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SCIENTIFIC NOTE

THE DISCOVERY OF WEST NILE VIRUS IN OVERWINTERING *CULEX PIPIENS* (DIPTERA: CULICIDAE) MOSQUITOES IN LEHIGH COUNTY, PENNSYLVANIA

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ABSTRACT. In February 2003, a pool of 6 *Culex pipiens* mosquitoes collected in Lehigh County, PA, tested positive for West Nile Virus (WNV). West Nile viral RNA was detected by reverse transcriptase-polymerase chain reaction. This is the first time in Pennsylvania and the third time in North America that WNV has been found in overwintering adult mosquitoes.

KEY WORDS *Culex pipiens*, West Nile Virus, overwintering mosquitoes, Pennsylvania

Following the West Nile Virus (WNV) outbreak in New York City in 1999, the Commonwealth of Pennsylvania established a WNV surveillance and control program administered by the Pennsylvania Department of Environmental Protection (PADEP). The program was designed to function as a cooperative effort between the state and counties. The work presented here is a result of that program.

The primary method of trans-seasonal viral maintenance for WNV in North America has not been determined. It has been suggested that vertically infected *Culex* could survive the winter to initiate a WNV amplification cycle the following spring. Vertical transmission of WNV from female *Culex pipiens* L. to their progeny has been demonstrated in the laboratory (Dohm et al. 2002). The first overwintering adult mosquitoes to test positive for WNV were collected in New York City in 2000 (Nasci et al. 2001). A second WN positive pool of *Culex pipiens* was collected from Monmouth County, New Jersey in January 2003 (Ary Farajollahi, Rutgers University, personal communication).

Lehigh County, located in eastern Pennsylvania 80 miles southwest of New York City, has a population of 312,000 residing in 349 square miles of urban, suburban, and rural areas. From January to December 2001, 718 mosquito pools were tested for WNV. No mosquito pools tested positive. Four of 35 birds tested were positive for WNV. In 2002, 39 birds of 89 tested were positive. From January to December 2002, 856 pools consisting of 18,667 mosquitoes were tested and WNV was detected in 5 pools. Four of the positive pools consisted of *Culex pipiens*, and the minimum infection rate (MIR) in this species was 0.003 (4/1,492). A single positive pool was detected in 87 pools consisting of 511 *Culex restuans* (MIR = 0.002).

The Centers for Disease Control has recommended that the overwintering mechanism for WNV in *Culex* and *Aedes* species should be investigated (Centers for Disease Control and Prevention, 1999). To explore the mechanism of WNV survival during cold environmental conditions, a survey of likely hibernacula was conducted to collect overwintering mosquitoes. Typical survey areas were subterranean locations, such as root or ground cellars, basements of abandoned buildings, springhouses, bank barns, tunnels, and industrial sites. Conditions in all of these microhabitats were similar in temperature and humidity. They were dark, relatively undisturbed, and protected from wind and weather. The average temperature in the hibernacula was 1.7°C with an average relative humidity (RH) of 43%. Mosquitoes were located using a flashlight and collected with a mechanical hand-held aspirator. Mosquitoes were removed from the aspirator collecting vials by allowing them to fly from the vials into a net from a standard ABC Trap Kit.

There was high mortality in the 2001–2002 samples, presumed due to desiccation because average RH in the holding area was only 23%. To alleviate this problem, a humidity chamber was constructed using a 13-in. × 30-in. × 38-in. cardboard box and 4-mil vinyl pane window covering. The box was fully enclosed and a container of water was placed on the bottom of the chamber.

Laboratory studies have shown that increased incubation temperature may enhance virus replication and allow for detection of virus if present (Dohm and Turell 2000). Conditions within the humidity chamber remained within 19–22°C with 40% average RH. Specimens were held for 48–72 h and offered a 5% sucrose solution. Samples were then frozen using dry ice and shipped on dry ice overnight to the PADEP laboratory in Harrisburg for identification. After processing, the mosquito pools were sent to the Pennsylvania Department of Health laboratory in Lionville, PA, for West Nile viral RNA testing by a TaqMan reverse transcription-

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Table 1. Numbers of specimens collected December–April.

Species	2001–2002	2002–2003
Culex pipiens	724[1]	501[2]
Anopheles punctipennis	75	98
An. quadrimaculatus	1	2
Uranotaenia sapphirina	4	
Culex erraticus		1
Cu. territians		1
Total mosquitoes	804	603

[1] Forty-seven pools tested.
[2] Twenty-seven pools tested; 1 pool WNV positive, February 2003.

polymerase chain reaction (RT-PCR) using procedures established by Lanciotti et al. (2000).

Overwintering collections were taken December–April in 2001–2003 (Table 1). In the 2001–2002 season, collections were taken from 32 sites. No adult mosquitoes tested positive for WNV. In 2002–2003, 39 sites were visited. One pool of 6 *Cx. pipiens* taken on February 3, 2003, tested positive by RT-PCR. Subsequent Vero cell culture was negative. In this study, mosquitoes were held between 19°C and 22°C for only 2–3 days. This may not have been sufficient temperature or time for the virus to replicate to detectable titers.

Studies done by Dohm et al. (2002) with *Cx. pipiens* indicate that vertical transmission of WNV may be an important mechanism for the maintenance of the virus in hibernating mosquito populations. The discovery of overwintering positive mosquitoes in New York in 1999, in New Jersey in 2003, and in Lehigh County, PA, in 2003 provides evidence that WNV is maintained in overwintering adult mosquitoes in the northern latitudes. Continued research is necessary to determine if this is the primary overwintering maintenance mechanism for WNV or if there is another yet undetected mechanism. An understanding of WNV transmission ecology will increase our ability to predict and/or manage WNV public health risk.

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