The Connecticut Agricultural Experiment Station (Experiment Station) in New Haven began its thirteenth year of mosquito collection and testing for encephalitis viruses during the first week of June 2009. “There have been nearly 30,000 cases in the United States and to date over 1,000 people have died from an encephalitis virus that was not even known in North America until just 10 years ago,” said Theodore G. Andreadis, chief medical entomologist and director of the state Mosquito Trapping and Testing Program.

**THE KEY VIRUSES:**

**EEE (Eastern Equine Encephalitis)** – According to Experiment Station scientists, EEE is largely unpredictable and it is unclear how the virus overwinters. The latest outbreak of EEE in New England occurred in 2005-2006, when seven cases were reported in New Hampshire and six in Massachusetts. In addition, EEE was identified in a flock of penguins at the Mystic Aquarium in 2003, highlighting its ability to infect birds.

**West Nile** – West Nile virus appears to overwinter in mosquitoes and is an annual threat to public health in the state. There is a strong correlation in time and place where the virus is found in mosquitoes and where subsequent human infections occur. West Nile virus has been detected by the Experiment Station in 21 species of mosquitoes, not all of which bite humans. In Connecticut, there have been 69 human cases, including three fatalities, caused by West Nile virus since 2000.

Other viruses known to cause human disease, and isolated one or more times over the years, include: Cache Valley, Jamestown Canyon, LaCrosse, and Trivittatus. One human case attributed to Jamestown Canyon virus occurred in Simsbury. LaCrosse virus was detected for the first time in Fairfield. It is a leading cause of mosquito-borne encephalitis in children in the nation’s Midwestern and Appalachian regions.

**HISTORY/TIMELINE:**

**Late summer 1996** – A large number of mosquitoes infected with eastern equine encephalitis (EEE) were detected in Westerly, RI, just over the Connecticut border. Andreadis and then Station Director and CASE member John F. Anderson created an emergency program of mosquito collection, identification, and testing.

**September 5, 1996** – Trapping began and continued until October 18 at 80 locations in 50 towns. A paper about the emergency surveillance reported that 6,440 female mosquitoes representing 16 species were collected, with 36 EEE virus isolations from eight mosquito species. Upon learning of the discovery of EEE, the governor and officials from the Connecticut Department of Public Health (DPH) notified the public, and some areas were sprayed to suppress mosquitoes. No human cases occurred (30% of EEE virus victims usually die and many survivors suffer permanent neurological damage).

**1997** – The governor requested and the General Assembly funded an arbovirus (viruses transmitted by arthropods) surveillance program at the Experiment Station under the direction of Andreadis. Trapping took place at 36 locations, mainly in eastern Connecticut, a focal area for EEE in horses.

**1998** – The Experiment Station’s tick laboratory was upgraded and certified by DPH and the Centers for Disease Control and Prevention (CDC) as a Biosafety Level 3 laboratory where testing for live encephalitis viruses could be done. By then, over 50,000 mosquitoes were being tested annually.

**September 1999** – Over Labor Day weekend, the CDC Division of Vector-Borne Infectious Diseases announced what it believed to be an encephalitis outbreak in New York City. Anderson collected mosquitoes in Greenwich, the town closest to New York City. The Experiment Station’s surveillance is noted in US General Accounting Office (GAO) review of the key events of the initial West Nile outbreak.

**September 25, 1999** – The CDC reported, “West Nile virus confirmed in (bird) specimens obtained from The Connecticut Agricultural Experiment Station.” The December 17, 1999 issue of Science, published paper co-authored by Anderson, Andreadis, Charles R. Vossbrinck from the Experiment Station and colleagues at Yale University and the University of Connecticut, containing the first peer-reviewed reports on West Nile virus in the United States.

**2003** – The Experiment Station completed construction and put into operation a new, state-of-the-art Biosafety Level 3 laboratory with expanded capacity to handle mosquitoes and arboviruses.

**2008** – 211,657 mosquitoes were tested. West Nile virus was found in 191 of the 15,108 pools of mosquitoes tested, but no EEE was isolated.

(continued on next page)
Two types of traps are set each year to increase the likelihood of collecting a wide range of species—one baited with a small incandescent bulb and carbon dioxide in the form of dry ice and the other baited with a hay infusion.

Field assistants set new traps daily and collect mosquitoes caught the previous night at some of the 91 trapping locations throughout the state. Trapping generally occurs at a particular site about once each week.

Identification is made using stereo dissecting microscopes and diagnostic keys written by Andreas and technicians Michael Thomas and John Shepard and published in an Identification Guide to Mosquitoes of Connecticut.

Mosquitoes are placed into flasks containing vero cells—cultured monkey cells—in which viruses grow.

Flasks are incubated at 35°C in the presence of 5% carbon dioxide and examined daily for virus growth for up to seven days. (Virus typically appears in 3-5 days. The contents of the cell culture appear cloudy when virus is present and under a microscope, destroyed cells can be seen.)

Experiment Station virologist Philip Armstrong has developed an array of molecular techniques using polymerase chain reaction amplification (PCR) to identify nine different viruses that are known to circulate in Connecticut. Depending upon location and time of year, a sample will be tested successively for the most likely virus until positive identification is made.

If EEE or West Nile virus is found, results are immediately reported to the state DPH. In addition, sampling is stepped up to two to three times per week at the location where the virus was found.

Decisions about whether to initiate mosquito control are made by local officials and the state Mosquito Management Team, which includes scientists from the Experiment Station and officials of the DPH and the Department of Environmental Protection.

The Experiment Station maintains an aggressive research program on mosquitoes and mosquito-associated diseases. Researchers use PCR methods to amplify DNA to identify the source of blood found in engorged mosquitoes. From this information, the potential of a particular species to serve as a transmitter of encephalitis viruses can be further estimated. This type of testing determined that the American Robin is the major avian reservoir of West Nile virus in this region, not the crow as originally suspected after deaths of thousands of crows occurred simultaneously with the appearance of West Nile virus in the state. Discover magazine recognized this finding by the Experiment Station as one of the top 100 science stories of 2005.

From 1996 to 2008, West Nile virus was isolated 767 times and EEE was isolated 939 times. Over the years, the Experiment Station has tested more than 1.7 million mosquitoes for viruses.

[Read more at www.ctcase.org/bulletin/24_2/24_2.pdf]