Avian Host and Mosquito (Diptera: Culicidae) Vector Competence Determine the Efficiency of West Nile and St. Louis Encephalitis Virus Transmission

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Acquired immune competence of the avian host plays an important role in establishing and maintaining the viremia that is necessary for mosquito vector competence. The ability of the invading NY99 strain of West Nile virus (WNV) to elicit an elevated viremia response in California passerine birds was critical for the effective infection of *Culex* mosquitoes. Of the bird species tested, Western scrub jays, *Aphelocoma coerulescens*, produced the highest viremia response, followed by house finches, *Carpodacus mexicanus*, and house sparrows, *Passer domesticus*. Most likely, few mourning, *Zenaidura macroura*, or common ground, *Columbina passerina*, doves and no California quail, *Callipepla californica*, or chickens would infect blood-feeding *Culex* mosquitoes. All Western scrub jays and most house finches succumbed to infection. All avian hosts produced a lower viremia response and survived after infection with an endemic strain of St. Louis encephalitis virus. *Culex* species varied in their susceptibility to infection with both viruses, with *Culex stigmatosoma* Dyar generally most susceptible, followed by *Culex tarsalis* Coquillett, and then *Culex p. quinquefasciatus* Say. Populations within *Culex* species varied markedly in their susceptibility, perhaps contributing to the fociality of WNV amplification. Transmitting female *Cx. tarsalis* expectorated from six to 3,777 plaque-forming units (PFU) of WNV during transmission trials, thereby exposing avian hosts to a wide range of infectious doses. Highly susceptible house finches and moderately susceptible mourning doves were infected by subcutaneous inoculation with decreasing concentrations of WNV ranging from 15,800 to <0.3 PFU. All birds became infected and produced comparable peak viremias on days 2–3 postinoculation; however, the rise in viremia titer and onset of the acute phase of infection occurred earliest in birds inoculated with the highest doses. WNV virulence in birds seemed critical in establishing elevated viremias necessary to efficiently infect blood-feeding *Culex* mosquitoes.

**KEY WORDS** West Nile virus, mosquito vector competence, avian host competence, St. Louis encephalitis virus, transmission
In vitro studies controlled the infectious virus dose, and mosquitoes were induced to feed on hanging droplets containing low and high doses of 5 and 7 log_{10} PFU/ml, respectively (Goddard et al. 2002). Artificial meals typically require more virus than vertebrate donor viremias to attain similar infection rates (Weaver et al. 1993); however, three populations of three different Culex species were readily infected at the low in vitro dose, indicating inter- and intraspecific variability in vector competence, as well as greater susceptibility to infection than eastern Cx. p. pipiens. Although both research groups felt that the mesenteronal barrier (Hardy et al. 1985) was the primary impediment to infection, no attempts have been made to estimate the median dose of virus required for infection. An expression between infectious oral dose and resulting mosquito infection has been described previously (Komar et al. 2003), using data from Cx. p. pipiens from the eastern United States. Therefore, a second objective was to determine dose-infection response curves for California Culex. Although the vector competence of California Culex to infection with SLEV has been well investigated (Meyer et al. 1983; Hardy et al. 1985; Hardy and Reeves 1990), populations vary in their response to infection over time and space (Hardy et al. 1990, Reisen et al. 1996), and therefore we felt it useful to compare susceptibility of infection with WNV to SLEV by using the same methods, virus strain, and populations of Culex. These data may provide critical insight into how these viruses may coexist in southeastern California.

The quantity of encephalitis virus excreted by infectious blood-feeding mosquitoes seems to vary over several orders of magnitude (Hayles 1976, Reisen et al. 2000). Recently, reverse transcriptase-polymerase chain reaction (RT-PCR) methods estimated that the quantity of WNV excreted into capillary tubes filled with immersion oil by Culex p. quinquefasciatus Say ranged from \( \sim 1-5 \log_{10} \) PFU (Vanlandingham et al. 2004). However, with the exception of early studies that used very different virus assay methods (Chamberlain et al. 1957), few modern studies have described the course of avian infection after inoculation with varying doses of flaviviruses (Reisen et al. 2004a). Therefore, a third objective of the current research was to estimate the quantity of virus excreted by California mosquitoes and describe the impact of this dose range on the infection response by representative avian hosts.

Materials and Methods

Viruses and Assays. The NY strain of WNV isolated from a Flamingo that died in the Bronx Zoo (strain 35211 AAF 9/23/99) was passaged twice in Vero cells and used for both avian and mosquito studies. The Kern217 strain of St. Louis encephalitis virus (SLEV) isolated from Culex tarsalis Coquillett collected in Bakersfield in 1989 was used for comparison. Both low passage strains have been used extensively in vector and host competence studies in our laboratory (Goddard et al. 2002, Reisen et al. 2003). The quantity of virus in mosquito or avian samples was determined by standard plaque assays on Vero cells by using single and double overlay systems, respectively (Chiles et al. 2004).

Mosquitoes. The F1 adult progeny of field-collected Cx. tarsalis, Cx. p. quinquefasciatus, or Culex stigmatosoma Dyar reared under insectary conditions (22°C and a photoperiod of 16:8 [L:D] h, three egg rafts per pan) or adults emerging from field-collected immatures were used for experimentation when they were 3–5 d old. Populations from Riverside (Coachella Valley), Los Angeles, and Kern counties, California, were sampled opportunistically during summer 2003. Previous genetic studies have shown that populations of Cx. tarsalis and Cx. p. quinquefasciatus within these three areas are panmictic (Urbanelli et al. 1997, Ginnig et al. 1999). Recent laboratory colonies (<2 yr old) from Indio, Riverside County (COAV), and the Kern National Wildlife Refuge (KNWR), Kern County, were included for comparison. Adults were held under insectary conditions on 10% sucrose until the day before blood feeding. Females were starved for 24 h and then allowed to feed on restrained viremic adult house finches, house sparrows, or chickens (<1 wk old). House finches and house sparrows were infected by subcutaneous inoculation with \( \sim 1,000 \) PFU, whereas chicks were infected by inoculation of stock virus into the jugular vein. Time postinfection when donor birds were exposed to mosquitoes is shown in Table 1. Field mosquitoes were exposed to avian hosts for <4 h during the crepuscular/early evening period, whereas colonized mosquitoes were exposed for <2 h during the diurnal period. A blood sample was taken from donor birds immediately after mosquitoes were removed to estimate the quantity of virus to which mosquitoes were exposed. Previously, Western equine encephalomyelitis (WEEV) titers in chicks infected by i.v. inoculation were found not to change for a 90-min period (Mahmood et al. 2004b). Alternatively, mosquitoes were allowed to engorge for a 1-h period on a 10-fold dilution series of virus mixed with either mechanically defibrinated rabbit or heparinized chicken blood (collected in 10-m1 vacutainers containing 143 freeze-dried USP units of sodium heparin per tube, BD Biosciences, Franklin Lakes, NJ) sweetened to 2% by volume with sucrose and presented on cotton pledgets. Mosquitoes blood fed on either birds or pledges were transferred to 0.67-liter (1-pint) cages and then maintained on 10% sucrose at 26°C for 2 wk. After incubation, females that blood fed on birds or the highest dose of virus on pledges were anesthetized with triethylamine and their ability to excrete virus assessed by inserting their proboscis into a capillary tube filled with a 1:1 by volume mixture of 10% sucrose and fetal calf serum (Attken 1977). After 10–20 min, tube contents were expelled into 0.3 ml of virus diluent (phosphate-buffered saline plus 20% fetal bovine serum and antibiotics [100 U of penicillin, 100 U of streptomycin, and 200 U of nystatin]), and the mosquito body and expectorate frozen at \(-80°C\) until tested for virus. In addition eight to 25 surviving fe-
males that fed on pledges from the remaining doses were frozen in individual cryovials and later tested for virus infection.

**Birds.** House finches, *Carpodacus mexicanus*; house sparrows, *Passer domesticus*; mourning doves, *Zeniadura macroura*; California quail, *Callipepla californica*; and Western scrub jays, * Aphelocoma coerulescens* were collected by grain-baited traps near Bakersfield, Kern County. Common ground doves, *Columbina passerina*, were the progeny of birds collected in Coachella Valley. All birds were banded, bled to determine antibody status, and maintained for 1–2 wk to observe general health and adaptation to confinement. Sera taken before infection were tested for antibodies against WEEV (*Togaviridae: Alphavirus*) and Flavivirus antigens by using an enzyme immunoassay (Chiles and Reisen 1998), with negative findings. Birds were fed mixed bird seed and housed in mosquito-proofed and air-conditioned infection units. Birds were inoculated subcutaneously with virus diluent. Data describing WNV infection in American crows, *Corvus brachyrhynchus*; Western scrub jays, *family Corvidae*; were most susceptible to infection, producing the highest viremia and all dying by days 5 or 6 postinoculation, similar to American crows (included in Fig. 1A for comparison; Komar et al. 2003). House finches and house sparrows were susceptible, with long-duration viremias but relatively few days when titers averaged >5 log10 PFU/ml (Fig. 1A); 63 and 16% of these birds died after infection, respectively. The remaining taxa produced variable titered viremias and all survived for >6 wk postinfection. Infections with the Kern217 strain of SLEV produced lower viremias than when the same species were infected with WNV, and all these birds survived infection (Fig. 1B).

**Vector Competence: Avian Donors.** Several populations of two *Culex* species were evaluated for vector competence by feeding on avian hosts with moderate viremias ranging from 4.5 to 7.3 log10 PFU/ml for WNV and from 2.2 to 4.3 log10 PFU/ml for SLEV (Table 1). There was no significant difference in WNV infection and transmission rates among females from three *Culex* tarsalis populations (*P* > 0.05). Interestingly, females from the KNWR colony feeding on house sparrows with a 7.3 log10 PFU/ml viremia all became infected and 73% transmitted, whereas only 20% of females from this colony feeding on a house sparrow with a 5.9 log10 PFU/ml viremia became infected, perhaps indicating that this colony may be less susceptible to infection than field populations. In contrast, there was a significant difference χ² = 7.6, df = 2, *P* = 0.02) in
SLEV infection rates among populations, being lowest for females collected in Los Angeles. Overall, females collected from the same populations had similar infection (93 versus 82%, $P > 0.05$) but significantly greater transmission (58 versus 20%, $\chi^2 = 16.5$, df = 1, $P < 0.001$) rates after feeding on house finches infected with WNV than SLEV, respectively. This may have been related to the lower viremias expressed in donor birds infected with SLEV than WNV; however, these data were representative of the viremia response of these natural host species (Fig. 1).

Southern California populations of Cx. p. quinquefasciatus were compared for their vector competence for WNV and SLEV by feeding on viremic chicks either 30 min or 1 d after intravenous inoculation (Table 1). If WNV titers were $> 5 \log_{10}$ PFU/ml, there was little difference ($P > 0.05$) in the infection rates among populations, but none of the infected females transmitted. A 1 $\log_{10}$ decrease in virus titer decreased the infection rate in the Kern population 10-fold, from 40 to 4%. In contrast to Cx. tarsalis, infection rates of Cx. p. quinquefasciatus feeding on chicks infected with SLEV were significantly higher ($\chi^2 = 4.0$, df = 1, $P = 0.04$) than feeding on chicks infected with WNV. In addition, 17% of these females were able to transmit SLEV after a 2-wk extrinsic incubation period, whereas none transmitted WNV.

**Vector Competence: Pledgets.** Because avian viremias and Culex susceptibility varied markedly among taxa, groups of 15–30 females of each Culex species population were infected by feeding on 10-fold dilution series of virus mixed with sweetened avian blood

![Viremia profiles for California birds infected with WNV (A) or SLEV (B) by syringe inoculation.](https://bioone.org/journals/Journal-of-Medical-Entomology/terms-of-use)
and presented on gauze pledgets. Inspection of the resulting curves delineated three responses to increasing WNV concentration (Fig. 2): 1) susceptible: *Cx. tarsalis* from Coachella Valley and Kern County and *Cx. stigmatosoma* from Los Angeles; 2) moderately susceptible: *Cx. tarsalis* and *Cx. p. quinquefasciatus* Los Angeles; and 3) refractory: *Cx. p. quinquefasciatus* from Kern County. Susceptible females required the least amount of virus to infect 50% of the population (Table 3). With the exception of *Cx. stigmatosoma* from Los Angeles, these populations seemed refractory to infection with SLEV (Fig. 2), and in agreement, SLEV was not detected in California during 2004.

Females feeding on the highest dose of virus (including samples from populations where too few were collected for an entire dilution series) were evaluated for their ability to expectorate virus after a 2-wk incubation period at 26°C (Table 2). Infection rates of *Cx. tarsalis* with WNV varied significantly among populations (χ² = 18.3, df = 5, P = 0.003), being highest for Coachella–West Wind Duck Club (WWDC), Kern–Bakersfield and Yolo, and lowest for the Kern–KNWR. In addition, the proportion of infected females that expectorated virus varied among populations (χ² = 10.9, df = 5, P = 0.05), being highest for Coachella–WWDC and Kern–Bakersfield. Populations with highest infection and transmission rates were exposed to the highest concentrations of WNV. Despite being fed the same concentration of WNV, infection rates of *Cx. p. quinquefasciatus* varied significantly (χ² = 8.05, df = 3, P = 0.04) among populations, being highest for the F₁ female progeny of females collected from several gravid traps from metropolitan Los Angeles. Transmission rates did not vary significantly among populations (P > 0.05). When data were pooled over species, infection rates varied significantly (χ² = 15.4, df = 2, P < 0.001), being highest for *Cx. stigmatosoma* (90%, n = 19) and lowest for *Cx. tarsalis* (43%, n = 122); *Cx. p. quinquefasciatus* was intermediate (57%, n = 99). Transmission rates by infected females did not vary among species (P > 0.05), being 30% (n = 53) for *Cx. tarsalis*, 14% (n = 57) for *Cx. p. quinquefasciatus*, and 18% (n = 17) for *Cx. stigmatosoma*.

Overall, infection rates for *Cx. tarsalis* with SLEV (25%, n = 67; χ² = 6.05, df = 1, P = 0.01) were lower than for *Cx. tarsalis* with WNV (43%, n = 122); transmission rates among infected females were similar (30%, n = 57 for WNV and 18%, n = 17 for SLEV, P > 0.05). A comparable pattern was seen with *Cx. p. quinquefasciatus*, with infection rates lower for SLEV (15%, n = 47) than WNV (57%, n = 99; χ² = 20.2, df = 1, P < 0.001), but transmission rates among infected females were comparable for both viruses (14%, n = 7 for SLEV; 14%, n = 57 for WNV; P > 0.05). In contrast there was no difference (P > 0.05) seen between infection and transmission rates for *Cx. stigmatosoma* infected with WNV or SLEV.

**Vector Competence: Quantity of Virus Expectorated.** The quantity of WNV expectorated by 30 transmitting female *Cx. tarsalis* from the KNWR colony infected by feeding on a viremic house sparrow was estimated by plaque assay on Vero cells. Because we used a logarithmic dilution series to estimate titer, estimates of 130 PFU for SLEV (Reisen et al. 2000), although the range was considerably broader for WNV, with a maximum of 3,777 PFU compared with SLEV with a maximum of 222 PFU.

**Host Competence: Dose Response.** Estimates of the quantity of virus expectorated by *Cx. tarsalis* or *Cx. p. quinquefasciatus* (Vanlandingham et al. 2004) indicated that avian hosts potentially are inoculated with a wide range of virus doses during mosquito blood feeding. To determine whether there was a threshold for avian infection, we inoculated replicated groups of highly (house finch) and moderately (mourning dove) susceptible birds with a dilution series of WNV (Fig. 3). The pattern for both species indicated that...
there was no lower or minimum infection threshold within the range of inocula we used. Mean viremia for house finches during days 1–6 postinoculation varied significantly as a function of days after inoculation ($F = 30.5$; $df = 5, 69; P < 0.001$) and dose $\times$ day interaction ($F = 2.92; df = 20, 69; P < 0.001$), but not as a function of virus dilution ($F = 1.19; df = 4, 15; P > 0.05$) when tested by repeated measures analysis of variance (ANOVA) (Hintze 1998). There was a 1-d lag in viremia increase for birds given low doses, and a faster time to viremia decrease for birds given higher doses; however, mean viremia for all groups peaked on day 3 postinoculation (Fig. 3A). Overall, survival in house finches seemed independent of dose ($\chi^2 = 8.0, df = 4, P = 0.09$), with one, zero, zero, three, and one of four individuals surviving at each of the doses ranging from 4 to $<0.3 \log_{10} PFU$, respectively. Results for mourning doves were similar to house finches (Fig. 3B), except that all birds survived all WNV doses. During days 1–3 postinoculation, mean viremia increased as a function of inoculum dose ($F = 3.73; df = 4, 15; P < 0.05$) and was highest on days 2–3 postinoculation ($F = 20.7; df = 2, 30; P < 0.05$). In addition, viremia peaked earliest and decreased soonest for birds given the highest dose, resulting in a significant interaction term in the ANOVA ($F = 7.92; df = 8, 30; P < 0.001$). Samples from $<0.3$ to $2.3 \log_{10} PFU$ groups for day 4 were compromised, resulting in some missing values in Fig. 3B.

**Discussion**

Elevated WNV viremias in avian hosts seemed critical for establishing infections in *Culex* mosquitoes. The proportion of *Culex* females infected with WNV was strongly dose dependent and varied significantly among species and species populations tested. Avian viremia responses generally were greater when the same species were infected with WNV than SLEV. Low viremia responses by adult birds infected with SLEV previously led us to emphasize the importance of nestling infections in SLEV epidemiology (Mahmood et al. 2004a). However, the proportion of *Culex* females infected was greater at lower donor host viremias with SLEV than WNV, indicating possible co-evolution between avian viremia and vector susceptibility. Previously, coevolution among SLEV strains and regional *Culex* vector species has been related to virus genetics and mammalian virulence (Monath et al. 1980, Trent et al. 1980). Therefore, although highly susceptible hosts such as corvids succumb to WNV infection, their extremely elevated viremias ensure that most *Culex* feeding on them become infected. This may be especially important during the final day of life when birds are acutely ill, highly viremic, and relatively immobile. This requirement of virulence for effective amplification seems counterintuitive to the argument that a good reservoir host does not succumb to infection (Hammon et al. 1943) and perhaps reflects differences between endemic and invading viruses. Recent studies have indicated a possible trend toward attenuation over time (Beasley et al. 2004a, b); however, this does necessarily not seem mandatory for viral persistence (Levin 1996). Other passeriform hosts such as house finches and house sparrows produced peak viremia titers significantly lower than corvids and exhibited lower mortality rates. Although these species are more numerous and evenly dispersed throughout the environment, mosquito populations (especially *Cx. p. quinquefasciatus*) would have to become more susceptible to acquire infection efficiently from these donor host populations.
Culex species and populations varied markedly in susceptibility to infection with WNV and SLEV. In general, median infectious doses estimated during 2003 indicated that *Cx. stigmatosoma* was most susceptible, followed by *Cx. tarsalis* and *Cx. p. quinquefasciatus*. Interestingly, field infection rates measured from these same areas of California during the summer 2004 epidemic did not reflect this pattern of susceptibility, perhaps because many less susceptible mosquitoes were infected by feeding on highly viremic crows. For example, the field infection rates for *Cx. p. quinquefasciatus* from Coachella Valley where there were few corvids was 1.29 per 1,000, whereas the infection rates for the same species from Los Angeles where there were several large American crow roosts was 8.09 and significantly greater. Estimates from Kern County where there are relatively few American crows but a large Western scrub jay population were intermediate (Table 3). Interestingly, infection rates in more susceptible *Cx. tarsalis* did not vary significantly among these three areas.

In general, our mosquito infection and transmission rates were lower than previously published results, but the general pattern of species susceptibility was similar (Goddard et al. 2002). However, patterns among populations within species were not similar to our previous survey. For example, one Los Angeles *Cx. p. quinquefasciatus* population was highly susceptible in the current study, whereas previous studies concluded that southern California populations may be relatively refractory (Goddard et al. 2002). In addition, our current collection of *Cx. tarsalis* from Yolo County was markedly less susceptible than the population evaluated previously. We are continuing to monitor changes in susceptibility in these populations in an attempt to resolve these discrepancies.

The quantity of virus expectorated by transmitting *Cx. tarsalis* was estimated to range from 6 to 3,777 PFU by using a plaque assay evaluation system. This range was similar for *Cx. p. quinquefasciatus* and *Cx. stigmatosoma* (unpublished data); however, our sample sizes for these species currently were too low for publication. The maximum quantity of WNV expectorated by *Cx. tarsalis* was an order of magnitude less than estimated for *Cx. p. quinquefasciatus* by using an RT-PCR system (Vanlandingham et al. 2004). Previously, salivary glands of *Cx. p. quinquefasciatus* were photographed with arrays of SLEV (Whitfield et al. 1973), but this does not necessarily define the quantity of virus expectorated during blood feeding. Excessive

Fig. 3. Mean WNV viremia response in log_{10} PFU per milliliter for four house finches (A) or mourning doves (B) on each day after being inoculated with five logarithmically decreasing doses of WNV (viral doses for each group within insets). Horizontal line shows minimum assay sensitivity. Some mourning dove samples from day 4 compromised and not included.

Table 3. Relationship between susceptibility to infection expressed as the median infectious dose (ID_{50}) in PFU per milliliter measured during 2003 and the field infection rate in infected females per 1000 tested, with the lower and upper 95% confidence intervals measured during May–September 2004 and calculated using a maximum likelihood approach (Biggerstaff 2003)

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<th>Infection rate/1000</th>
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nd, not done.
virus replication could damage the salivary glands and inhibit transmission.

Although the quantity of virus estimated to be expectorated by mosquitoes frequently was very low, there did not seem to be a safe dose of WNV for house finches or mourning doves. When inoculated subcutaneously with <1 PFU of virus (or a seven-fold dilution of stock WNV NY99 with 7.1 log_{10} PFU/0.1 ml titer), all birds became infected. These data were similar to our recent studies with SLEV in house finches (Reisen et al. 2004a) but differed markedly from SLEV infection studies with house sparrows; brown-headed cowbirds, Molothrus ater; and red-winged blackbirds, *Agelaius phoeniceus*, where low infectious doses apparently failed to produce a detectable viremia (Chanlerlain et al. 1957). The course of infection was modified slightly by dose, with high infectious doses resulting in a rapid onset of elevated viremia and an early acute phase that resulted in either recovery or death. House finch survival was not dose dependent, and more birds survived a <0.3 log_{10} PFU dose (three alive of four) than either 0.6 (zero of four) or <0.3 log_{10} PFU (one of four) doses. In addition, the viremia response in birds inoculated with the same WNV dose was variable, confounding attempts to compare mosquito populations using this natural host system.

In summary, avian virulence and associated elevated viremias in several passerine species (especially within the Corvidae) seemed to be a critical factor enabling *Culex* infection and effective WNV transmission. Corvids produced the most elevated viremias (Komar et al. 2003) and the epidemiology of WNV in suburban/urban habitats seems to be closely associated with their communal roosts and sickness/death in adjacent neighborhoods (Eidson et al. 2001, Nasci et al. 2002, Julian et al. 2002). House sparrows and house finches typically are more abundant, evenly distributed, and may be important hosts for infecting mosquitoes over a wide range of rural and urban habitats (Komar et al. 2001). However, local infection and transmission rates among *Culex* mosquitoes will be heavily dependent upon the viremia response of these birds and the susceptibility patterns of the local mosquito populations. Mosquitoes imbibing high titers of SLEV during peak nesting viremias developed high body titers and frequently expectorated more virus than mosquitoes feeding when viremia titers were lower (Mahmood et al. 2004a), thereby contributing more to virus amplification. These data collectively detail the quantitative intricacies of host–vector–virus interaction necessary for efficient amplification by invading WNV.

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