SHORT COMMUNICATION

# Mosquitoes Infected with West Nile Virus in the Florida Keys, Monroe County, Florida, USA

LAWRENCE J. HRIBAR,[1] JOSHUA J. VLACH,[2] DAVID J. DEMAY,[3] LILLIAN M. STARK,[4] ROBIN L. STONER,[4] MARVIN S. GODSEY,[5] KRISTIN L. BURKHALTER,[5] MICHAEL C. SPOTO,[2] SHANNON S. JAMES,[2, 6] JENNIFER M. SMITH,[2] AND EDSEL M. FUSSELL[2]

J. Med. Entomol. 40(3): 361–363 (2003)

**ABSTRACT** More than 30,000 mosquitoes in 22 species or species groups were collected from the Florida Keys, Monroe County, FL, USA, in dry ice-baited light and gravid traps. Dry ice-baited traps collected more mosquitoes than did gravid traps. West Nile virus was detected in pools of *Anopheles atropos* Dyar & Knab, *Deinocerites cancer* Theobald, and *Ochlerotatus taeniorhynchus* (Wiedemann).

**KEY WORDS** West Nile virus, *Anopheles atropos*, *Deinocerites cancer*, *Ochlerotatus taeniorhynchus*, Florida Keys



WEST NILE VIRUS (WN) appeared in the Florida Keys, Monroe County, FL, late in 2001. The first confirmed human case was reported in August in an elderly woman from Sarasota who had visited the Florida Keys in July 2001. Additional human cases were soon confirmed in the Florida Keys. Public health and mosquito control personnel were taken by surprise, because the distribution of WN was thought to be limited to northern Florida (http://www9.myflorida.com/Disease_ctrl/epi/htopics/arbo/index.htm). An intense mosquito surveillance effort was initiated to determine what mosquito species might be serving as the vector(s) of WN in the Florida Keys.

## Materials and Methods

Collections were made with carbon dioxide-baited ABC traps and manure or grass infusion-baited gravid traps from September to December 2001. Collections were made from Big Pine Key, Cross Key, Grassy Key, Indian Key, Key Largo, Key West, Lignumvitae Key, Long Key, No Name Key, Plantation Key, Sugarloaf Key, Stock Island, Vaca Key, and Windley Key. Traps were deployed in mid- to late afternoon. Mosquitoes were collected the following morning, taken to the laboratory, and placed in a freezer. Sorting and identification were done on a chill table, after which specimens were stored at −75°C. Mosquitoes were pooled by species, location, and collection date in groups of up to 50 individuals and shipped on dry ice to either the Florida Department of Health Laboratory in Tampa or the CDC Division of Vector-borne Infectious Diseases Laboratory in Ft. Collins, CO. Pools of specimens sent to Tampa were homogenized in 1 ml cell culture diluent using a Qiagen Mixer Mill. This medium was removed and replaced by 1 ml lysis buffer and homogenization repeated. RNA was extracted from the lysate and assayed for WN by reverse transcriptase-polymerase chain reaction (PCR) (RT-PCR) in an ABI Prism 7700 Sequence Detection system using TaqMan reagents. Primers and probe sequences were from Lanciotti et al. (2000). The cell culture diluent was centrifuged, passed through a pretreated 0.22 micron sterilizing filter, and inoculated onto confluent monolayers of VERO cell culture in 25 $cm^2$ flasks. Cultures were observed periodically for 14 d postinoculation. RNA was extracted from cultures exhibiting a cytopathic effect and was assayed by RT-PCR as described above. Pools of specimens sent to Ft. Collins were tested for virus by VERO cell plaque assay (Beaty et al. 1989). Virus isolates were identified as WN by RT-PCR assay (Lanciotti et al. 2000).

Mosquito collections from Cross Key, Indian Key, Key Largo, Lignumvitae Key, and Windley Key were made with ABC traps and gravid traps operated on the same night at substantially the same location. These data were analyzed by a two-tailed paired sample *t*-test ($\alpha = 0.05$) to determine which trapping method collected more mosquitoes.

[1] Florida Keys Mosquito Control District, 506 106th Street Gulfside, Marathon, FL 33050 (e-mail: gringo1122@hotmail.com).
[2] Florida Keys Mosquito Control District, 5224 College Road, Key West FL 33040.
[3] Florida Keys Mosquito Control District, 100701 Overseas Highway, Key Largo FL 33037.
[4] Florida Department of Health, Bureau of Laboratories, Tampa Branch Laboratory, 3602 Spectrum Boulevard, Tampa, FL 33612.
[5] Centers for Disease Control and Prevention, National Center for Infectious Diseases, Foothills Campus, Fort Collins, CO 80522.
[6] Present address: USDA-APHIS-PPQ-CPHST, Soil-Inhabiting Pests Laboratory, 3505, 25th Avenue, Building 1, Gulfport, MS 39501.

0022-2585/03/0361–0363$04.00/0 

**Table 1. Mosquito pools collected in Monroe County, Florida, 2001**

| Species | Number of pools | Positive pools |
|---|---|---|
| *Aedes aegypti* Linnaeus | 93 | 0 |
| *Ae. vexans* (Meigen) | 1 | 0 |
| *Anopheles atropos* Dyar & Knab | 110 | 3 |
| *An. crucians* complex | 17 | 0 |
| *An.* spp. | 7 | 0 |
| *Culex atratus* Theobald | 3 | 0 |
| *Cx. bahamensis* Dyar & Knab | 15 | 0 |
| *Cx. erraticus* (Dyar & Knab) | 3 | 0 |
| *Cx. nigripalpus* Theobald | 214 | 0 |
| *Cx. peccator* Dyar & Knab | 2 | 0 |
| *Cx. quinquefasciatus* Say | 187 | 0 |
| *Cx.* spp. | 58 | 0 |
| *Deinocerites cancer* Theobald | 246 | 2 |
| *Ochlerotatus infirmatus* (Dyar & Knab) | 57 | 0 |
| *Oc. taeniorhynchus* (Wiedemann) | 325 | 2 |
| *Oc. thelcter* (Dyar) | 1 | 0 |
| *Oc. tortilis* (Theobald) | 48 | 0 |
| *Oc. triseriatus* (Say) | 8 | 0 |
| *Psorophora columbiae* (Dyar & Knab) | 9 | 0 |
| *Ps. johnstonii* (Grabham) | 2 | 0 |
| *Ps.* spp. | 2 | 0 |
| *Uranotaenia lowii* Theobald | 14 | 0 |
| Total pools | 1,422 | 7 |

## Results and Discussion

Over 30,000 mosquitoes in 22 species or species groups were collected during September through December 2001 (Table 1). WN was detected in pools of *Anopheles atropos* Dyar and Knab, *Deinocerites cancer* Theobald, and *Ochlerotatus taeniorhynchus* (Wiedemann) (Table 2). All positive mosquito pools were captured in $CO_2$-baited traps. More mosquitoes were collected in ABC traps than in gravid traps for all species analyzed ($t = 4.21$, df = 162, $P < 0.00004$) (Table 3).

*Deinocerites cancer* Theobald feeds primarily on birds (Edman 1974), although it occasionally will bite humans (Haeger and Phinizee 1959). Another *Deinocerites* species, *De. pseudes* Dyar and Knab, has been found naturally infected with Saint Louis encephalitis (SLE) virus and Venezuelan equine encephalitis (VEE) virus and is an efficient laboratory vector of VEE (Grayson et al. 1967, Tempelis and Galindo 1970, Grayson and Galindo 1972, Martin et al. 1972). However, *De. cancer* has a delayed blood-feeding behavior that may lessen its potential role as a disease vector (O'Meara and Mook 1990).

**Table 2. Mosquitoes positive for West Nile virus in Monroe County, Florida**

| Species | Island | Date collected | Number in pool |
|---|---|---|---|
| *An. atropos* | Big Pine Key | 9/26/01 | 4 |
| | Cross Key | 10/8/01 | 5 |
| | Key West | 12/10/01 | 23 |
| *De. cancer* | Key West | 10/23/01 | 1 |
| | Sugarloaf Key | 11/26/01 | 1 |
| *Oc. taeniorhynchus* | Grassy Key | 10/3/01 | 30 |
| | Stock Island | 9/25/01 | 40 |

**Table 3. Mosquitoes collected by carbon dioxide-baited light traps and gravid traps on Cross Key, Indian Key, Key Largo, Lignumvitae Key, and Windley Key, Monroe County, Florida**

| Species | $CO_2$-baited light trap | Gravid trap |
|---|---|---|
| *Ae. aegypti* | 32 | 0 |
| *An. atropos* | 1,877 | 2 |
| *Cx. nigripalpus* | 470 | 86 |
| *De. cancer* | 41 | 1 |
| *Oc. infirmatus* | 3 | 0 |
| *Oc. taeniorhynchus* | 6,608 | 6 |
| *Oc. triseriatus* | 31 | 0 |
| *Ur. lowii* | 12 | 7 |
| Total | 9,074 | 102 |

*Ochlerotatus taeniorhynchus* (Wiedemann) feeds primarily on mammals, but also feeds on birds (Edman 1971, O'Meara and Edman 1975). This species has been found naturally infected with SLE and Everglades (EVE) viruses (Hodapp et al. 1966, Chamberlain et al. 1969, Sudia et al. 1969), but is relatively refractory to infection with WN (Turell et al. 2001a). Nevertheless, in the Florida Keys *Oc. taeniorhynchus* is the most abundant mosquito species, where as many as 15,000 individuals may be collected during a single trap-night (Hribar 2002).

*Anopheles atropos* Dyar and Knab will feed on both avian and mammalian hosts, but most frequently selects mammals (Cupp and Stokes 1973). It is known to bite humans (Komp 1926, Griffitts 1927).

Carbon dioxide-baited light traps typically collect more mosquitoes than do gravid traps (Nayar et al. 2001), but most *Culex* spp. females collected in the light traps are unfed nullipars, making the collection of large numbers of mosquitoes necessary for virus surveillance (Reisen and Pfuntner 1987). Gravid traps often collect small numbers of mosquitoes but these specimens may be better suited for virus surveillance work because the females have ingested and digested at least one blood meal.

Interestingly, no *Culex* spp. were found infected with WN during this study. *Culex* spp. mosquitoes have been incriminated as vectors of WN in other parts of the United States, and because of their life cycles and association with SLE are believed to be important potential vectors of WN (Turell et al. 2001b). *Culex quinquefasciatus* Say and *Cx. nigripalpus* Theobald are common around residences in the Florida Keys (Hribar et al. 2001), and therefore remain of public health concern. Mosquito collections are continuing throughout the Florida Keys.

## Acknowledgments

We thank K. Hall (Florida Keys MCD) for some mosquito collections and R. Oliveri (Florida Department of Health) for her assistance. J. Burgess (Lee County Mosquito Control District), J. Day and G. O'Meara (University of Florida), D. Shroyer (Indian River Mosquito Control District), and R. Xue (USDA, Gainesville) provided copies of important literature. Access to some trapping sites was provided by Laura Quinn (Wild Bird Center, Tavernier, FL) and Pat

Wells and Danny Jones (Florida Department of Environmental Protection, Division of Recreation and Parks).

## References Cited

**Beaty B. J., C. H. Calisher, and R. S. Shope. 1989.** Arboviruses, pp. 797–856. *In:* Schimdt NJ, Emmons RW [eds.], Diagnostic procedures for viral, rickettsial and chlamydial infections. American Public Health Association, Washington, DC.

**Chamberlain, R. W., W. D. Sudia, T. W. Work, P. H. Coleman, V. F. Newhouse, and J. G. Johnston, Jr. 1969.** Arbovirus studies in south Florida, with emphasis on Venezuelan equine encephalomyelitis virus. Am. J. Epidemiol. 89: 197–210.

**Cupp, E. W., and G. M. Stokes. 1973.** Identification of bloodmeals from mosquitoes collected in light traps and dog-baited traps. Mosq. News. 33: 39–41.

**Edman, J. D. 1971.** Host-feeding patterns of Florida mosquitoes. I. *Aedes, Anopheles, Coquillettidia, Mansonia,* and *Psorophora.* J. Med. Entomol. 8: 687–695.

**Edman, J. D. 1974.** Host-feeding patterns of Florida mosquitoes. IV. *Deinocerites.* J. Med. Entomol. 11: 105–107.

**Grayson, M. A., and P. Galindo. 1972.** Experimental transmission of Venezuelan equine encephalitis virus by *Deinocerites pseudes* Dyar and Knab, 1909. J. Med. Entomol. 9: 196–200.

**Grayson, M. A., S. Srihongse, and P. Galindo. 1967.** Isolation of St. Louis encephalitis virus from *Deinocerites pseudes* in Panama. Mosq. News. 27: 204.

**Griffitts, T.H.D. 1927.** *Anopheles atropos* Dyar and Knab, a note on its breeding and other habits. US Publ. Health Rpts. 42: 1903–1905.

**Haeger, J. S., and J. Phinizee. 1959.** The biology of the crabhole mosquito, *Deinocerites cancer* Theobald. Proc. Florida Anti-Mosq. Assoc. 30: 34–37.

**Hodapp, C. J., W. D. Hillis, and E. V. Dahl. 1966.** Isolation of two arboviruses from *Aedes taeniorhynchus* Wiedemann. J. Med. Entomol. 3: 44–45.

**Hribar, L. J. 2002.** Mosquito (Diptera: Culicidae) collections in the Florida Keys, Monroe County, Florida, USA. Studia Dipterologica 9: 679–691.

**Hribar, L. J., J. M. Smith, J. J. Vlach, and T. N. Verna. 2001.** Survey of container-breeding mosquitoes from the Florida Keys, Monroe County, Florida. J. Am. Mosq. Control Assoc. 17: 245–248.

**Komp, W.H.W. 1926.** Observations on *Anopheles walkeri* and *Anopheles atropos* (Diptera, Culicidae). Ins. Ins. Mens. 14: 168–176.

**Lanciotti, R. S., A. J. Kerst, R. S. Nasci, M. S. Godsey, C. J. Mitchell, H. M. Savage, N. Komar, N. A. Panella, B. C. Allen, K. E. Volpe, B. S. Davis, and J. T. Roehrig. 2000.** Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J. Clin. Microbiol. 38: 4066–4071.

**Martin, D. H., G. A. Eddy, W. D. Sudia, W. C. Reeves, V. F. Newhouse, and K. M. Johnson. 1972.** An epidemiological study of Venezuelan equine encephalomyelitis in Costa Rica. Am. J. Epidemiol. 95: 565–578.

**Nayar, J. K., N. Karabatsos, J. W. Knight, M. Godsey, J. Chang, and C. J. Mitchell. 2001.** Mosquito hosts of arboviruses from Indian River County, Florida, during 1998. Fla. Entomol. 84: 376–379.

**O'Meara, G. F., and J. D. Edman. 1975.** Autogenous egg production in the salt-marsh mosquito *Aedes taeniorhynchus.* Biol. Bull 149: 384–396.

**O'Meara, G. F., and D. H. Mook. 1990.** Facultative bloodfeeding in the crabhole mosquito, *Deinocerites cancer.* Med. Vet. Entomol. 4: 117–123.

**Reisen, W. K., and A. R. Pfuntner. 1987.** Effectiveness of five methods for sampling adult *Culex* mosquitoes in rural and urban habitats in San Bernardino County, California. J. Am. Mosq. Control Assoc. 3: 601–606.

**Sudia, W. D., V. F. Newhouse, and W. A. Chappell. 1969.** Venezuelan equine encephalitis virus-vector studies following a human case in Dade County, Florida, 1968. Mosq. News. 29: 596–600.

**Tempelis, C. H., and P. Galindo. 1970.** Feeding habits of five species of *Deinocerites* mosquitoes collected in Panama. J. Med. Entomol. 7: 175–179.

**Turell, M. J., M. L. O'Guinn, D. J. Dohm, and J. W. Jones. 2001a.** Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile Virus. J. Med. Entomol. 38: 130–134.

**Turell, M. J., M. R. Sardelis, D. J. Dohm, and M. L. O'Guinn. 2001b.** Potential North American vectors of West Nile virus. Ann. NY Acad. Sci. 951: 317–324.

*Received for publication 6 August 2002; accepted 24 January 2003.*