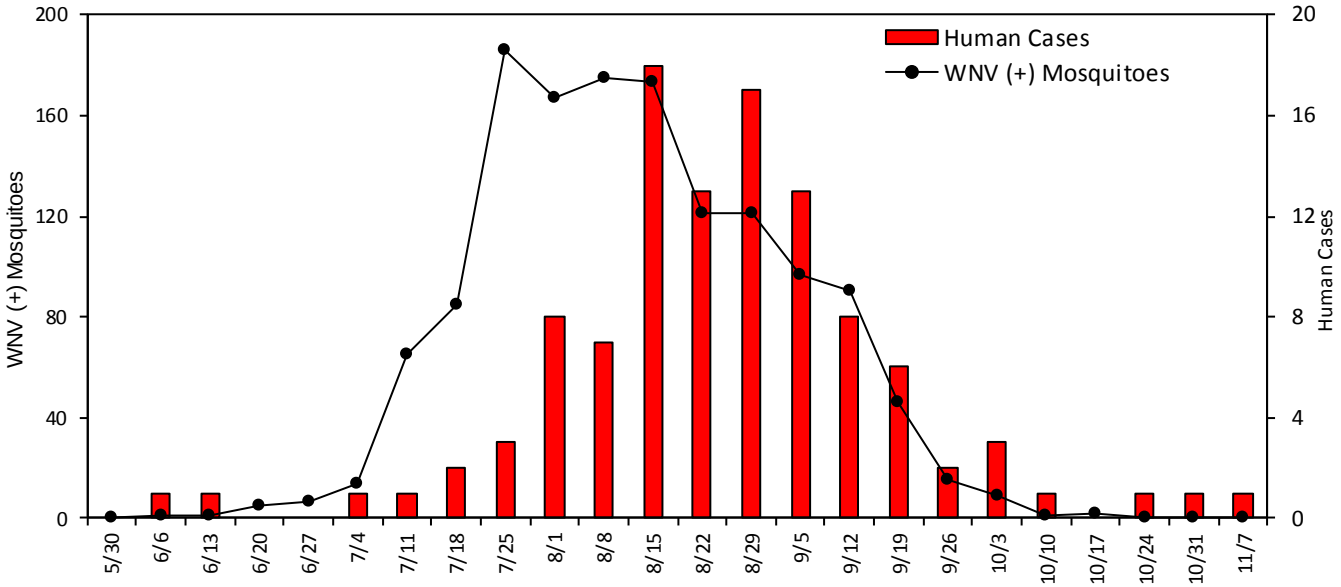
Two trap types are used at all trapping stations – a CO_2-baited CDC Light Trap, designed to trap host-seeking adult female mosquitoes (all species), and a Gravid Mosquito Trap with hay infusion, designed to trap previously blood-fed adult female mosquitoes (principally *Culex* that transmit WNV).

Gravid Mosquito Trap

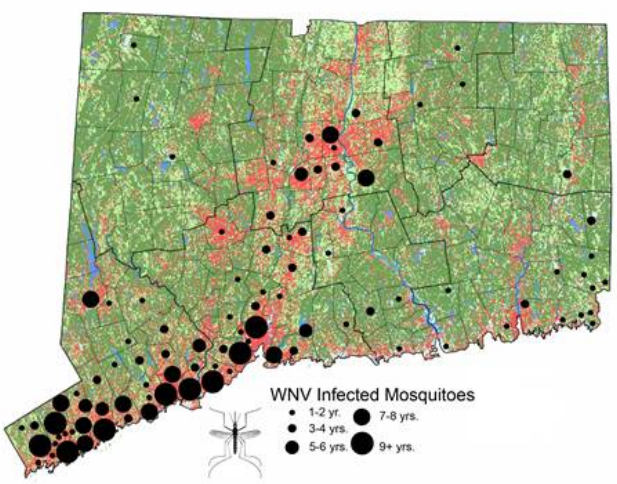
Mosquitoes are transported alive to the laboratory each morning where they were identified to species. Mosquitoes are grouped (pooled) according to species, collecting site, and date and frozen at –80°C. A maximum of 50 female mosquitoes are included in each pool. Aliquots of each mosquito pool are inoculated into Vero cell cultures (African Green Monkey cells) and screened daily for detection of WNV, EEE and other mosquito-borne arboviruses of public health importance. Virus isolates from mosquito pools are tested for WNV, EEE, Jamestown Canyon (JC), Cache Valley (CV), Trivittatus (TVT), Highlands J (HJ), LaCrosse (LAC), and Potosi (POT) viruses. Isolated viruses are identified by Real Time (TaqMan) polymerase chain reaction (PCR) or standard RT-PCR using virus-specific primers, plaque reduction neutralization (PRNT) and/or an enzyme-linked immunosorbent assay (ELISA) with specific reference antibodies. All of the virus isolation work is conducted in the certified Bio-Safety Level 3 laboratory at the CAES. Complete processing of mosquitoes is usually completed within 7days. Weekly test results are immediately reported to the CDC electronically via ArboNet and to the DPH for dissemination to other state agencies, local health departments, the media, and neighboring states.

Since 1996, nearly than 2.8 million mosquitoes have been trapped and tested and specific geographic localities in the State and time of the season associated with increased risk of human exposure to EEE and WNV have been identified. A total of 1,402 isolations of WNV have been made from 21 different species of mosquitoes, and a total of 341 isolations of EEE have been made from 18 species of mosquitoes. There have been 110 human cases of WNV in the state with 3 fatalities and one human case (fatal) of EEE. The principal foci of WNV activity in Connecticut have been identified as densely populated residential communities in coastal Fairfield and New Haven Counties. The principal foci for EEE activity are in more rural locales located in the southeastern corner of the state. We have observed a correlation both temporally and spatially between the isolation of WNV and EEE from field-collected mosquitoes and the elevated risk of human infection that typically extends from late July through September in Connecticut.

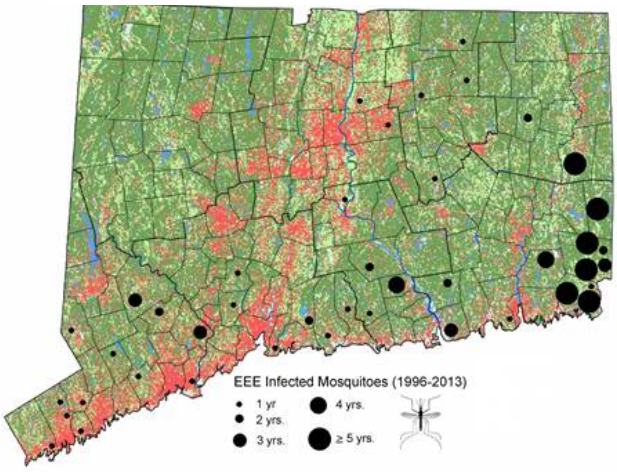
Seasonal Activity of West Nile Virus

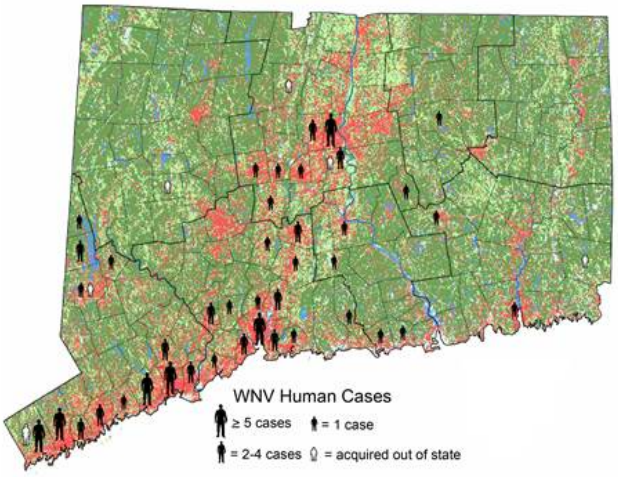


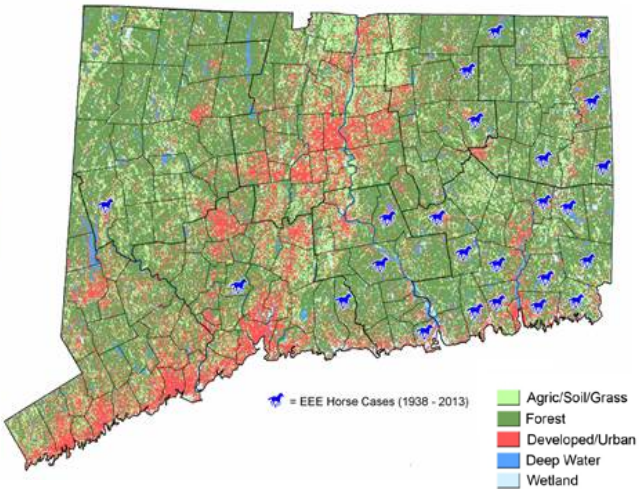
West Nile Virus Activity
1999 - 2013



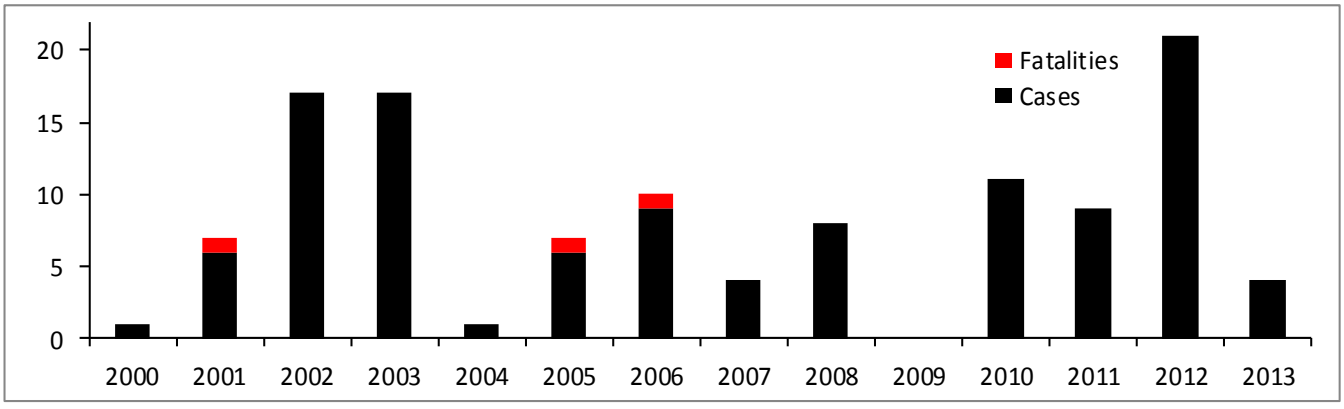
Eastern Equine Encephalitis Virus Activity
1996 - 2013







Human Cases of West Nile Virus in Connecticut 2000 - 2013



Mosquito Collection and Arbovirus Testing Summary 1996-2013

Year	No. Mosquitoes Trapped	No. Pools Tested	No. Arboviruses Isolated							
			CV	EEE	HJ	JC	LAC	PV	TVT	WNV
1996	6,440	620	-	36	19	-	-	-	-	-
1997	45,556	3,268	-	2	22	7	-	-	-	-
1998	66,383	3,981	22	8	23	5	-	-	2	-
1999	45,391	3,524	-	-	1	9	-	-	1	2
2000	137,199	9,085	1	10	11	24	-	20	3	14
2001	124,414	10,135	3	14	31	6	-	-	7	30
2002	119,950	10,643	-	-	-	16	-	-	4	73
2003	255,334	15,447	72	87	20	56	-	73	16	72
2004	156,409	12,521	-	37	-	12	-	-	1	43
2005	111,731	9,843	1	-	-	22	1	-	4	34
2006	197,793	12,661	4	3	5	23	-	3	7	219
2007	157,476	11,233	-	5	2	42	-	-	-	69
2008	211,657	15,108	13	-	13	20	-	163	9	191
2009	291,641	16,895	1	122	61	43	-	-	29	35
2010	115,377	10,580	6	4	1	22	-	-	-	220
2011	333,334	19,132	43	4	21	64	-	2	9	166
2012	189,379	14,058	-	9	3	14	-	6	-	234
2013	192,172	13,601	-	58	9	15	-	3	10	90
TOTAL	**2,759,025**	**192,568**	**163**	**399**	**234**	**400**	**1**	**270**	**102**	**1,493**

CV = Cache Valley, **EEE** = Eastern Equine Encephalitis, **HJ** = Highlands J, **JC** = Jamestown Canyon, **LAC** = LaCrosse, **PV** = Potosi, **TVT** = Trivittatus, **WNV** = West Nile Virus

Current Research Projects on Mosquitoes and Mosquito-Borne Diseases

The Connecticut Agricultural Experiment Station has a distinguished history of research on mosquitoes, ticks and associated disease-causing agents. Our scientists made the first isolation of WNV in North America and were instrumental in assisting the CDC Division of Vector-Borne Infectious Diseases during the initial outbreak (GAO/HEHS-00-180, West Nile Virus Outbreak: Lessons for Public Health Preparedness, September 2000) Since then, we have remained on the forefront of efforts to investigate and monitor mosquito-borne viruses and tick associated diseases throughout the Northeast through research and surveillance activities conducted at our Center for Vector Biology & Zoonotic Diseases.

Since its introduction in 1999, WNV virus has sickened nearly 30,000 people across the US and Canada resulting in over 1,500 fatalities. It is estimated that in the US alone, more than 1.6 million people have been infected and new evidence indicates that the virus can persist for years in convalescing patients. In 2012 we experienced the largest outbreak ever recorded in the State with 234 WNV positive mosquito pools recovered from 51 different locations in 44 towns in the state and a record 21 human cases. Meanwhile, we have witnessed a resurgence of EEE virus activity throughout the northeastern US, including regions where it had not been previously detected, resulting in 26 human cases and 9 fatalities. Our surveillance activities have been integral to the public health response to these viruses and have provided an early warning system that has directed targeted intervention strategies and prevented transmission of mosquito-borne diseases to humans. More than two and a half million mosquitoes have been trapped and tested and specific geographic localities in the state and time of the season associated with increased risk of human exposure have been identified. Work in our Select Agent Laboratory, where a worldwide reference collection of arboviruses is currently held, has resulted in the isolation and molecular characterization of seven mosquito-borne viruses that cause human disease, including two not previously recognized in the state, LaCrosse and Potosi. This work highlights our capacity to detect and respond to the potential threat to the U.S. from mosquito transmitted diseases such as dengue, chickungunya and Rift Valley fever viruses which affect millions of people globally. Through our research initiatives, we have further:

- Elucidated the natural history and epidemiology of WNV in the northeastern US including the role of various mosquitoes and birds
- Evaluated the competence of mosquitoes to transmit and serve as over-wintering hosts for the virus
- Examined the feeding and biting behavior of the primary mosquito vectors of WNVand other mosquito-borne viruses
- Developed more sensitive and rapid molecular diagnostic techniques to identify viruses
- Documented the introduction and establishment of two invasive mosquitoes from Asia
- Developed and tested novel mosquito trapping methods to enhance the early detection of mosquito-borne viruses
- Evaluated the efficacy of new and established biological agents to control mosquitoes.

The findings from these investigations have resulted in the publication of over 95 scientific articles in 26 different peer-reviewed journals since 1999.

A comprehensive surveillance program complemented with science-based mosquito control and a well-developed and timely outreach network continue to be the most effective ways to educate and protect the public. The recent establishment of invasive mosquitoes (*Aedes albopictus* and *Aedes japonicus*) from Asia in the continental U.S. including Connecticut, and the potential for the introduction of exotic mosquito-borne viruses are cause for concern due to increased global trade and travel. The detection of emerging threats from abroad in our Select Agent Virus Laboratory is further strengthened by our strategic location in close proximity to the nation's busiest ports and travel hubs, New York and Boston. We are the only regional agency in the northeastern US with the facilities, biological reference collections, technical scientific expertise, trained personnel and infrastructure to conduct these investigations from one central location. Our research findings have national significance and we are collaborating with researchers and agencies throughout the nation including: California, Delaware, Florida, Georgia, Illinois, Kentucky, Massachusetts, Michigan, New Hampshire, New Jersey, New Mexico, New York, North Carolina, and North Dakota.

Current Research Projects include:

West Nile Virus

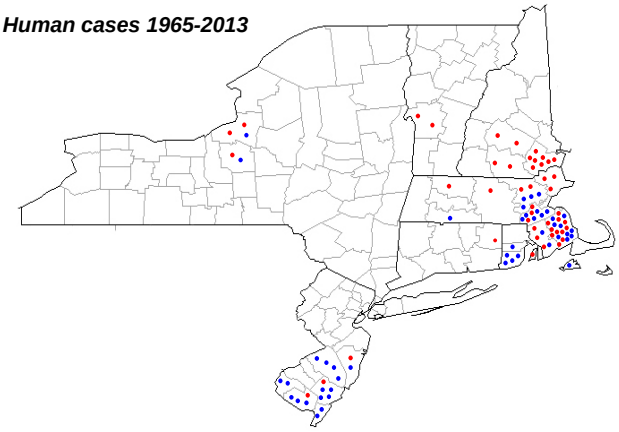
- Continuing to monitor the ecology and epidemiology of WNV in the northeastern US
- Tracking changes in mosquito populations and virus activity patterns that may be related to global climate change
- Examining the role of various mosquitoes in supporting enzootic virus transmission among avian hosts and epidemic "bridge" transmission to humans
- Investigating virus titer variation in field-collected mosquitoes to evaluate their capacity to acquire, replicate and transmit the virus
- Defining genetic mutations in WNV that may impact virulence, host-range, and infectivity through whole genome sequencing

Eastern Equine Encephalitis

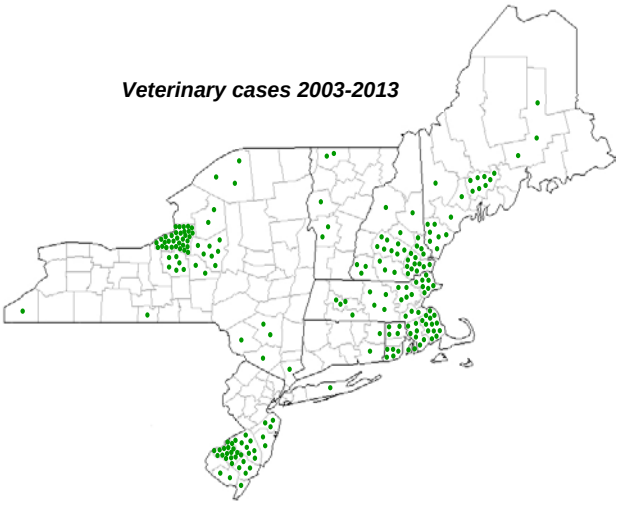
- Investigating the re-emergence and expansion of eastern equine encephalitis virus in the northeastern US
- Examining the role of *Culiseta melanura* and wild avian hosts in supporting local enzootic virus transmission (amplification) in several recognized freshwater foci in Connecticut (Chester, Killingworth, Madison, North Stonington) and epidemic "bridge" transmission to mammals
- Examining regional differences in the epidemiology of EEE virus activity in the northeast. Have cooperative projects with Vermont, Massachusetts and New York
- Studying the fine scale-scale population structure of EEE virus along the eastern seaboard through molecular phylogenetic analyses to infer virus introduction, spread, overwintering and extinction in the region

- Examining the ecological and climatic factors associated with the re-emergence of eastern equine encephalitis virus in the northeast. Cooperative project with Dr. Heidi Brown, University of Arizona
- Examining the genetic structure of *Culiseta melanura* populations throughout the east coast using novel DNA-based nucleotide polymorphism markers (microsatellites) to determine if population differences exist that may be associated with vector competence
- Identified a unique mosquito-specific flavivirus within *Culiseta melanura* populations in Connecticut. Studies are underway to characterize this virus through molecular and ultrastructural analyses and assess its distribution among geographically isolated populations

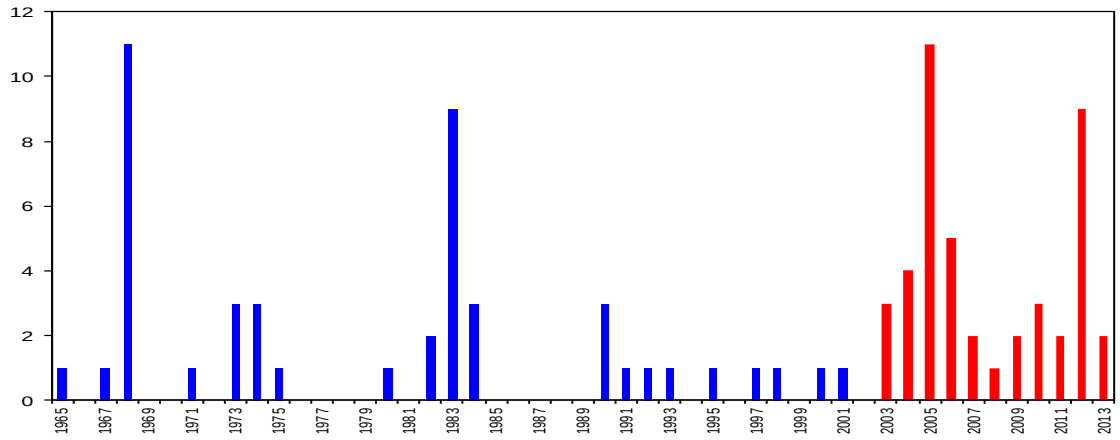
Human cases 1965-2013



Veterinary cases 2003-2013



Human Cases of EEE in the Northeastern US 1965 – 2013



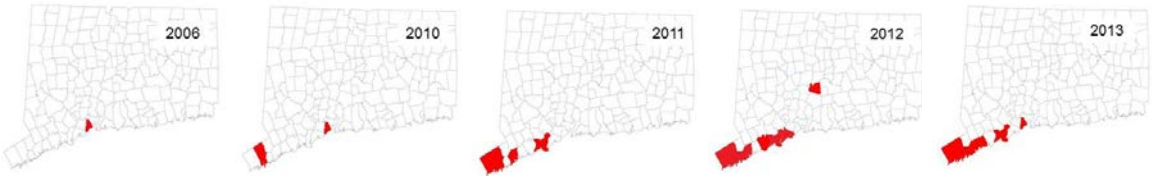
Exotic Invasive Mosquitoes

Ochlerotatus (Aedes) japonicus

- 1998 - First detected in State, likely introduced in mid-1990's
- Shown to be established throughout State
- Evaluating the competitive impact of the species on native mosquito species in natural and artificial container habitats
- Evaluating the biological control potential of a novel recently described microsporidian parasite from Japan, *Takaokaspora nipponicus*
- Evaluating its role as a local vector of WNV and other arboviruses

Aedes albopictus

- 2006 - first adults detected in Stratford and at a tire recycling plant in Sterling, but unable to overwinter
- 2012 - Overwintering populations confirmed in Stratford for first time
- Tracking its dispersal throughout coastal Fairfield and New Haven Counties. Current distribution extends from Greenwich to West Haven.



PUBLICATIONS ON MOSQUITOES AND MOSQUITO-BORNE DISEASES 1999-2014

1999

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