Short Report

Vector Competence of Culex tarsalis from Orange County, California, for West Nile Virus

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ABSTRACT

To evaluate the vector competence of Culex tarsalis Coquillett for West Nile virus (WN), females reared from larvae collected in Huntington Beach, Orange County, CA, were fed on 2–3-day-old chickens previously inoculated with a New York strain (Crow 397-99) of WN. The Cx. tarsalis mosquitoes were efficient laboratory vectors of WN, with estimated transmission rates of 81% and 91% for mosquitoes that ingested 10^{6.5} or 10^{7.3} plaque-forming units of WN/mL of blood, respectively. Based on efficiency of viral transmission and the role of this species in the transmission of the closely related St. Louis encephalitis virus, Cx. tarsalis should be considered a potentially important vector of WN in the western United States. Key Words: West Nile virus—Culex tarsalis mosquitoes—Vector competence.

INTRODUCTION

Since its first detection in the New York Metropolitan Area in 1999 (Centers for Disease Control and Prevention 1999, Lanciotti et al. 2000), West Nile virus (WN) has expanded its range in the United States. By October 2002, it had spread to at least 43 states and was responsible for >3,200 cases of human illness (Centers for Disease Control and Prevention 2002). In North America, WN has been isolated from birds, principally crows, and has been responsible for encephalitis and death in humans and horses (Centers for Disease Control and Prevention 2001). WN is a member of the Japanese encephalitis virus serogroup in the genus Flavivirus, family Flaviviridae, and is closely related to St. Louis encephalitis virus (SLE). Both viruses share a similar epidemiology, with transmission between ornithophagic mosquitoes of the genus Culex and various avian hosts. Because of the importance of Culex tarsalis Coquillett in the epidemiology of SLE in the western United States (Tsai and Mitchell 1989), there is concern that this species might be an important vector of WN as its range expands to areas where Cx. tarsalis is found. Therefore, we evaluated the vector competence of Cx. tarsalis from California for WN under laboratory conditions.

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MATERIALS AND METHODS

*Cx. tarsalis* mosquitoes, collected as larvae during March and April 2001 in the Bolsa Chica wetlands, Huntington Beach, Orange County, CA, were reared to adults and transported to a biological safety level-3 laboratory at the U.S. Army Medical Research Institute of Infectious Diseases (Fort Detrick, MD).

Adult mosquitoes were kept in 3.8-L cartons with netting over the open end, placed in an incubator maintained at 26°C with a 16:8-h photoperiod, and provided apple slices and water-soaked gauze pads. Groups of 20–50 female mosquitoes were transferred to 0.5-L water-soaked gauze pads. Groups of 20–50 1–2-day-old chickens that had been inoculated with netting over the open end, placed in an incubator maintained at 26°C with a 16:8-h photoperiod, and provided apple slices and water-soaked gauze pads. Groups of 20–50 female mosquitoes were transferred to 0.5-L water-soaked gauze pads. Groups of 20–50 1–2-day-old chickens that had been inoculated with 10^4 plaque-forming units (PFU) of WN 24 or 48 h previously. Immediately after mosquito feeding, a 0.1-mL blood sample was obtained from the jugular vein of each chicken and diluted in 0.9 mL of diluent (10% heat-inactivated fetal bovine serum in Medium 199 with Earle’s salts, NaHCO_3, and antibiotics) plus 10 units of heparin/mL. This sample was used to determine the WN viremia level of the chickens at the time of mosquito feeding. Engorged mosquitoes were transferred to 3.8-L cages and maintained in an incubator at 26°C.

The Crow 397-99 strain of WN was used throughout. This strain was isolated from a dead crow found in the Bronx, New York City, during an epizootic in 1999 (Turell et al. 2000) and had been passaged once in Vero cell culture. Viral stocks, triturated mosquito suspensions, and chicken blood samples were tested for infectious virus by plaque assay on Vero cells as described by Gargan et al. (1983), except that the second overlay, containing neutral red stain, was added 2 days after the first overlay.

To determine infection and dissemination rates, mosquitoes were killed by freezing at −20°C for ~5 min 12–14 days after the infectious blood meal, and their legs and bodies were triturated separately in 1 mL of diluent. These suspensions were stored at −70°C until tested for virus by plaque assay. Presence of virus in a mosquito’s body indicated infection, whereas virus in the legs indicated that the mosquito had a disseminated infection (Turell et al. 1984). To determine the percentage of *Cx. tarsalis* with a disseminated infection that could transmit WN by bite, we inoculated (Rosen and Gubler 1974) some of the original *Cx. tarsalis* with 0.3 μL of a suspension containing 10^{14.2} PFU of WN/mL (10^{0.7} PFU per mosquito). These mosquitoes were allowed to feed individually on 1–2-day-old chickens 7 or 8 days later, and the chickens were bled the following day to determine if they were infected.

We defined the infection rate as (the number of infected mosquitoes/total tested) × 100 and the dissemination rate as (the number of mosquitoes with virus in their legs/total tested) × 100. To estimate transmission rates, we multiplied the percentage of mosquitoes that developed a disseminated infection after ingesting WN by the percentage of mosquitoes with a disseminated infection that transmitted virus by bite.

RESULTS AND DISCUSSION

Viremias in the five chickens at the time of mosquito blood-feeding ranged from 10^{6.5} to 10^{7.5} PFU/mL of blood. Virtually all of the *Cx. tarsalis* that ingested blood from chickens with these viremias became infected (Table 1). There was no significant difference (Fisher’s exact test, $p \geq 0.96$) in infection (94% and 97%) or dissemination (81% and 83%) rates between those mosquitoes that ingested 10^{6.5}–10^{7.5} PFU/mL, respectively. All six WN-inoculated *Cx. tarsalis* that fed on uninfected chickens transmitted virus by bite. Although none of the orally exposed *Cx. tarsalis* fed on a susceptible chicken, previous studies indicate that transmission rates for *Culex*, *Aedes*, and *Ochlerotatus* mosquitoes with a disseminated infection after ingesting a WN viremic blood meal are nearly identical to those for the same species that had been inoculated with WN (Sardelis and Turell 2001). Therefore, the observed transmission rate for WN-inoculated *Cx. tarsalis* (100%) should approximate the rate that would occur in orally exposed individuals with a disseminated infection. Because there does not appear to be a salivary gland barrier for WN in *Cx. tarsalis*, we expect that ~81% of *Cx. tarsalis*
would be able to transmit WN by day 12 after oral exposure to $10^{6.5}$ PFU.

Our experiment documented that a California population of *Cx. tarsalis* has the potential to serve as a vector for WN based on its susceptibility to infection and ability to transmit WN efficiently. This finding is consistent with previous laboratory transmission studies that showed that WN is transmitted by a broad range of North American mosquito species, including a number of *Culex* and *Aedes* species (Turell et al. 2000, 2001, Sardelis and Turell 2001, Sardelis et al. 2001). Unlike most of the other North American *Culex* (*Culex*) species tested (Turell et al. 2000, 2001, Sardelis et al. 2001), there was little evidence of a midgut escape barrier in *Cx. tarsalis*, and this species was among the most efficient species tested in the laboratory. In general, while floodwater *Aedes* and *Ochlerotatus* species had low infection rates, usually <20%, container-breeding *Aedes* and *Ochlerotatus* species were highly susceptible to infection with WN, with infection rates of >60% (Sardelis and Turell 2001, Turell et al. 2001; M.J.T., unpublished data).

Also, because all six individuals with a disseminated infection tested transmitted WN by bite, a salivary gland barrier for WN did not appear to be important in this species. This is consistent with the lack of a salivary gland barrier in other *Culex* species tested (Turell et al. 2000, 2001, Sardelis et al. 2001).

The viremias used in our study, $10^{6.5}$–$7.5$ PFU/mL of blood, were consistent with low to moderate viremias for hooded crows and house sparrows in Egypt (Work et al. 1955) and experimentally infected North American house sparrows and crows (M.J.T., unpublished data). Therefore, our results should reflect what would happen when *Cx. tarsalis* mosquitoes feed on birds with a similar concentration of virus in nature.

Although *Cx. tarsalis* was among the most efficient laboratory vectors of WN tested to date, various aspects of the mosquito’s bionomics must be considered to effectively evaluate the importance of this species in the transmission of WN in nature. *Cx. tarsalis* is considered an ornithophilic feeder, taking blood meals mostly from birds, but feeding on mammals in late summer (Tempelis et al. 1967, Tempelis 1975). The results of our study, combined with evidence of the distribution and bionomics of *Cx. tarsalis*, indicate that this species could function both as a maintenance as well as a bridge vector for WN. Because of intraspecific variation in vector competence (Hardy and Reeves 1990), additional strains of *Cx. tarsalis* need to be tested to determine their transmission efficiency to best estimate the role this species may play in the epidemiology of WN in a particular area.

**ACKNOWLEDGMENTS**

We thank R. Meyer, S. Bennett, and R. Cummings for their assistance in capturing and rearing the *Cx. tarsalis* used in these studies, and G. Ludwig, R. Leon, J. Blow, and K. Kenyon for their critical reading of the manuscript.

The views of the authors do not necessarily reflect the position of the Department of Defense or the Department of the Army.

In conducting research using animals, the investigators adhered to the *Guide for the Care and Use of Laboratory Animals*, as prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication 86-23, Revised 1996). The facilities are

<table>
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<th>Infectious dose (log&lt;sub&gt;10&lt;/sub&gt; PFU/mL)</th>
<th>Number tested</th>
<th>Infection rate (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Dissemination rate (%)&lt;sup&gt;2&lt;/sup&gt;</th>
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<tr>
<td>$6.5 \pm 0.1$</td>
<td>36</td>
<td>$94 (81–99)^a$</td>
<td>$81 (64–92)^a$</td>
</tr>
<tr>
<td>$7.3 \pm 0.2$</td>
<td>35</td>
<td>$97 (85–99)^a$</td>
<td>$83 (66–93)^a$</td>
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<sup>1</sup> Percentage of mosquitoes containing virus in their bodies (95% confidence interval). Infection rates followed by the same letter are not significantly different at $\alpha = 0.05$ by a Fisher’s exact test.

<sup>2</sup> Percentage of mosquitoes containing virus in their legs (95% confidence interval). Dissemination rates followed by the same letter are not significantly different at $\alpha = 0.05$ by a Fisher’s exact test.
fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

ABBREVIATIONS

PFU, plaque-forming units; SLE, St. Louis encephalitis virus; WN, West Nile virus.

REFERENCES


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