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AN EVALUATION OF THE AVIAN SURVEILLANCE SYSTEM
FOR WEST NILE VIRUS IN CONNECTICUT, 2000

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B.S., University of Hartford, 1994

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AN EVALUATION OF THE AVIAN SURVEILLANCE SYSTEM
FOR WEST NILE VIRUS IN CONNECTICUT, 2000

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INTRODUCTION

West Nile virus (WNV) first appeared in the northeastern United States in the summer of 1999. Prior to this time, it had not been identified in the Western Hemisphere. The mosquito-borne, viral epidemic that ensued resulted in 61 human cases, including 7 deaths, in the New York city area.\(^1\) A simultaneous epizootic in birds, also attributed to WNV, resulted in a large die off in the surrounding areas including Connecticut. Although the 1999 epidemic and epizootic ceased when cold weather brought an end to the mosquito season, the virus was expected to, and did, reemerge in 2000, possibly due to locally hibernating infected mosquitoes or reintroduction by migrating birds.\(^2\) Due to this threat, Connecticut developed a multifaceted surveillance system, including an avian mortality component, to detect WNV’s presence when it resurfaced. The goal of tracking locations and dates of bird deaths, in addition to viral testing of select wild birds, was to help assess the geographic distribution of WNV in the state and determine areas of potential human risk. This paper will describe the avian surveillance system used in Connecticut in 2000 and evaluate whether it has the potential to meet this goal.

The main objectives of this paper are to: 1) evaluate the usefulness of avian surveillance, 2) evaluate the ability of data collection methods to gather pertinent avian data, 3) compare the value of avian mortality surveillance to that of avian viral testing, 4) compare the value of avian surveillance methods to non-avian surveillance methods, 5) evaluate the ability of avian surveillance to track the
geographic distribution of WNV in Connecticut and 6) discuss the potential of avian surveillance to serve as an early indicator of potential human risk.
BACKGROUND

History of WNV

West Nile virus is a member of the Japanese encephalitis antigenic complex of the genus *Flavivirus*, family *Flaviviridae*. Members of this arboviral complex that are important causes of illness in people include St. Louis encephalitis (SLE), Japanese encephalitis, and Kunjin, among others. West Nile virus is the most widespread *Flavivirus*, extending throughout Africa, the Middle East, southern Europe, and Asia.² Other members of the *Flavivirus* genus found in North America include the mosquito-borne viruses SLE and Dengue fever, and the tick-borne Powassan virus. St. Louis encephalitis and Powassan are the only members of the *Flaviviridae* family that have been found in the northeastern United States besides WNV.³⁴

The virus was first isolated in the West Nile district of Uganda in 1937. In the years following this initial human isolation numerous epidemics have occurred. The first documented were in Israel in 1950-54 and 1957. Following these, other epidemics occurred in France in 1962, and Romania in 1996-97.⁵ The Romanian outbreak involved more than 500 clinical cases with a case-fatality rate near 10%.² Most recently, a 1999 outbreak in Russia resulted in nearly 500 probable cases and 40 deaths, and a nationwide outbreak in Israel in 2000 lead to approximately 400 confirmed cases and 35 deaths.⁶⁷ The largest known human epidemic occurred in 1974 in South Africa with approximately 3000 clinical cases. The virus’s endemic nature in certain areas is supported by a human serosurvey completed in the Nile
Delta of Egypt in 1950, in which 40% of the population was estimated to be seropositive for WNV.8

The first isolations of WNV from birds and mosquitoes in Egypt occurred during the 1950s.2 Since then it has been isolated from over 100 different avian species, most of which survive infection.9 Numerous virus isolations have been made from horses with neurologic illness both in the United States and in other parts of the world.2,10 Virus isolation from dogs and cats is rare worldwide.2,9 However, a dog serosurvey conducted in New York City in 1999 demonstrated that dogs are frequently infected although disease has not been documented.9 Other mammals with documented infection in the United States include fourteen bats, two raccoons, three rabbits, four rodents, and a skunk.10 In Europe other mammals with documented infection include cattle, sheep, goats, rodents and hares.2

**Transmission of WNV**

Transmission of WNV most commonly occurs through a bird-mosquito cycle. High viremia of a duration long enough to infect mosquito vectors has been found in infected birds.3 Approximately two weeks after a mosquito feeds on an infected bird, that mosquito is able to pass on the virus to humans or animals while biting to take blood.11

Although the virus has been isolated from 43 different species, mosquitoes of the *Culex* genus are most commonly infected.2 *Culex* species, in particular *C. pipiens* and *C. restuans*, are thought to be the main vectors responsible for WNV amplification in the northeastern United States due to their preference for feeding
Environmental factors, such as temperature and rainfall, also play a roll in transmission. WNV, and other flaviviruses, are transmitted more efficiently at higher temperatures. $^{13}$ Culex pipiens are most abundant in Connecticut in August when temperatures are typically highest. $^{12}$ In Africa and the Middle East *C. univittatus* is the main vector while in Europe *C. pipiens* is. $^{2}$ Mosquito species testing positive in the 1999 New York epidemic were *C. pipiens* and *Aedes vexans*. $^{14}$

The risk of human WNV infection depends upon the mosquito species carrying the virus. *C. pipiens* prefer birds as hosts, although they have been known to feed on humans and other mammals in urban areas. $^{12}$ *Aedes vexans* will feed on humans and may serve as a bridge vector, carrying the virus from the bird-mosquito cycle into one that includes humans and other mammals. *Culex salinarius* and *Aedes japonicus* are thought to be two of the most important species involved in transmission of the virus to mammals in North America due to their high ability to transmit the virus and potential to serve as bridge vectors. $^{13}$ Thus, determining the species of mosquito carrying WNV virus is an important element in predicting human risk of infection.

**Symptoms and treatment**

Human WNV infection is usually unapparent or causes only mild flu-like symptoms. The typical incubation period is from 3-12 days. $^{11}$ Symptoms include fever, headache, malaise, rash, arthalgia, myalgia, and occasionally nausea and
vomiting. Fevers typically last 3 to 5 days. Encephalitis and acute aseptic meningitis occur in less than 15% of cases but can be life threatening. People most at risk of these severe complications are those over 50 years of age. Case-fatality rates range from 3-15% with the elderly being at the most risk. No specific cure is available once the virus is contracted so supportive medical therapy is the only option. A vaccine is not available to prevent infection.

**WNV activity in the United States in 1999**

In late August 1999, a cluster of patients with arboviral induced encephalitis was identified in northern Queens, New York. Initial serologic tests of cerebrospinal fluid (CSF) and serum samples by virus specific IgM-capture enzyme-linked immunosorbent assay (ELISA), were positive for SLE.

Although the relevance was not recognized at the time, avian species in the area began experiencing an unusually high number of fatalities shortly before the cluster of human cases was identified. Necropsies performed in early September on several exotic birds that died at the Bronx Zoo showed encephalitis. In Connecticut, a dead crow with encephalitis tested positive for SLE. This finding was questionable for a crow since SLE is not known to be virulent in most native North American birds as most have developed antibodies to it.

Further testing at the Centers for Disease Control and Prevention (CDC) on isolates from bird tissues and a human brain specimen from a patient with encephalitis using reverse transcriptase polymerase chain reaction (RT-PCR) found the true causative agent to be WNV. Reverse transcriptase polymerase chain
reaction is a more specific test than ELISA as it can identify the genetic material of a specific virus while ELISA can only detect viral antigens. Since WNV is antigenically related to SLE, cross-reactions can occur with ELISA. Upon reevaluation of available serologic evidence it was found that all reacted more positively to WNV than SLE. In addition, a number of samples considered to be borderline or negative for SLE were positive for WNV.\textsuperscript{5}

In response to these findings, health departments in New York, Connecticut, and other surrounding states initiated surveillance systems to track the geographic distribution of human, avian, and veterinary cases. Trapping and testing of mosquito pools was also done. Collected information was used to guide decisions about the need for using larvicide and spraying pesticides to control the mosquito population and to decrease the risk of transmission to humans.

Surveillance for avian cases concentrated primarily on crows because of their high case fatality rate. Local health departments tracked reports of crow deaths called in by local citizens. Due to the large die-off, not all dead crows reported were tested. Instead, most dead crows counted by the systems were considered likely to have WNV based upon the geographic distribution of crows that did test positive. In Connecticut, over 1000 crow deaths were reported to the Department of Public Health (DPH). Health departments in seven of the eight Connecticut counties received dead bird sightings including six with dead crows. Ninety percent of reported bird deaths were from Fairfield County. Of 97 dead crows tested, 73 (75\%) were positive for WNV.\textsuperscript{6} Although no evidence of
transmission to humans was found in Connecticut, a human serosurvey conducted in October 1999 around the epicenter of the outbreak in northern Queens estimated that 500 to 1900 (1.2-4.1%) people might have been infected out of a population of 46,000. 

Although new to North America, a recurrence of WNV in 2000 was considered likely. It was hypothesized that the virus would overwinter in hibernating *Culex* mosquitoes or be transmitted transovarially to their offspring. Migratory birds might also reintroduce it to the area. Support for these hypotheses were found when West Nile viral RNA was detected in three overwintering mosquito pools collected in January and February 2000 in Queens. In addition, WNV was isolated from a red-tailed hawk that died in February in Westchester county, New York.

Surveillance in wild birds, in addition to humans and mosquitoes, was again begun in Connecticut in the spring of 2000. The Connecticut Mosquito Management Team is responsible for all surveillance and response plans related to WNV. The team consists of staff from 4 agencies; the Department of Environmental Protection (DEP) monitors mosquito breeding sights and provides municipalities with mosquito control assistance; the Connecticut Agricultural Experiment Station (CAES) is in charge of mosquito trapping and testing; the Department of Agriculture (DoAg) is responsible for surveillance for WNV in domestic animals; and the Department of Public Health (DPH) is in charge of human and avian surveillance. Wild bird mortality surveillance and viral testing
was done for four reasons: 1) to determine the spread of WNV activity in the state, 2) to focus mosquito trapping and testing locations, 3) to act as an early indicator of human risk, and 4) to guide larvicide/pesticide application decisions.
METHODS

The Connecticut avian mortality surveillance system coordinated by the DPH consisted of two parts. The first involved tabulating and mapping the location of each dead bird sighting in the state. The second part consisted of testing selected birds for the presence of WNV and mapping the location of those testing positive.

Wild bird mortality surveillance

Each local health department (LHD) in Connecticut was requested by the DPH to assist in the wild bird mortality surveillance system. Instructions for the reporting and the handling of dead birds were sent to each LHD in early April 2000. The LHDs were asked to compile reports of dead bird sightings in their area and to locally publicize the avian mortality system so citizens would be aware of the need and method of reporting dead bird sightings. Private citizens were instructed to report dead birds to their LHD. The LHD entered these reports into a line list consisting of the date of the report, date of bird sighting, number and type of bird, street address of sighting, town, and zip code. The line list of dead birds was faxed on a weekly basis to the DPH beginning April 25, 2000. Weekly logs were collected even when no dead bird sightings were reported and continued through November 3, 2000. All of the information from the logs was entered and summarized weekly in an Access database by the DPH. MatchMaker/2000, a geocoding program, was used to determine longitude and latitude coordinates for the address of each sighting. These coordinates were then plotted on a map of the state using ArcView.
Viral testing of selected birds

Three main criteria were used to determine which crows would be submitted for WNV testing. First, death of the crow within the previous 24 hours as evidenced by no obvious signs of decomposition. Second, encephalitis was a potential cause of death of the crow. There could be no overt signs of other disease or traumatic injury. Third, there must have been a history of numerous crow deaths, defined as two or more crow deaths within one mile of each other within a 72-hour period, in the area. Numerous deaths of other bird species were considered for WNV testing on an individual basis. No more than 5 birds from a single town could be submitted for testing at any one time and no more than 10 from a LHD with more than one town in its district.

Birds deemed appropriate for WNV testing were picked up by the DEP and transported to the University of Connecticut (UCONN). For transport, a metallic wing tag with a unique specimen number was attached to each bird. This number corresponded with that on a submission form detailing the exact location and date of collection of the bird. At UCONN necropsies were performed and brain tissue samples of suitable specimens were prepared for testing for WNV. Tissue samples were then sent to the DPH laboratory for viral testing. Initially the virus was isolated on Vero cell culture and tested for the presence of WNV by fluorescent-antibody (FA) testing using reagents supplied by the CDC. After the presence of WNV was confirmed in the state, testing was done by RT-PCR with FA used only to confirm negative findings. Results of all viral testing were projected to be
available 10 days after bird pickup and were reported to the LHD by the Epidemiology Program at the DPH.

**Dissemination of surveillance data**

Statewide bird mortality information was tabulated and disseminated weekly to the media by the DEP and made available on the DEP website. Information was reported as the total number of bird deaths per town, per week and cumulative to date. Birds deaths were separated into crows, other species, and unknown species. Birds testing positive for WNV were first reported to the submitting LHD by the DPH and then to the media by the DEP.

Weekly surveillance data submitted electronically to the CDC included the number of bird deaths by county, species, and week of sighting. As part of the national ArboNET surveillance system, this data was combined with that from 19 other states in the eastern US and the District of Columbia.20

**Data Analysis**

Local health department cooperation was evaluated by calculating the frequency of submitted weekly bird mortality logs in relation to state land area, population represented, LHD health director employment status, and LHD location. Overall and weekly bird deaths per square mile and per 100,000 were calculated statewide, by county, and by town. The overall and weekly percents of crows testing positive for WNV were calculated in total and per square mile statewide, by county and by town. The correlation between crow sightings per square mile and
percent tested crows positive was calculated by the Pearson correlation coefficient.

Land area and population of nonreporting towns were excluded from the analysis.
RESULTS

Wild bird mortality surveillance

Local Health Department Response

Submission of weekly bird mortality logs by LHDs to the DPH started following the first week of surveillance, beginning April 16, 2000, and continued through the week beginning October 29, 2000 for a total of 29 weeks. The median proportion of the state’s land area covered by these logs each week was 67%, with a range of approximately 38% to 78%. The median proportion of the state’s population represented by logs each week was 76% with a range of 35% to 88% (Figure 1). Ninety-three (87%) of the 107 LHDs in the state reported dead bird sightings during the surveillance period (Table 1). Approximately 26% of LHDs submitted logs for greater than 90% of the surveillance weeks, representing 41% of the state’s land area and 44% of the population. LHDs submitting logs for greater than 75% of the surveillance weeks (43%) represented close to 60% of the state’s land area and 65% of the population. The 14 LHDs that did not submit logs (13%) represented approximately 7% of the state’s land area and 2% of the population.

Local health departments submitting logs for less than 25% of surveillance weeks tended to be located in areas with lower population densities than those submitting more frequently (Table 1). When analyzed by location, it was found that LHDs reporting most frequently represented towns in the western, central, and northeastern portions of the state while LHDs reporting less frequently represented towns in the southeastern portion of the state (Figure 2).
When the frequency of submitted weekly bird mortality logs was compared to the status of each LHD’s health director, the frequency of submitted logs was found to decrease as the percent of part-time health directors increased (Table 2). Thus, LHDs reporting 75% or more of the surveillance weeks had approximately 25% part-time health directors while LHDs reporting less than 25% of the time had over 85% part-time health directors. Each of the fourteen LHDs that did not submit logs had part-time health directors.

Wild Bird Mortality

A total of 10,744 dead birds were reported, of which the species was identified for 8952 (83%). Crows accounted for 4340 (49%) of those identified. Numerous other species accounted for the remainder of the birds. The most frequent being sparrows with 1181 (13%) sightings, blackbirds with 538 (6%) sightings, and blue jays with 521 (6%) sightings. Doves, pigeons, robins and starlings were the next most commonly reported species. Unknown species accounted for 1792 (17%) of the total reported deaths. From the beginning of surveillance through the end of August the percent of identified dead bird sightings accounted for by crows fluctuated between 20% and 40%. Beginning in September and lasting through the last week of surveillance, the percent of total dead bird sightings identified as crows increased to between 50% and 65% each week (Figure 3).

Approximately 0.03 to 0.32 dead birds per square mile were reported each week statewide, with an average of 0.12. Reports of bird deaths of all species
peaked the week of 7-23-2000 (Figure 4). Dead crow sightings ranged from approximately 0.01 to 0.13 crows per square mile statewide, with an average of 0.05. During the period from 7-9-2000 to 10-15-2000 crow deaths remained above 0.05 per square mile each week, peaking during the week of 9-17-2000. The death rate among non-crow species also averaged 0.05 birds per square mile. The peak death rate among non-crow species of 0.17 deaths per square mile occurred during the week of 7-23-2000, two months earlier than the peak for crows.

Counties with the highest population density had the greatest number of reported dead bird sightings. Fairfield County is the most densely populated county in the state and it also had the greatest number of dead crows reported per square mile (0.16) as compared to any other county (Table 3). New Haven and Hartford Counties are slightly less densely populated and had 0.07 and 0.04 dead crow reports per square mile respectively. The remaining counties had 0.02 or fewer dead crows per square mile. Dead crow sightings per square mile in Fairfield, Hartford, and New Haven counties increased gradually through mid-September to peak the week of 9-17-2000 (Figure 5). After this date crow deaths dropped off sharply, especially in Fairfield County. Dead crow sightings per 100,000 population followed a similar pattern, peaking the week beginning 9-17-2000 in Fairfield County (Figure 6). Sightings peaked slightly earlier in New Haven County and later for Hartford County.

Dead crow sightings were most often reported in towns located along the coastline of southwestern Connecticut. Overall, the towns of Darien and Milford
had the highest number of dead crow sightings in the state averaging 0.51 and 0.52 crows per square mile per week. During select weeks of surveillance when WNV activity was highest, Darien had as many as 2.25 dead crow sightings per square mile and 148 dead crow sightings per 100,000 population (Table 4).

**Viral testing of selected birds**

A total of 1763 crows were submitted for viral testing during the surveillance period. Of the 1574 tested, 1095 (70%) were positive for WNV and 479 were negative. The remaining 189 were not suitable candidates for testing primarily due to the condition of the specimen. Non-crow species were tested only in the first two months of the surveillance period. All were negative for WNV and after this time period they were not submitted for testing and are not included in analyses.

The first positive crow was collected during the week of 7-2-2000 from the town of Stamford in Fairfield County. From that week on, positive crows were found each week through the end of the surveillance period. Seventeen percent of crows collected statewide and tested during the week of 7-2 were positive (Figure 7). This percentage increased steadily through mid-September to a high of 89% statewide. The percent of tested crows positive for WNV remained above 80% statewide each week from the beginning of September through mid-October. After this time data fluctuated with only 38% of tested crows positive during the week of 10-22 and 78% of tested crows positive the following week. The number of dead
crow sightings per square mile corresponded well with the rate of crows testing positive (R=0.77, p<0.001) (Figure 7). Both peaked in mid-September.

When the data were examined by individual county, a similar pattern to that found statewide was seen in the percent of crows testing positive. Fairfield County showed the most consistent increasing trend and the percent of tested crows positive for WNV remained at 80% or higher from mid-August through mid-September (Figure 8). Both New Haven and Tolland Counties submitted crows that tested positive the week of 7-9. Hartford County’s first positive crow was collected during the week of 7-30 and the remaining counties did not have confirmed viral activity in crows until mid to late August.

During the surveillance period, an average of 0.29 positive crows per square mile were found statewide (Table 5). Areas with the highest numbers of positive crows were located in the southwestern part of the state in Fairfield and New Haven counties (Figure 9). Fairfield had more positive crows per square mile (0.84) than any other county (Table 5). A total of 510 positive crows were confirmed in the county comprising 74% of 687 crows tested in the county and 47% of all positives in the state. Approximately 0.38 positive crows per square mile were found in New Haven County. It submitted 338 crows for testing of which 239 (71%) were positive for WNV representing 22% of all positives in the state. Hartford County had 0.31 positive crows per square mile. The remaining counties had less than 0.2 positive crows per square mile. The town of Darien, located in Fairfield County,
had the highest WNV activity in crows of all towns in the state with 3.8 positive per square mile.

**Dissemination of surveillance data**

The DEP began issuing weekly press releases summarizing both the dead bird sightings for the preceding week and viral testing results on May 12. Dead bird sightings were initially reported in entirety with results by town for the preceding week as well as cumulative totals and species sighted. Later in the season this aspect of the surveillance system was reported in abbreviated form as the weekly total, percent of total from the heaviest reporting county, and the frequency of crow sightings. Viral testing results were initially presented in entirety by town found, species, date of collection, and test result status for all birds in the testing system whether they were negative, positive, pending, or not tested. Later in the season, only the number of newly positive birds in a particular town were reported in the press releases. These press releases were available for public viewing on the DEP’s website and local television and newspapers also reported the details.

Bird mortality sighting and viral testing results were sent to the CDC weekly where the data was compiled with that of other states participating in ArboNet, the national surveillance system. This information was then disseminated through the National Atlas of the United States website (www.nationalatlas.gov/virusmap.html) on maps showing the geographic distribution and temporal spread of WNV in the eastern US.
DISCUSSION

The WNV surveillance system established in Connecticut in 2000, along with those in other states, demonstrated that the geographic distribution of WNV in the United States had spread as compared to 1999. Only 4 states had confirmed viral activity in 1999 as compared to 12 states and the District of Columbia (DC) in 2000. The spread of the virus is likely due to migrating birds introducing it into new areas and is likely to continue. Evaluation of the usefulness of wild bird mortality surveillance systems is critical as these systems may provide one of the timeliest methods of determining areas of potential human risk.

The wild bird mortality surveillance system is dependent upon cooperation of LHDs and local citizens with the request to submit weekly logs of dead bird sightings. Compliance with the request was high with more than 60% of the state’s land area and 70% of the state’s population typically represented by the logs each week through mid-September. Local health departments representing towns located in the two most densely populated counties, Fairfield and New Haven, tended to submit mortality reports more frequently than LHDs in other sections of the state. This likely reflects the fact that these areas had the only confirmed WNV activity in the state in 1999. Thus, a heightened awareness of the health risks associated with WNV among local citizens and LHD officials in these areas as compared to those in other areas of the state may have led to more consistent reporting. A difference in frequency of submitted bird mortality reports was also seen when comparing the status of the LHDs health directors. Those with full-time
health directors tended to submit reports more frequently than those with part-time health directors, although this finding may be confounded by the fact that towns with lower population density tend to have part-time health directors. Less participation by part-time LHDs may reflect lack of adequate staff to keep track of sightings reported by citizens and/or lack of publicity in the local area of the need to and where to direct sighting reports. The fact that each of the 14 LHDs not submitting bird mortality reports were represented by part-time health directors supports the claim that lack of staff/resources may have contributed to the lack of participation by these LHDs.

The finding of a connection, by both date and location, between crow deaths and confirmed WNV activity in crows is an important one that warrants further study. Without viral testing it would be difficult to interpret the meaning of large numbers of crow deaths since there is no baseline for comparison and crow mortality may be due to other avian diseases or environmental factors. Reports of crow deaths proved to be a good indicator of WNV activity as evidenced by several pieces of information. First, reports of crow deaths were directly related to the percent of crows testing positive. Crow death reports per square mile rose steadily through their peak in mid-September, as did the percent of crows testing positive. Second, rates of dead crow reports and WNV activity in crows were associated by specific geographic locations within the state. Fairfield and New Haven counties had both the highest rate of crow deaths and the highest rate of crows testing positive. This was consistent with town data also. The towns of Darien and
Milford had both the greatest number of reported crow deaths per square mile and the greatest number of positive birds per square mile of any towns in the state. Crow death reports may be particularly important as an early warning system of WNV activity since they are timelier than crow viral testing. Death reports for a particular area were tabulated and typically reported within a week of the sighting. Crow viral testing results take more time due to the need to transport and test the birds. Testing also requires additional resources, such as laboratory staff and equipment, while tabulating crow death reports can be done with existing staff. Once an area is found to have a high level of positive crows it may not be necessary to expend the resources to test each dead bird but rather rely on dead crows sightings to determine the extent of WNV activity in the area.

It was not surprising to have the majority of the sightings from Fairfield and New Haven counties for a number of reasons. First, these areas, especially Fairfield county, are where the only confirmed WNV activity in birds and mosquitoes was found in the state in 1999. Infected overwintering mosquitoes are thought to be a potential source for WNV reemergence in an area. In New York City, an area with widespread WNV activity in 1999, mosquito pools tested during the winter of 2000 were found to have WNV RNA and live WNV. Although positive overwintering pools were not found in Connecticut it seems likely that a similar situation would exist in mosquitoes overwintering there. This and the fact that crows tend not to travel far from their nests in early summer suggests that the finding of positive crows and mosquitoes in early July in Fairfield County was due
to local transmission of the virus rather than reintroduction from New York in the early summer of 2000.¹²

Second, the close proximity of these counties to the New York City epicenter of the 1999 outbreak focused a lot of media attention on the area. This would tend to greatly increase public awareness of the threat of WNV as well as increase knowledge of the bird mortality surveillance system. Fairfield and New Haven Counties, along with Hartford County, contain the highest population density in the state. The probability of a dead bird being sighted is likely to increase in areas of high population density. This, combined with the heightened awareness of WNV due to media attention, may lead citizens to place more importance on reporting bird mortality to their LHD. Counties located in the northeastern and northwestern portions of the state had the fewest sightings. These areas are more rural and have less population density. This may decrease the likelihood that a dead bird will be sighted. In addition no WNV activity was found in those areas of the state during 1999. Thus, local citizens there did not have the increased awareness of the WNV threat that those in the southwestern portion of the state did and may not have been aware of the need to report dead bird sightings.

The effect of media attention on the number of dead bird sightings can be seen by looking at the frequency of sightings in relation to the date of WNV related press releases. For example, on July 21, 2000 a statement was released to the media by the DEP stating that C. restuans mosquitoes isolated in Stamford had tested positive for WNV.¹⁰ This was the first test result that confirmed the presence
of WNV in the state in 2000. Although positive crows collected prior to this date were later found, their viral testing results were still pending at this point. The number of dead bird reports increased by 378 (55%) in the week following the release of this information to the public. This was the largest weekly increase seen during the surveillance period. Thus, determining the geographic distribution of WNV in an area based upon bird data is limited by the extent of public awareness of the surveillance system and willingness to participate in it, population density, and the amount of media attention.

As in 1999, crows were again the most common avian species in mortality reports in 2000 and were also the only species from which WNV was isolated in Connecticut as part of the DPH surveillance system. For unknown reasons crows seem to be more susceptible to death from WNV than other species. This and the fact that crows are easily recognized by most people and are common to the area makes them a key species to be used in determining the geographic distribution of WNV. However, it is unknown if crows will remain as susceptible to death by WNV in future years. In 1999, crows shot by hunters in Connecticut were tested for WNV. Ten percent of 33 tested were found to have antibodies to WNV indicating that some crows have immunity to WNV and do not die as a result of infection. The crow population may adapt and develop more widespread immunity to WNV in future years thereby decreasing the usefulness of the species in a sentinel surveillance system.
The impact of WNV on other species should not be ignored and deserves further study. Testing of non-crow species in Connecticut in 2000 was limited to the first two months of surveillance. Thus, the impact of WNV on other species was not adequately determined. However, 10 non-crow species submitted directly to the Connecticut Agricultural Experiment Station (CAES) outside of the DPH surveillance system tested positive for WNV including blue jays (3) and robins (2). Crows were again found to be the species with the most susceptibility to WNV in 2001 but additional isolates were also made from blue jays, doves, and sparrows. New York State isolated WNV from 19 non-crow species in 1999 and from 62 non-crow species in 2000. In an avian serosurvey conducted on live birds in New York in 1999 approximately 33% (142) of 430 birds tested had WNV-neutralizing antibodies. Rock doves and house sparrows were determined to be good reservoir hosts due to both their high seropositivity for WNV and their relative abundance in the study area. Testing live free birds of these other common species for WNV antibodies might prove to be another useful indicator of WNV activity, especially in areas where the crow population is not as abundant.

Since mosquitoes serve as the vector causing human and avian illness, it was expected that bird mortality and viral testing results would correspond geographically with WNV activity in mosquitoes. During 2000, confirmed WNV activity in mosquitoes was first identified in Culex restuans mosquitoes collected in Stamford on July 11th, approximately one week after a positive crow was identified in this same town. Additional positive mosquitoes pools continued to be collected
through early October in *C. restuans*, *C. pipiens*, and *Culiseta melanura* mosquitoes, all bird biting species, and from *C. salinarius* which will bite both birds and mammals. A total of 14 positive isolations were made from the towns of Stamford, Norwalk, Greenwich, Fairfield, Shelton, and Westport, all of which are located in Fairfield County. Isolations were also made in the towns of Milford and Meriden in New Haven County.12 With the exception of Meriden, the seven remaining towns with positive mosquito isolations were among the towns with the most dead bird sightings per square mile and with the highest levels of confirmed WNV activity in birds. The town of Darien had the second highest number of dead bird reports per square mile and the highest number of positive crows per square mile of any town in the state. Although no positive mosquito isolations were made in Darien, it is located in close proximity to towns with positive mosquito isolations. Thus, it is likely that positive mosquitoes were in Darien also.

Although the avian surveillance system can identify geographic areas where WNV activity exists, it can only supplement mosquito surveillance, not replace it. Testing mosquito pools is a needed tool in determining the intensity of WNV activity and the threat to people. It is especially important because not all mosquito species will bite humans. WNV can exist in a bird-mosquito cycle and not pose high risk to humans. Identification of WNV in a human-biting mosquito species suggests an increased level of potential human risk in an area. However, the extent of mosquito trapping is limited by financial resources available to place and collect traps, sort and speciate the mosquitoes, and test the mosquitoes. The majority of
the mosquito traps were placed in the southwestern portion of the state in areas with the highest WNV activity in 1999. Very few were placed in the northwest and northeast portions of the state due to lack of confirmed WNV activity in the prior year and low population density in these areas. West Nile virus activity in mosquitoes cannot be definitively ruled out in areas without mosquito traps since mosquitoes tend to fly short distances. Thus, even areas located just several miles from a mosquito trap site may have had undetected WNV activity in mosquitoes. However, judging from the high crow mortality and WNV activity in crows seen in areas in close proximity to positive trap sites it is likely that areas without significant bird mortality reports also had less mosquito activity. Collection and viral testing of trapped mosquitoes takes time. Positive results may not be able to be provided to the public in a manner timely enough to serve as an early warning system. Since a link has been found between WNV activity in mosquitoes and WNV activity in crows, tracking of crow deaths can be used as a surrogate for determining if the potential for human risk exists when it is not feasible to have traps in an area. Analysis of crow death reports requires less time and resources. Since crows are widespread in the state, deaths can be analyzed statewide wherever there are people to report them.

One limitation of avian surveillance is that it relies upon having people available to see and report dead bird sightings. Areas with low population density would be expected to have fewer sightings reported because of this. Determining if WNV is present in areas of low population density is more difficult, especially in
those areas without mosquito traps. Equine surveillance may be the first indicator
of WNV activity in areas like these. In addition, equine surveillance is useful
because WNV activity in horses is a sign of virus activity outside the bird-mosquito
cycle. It can indicate potential human risk since the same mosquito species that
bite horses will also bite humans. During the 2000 surveillance season, seven
horses believed infected in Connecticut were confirmed to have WNV with onset
dates ranging from late August to mid-October.27 Only one of these horses, stabled
in Milford, was in close proximity to a positive mosquito trap site. This trap had
been placed in response to the confirmation of WNV in that horse. The onset of
illness in horses did correspond temporally with the peak number of positive
mosquito pools.27 Although WNV infection in horses does signify activity outside
the bird-mosquito cycle, they were not found to be a useful sentinel species to
predict human risk. A comparison of WNV illness onset dates between humans
and horses in all states conducting surveillance in 2000 showed that human onset
dates peaked in mid-August while horse onset dates peaked approximately one
month later.10 This may reflect the fact that human exposure to mosquitoes
decreases in the fall when cooler temperatures arrive. People tend to remain inside
more and wear long sleeve clothing if outdoors during the evening hours. Thus,
although equine surveillance has value in geographic areas where other surveillance
indicators are lacking, it cannot replace avian surveillance due to its inability to
serve as a timely indicator of human risk.
Only one confirmed human case of WNV in Connecticut was found in a patient experiencing mild symptoms. She resided in the town of Norwalk in Fairfield County and reportedly spent evenings outside in the town. The onset of her symptoms indicates that she was most likely infected in mid-August. West Nile virus was isolated from two pools of *C. restuans* mosquitoes collected on August 8th in Norwalk. This is primarily a bird-biting species although some research has indicated that under certain circumstances it will bite humans. There was also considerable activity in birds in the time period before she was likely to have been infected. Between 20 and 40 dead bird sightings were reported in Norwalk each week from early July through the end of August. Of the 22 crows submitted for viral testing during July and August, only one tested negative. High numbers of bird deaths, high numbers of positive birds, and positive mosquito pools preceded and were concurrent with human infection. This demonstrates the capability of these surveillance systems for indicating the potential for human infections. However, this capability relies on the ability to collect and analyze surveillance results in a timely manner. The viral testing results of the two positive mosquito pools in Norwalk were not available until mid to late August, too late to have served as a warning to the infected woman. However, bird mortality counts and positive viral testing of birds in the area were available before the time she was likely to be infected.

Finding only one confirmed human WNV case in the state was surprising due to high levels of avian disease and viral activity in human-biting mosquitoes.
Surveillance evidence pointed to high levels of human risk in certain areas. Since most WNV infections in humans are unapparent or cause only mild symptoms\textsuperscript{11}, it was suspected that more human cases might have occurred in the state in people that had not sought medical care. A serosurvey was conducted in early October 2000 in the Fairfield County towns of Stamford and Greenwich in order to determine the extent of unapparent WNV infections. This area of the state was chosen because it had considerable WNV activity in birds and mosquitoes. A total of 730 people from 645 households were tested. None of the samples were positive for WNV antibodies (95\%CI 0.01\%-0.52\%).\textsuperscript{29} In a similar serosurvey conducted in 1999 in the epicenter of the New York City outbreak it was estimated that 2.6\% of the population had been infected with WNV although the rate of severe illness requiring hospitalization was much less at 18.2 per 100,000 population.\textsuperscript{27}

High WNV activity in birds and mosquitoes can only indicate the potential for human risk. The use of personal control measures may play a part in reducing human risk of infection. Among those questioned as part of the 1999 serosurvey in New York City, people with the highest infection rates were those that reported spending time outdoors between dusk and dawn without using mosquito repellent. Seventy percent of all New York City residents surveyed reported never applying repellent as compared to 56\% of Connecticut residents questioned during the 2000 serosurvey.\textsuperscript{29}
RECOMMENDATIONS

Avian mortality surveillance, supplemented by viral testing, may be a highly useful early indicator of WNV activity. It appears to track the progression of WNV both geographically and temporally. However, every surveillance system should be evaluated periodically to assess areas in which effectiveness can be improved. Key elements that should be considered include the system’s acceptability, simplicity, timeliness, flexibility, and representativeness.30

Acceptance of the avian surveillance system by LHDs, particularly those with full-time health directors, was high as indicated by the frequency of submitted avian mortality reports. However, a lower participation rate from LHDs with part-time health directors indicates that lack of staff resources may have hindered participation. In order to obtain more complete and representative reporting, particularly from LHDs with part-time health directors, simplifying the bird mortality reporting system so that it places less of a time constraint on LHD staff is needed. Data collection and tabulation also placed a significant burden on DPH staff. Entering each dead bird sighting individually into the Access database on a weekly basis proved to be a very time consuming task, especially from mid July through September when over 500 sightings had to be entered each week. In order to streamline the process for the 2001 surveillance period, LHDs were encouraged to enter bird mortality data directly into the DPH Local Health Directors web site. When utilized by the LHDs, this electronic system eliminated the need for DPH staff to type each sighting individually. This system may also prove to be less time
consuming for LHDs leading to greater participation in submitting bird mortality reports in the future. In addition, the new system permits LHDs to directly access pertinent surveillance findings for their district anytime, leading to more timely distribution of data. Another benefit of the new system is that it eliminated the need for DPH staff to manually check viral testing results because the database was linked to state laboratory files that were updated electronically each night.

Avian viral testing requires considerable financial resources and staff. Due to the high positivity rate in crows and the association found between crow mortality and viral activity repeated testing of large numbers of crows from the same geographic area may not be needed in future years. Dead bird surveillance may be able to be used to track WNV activity once avian viral activity has been confirmed. This would decrease the burden on staff and save a significant amount of financial resources. Further study of the geographic and temporal association found between crow mortality and viral testing is needed to see if the relationship holds true in future surveillance years.

It is unknown if crows will remain susceptible to WNV in future years or if they will adapt and develop immunity. The avian surveillance system needs to have the flexibility to allow for this. WNV activity in other species, possibly doves and sparrows, should be evaluated further to determine if they could serve as sentinels. These other species could easily be incorporated into the avian surveillance system if and when need arises.
CONCLUSION

The crow mortality surveillance system appears to be a valuable tool in tracking the distribution of WNV activity across the state when used in conjunction with crow and mosquito viral testing. The system relied on existing staff and resources and allowed data to be collected and analyzed in a timely manner. It served as an early warning of local amplification of the virus and in areas where crows are widely distributed acted as an indicator of wider geographic spread. It can serve as a potential trigger for where and when additional surveillance activities, such as mosquito trapping, should be conducted. However, additional modeling is needed to use the system to predict the risk of human WNV infection in a particular geographic area. Additional factors such as weather, stage of season, and personal behaviors, must be considered to determine risk of transmission to people. Retaining public interest in reporting dead bird sightings is necessary if the surveillance system is to be useful in future years.
REFERENCES


9. Centers for Disease Control and Prevention. Division of Vector-Borne Infectious Diseases. West Nile virus vertebrate ecology. Available at


30. CDC. Updates guidelines for evaluating public health surveillance systems.

Recommendations from the guidelines working group. MMWR 2001;50(RR13):1-35.
Table 1  Frequency of bird mortality log submissions by land area, population represented, and population density. Connecticut, April 16 - October 29, 2000

<table>
<thead>
<tr>
<th>Percent of weekly logs submitted*</th>
<th>Number (%) of LHDs Reporting (n=107)</th>
<th>Connecticut land area represented, sq mile (%)</th>
<th>Connecticut population represented x100,000 (%)</th>
<th>Population density in areas represented (people/sq mile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90-100</td>
<td>28 (26)</td>
<td>1996 (41.2)</td>
<td>15 (44.1)</td>
<td>752</td>
</tr>
<tr>
<td>75-89</td>
<td>18 (17)</td>
<td>839 (17.3)</td>
<td>7.2 (21.2)</td>
<td>858</td>
</tr>
<tr>
<td>50-74</td>
<td>21 (20)</td>
<td>811 (16.7)</td>
<td>6.3 (18.4)</td>
<td>777</td>
</tr>
<tr>
<td>25-49</td>
<td>12 (11)</td>
<td>336 (6.9)</td>
<td>3.5 (10.3)</td>
<td>1042</td>
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<tr>
<td>1-24</td>
<td>14 (13)</td>
<td>547 (11.3)</td>
<td>1.4 (4.0)</td>
<td>256</td>
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<tr>
<td>0</td>
<td>14 (13)</td>
<td>321 (6.6)</td>
<td>0.7 (2.0)</td>
<td>218</td>
</tr>
</tbody>
</table>

*based upon 29 weeks of surveillance
Table 2  Frequency of bird mortality log submissions by status of local health director.  Connecticut, April 16 - October 29, 2000.

<table>
<thead>
<tr>
<th>Percent of weekly logs submitted*</th>
<th>Number (%) of LHDs Reporting</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n=107)</td>
<td>Full-time health director</td>
<td>Part-time health director</td>
</tr>
<tr>
<td>90-100</td>
<td>28 (26)</td>
<td>20 (71)</td>
<td>8 (29)</td>
</tr>
<tr>
<td>75-89</td>
<td>18 (17)</td>
<td>14 (78)</td>
<td>4 (22)</td>
</tr>
<tr>
<td>50-74</td>
<td>21 (20)</td>
<td>8 (38)</td>
<td>13 (62)</td>
</tr>
<tr>
<td>25-49</td>
<td>12 (11)</td>
<td>4 (33)</td>
<td>8 (67)</td>
</tr>
<tr>
<td>1-24</td>
<td>14 (13)</td>
<td>2 (14)</td>
<td>12 (86)</td>
</tr>
<tr>
<td>0</td>
<td>14 (13)</td>
<td>0</td>
<td>14 (100)</td>
</tr>
</tbody>
</table>

*based upon 29 weeks of surveillance
Table 3: Cumulative reported bird deaths by county and population density, Connecticut 2000

<table>
<thead>
<tr>
<th>COUNTY</th>
<th>Population Density (persons/sq mile)*</th>
<th>All Species</th>
<th>Crows</th>
<th>Non-Crows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fairfield</td>
<td>1405</td>
<td>0.35</td>
<td>0.16</td>
<td>0.12</td>
</tr>
<tr>
<td>New Haven</td>
<td>1396</td>
<td>0.18</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Hartford</td>
<td>1165</td>
<td>0.11</td>
<td>0.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Middlesex</td>
<td>461</td>
<td>0.06</td>
<td>0.02</td>
<td>0.04</td>
</tr>
<tr>
<td>New London</td>
<td>467</td>
<td>0.05</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Tolland</td>
<td>332</td>
<td>0.03</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Litchfield</td>
<td>204</td>
<td>0.02</td>
<td>0.004</td>
<td>0.01</td>
</tr>
<tr>
<td>Windham</td>
<td>213</td>
<td>0.01</td>
<td>0.003</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Birds deaths are expressed per square mile. Land area and population of nonreporting towns not included in calculations.
Table 4: Highest weekly number of dead crow sightings per square mile and per 100,000 population in select towns, Connecticut 2000

<table>
<thead>
<tr>
<th>TOWN</th>
<th>SURVEILLANCE WEEK</th>
<th>NO. CROW SIGHTINGS</th>
<th>SQUARE MILES</th>
<th>NO. CROW SIGHTINGS per SQ MILE</th>
<th>POPULATION*</th>
<th>NO. CROW SIGHTINGS PER 100,000 POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Darien</td>
<td>All</td>
<td>189</td>
<td>12.9</td>
<td>0.51</td>
<td>19607</td>
<td>33</td>
</tr>
<tr>
<td>(Fairfield)</td>
<td>8/13</td>
<td>29</td>
<td>12.9</td>
<td>2.25</td>
<td>19607</td>
<td>148</td>
</tr>
<tr>
<td></td>
<td>8/6</td>
<td>19</td>
<td>12.9</td>
<td>1.47</td>
<td>19607</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>9/24</td>
<td>18</td>
<td>12.9</td>
<td>1.4</td>
<td>19607</td>
<td>92</td>
</tr>
<tr>
<td>Milford</td>
<td>All</td>
<td>343</td>
<td>22.6</td>
<td>0.52</td>
<td>52305</td>
<td>23</td>
</tr>
<tr>
<td>(New Haven)</td>
<td>9/3 and 9/10</td>
<td>39</td>
<td>22.6</td>
<td>1.73</td>
<td>52305</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>8/20</td>
<td>35</td>
<td>22.6</td>
<td>1.55</td>
<td>52305</td>
<td>67</td>
</tr>
<tr>
<td>Fairfield</td>
<td>All</td>
<td>321</td>
<td>30</td>
<td>0.37</td>
<td>57340</td>
<td>19</td>
</tr>
<tr>
<td>(Fairfield)</td>
<td>9/17</td>
<td>39</td>
<td>30</td>
<td>1.3</td>
<td>57340</td>
<td>68</td>
</tr>
</tbody>
</table>

*Population figures obtained from 2000 census
**Table 5** WNV positive crows in relation to land area in all counties and select towns, Connecticut 2000

<table>
<thead>
<tr>
<th>County*</th>
<th>Land Area</th>
<th>No. Crows Tested</th>
<th>No. Positive Crows (%)</th>
<th>No. Positive / Sq Mile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fairfield</td>
<td>604.1</td>
<td>687</td>
<td>510 (74)</td>
<td>0.84</td>
</tr>
<tr>
<td>New Haven</td>
<td>615.6</td>
<td>338</td>
<td>239 (71)</td>
<td>0.39</td>
</tr>
<tr>
<td>Hartford</td>
<td>605.8</td>
<td>299</td>
<td>190 (64)</td>
<td>0.31</td>
</tr>
<tr>
<td>New London</td>
<td>539.5</td>
<td>131</td>
<td>98 (75)</td>
<td>0.18</td>
</tr>
<tr>
<td>Middlesex</td>
<td>353.4</td>
<td>53</td>
<td>35 (66)</td>
<td>0.10</td>
</tr>
<tr>
<td>Tolland</td>
<td>308.8</td>
<td>27</td>
<td>14 (52)</td>
<td>0.05</td>
</tr>
<tr>
<td>Windham</td>
<td>395.1</td>
<td>21</td>
<td>5 (24)</td>
<td>0.01</td>
</tr>
<tr>
<td>Litchfield</td>
<td>373.5</td>
<td>18</td>
<td>4 (22)</td>
<td>0.01</td>
</tr>
<tr>
<td>All Counties</td>
<td>3795.8</td>
<td>1574</td>
<td>1095 (70)</td>
<td>0.29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Town (County)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Darien (F)</td>
<td>12.9</td>
<td>66</td>
<td>49 (74)</td>
<td>3.8</td>
</tr>
<tr>
<td>Milford (NH)</td>
<td>22.6</td>
<td>87</td>
<td>75 (86)</td>
<td>3.3</td>
</tr>
<tr>
<td>Fairfield (F)</td>
<td>30.0</td>
<td>104</td>
<td>85 (82)</td>
<td>2.8</td>
</tr>
<tr>
<td>Stamford (F)</td>
<td>37.7</td>
<td>134</td>
<td>105 (78)</td>
<td>2.8</td>
</tr>
<tr>
<td>Wethersfield (H)</td>
<td>12.4</td>
<td>43</td>
<td>34 (79)</td>
<td>2.7</td>
</tr>
<tr>
<td>West Hartford (H)</td>
<td>22.0</td>
<td>54</td>
<td>42 (78)</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*County analysis excludes land area of towns from which no crows were tested
Figure 1: Proportion of Connecticut's land area and population represented by local health departments submitting weekly bird mortality logs

[Line graph showing percentage over time with two lines labeled: Land area and Population]
Figure 3: Proportion of dead bird sightings identified as crows by surveillance week, Connecticut 2000.
Figure 4: Bird mortality sightings by species and surveillance week, Connecticut 2000

*Land area of nonreporting towns excluded from calculations*
Figure 5: Dead crow sightings by selected county and surveillance week, Connecticut 2000

*land area of nonreporting towns excluded from calculations
Figure 6: Dead crow sightings per population reporting by selected county and surveillance week, Connecticut 2000

*Population(s) of nonreporting towns excluded from calculations
Figure 7: Dead crow sightings and percent testing positive by surveillance week, Connecticut 2000.
Figure 8: Percent of crows testing positive for WNV by surveillance week in select counties, Connecticut 2000
Figure 9: WNV positive crows by town in relation to positive mosquitoes and horses, Connecticut 2000

*Figure from reference #27.