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

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**ABSTRACT** 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 passerine*, 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 focality 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

WEST NILE VIRUS (WNV) (*Flaviviridae: Flavivirus*) rapidly dispersed across the United States with little genetic change (Beasley et al. 2004b) and became established in southern California during summer 2003 (Reisen et al. 2004b). During 2004, transmission amplified to epidemic proportions in southern California, and WNV invaded the Central Valley. Our ongoing field and laboratory research attempts to elucidate factors enabling WNV transmission effectiveness. The current study focused on the interplay between avian host and mosquito vector competence and on the

importance that WNV virulence and elevated acute viremias play in amplification.

Avian host competence is determined by the amplitude and duration of the viremia period and can be expressed in relation to vector susceptibility to infection (Komar et al. 1999, 2003; Reisen et al. 2003). The competence of North American birds for WNV has been surveyed and expressed in relation to *Culex pipiens pipiens* L. susceptibility to per os infection (Komar et al. 2003). Because bird species and populations may vary in susceptibility to infection, one goal of our research was to describe the viremia profiles of representative California birds after infection with the NY99 strain of WNV.

The vector competence of eastern North American (Turell et al. 2000, 2001, 2002; Sardelis and Turell 2001) and Californian (Goddard et al. 2002) mosquitoes for the NY99 strain of WNV has been described using donor host and artificial blood meal infection methods, respectively. In the in vivo studies, chick donor viremias ranged from 6 to 7 log<sub>10</sub> plaque-forming units

The collection and infection of wild birds was done under Protocol 11184 approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, California Resident Scientific Collection Permit No. 801049-02 from the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit No. MB082812-0 from the Department of the Interior. Animal Use and Care Administrative Advisory Committee Protocol No. 11187 approved procedures for using wild birds and chickens for mosquito infection experiments. Use of arboviruses was approved under Biological Use Authorization #0554 by Environmental Health and Safety of the University of California, Davis, and USDA Permit #47901.

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(PFU)/ml (Turell et al. 2001). 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 mesenteron barrier (Hardy et al. 1983) 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 expectorated 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 expectorated into capillary tubes filled with immersion oil by *Culex p. quinquefasciatus* Say ranged from  $\approx 1$ – $5 \log_{10}$  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 expectorated 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 (God-

dard 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  $F_1$  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 3–8 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 were panmictic (Urbanelli et al. 1997; Gimmig 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  $\approx 1,000$  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-ml 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 pledgets 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 pledgets were anesthetized with triethylamine and their ability to expectorate 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 (Aitken 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  $-80^\circ\text{C}$  until tested for virus. In addition eight to 25 surviving fe-

**Table 1.** Vector competence of *Cx. tarsalis* and *Cx. p. quinquefasciatus* collected from Coachella Valley, Los Angeles, and Kern County after blood feeding on viremic house finches (HOFI) or house sparrows (HOSP) 2 or 3 d after subcutaneous inoculation or 7-d-old chickens (chick) 30 min or 1 d after intravenous inoculation with WNV

Virus	Bird species	Viremia (log <sub>10</sub> PFU/ml)	<i>Culex</i> species	Collection site	n	Infected <sup>a</sup> (%)	Transmission <sup>a</sup> (%)	
WNV	HOFI (2 d)	5.4	<i>tarsalis</i>	Coachella-WWDC	21	90	52	
	HOFI (2 d)	5.9	<i>tarsalis</i>	LA-Panaorama	16	94	25	
	HOFI (2 d)	5.4	<i>tarsalis</i>	Kern-KNWR	17	94	65	
	HOSP(3d)	7.3	<i>tarsalis</i>	KNWR-c	40	100	73	
	HOSP(3d)	6.0	<i>tarsalis</i>	KNWR-c	40	20	15	
	Chick (30 min)	5.7	<i>tarsalis</i>	Kern-Bakersfield	16	13	0	
	Chick (1 d)	5.0	<i>quinquefasciatus</i>	Coachella-Indio	25	64	0	
	Chick (1 d)	5.5	<i>quinquefasciatus</i>	LA-metro	25	60	0	
	Chick (30 min)	5.7	<i>quinquefasciatus</i>	Kern-Bakersfield	25	40	0	
	Chick (30 min)	4.8	<i>quinquefasciatus</i>	Kern-Bakersfield	25	4	0	
	SLEV	HOFI (2 d)	3.7	<i>tarsalis</i>	Coachella-WWDC	25	88	12
		HOFI (2 d)	4.0	<i>tarsalis</i>	LA-Panaorama	22	64	9
		HOFI (2 d)	3.9	<i>tarsalis</i>	Kern-KNWR	26	92	19
Chick (1 d)		4.3	<i>quinquefasciatus</i>	Coachella-Indio	25	96	20	
Chick (1 d)		2.2	<i>quinquefasciatus</i>	LA-metro	17	65	12	

KNWR-c, Kern National Wildlife Refuge colony.

<sup>a</sup> Percentage of number tested (n).

males that fed on pledgets 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, *Zenaidura 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* antigen 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 ≈1,000 PFU of virus in the cervical region. Previous studies have shown that comparable titers of SLEV delivered by syringe or infectious mosquito bite produced similar viremia and antibody responses (Reisen et al. 2000). Birds were bled daily for 6–7 d by jugular puncture (0.1 ml of blood taken by 28-gauge syringe and expelled into 0.4 ml of virus diluent). Data describing WNV infection in American crows, *Corvus brachyrhynchus* (Komar et al. 2003), and SLEV infection in the remaining species (Reisen et al. 2003) were included for comparison.

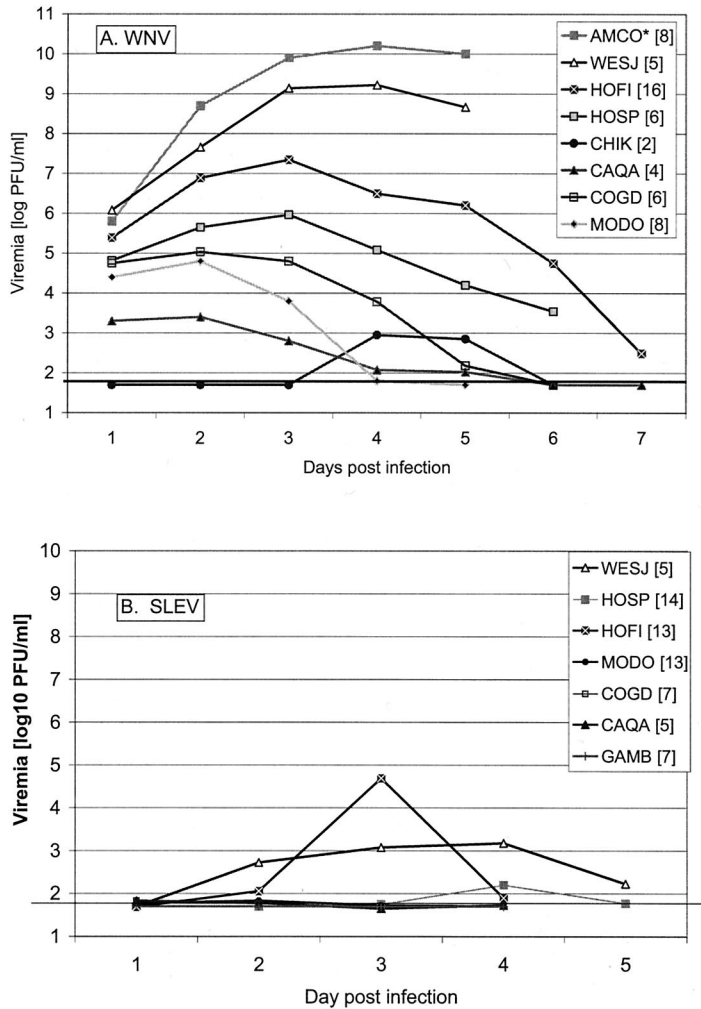
Because the amount of WNV excreted by transmitting female mosquitoes varies markedly (Vanlandingham et al. 2004), we evaluated the response (survival and viremia) of susceptible (house finch) and moderately refractory (mourning dove) hosts to varying infectious doses. Four house finches and mourning doves each were inoculated with 0.1 ml of diluent containing one of five 10-fold decreasing doses of WNV ranging from 15,800 to <1 PFU. Viremia re-

sponse was monitored daily for each bird using the same methods described above.

## Results

**Avian Viremia.** Viremia response of several common California birds to infection with WNV varied markedly among taxa (Fig. 1A), but generally agreed with previously published findings (Komar et al. 2003). 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 log<sub>10</sub> 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 4.8 to 7.3 log<sub>10</sub> PFU/ml for WNV and from 2.2 to 4.3 log<sub>10</sub> PFU/ml for SLEV (Table 1). There was no significant difference in WNV infection and transmission rates among females from three *Cx. tarsalis* populations ( $P > 0.05$ ). Interestingly, females from the KNWR colony feeding on house sparrows with a 7.3 log<sub>10</sub> 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 log<sub>10</sub> 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  $\chi^2 = 7.6$ ,  $df = 2$ ,  $P = 0.02$ ) in



**Fig. 1.** Viremia profiles for California birds infected with WNV (A) or SLEV (B) by syringe inoculation. Birds included American crow (AMCO, data from Komar et al. 2003), Western scrub jay (WESJ); house finch, (HOFI); house sparrow, (HOSP), 18-wk-old domestic chicken (CHIK), California quail (CAQA), Gambel's quail (GAMB), common ground dove (COGD), and mourning dove (MODO). Numbers in parentheses were individuals of each species tested. Data on SLEV redrawn (Reisen et al. 2003). Horizontal line shows minimum assay sensitivity.

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

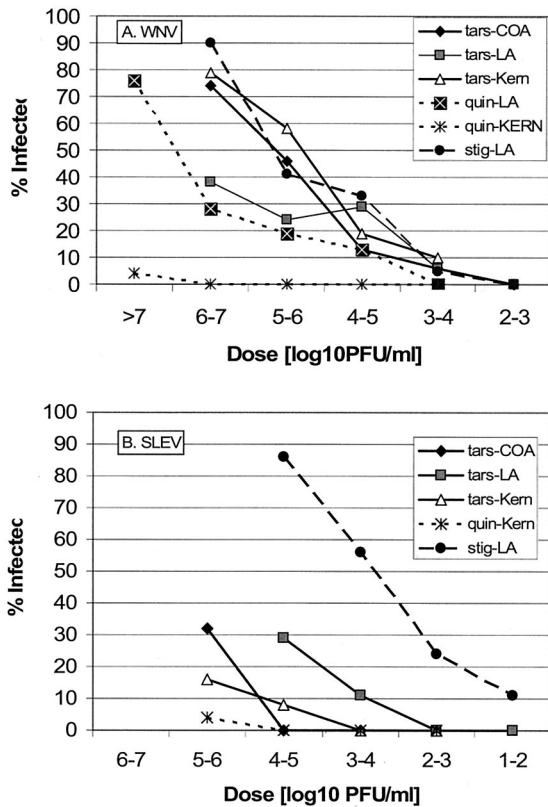


Fig. 2. Percentage of 15–30 female mosquitoes infected plotted as a function of the infecting dose in log<sub>10</sub> PFU/ml of WNV (A) or SLEV (B) viruses per milliliter of sweetened blood presented on gauze pledgets. Populations tested included *Cx. tarsalis* (tars), *Cx. p. quinquefasciatus* (quin), or *Cx. stigmatosoma* (stig) collected in Coachella Valley, Riverside County (COA); Los Angeles, Los Angeles County (LA); or Kern County (Kern), California.

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 ( $\chi^2 = 18.3$ ,  $df = 5$ ,  $P = 0.003$ ), 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 ( $\chi^2 = 10.9$ ,  $df = 5$ ,  $P = 0.05$ ), 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 ( $\chi^2 = 8.05$ ,  $df = 3$ ,  $P = 0.04$ ) among populations, being highest for the F<sub>1</sub> 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 ( $\chi^2 = 15.4$ ,  $df = 2$ ,  $P < 0.001$ ), 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%,  $n = 67$ ;  $\chi^2 = 6.05$ ,  $df = 1$ ,  $P = 0.01$ ) 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%,  $n = 99$ ;  $\chi^2 = 20.2$ ,  $df = 1$ ,  $P < 0.001$ ), 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 Expecto-rated.** 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, mean and standard error of the mean were calculated as a geometric mean to be 1.59 log<sub>10</sub> PFU (SE = 0.14). When backtransformed and adjusted for dilution, the mean was 117, median was 95, and the range was 6–3,777 PFU per mosquito. The mean PFU of WNV expectorated by *Cx. tarsalis* was similar to our previous 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

**Table 2.** Vector competence of *Cx. tarsalis*, *Cx. p. quinquefasciatus*, and *Cx. stigmatosoma* collected from Coachella Valley, Los Angeles (LA), Kern County, and Yolo County after feeding on cotton pledgets soaked with a mixture of stock virus diluted 1:10 in either sweetened defibrinated rabbit or heparinized chicken blood

Virus	<i>Culex</i> species	Collection site	Dose (log <sub>10</sub> PFU/ml)	n	Infected <sup>a</sup> (%)	Transmission <sup>a</sup> (%)
WNV	<i>tarsalis</i>	Coachella-WWDC	6.8	22	68	32
	<i>tarsalis</i>	Coachella-Indio	6.3	25	36	0
	<i>tarsalis</i>	LA-Panorama	5.8	24	38	4
	<i>tarsalis</i>	Kern-KNWR	6.6	15	7	0
	<i>tarsalis</i>	Kern-Bakersfield	6.8	15	47	33
	<i>tarsalis</i>	Yolo	6.6	19	47	16
	<i>tarsalis</i>	COAV-c	6.6	18	89	11
	<i>tarsalis</i>	KNWR-c	6.6	25	56	8
	<i>quinquefasciatus</i>	Coachella	7.3	25	44	8
	<i>quinquefasciatus</i>	LA-metro	7.3	25	76	8
	<i>quinquefasciatus</i>	LA-Machado Lake	7.3	24	67	8
	<i>quinquefasciatus</i>	LA-San Fernando	7.3	25	44	8
	<i>stigmatosoma</i>	LA-Panorama	5.8	19	90	16
	SLEV	<i>tarsalis</i>	Coachella-Indio	5.3	25	32
<i>tarsalis</i>		LA-Panorama	4.9	17	29	17
<i>tarsalis</i>		Kern-KNWR	5.4	25	16	0
<i>quinquefasciatus</i>		LA-metro	5.3	25	8	0
<i>quinquefasciatus</i>		LA-Machado Lake	5.3	22	23	5
<i>stigmatosoma</i>		LA-Panorama	4.9	21	86	29

Most localities abbreviations are described in text. COAV-c, colony from Coachella Valley originating from Indio; KNWR-c, colony from KNWR; LA-metro, several gravid trap sites in downtown Los Angeles.

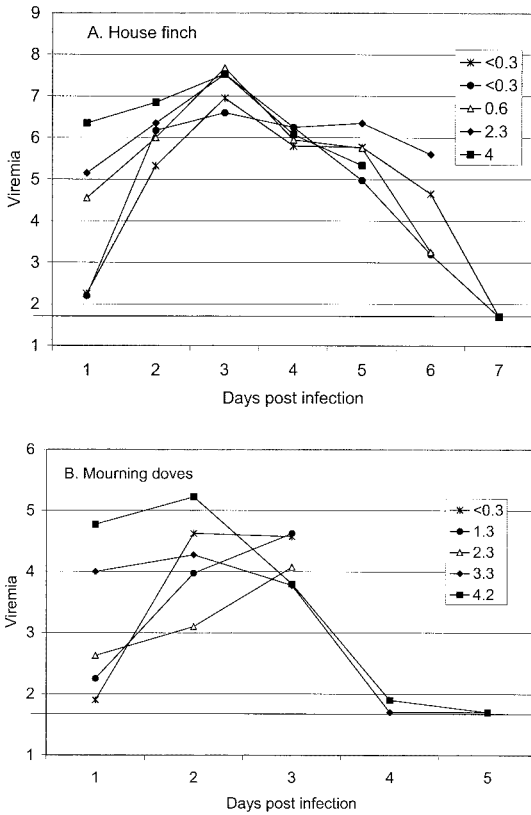
<sup>a</sup> Percentage of number tested (n).

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 a 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 coevolution 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.



**Fig. 3.** Mean WNV viremia response in log<sub>10</sub> 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.

*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

**Table 3.** Relationship between susceptibility to infection expressed as the median infectious dose (ID<sub>50</sub>) 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)

<i>Culex</i> species	Median infectious dose (log <sub>10</sub> PFU/ml)	Pools tested	Total mosquitoes tested	WNV positives	Infection rate/1000	Lower limit	Upper limit
Coachella Valley							
<i>quinquefasciatus</i>	nd	132	3,132	4	1.29	0.42	3.08
<i>tarsalis</i>	5.7	424	15,137	63	4.56	3.54	5.79
Kern County							
<i>quinquefasciatus</i>	>7.3	406	15,325	86	6.42	5.17	7.89
<i>tarsalis</i>	5.4	410	16,893	85	5.72	4.60	7.04
Los Angeles							
<i>quinquefasciatus</i>	6.8	1029	38,420	270	8.09	7.18	9.09
<i>tarsalis</i>	>5.8	135	4,411	18	4.34	2.68	6.70
<i>stigmatosoma</i>	5.6	37	613	6	10.22	4.32	20.89

nd, not done.



virus replication could damage the salivary glands and inhibit transmission.

Although the quantity of virus estimated to be excreted 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<sub>10</sub> 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 (Chamberlain 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<sub>10</sub> PFU dose (three alive of four) than either 0.6 (zero of four) or <0.3 log<sub>10</sub> 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 nestling viremias developed high body titers and frequently excreted 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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