

A new mosquito-borne disease caused by West Nile virus was isolated in Connecticut in September 1999, requiring a shift in mosquito surveillance efforts. In the end, new knowledge was gained and the state prepared itself for a possible recurrence in 2000.

## *Discovery of West Nile virus in Connecticut and what was learned during the first year*

By Paul Gough

On Saturday, September 4, 1999, while most people were enjoying the beginning of the Labor Day weekend, Experiment Station Director John F. Anderson was helping a chicken farmer deal with a fly infestation, and became aware, through news media reports, of mosquito-borne illnesses and deaths in New York.

Anderson, an expert on disease-carrying mosquitoes, was puzzled to hear of this outbreak of what was initially identified as the rare St. Louis encephalitis and its extent. He was very aware of such viruses as he and fellow entomologist and Head of the Department of Soil and Water Theodore G. Andreadis were already working on the state-wide surveillance for eastern equine encephalitis and other viruses, including St. Louis encephalitis. He began immediately to plan the Experiment Station's response to the deadly threat near Connecticut's southwestern border.

That evening, Dr. Matt Carter of the Department of Public Health asked Anderson to participate in a conference call with representatives of the Centers for Disease Control and Prevention (CDC) and New York and New Jersey health officials to learn first-hand what was then known about the virus threat. They learned where people were coming down with the disease, and Anderson took notice of the fact that a few of the suspected cases were in Westchester County, NY, adjacent to Fairfield County. After talking with and getting encouragement from Dr. James Hadler, Connecticut State Epidemiologist, on Sunday morning Anderson prepared six mosquito traps for placement in Greenwich, the closest Connecticut city to the area where the disease was reported.

Because the Experiment Station was already trapping mosquitoes at 37 sites throughout the state for an early warning on viruses, the equipment and expertise were available to increase the number of trapping locations to monitor a possible appearance of St. Louis encephalitis in Connecticut.

Sunday morning Anderson made some dry ice to bait the traps from carbon dioxide gas at the Experiment Station's main laboratories in New Haven and in the afternoon headed for Greenwich.

He first stopped at Nathaniel Wetherill, a home for the aged. He entered the lobby and explained his mission to Louise Comeau who called a supervisor who said a trap could be placed if the Greenwich Police Department approved. After a call to Officer Yasek, the first trap was set. It was raining as Anderson drove to the Greenwich Police

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Headquarters where Sargent Marr assigned Officer Gleason to guide Anderson to five more Greenwich locations. As the day ended, Anderson had placed the last trap at the Montgomery Pinetum, a place where station entomologist Mark McClure had studied scale insects.

As darkness came and the usual overnight period of mosquito activity began, the cone-shaped traps emitted light and carbon dioxide to attract the insects into a cloth mesh enclosure which would prevent their escape.

Labor Day, the next morning, Anderson returned to Greenwich, gathered the six traps, and cooled the captured mosquitoes with ice packs. Tuesday morning, in a laboratory in New Haven, Andreadis and technician John Shepard identified all mosquitoes and separated them into batches of up to 50 of each species for virus testing, which was conducted in another laboratory at the Station by Jodie Correia and Bonnie Hamid.

Also on Tuesday, Anderson called two officials in Greenwich, Caroline Baisley, Health Director, and Larry Cooper, Superintendent of Parks and Trees, to make plans for more trapping. Bob Wagner of the Parks Department was assigned to help Anderson find more likely sites. Anderson also called Phyllis and Paul Mazik, friends of the Experiment Station, who were both retired from the Stamford Health Department, and obtained their help in locating trapping sites in Stamford, the next town from the New York border.

The sites sought were prime mosquito habitat: near marshes and wooded areas where stagnant water allowed mosquito eggs to hatch into larvae and transform into adults in as little as 10 days.

By this time the Connecticut news media were covering the epidemic and the developing response. In a fortuitous circumstance, Tony Campanella, working under Course Superintendent Patrick Lucas of the Innis Arden Golf Club on the Greenwich-Stamford border, who had read one of

these reports, called Anderson about a place on the golf course where mosquitoes were abundant and suggested that a trap could be put there. Anderson, with the aid of Pat Lucas and his staff, placed a trap in the woods near the 4th tee overlooking a pond.

It was there, on September 14, where virus-infected mosquitoes were captured. The virus was isolated from two different species at the Experiment Station using the same laboratory and techniques employed in the surveillance for eastern equine encephalitis. It was tentatively identified as St. Louis encephalitis virus because of its reaction to antiserum to mice infected with St. Louis encephalitis virus and the confirmation by CDC of residents infected with St. Louis encephalitis in New York. These mosquitoes were the only ones to test positive for the virus in Connecticut during 1999.

Although there had never been a reported human case of mosquito-borne encephalitis in Connecticut, a potential killer virus had been found and the public health implications were clear. If that wasn't sufficient to warrant immediate warnings and action, another unusual event was about to be discovered.

In the middle of September reports started to come to health departments and the Experiment Station of an unusual number of dead birds—mainly crows—being found in Fairfield County towns near New York. The first such crow, found on the bank of the Saugatuck River in Westport, was sent for examination to Edward M. Wakem, Richard A. French, Antonio E. Garmendia, and Herbert J. Van Kruiningen at the Pathobiology Department at the University of Connecticut. Its brain was removed and was picked up by Andreadis's technician Colleen Scott, stored in dry ice, and brought it to the Experiment Station for virus testing.

Anderson, who in 1998 had isolated eastern equine encephalitis virus from the brain of a donkey that died in the northeastern Connecticut town of Canterbury, took seven pieces of brain tissue and placed them on Vero cells. Five days later, all seven samples yielded virus. This virus isolate also reacted with antiserum from mice infected with St. Louis encephalitis virus. So now, the virus had been isolated by the Experiment Station from both mosquitoes and a bird and the viruses seemed identical.

At virtually the same time technician Jodie Correia and Shirley Terrell of Yale University confirmed presence of the virus in the crow on September 21, Andreadis was attending a news conference with Governor John G. Rowland and health officials in Greenwich where it was being announced that the Department of Environmental Protection would initiate mosquito control.

Contacted just as the news conference ended, Andreadis informed the Governor's staff and went to the podium to announce the finding to the media. Plans were also made to expand the mosquito trapping in lower Fairfield County.

Two days later, the CDC announced that the virus had been identified as West Nile-like, based on an isolate of the virus made from a bird at the Bronx Zoo, although a definite link had not been made to the illnesses. This finding did, however, clear up questions about the St. Louis virus because it would have been highly unusual for that virus to kill birds.

The Centers for Communicable Diseases and Prevention requested samples of the viruses isolated from the mosquitoes and the crow. The samples, packed in dry ice, were sent from the Experiment Station to the CDC overnight.

On Sunday September 26, Andreadis received a call from the CDC indicating that the virus isolated from the crow and the mosquitoes in Connecticut was identical to the virus identified from a bird at the Bronx Zoo. By September 29, the West Nile-like virus was reported present in the human victims.

With the virus isolated, the research was taking a new direction aimed at finding the exact identity of the virus, determining its action, perhaps even discovering its origin, and looking for the virus in mosquitoes and crows collected elsewhere in Connecticut.

Charles Vossbrinck, working with Anderson and Andreadis, extracted the RNA from the Connecticut virus isolate, converted it to DNA, and with the assistance of the

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Keck Biotechnology Center at Yale University, the sequence DNA of the virus was determined. Using a database of genes, Vossbrinck searched for similar sequences of DNA. The closest match he found was a strain of West Nile virus from Romania.

West Nile virus is widely distributed in Africa, south-western Asia and the Mediterranean area, and southern and central Europe. It had not previously been identified in North America.

Library research indicated that West Nile virus could kill birds, but there was no indication that this virus killed large numbers of birds as observed in Connecticut. The reports gave insight into the mosquito species that spread it and the birds that may harbor it. All of this information would be helpful in formulating plans for early detection and for control of mosquitoes. It was time to identify the specific strain to give additional insight into the outbreak and how it might be controlled.

The Experiment Station had virus from four species: two from the mosquitoes captured in Greenwich, one from the crow from Westport, and one from a Cooper's hawk that had been found alive in East Haven by wildlife rehabilitator Karen Hannon but had subsequently died.

After frost came and the last mosquito was tested, Anderson, Andreadis, Vossbrinck, and several colleagues at Yale and the University of Connecticut wrote a scientific paper documenting the West Nile virus in Connecticut. This paper was published in December 1999 in the same issue of the journal *Science* with another by scientists at the Centers for Communicable Diseases and Prevention detailing the outbreak in New York. The CDC scientists reported the virus matched an unpublished strain recovered from a goose from Israel. These first reports of West Nile virus in the New World will provide the basis of knowledge of the outbreak of 1999 for years to come. The Experiment Station's report in

Science concluded, "Our isolation of WN virus from mosquitoes and birds conclusively documented the presence of this virus in Connecticut during September 1999. If established in North America, WN virus likely will continue to have severe effects on human health and on avian populations, such as American crows and raptors, which heretofore have never been exposed to this virus."

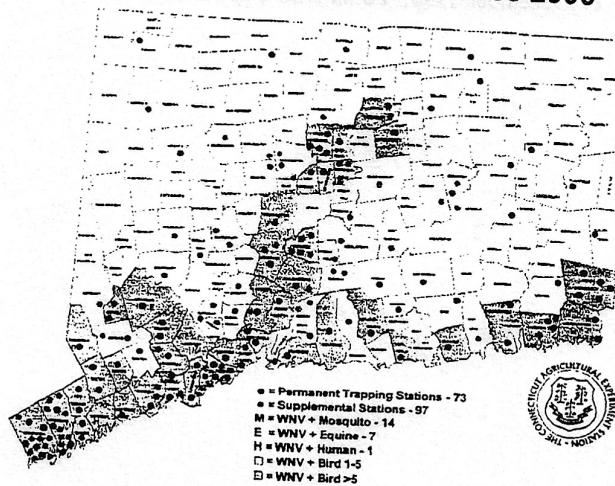
During the winter of 1999-2000, The Experiment Station joined the State Departments of Environmental Protection, Public Health, and Agriculture, and the Pathobiology Department at the University of Connecticut, in outlining a state-wide response plan for surveillance and mosquito control to be followed in 2000.

Mosquitoes have been picked up at 73 surveillance sites throughout Connecticut beginning June 1, 2000 for testing for West Nile virus. In addition, hundreds of birds and bird sera collected by Michael Vasil and Ira Kettle have been tested for the virus. The first West Nile virus of the year from mosquitoes was found in specimens collected in Stamford on July 11, 2000. On July 21, 2000 the finding of the first West Nile virus in a crow from Stamford was announced by the Experiment Station.

The finding of the virus in Stamford, along with reports of the virus in mosquitoes and birds in Massachusetts, New

York, and New Jersey documented the fact that the virus had successfully survived the winter in the Northeast.

## West Nile Virus Surveillance 2000



Map of Connecticut showing where West Nile virus was found during 2000. Data from Experiment Station, Department of Public Health, and Department of Pathobiology at UCONN.

## West Nile Virus Facts (September 1999-October 2000)

West Nile virus was found in 28 of 31 crows tested in a 62 mile stretch of Connecticut's coast from Greenwich to Madison and 15 miles inland during 1999. Other bird species testing positive for West Nile virus were a Cooper's hawk from East Haven and a sandhill crane from Bridgeport.

Number of mosquitoes tested during 1999: 45,391. Number of sites where mosquitoes were trapped: 73. The two mosquito species from which WNV was isolated by the Experiment Station during 1999: *Culex pipiens*, the most common suspect vector, and *Aedes vexans*, the floodwater mosquito. *Culex pipiens* prefers stagnant, organic or polluted water such as can be found in catch basins and a variety of backyard habitats.

The towns and the number of trapping sites in each town in Fairfield and New Haven Counties where West Nile virus activity was considered most likely during 1999: An asterisk indicates a bird with WNV was found during 1999. Fairfield County: Greenwich (3\*), Stamford (3\*), Darien (2\*), New Canaan (2\*), Norwalk (2\*), Wilton (2), Ridgefield (1), Westport (2\*), Weston (1\*), Redding (1\*), Fairfield (2\*), Easton (1), Bridgeport (2\*), Trumbull (1), Monroe (1), Newtown (1), Stratford (2\*), and Shelton (1). New Haven County: Milford (1\*), Orange (1\*), Woodbridge (1\*), Bethany, West Haven, New Haven (1\*), Hamden (1\*), East Haven (1\*), North Haven (1\*), Branford (1), North Branford (1), Guilford (1), and Madison (1\*).

Over the winter 1999-2000, over 200 mosquitoes, blood from over 1000 songbirds, and serum from over 900 birds were tested for WNV at the Experiment Station, but no virus was found. However, virus was found in New York in mosquitoes by the CDC and the University of Connecticut found, and the Experiment Station confirmed, WNV from a red-tailed hawk that died in February in Westchester County, NY. Virus-infected birds were also reported during early summer 2000 by health authorities in New York and New Jersey.

The first West Nile virus of the year from mosquitoes was found in specimens collected in Stamford on July 11, 2000. At the end of the testing, October 31, 2000, a total of 137,199 mosquitoes had been collected and tested. The virus has been found in four mosquito species: *Culex pipiens*, *Culex restuans*, *Culex salinarius*, and *Culiseta melanura*. Towns where the virus was found in mosquitoes and the number of times collected were: Greenwich (1), Stamford (3); Norwalk (2), Fairfield (1), Milford (3), Shelton (1), Meriden (1), and Westport (1). In addition West Nile virus affected horses in Bethel/Danbury, Cheshire, Glastonbury, Hebron, Middlefield, Milford, and Norwalk. A total of 1,105 birds tested positive for West Nile virus in 109 communities in all eight counties.

## Isolation of West Nile Virus from Mosquitoes, Crows, and a Cooper's Hawk in Connecticut

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