Research Paper

A Comparison of West Nile Virus Transmission by Ochlerotatus trivittatus (COQ.), Culex pipiens (L.), and Aedes albopictus (Skuse)

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ABSTRACT

Transmission of West Nile virus (WNV) by Ochlerotatus trivittatus, Culex pipiens, and Aedes albopictus were compared 14 days after taking blood meals from viremic chickens with titers ranging from $10^{2.5}$ to $10^{6.5}$ cell infective dose 50s (CID50s)/mL serum. Transmission occurred in one of four (25%) Oc. trivittatus and one of 25 (4%) Cx. pipiens that fed on chickens with titers of $10^{5.5}$ CID50s/mL. No transmission occurred among two of 16 (13%) Oc. trivittatus or one of 25 (4%) Cx. pipiens that became infected after blood meals with titers of $10^{4.0}$ and $10^{4.5}$ CID50s/mL, the next lowest blood meal titers evaluated. Seventeen of 28 (61%) Ae. albopictus transmitted WNV after blood meals with titers of $10^{7.0}$ CID50s/mL, but no infection or transmission was observed among 21 Ae. albopictus that fed on chickens with titers of $10^{5.0}$ CID50s/mL, the next lowest titer evaluated. Transmission by all three species increased dramatically after blood meals with WNV titers of $>10^{7.0}$ CID50s/mL. No significant differences occurred in dissemination and transmission rates of the three species after taking blood meals with titers of $>10^{7.0}$ CID50s/mL. The cumulative mean ± SE transmission rates of Oc. trivittatus, Cx. pipiens, and Ae. albopictus after blood meals with titers of $>10^{7.0}$ CID50s/mL were 45.5 ± 4.1%, 46.8 ± 4.5%, and 72.4 ± 5.5%. The cumulative mean dissemination rates of the three species were 78.3 ± 6.7%, 74.8 ± 2.6%, and 88.6 ± 2.1%. The rates of transmission by the three species that developed disseminated infections after blood meals with titers of $>10^{7.0}$ CID50s/mL were 58.8 ± 4.4%, 62.6 ± 5.8%, and 81.6 ± 5.4%, respectively. In a previous study, we found that susceptibility of the three species to WNV was essentially the same when fed on chickens with WNV titers of $>10^{7.0}$ CID50s/mL, but Oc. trivittatus and Cx. pipiens were more susceptible than Ae. albopictus to WNV at lower virus titers. The current study strongly suggests that Ae. albopictus is a more efficient vector than Oc. trivittatus and Cx. pipiens when fed blood meals with titers of $>10^{7.0}$ CID50s/mL. However, Oc. trivittatus and Cx. pipiens might be more efficient as vectors when infected by blood meals with titers of $<10^{7.0}$ CID50s/mL. Key Words: West Nile virus—Ochlerotatus trivittatus—Aedes albopictus—Culex pipiens—Transmission—Vector competence. Vector-Borne Zoonotic Dis. 5, 40-47.

INTRODUCTION

WEST NILE VIRUS (WNV), a member of the Flavivirus genus of the Flaviviridae, continues to be a significant veterinary and public health problem. Since its emergence in New York City in 1999, it has spread and established itself throughout much of North America (Weese et al. 2003, Blitvich et al. 2003, Ulloa et al. 2003). The virus has been isolated from at

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least 60 different species of mosquitoes representing 11 genera (CDC 2005). The commonly accepted paradigm of WNV is that it is maintained in nature by the bird-mosquito-bird cycle and that bridge vectors transmit the virus to humans, horses, and other mammals that typically develop levels of viremia that seldom exceed $10^{4.0}$ plaque forming units (pfu)/mL. Consequently, mammals are considered to be dead-end hosts. Species in which low titers of WNV have been described include mice, cats, dogs, and horses (Odelola and Oduye, 1977, Halevy et al, 1994, Ben-Nathan and Feuerstein, 1990, Ben-Nathan et al, 1996, Austgen et al, 2002). In contrast, WNV viremias in the adult golden hamster (Mesocricetus auratus) can exceed $10^{5.5}$ TCID$_{50s}$/mL (Xiao et al. 2001). Similar observations in cottontail rabbits (Sylvilagus floridanus) and pigs have been made in our laboratory (unpublished data). These observations raise the question of whether or not enzootic cycles involving mammals exist.

In a recent study, Tiawsirisup et al. (2004) compared the susceptibility of Ochlerotatus trivittatus (Coq.) to infection by WNV to that of Culex pipiens (L) and Aedes albopictus (Skuse). They demonstrated that Oc. trivittatus and Cx. pipiens were essentially as susceptible to WNV as Ae. albopictus after blood meals with titers of $>10^{7.0}$ cell culture infective dose$_{50s}$ (CID$_{50s}$)/mL. They also demonstrated that Oc. trivittatus and Cx. pipiens could be infected by blood meals with titers as low as $10^{4.5}$ CID$_{50s}$/mL. The lowest infective blood meal titer for Ae. albopictus was $10^{5.5}$ CID$_{50s}$/mL. The susceptibility of Oc. trivittatus and Cx. pipiens to relatively low virus titers raises the question of their potential importance in possible enzootic cycles of WNV involving mammals.

Ochlerotatus trivittatus is anthropophilic but will, like Ae. albopictus, feed on mammalian and avian species (Pinger and Rowley, 1975, Niebyski et al. 1994, Gomes et al. 2003, Samui et al. 2003). While Cx. pipiens is primarily ornithophilic, genetically distinct populations have been described that will readily feed on mammals (Fonesca et al. 2004). These observations suggest that both Oc. trivittatus, which is one of the most abundant mosquito species in the North and North Central United States (Carpenter 1968, 1970, Howard et al. 1917, Rowley et al.1973, Trimbel 1972), and zoophilic forms of Cx. pipiens, have the potential to serve as vectors within enzootic WNV cycles involving mammalian species such as cottontail rabbits. Whether or not Oc. trivittatus is an important vector is dependent on the efficiency by which it transmits the virus. In the present study, we compared the ability of Oc. trivittatus to transmit WNV to that of Cx. pipiens, considered to be a primary amplifying vector of WNV (Andreadis et al. 2001) and Ae. albopictus, a possible WNV bridge vector (Sardelis et al. 2002).

**MATERIALS AND METHODS**

**Experimental design and data analysis**

Infection, dissemination and transmission rates for Oc. trivittatus, Ae. albopictus, and Cx. pipiens were determined by calculating the percentage of blood feeding mosquitoes with virus in torsos, legs and saliva 14 days after taking blood meals from chickens with WNV titers ranging from $10^{2.5}$ to $10^{9.5}$ CID$_{50s}$/mL serum. This broad range of titers was generated by injecting groups of 2-3-day-old chickens with WNV doses ranging from $10^{2.0}$ to $10^{4.0}$ CID$_{50s}$. At 12–72 h later, chickens were used as blood meal sources for mixed and homogenous groups of Oc. trivittatus, Ae. albopictus, and Cx. pipiens. Mosquitoes that fed to repletion were removed from the pools by species and maintained separately. Serums were collected from chickens and assayed immediately after blood-feeding. Some mosquito susceptibility data representing the three species in this study had been incorporated with other mosquito susceptibility data in an earlier companion study on susceptibility (Tiawsirisup et al. 2004).

Data were analyzed by JMP version 5.0 (SAS Institute Inc., Cary, NC). First, omnibus protected pairwise tests were used to control type I error inflation (Ramsey and Schafer 2002). Differences in infection, dissemination, and transmission rates within species among titers were tested using an omnibus chi-square test for an overall effect of species. When statistically significant, Fisher’s exact test ($p \leq 0.05$) was used.
for pairwise comparisons. No significant differences in the three parameters were observed within species at blood meal titers of \( \geq 10^{7.0} \) CID<sub>50</sub>/mL except for two dissemination values. Consequently infection, dissemination and transmission rates of each species feeding on chickens with titers of \( \geq 10^{7.0} \) CID<sub>50</sub>/mL were collapsed and compared by one-way ANOVA used as an omnibus test to protect against type I error inflation. When the ANOVA was statistically significant, differences between mean infection and transmission rates, and between mean dissemination and transmission rates by mosquito species were compared by pairwise t-tests using the Tukey-Kramer HSD adjustment with data collapsed over chicken/virus titer repetitions.

**Chickens**

Two-day-old WNV-specific antibody-free broiler chickens (Ross × Ross) were obtained from a commercial hatchery and housed in biosafety level 3 facilities.

**Mosquitoes**

*Ochlerotatus trivittatus* were first generation mosquitoes derived from adults collected in Iowa in 2002. *Aedes albopictus* were the 10th to 20th generations of mosquitoes originally collected in Missouri and colonized by the Illinois Natural History Survey. *Culex pipiens* were the 8th to 10th generations of mosquitoes originally collected in Iowa and colonized at Iowa State University in 2002. All mosquitoes were maintained in controlled environmental conditions (27 ± 1°C and 80 ± 5% RH with a 16:8-h photoperiod) and fed a 10% sucrose solution. Mosquitoes were deprived of sucrose for 48 h before blood-feeding on viremic chickens or imbibing a 5% (w/v) sucrose solution in phosphate-buffered saline, pH 7.0, with 0.5% fetal bovine serum (FBS) contained in capillary tubes.

**Cells and medium**

Vero-76 cells were used for virus propagation and assay using cell culture media prepared as previously described (Tiawsirisup et al. 2004). Growth medium was supplemented with 10% fetal bovine serum (FBS). Maintenance medium (MM) was supplemented with 1% FBS. Grinding diluent (GD) used to triturate mosquito specimens for virus isolation was MM supplemented with 20% FBS.

**Virus and virus assays**

West Nile virus (NY 1999–crow) was supplied by the National Veterinary Services Laboratory (Ames, IA). The virus was passed six times in Vero-76 cells before being propagated in *Aedes albopictus* as previously described (Tiawsirisup et al. 2004).

Mosquito propagated virus and chicken sera were assayed for WNV on Vero-76 cells by the quantal method as described by Tiawsirisup et al. (2004). Assays were expressed as CID<sub>50</sub>/mL and verified by RT-PCR as previously described (Tiawsirisup et al. 2004).

**Virus isolation**

Virus was detected in torsos and legs of individual mosquitoes by triturating separately in 300 μL of cold GD. The volume of each sample was then increased to 2 mL using cold MM. Virus in saliva of individual mosquitoes was detected by depositing contents of individual capillary tubes into 2 mL of MM following a 20-min feeding period. These preparations were passed through 450-nm filters directly into 25-cm<sup>2</sup> cell culture flasks containing cell monolayers from which medium was removed. An additional 5 mL of MM was added to individual flasks after a 1-h incubation period. Cell cultures were observed for CPE for up to 8 days and assays verified by RT-PCR.

**RESULTS**

Infection, dissemination, and transmission rates are summarized by WNV blood meal titers (BMT’s) for *Oc. trivittatus*, *Cx. pipiens*, and *Ae. albopictus* in Tables 1–3, respectively. The lowest infective titer of blood meals for *Oc. trivittatus* and *Cx. pipiens* that was tested was \( 10^{4.5} \) CID<sub>50</sub>/mL. The infection rates were 13% (2/16) and 4% (1/25), respectively. None of 21 *Ae. albopictus*
were infected after blood meals with titers of
10^{5.0} \text{ CID}_{50}/\text{mL}, but 89\% (25/28) of \textit{Ae. albopictus} were infected by blood meals with titers of
10^{7.0} \text{ CID}_{50}/\text{mL}, the next highest BMT evaluated. There were no significant differences in in-
fec tion rates within individual species after
blood meals with titers of \( \geq 10^{7.0} \text{ CID}_{50}/\text{mL}. \) The cumulative mean infection rates represent-
ing all BMT’s of \( \geq 10^{7.0} \text{ CID}_{50}/\text{mL} \) for \textit{Oc. trivittatus}, \textit{Cx. pipiens}, and \textit{Ae. albopictus} were 95.5 \pm
3.0\%, 97.4 \pm 1.1\%, and 92.2 \pm 1.6\%, respectively
(Table 4).

The lowest infective BMT’s that resulted in
disseminated infections for \textit{Oc. trivittatus}, \textit{Cx. pipiens}, and \textit{Ae. albopictus} were 10^{5.5}, 10^{5.5}, and
10^{7.0} \text{ CID}_{50}/\text{mL}, respectively. The dissemi-
nated infection rates corresponding to these
BMT’s were 75\% (three of four), 8\% (two of 25),
and 82 (23 of 28), respectively. No disseminated
infections were observed in the two \textit{Cx. pipiens}

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**Table 1.** Infection, Dissemination, and Transmission Rates of West Nile Virus
in \textit{Ochlerotatus trivittatus} 14 Days after Feeding on Viremic Chickens

<table>
<thead>
<tr>
<th>Blood meal virus titer</th>
<th>No. tested mosquitoes</th>
<th>Percent infection</th>
<th>Percent dissemination</th>
<th>Percent transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5\textsuperscript{a}</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.5</td>
<td>16</td>
<td>13\textsuperscript{1}</td>
<td>75\textsuperscript{1,2}</td>
<td>25\textsuperscript{1,2}</td>
</tr>
<tr>
<td>5.5</td>
<td>4</td>
<td>72\textsuperscript{3}</td>
<td>42\textsuperscript{1,3}</td>
<td>15\textsuperscript{2}</td>
</tr>
<tr>
<td>6.5</td>
<td>26</td>
<td>81\textsuperscript{2,3}</td>
<td>56\textsuperscript{1,4}</td>
<td>38\textsuperscript{1,2}</td>
</tr>
<tr>
<td>7.0</td>
<td>16</td>
<td>95\textsuperscript{2}</td>
<td>77\textsuperscript{2,4}</td>
<td>42\textsuperscript{1}</td>
</tr>
<tr>
<td>7.5</td>
<td>43</td>
<td>97\textsuperscript{2}</td>
<td>86\textsuperscript{2}</td>
<td>52\textsuperscript{1}</td>
</tr>
<tr>
<td>8.0</td>
<td>29</td>
<td>100\textsuperscript{2,3}</td>
<td>63\textsuperscript{2,3,4}</td>
<td>38\textsuperscript{1,2}</td>
</tr>
<tr>
<td>8.5</td>
<td>8</td>
<td>100\textsuperscript{2,3}</td>
<td>88\textsuperscript{2,4}</td>
<td>63\textsuperscript{1}</td>
</tr>
<tr>
<td>9.0</td>
<td>8</td>
<td>100\textsuperscript{2}</td>
<td>100\textsuperscript{2}</td>
<td>40\textsuperscript{1,2}</td>
</tr>
<tr>
<td>9.5</td>
<td>10</td>
<td>100\textsuperscript{2}</td>
<td>100\textsuperscript{2}</td>
<td>40\textsuperscript{1,2}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Titer expressed as log\textsubscript{10} \text{ CID}_{50}/\text{mL} serum.

\textsuperscript{b} Percent infection defined as percent of blood-fed mosquitoes with virus in torsos.

\textsuperscript{c} Percent dissemination defined as percent of blood-fed mosquitoes with virus in the hemocoel as indicated by detecting virus in legs.

\textsuperscript{d} Percent transmission defined as the percent blood-fed mosquitoes with virus in saliva.

Values within each category that have a numerical superscript in common are not significantly different as determined by Fisher’s exact test (\( p \leq 0.05 \)). Comparison of zero values not shown.

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**Table 2.** Infection, Dissemination, and Transmission Rates of West Nile Virus
in \textit{Culex pipiens} 14 Days after Feeding on Viremic Chickens

<table>
<thead>
<tr>
<th>Blood meal virus titer</th>
<th>No. tested mosquitoes</th>
<th>Percent infection</th>
<th>Percent dissemination</th>
<th>Percent transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5\textsuperscript{a}</td>
<td>25</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4.5</td>
<td>25</td>
<td>4\textsuperscript{1}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
<td>25</td>
<td>4\textsuperscript{1}</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.5</td>
<td>25</td>
<td>16\textsuperscript{1}</td>
<td>81\textsuperscript{1}</td>
<td>41\textsuperscript{1}</td>
</tr>
<tr>
<td>6.5</td>
<td>13</td>
<td>77\textsuperscript{2}</td>
<td>31\textsuperscript{1,2}</td>
<td>81\textsuperscript{1,2}</td>
</tr>
<tr>
<td>7.0</td>
<td>21</td>
<td>95\textsuperscript{2,3}</td>
<td>67\textsuperscript{2,3}</td>
<td>43\textsuperscript{2,3}</td>
</tr>
<tr>
<td>7.5</td>
<td>25</td>
<td>96\textsuperscript{2,3}</td>
<td>76\textsuperscript{3}</td>
<td>32\textsuperscript{2,3}</td>
</tr>
<tr>
<td>8.0</td>
<td>24</td>
<td>100\textsuperscript{3}</td>
<td>71\textsuperscript{3}</td>
<td>54\textsuperscript{3}</td>
</tr>
<tr>
<td>8.5</td>
<td>26</td>
<td>96\textsuperscript{2,3}</td>
<td>81\textsuperscript{3}</td>
<td>58\textsuperscript{3}</td>
</tr>
<tr>
<td>9.5</td>
<td>19</td>
<td>100\textsuperscript{2,3}</td>
<td>79\textsuperscript{3}</td>
<td>47\textsuperscript{3}</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Titer expressed as log\textsubscript{10} \text{ CID}_{50}/\text{mL} serum.

\textsuperscript{b} Percent infection defined as percent of blood-fed mosquitoes with virus in torsos.

\textsuperscript{c} Percent dissemination defined as percent of blood-fed mosquitoes with virus in the hemocoel as indicated by detecting virus in legs.

\textsuperscript{d} Percent transmission defined as the percent blood-fed mosquitoes with virus in saliva.

Values within each category that have a numerical superscript in common are not significantly different as determined by Fisher’s exact test (\( p \leq 0.05 \)). Comparison of zero values not shown.
that took infective blood meals with titers of $10^{4.5}$ and $10^{5.0} \text{CID}_{50}\text{s/mL}$, nor in two *Oc. trivittatus* that were infected by blood meals with a titer of $10^{4.5} \text{CID}_{50}\text{s/mL}$. Dissemination rates of *Cx. pipiens* and *Ae. albopictus* did not vary significantly after feeding on chickens with WNV titers of $\geq 10^{7.0} \text{CID}_{50}\text{s/mL}$. Similarly, no significant differences occurred among WNV dissemination rates of *Oc. trivittatus* after blood meals with titers of $\geq 10^{7.5} \text{CID}_{50}\text{s/mL}$. The cumulative mean dissemination rates representing all BMT’s of $\geq 10^{7.0} \text{CID}_{50}\text{s/mL}$ were 78.3 ± 6.7%, 74.8 ± 2.6%, and 88.6 ± 2.1% for *Oc. trivittatus*, *Cx. pipiens*, and *Ae. albopictus*, respectively (Table 4).

Transmission of WNV by *Oc. trivittatus* and *Cx. pipiens* was first observed after infection by blood meals with titers of $10^{5.5} \text{CID}_{50}\text{s/mL}$. The rates of transmission by the two species at these levels were 25% (1/4) and 4% (1/25), respectively. No WNV transmission by either species was observed after infection by blood meals with titers as low as $10^{4.5} \text{CID}_{50}\text{s/mL}$. Transmission of WNV by *Ae. albopictus* was first observed after infection by blood meals with a titer of $10^{7.0} \text{CID}_{50}\text{s/mL}$. Seventeen of 28 mosquitoes (61%) transmitted virus. No transmission was observed among 21 *Ae. albopictus* after a blood meal with a titer of $10^{5.0} \text{CID}_{50}\text{s/mL}$.

### Table 3. Infection, Dissemination, and Transmission Rates of West Nile Virus in *Aedes albopictus* 14 Days After Feeding on Viremic Chickens

<table>
<thead>
<tr>
<th>Blood meal virus titer</th>
<th>No. tested mosquitoes</th>
<th>Percent infection</th>
<th>Percent dissemination</th>
<th>Percent transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5.0</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7.0^a</td>
<td>28</td>
<td>89^1</td>
<td>82^1</td>
<td>61^1</td>
</tr>
<tr>
<td>7.5</td>
<td>40</td>
<td>98^1</td>
<td>95^1</td>
<td>75^1</td>
</tr>
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<td>8.0</td>
<td>30</td>
<td>93^1</td>
<td>87^1</td>
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<td>8.5</td>
<td>55</td>
<td>91^1</td>
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<td>76^1</td>
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<tr>
<td>9.5</td>
<td>10</td>
<td>90^1</td>
<td>90^1</td>
<td>90^1</td>
</tr>
</tbody>
</table>

^aTiter expressed as Log_{10} \text{CID}_{50}\text{s/mL} serum.

^bPercent infection defined as percent of blood-fed mosquitoes with virus in their torsos.

^cPercent dissemination defined as percentage blood-fed mosquitoes with virus in the hemocoel as indicated by detecting virus in legs.

^dPercent transmission defined as the percent blood-fed mosquitoes with virus in their saliva.

^eValues within each category that have a numerical superscript in common are not significantly different as determined by Fisher's exact test (p < 0.05). Comparison of zero values not shown.

### Table 4. Cumulative Infection, Dissemination, and Transmission Rates of West Nile Virus by *Ochlerotatus trivittatus*, *Culex pipiens* and *Aedes albopictus* 14 Days After Feeding on Chickens with WNV Titers of $\geq 10^{7.0} \text{CID}_{50}\text{s/mL}$

<table>
<thead>
<tr>
<th></th>
<th>Ochlerotatus trivittatus</th>
<th>Culex pipiens</th>
<th>Aedes albopictus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number tested</td>
<td>114</td>
<td>115</td>
<td>163</td>
</tr>
<tr>
<td>Infection rate^b,c</td>
<td>95.5 ± 3.0^1</td>
<td>97.4 ± 1.1^1</td>
<td>92.2 ± 1.6^1</td>
</tr>
<tr>
<td>Dissemination rate^b,c</td>
<td>78.3 ± 6.7^1</td>
<td>74.8 ± 2.6^1</td>
<td>88.6 ± 2.1^1</td>
</tr>
<tr>
<td>Transmission rate^b,c</td>
<td>45.5 ± 4.1^1</td>
<td>46.8 ± 4.5^1</td>
<td>72.4 ± 5.5^2</td>
</tr>
</tbody>
</table>

^aViremias of chickens ranged from $10^{7.0}$ to $10^{9.5} \text{CID}_{50}\text{s/mL}$ serum.

^bInfection, dissemination, and transmission rates = % ± SE of blood-fed mosquitoes with WNV in their torsos and legs, and those depositing WNV-containing saliva in capillary tubes, respectively.

^cAll values by category across species with numerical superscripts in common are not different (p ≤ 0.05) as determined by one-way ANOVA. All values within species with uppercase letter subscripts in common are not different as determined by pairwise t-tests using the Tukey-Kramer HSD adjustment.
and *Ae. albopictus* after blood meals with titers of \(\geq 10^{7.0}\) CID_{50}/mL were 45.5 \(\pm\) 4.1\%, 46.8 \(\pm\) 4.5\%, and 72.4 \(\pm\) 5.5\%, respectively. The cumulative rates of transmission by the three species that developed disseminated infections after blood meals with titers of \(\geq 10^{7.0}\) CID_{50}/mL were 58.8 \(\pm\) 4.4\%, 62.6 \(\pm\) 5.8\%, and 81.6 \(\pm\) 5.4\%, respectively.

**DISCUSSION**

*Ochlerotatus trivittatus* is a ubiquitous mosquito that is widely distributed throughout North America, and parts of Mexico and Panama (Carpenter 1968, 1970, Trimbel 1972, Howard et al. 1917). It is also one of the most abundant species in the North and North Central regions of the United States. Earlier studies in our laboratory (Tiawsirisup et al. 2004), demonstrated that the susceptibility of *Oc. trivittatus* to WNV was essentially the same as the susceptibility of *Cx. pipiens* and *Ae. albopictus* after blood meals with titers of \(\geq 10^{7.0}\) CID_{50}/mL, a level that is commonly observed in avian species. *Ochlerotatus trivittatus* feeds predominately on small mammals but is opportunistic and will also feed on humans and birds. Unpublished data generated in our laboratory demonstrates that this mosquito is also capable of transmitting WNV to cottontail rabbits (*Sylvilagus floridanus*), which raises the question of how important *Oc. trivittatus* might be, not only as a bridge vector but also as a contributor to a possible enzootic cycle of WNV in wild mammals. Accordingly the efficiency of transmission by *Oc. trivittatus* was compared to the efficiency of transmission by an Iowa strain of *Cx. pipiens* and *Ae. albopictus*. Results of this comparison are summarized in Table 4 and clearly demonstrate that *Oc. trivittatus* can efficiently transmit WNV especially after blood meals with titers of \(\geq 10^{7.0}\) CID_{50}/mL.

The higher rate of WNV transmission by *Ae. albopictus* observed in the present study might have been due to a combination of differences in midgut and salivary gland escape barriers because infection rates for all three species appeared to be essentially equal after blood meals with titers of \(\geq 10^{7.0}\) CID_{50}/mL. The near identical infection and dissemination rates of *Ae. albopictus* (92.2 \(\pm\) 1.6\% and 88.6 \(\pm\) 2.1\%) observed in the present study (Table 4) suggests the absence of an efficient midgut escape barrier (MEB) in this species. Similar observations were made by Turell et al. (2001), who reported a 90\% infection rate and 85\% dissemination rate in the Oahu strain of *Ae. albopictus* that blood-fed on chickens with a titer of \(10^{7.2} \pm 0.3\) pfu/mL, and by Sardelis et al. (2002), who observed near identical WNV infection and dissemination rates among the FRED, CHEV, TAMU, and Oahu strains of *Ae. albopictus* that blood-fed on a chicken with a titer of \(10^{6.8}\) pfu/mL. The average difference between infection and dissemination rates of these four *Ae. albopictus* strains was 4.8\%. In contrast, a significant difference of 22.6\% between the infection and dissemination rates of *Cx. pipiens* (Table 4) suggests the presence of a more efficient MEB. The difference between infection and dissemination rates of *Oc. trivittatus* was 4.8 times greater than the 3.6\% difference between the infection and dissemination rates of *Ae. albopictus* but was not significant.

The differences between the WNV dissemination and transmission rates of the three species suggest that the salivary gland barriers (SGB’s) of *Oc. trivittatus* and *Cx. pipiens* might
be more efficient than the SGB of *Ae. albopictus* (Table 4). Significant ($p < 0.05$) differences of 33.0% and 27.9% were observed for *Oc. trivittatus* and *Cx. pipiens*. In contrast, the difference between the dissemination and transmission rates of *Ae. albopictus* was 16.2%, which was not significant.

The mean cumulative transmission rate ($72.4 \pm 5.5\%$) of *Ae. albopictus* observed in the present study is essentially identical to the mean estimated transmission rate of 73% reported by Turell et al. (2001) for the Oahu strain of *Ae. albopictus*. It also appears to be similar to the mean transmission rates of the CHEV, TAMU, and Oahu strains reported by Sardelis et al. (2002), but might be different from the FRED strain. The means and 95% confidence intervals of these rates were 50% (21–79), 92% (62–99), 83% (52–99), and 36% (11–69) respectively. The transmission rate (46.8%) of an Iowa strain of *Cx. pipiens* used in our study appears to be different from the estimated transmission rate (20%) of a New York strain of *Cx. pipiens* (Turell et al. 2001) and a California strain of *Cx. pipiens* (70%) (Goddard et al. 2002). The range of differences among both species of mosquitoes suggests intra species variation. No reports of WNV transmission rates by *Oc. trivittatus* are available for comparison.

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