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Source: Journal of Medical Entomology, 49(4):942-946. 2012.
Published By: Entomological Society of America
DOI: http://dx.doi.org/10.1603/ME11293
URL: http://www.bioone.org/doi/full/10.1603/ME11293

Vector Competence of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) for Dengue Virus in the Florida Keys

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J. Med. Entomol. 49(4): 942–946 (2012); DOI: http://dx.doi.org/10.1603/ME11293

ABSTRACT In 2009–2011, Monroe County in southern Florida experienced locally acquired and traveler-imported focal dengue outbreaks. *Aedes aegypti* (L.) is the primary vector of dengue virus (DENV) worldwide, is prevalent in Monroe County, and is the suspected vector in Florida. *Ae. albopictus* (Skuse) is also known to be an important vector of DENV and this species is ubiquitous in Florida; however, it is not yet established in Monroe County. Florida *Ae. aegypti* (Key West and Stock Island geographic colonies) and *Ae. albopictus* (Vero Beach geographic colony) were fed blood containing 3.7 Log_{10} plaque-forming unit equivalents of DENV serotype 1 isolated from a patient involved in the Key West, FL, outbreak in 2010. Mosquitoes were maintained at extrinsic incubation temperatures of 28 or 30°C for an incubation period of 14 d. Vector competence was assessed using rates of infection (percent with virus-positive bodies), dissemination (percent infected with virus-positive legs), and transmission (percent infected with virus-positive saliva). No significant differences were observed in rates of infection or dissemination between *Ae. aegypti* or *Ae. albopictus* at either extrinsic incubation temperature. Transmission was observed only at 28°C in both *Ae. aegypti* (Key West) and *Ae. albopictus*. The assessment of local mosquito populations for their DENV vector competence is essential and will aid mosquito control operators interested in pinpointing specific vector populations for control. The extent to which vector competence is affected by seasonal changes in temperature is discussed and provides baseline risk assessment data to mosquito control agencies.

KEY WORDS vector competence, dengue virus, Florida, *Aedes*

Dengue virus (DENV, family *Flaviviridae*, genus *Flavivirus*) is a globally important pathogen primarily transmitted to humans by *Aedes aegypti* (L.). All four DENV serotypes are prevalent in the Americas, including the Caribbean (Pinheiro and Corber 1997, San Martin et al. 2010), and the numbers of DENV cases have shown an increasing trend in Latin America (Guzman and Kouri 2003, Teixeira et al. 2009). As DENV is so widespread and humans are the reservoir hosts, traveler-imported cases remain an issue for its continued range expansion (e.g., Centers for Disease Control and Prevention [CDC] 1998, 1999, 2006; Franco et al. 2010). This issue is especially important in areas where there are competent mosquito vectors for DENV (Mohammed et al. 2009).

Florida has a history of epidemic DENV transmission demonstrated in 1934 when ca. 15,000 people were sickened primarily in Tampa (Hillsborough County) and Miami (Miami-Dade County), showing that imported cases can lead to large-scale epidemic transmission (Griffitts 1935, MacDonnell 1935, Hanson 1935). This Florida DENV epidemic affected people of all ages in 31 counties from ca. July through November (Griffitts 1935). In 2009, Florida experienced a focal DENV outbreak in Key West (Monroe County) where 9/21 (43%) physician-submitted cases and 13/240 (5%) residents randomly surveyed by the CDC and Florida Department of Health (FDOH) were positive for DENV or DENV antibodies (CDC 2010, FDOH 2009). The duration of reported cases occurred from July through October. *Ae. aegypti* is the suspected vector as other potential vectors such as *Aedes albopictus* (Skuse) are not found in Monroe County (O'Meara 1997: revised 2005). Out of 407 *Ae. aegypti* pools tested in Key West from September through December 2009, two were DENV-1-positive (CDC 2010). In 2010, Florida experienced another DENV outbreak in Monroe County with 63 locally acquired cases reported (FDOH 2010). In the same county, 3 out of 1,178 *Ae. aegypti* pools tested in 2010 were DENV-1-positive (Graham et al. 2011). The DENV-1 serotype has also been identified in infected patients in Key West (CDC 2010) and shows similarity to Central American strains, specifically from Nicaragua (Graham et al. 2011). In 2011, there were no confirmed cases of locally acquired infections in Monroe County; however, six people from four different counties (Miami-Dade, Martin, Hillsborough, and Palm Beach Counties) across Florida became infected

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with DENV from local mosquito populations (FDOH 2011).

Moncayo et al. (2004) reports variation in vector competence for DENV serotype 2 (DENV-2) in *Ae. aegypti* and *Ae. albopictus* from different geographic areas. The same study demonstrates equivalent vector competence of both species to some DENV-2 strains, but differential competence to other strains. Furthermore, higher environmental temperatures may reduce the duration of the extrinsic incubation period required for virus transmission as has been observed for *Ae. aegypti* and DENV (Rohani et al. 2009), as well as *Culex* spp. and other flaviviruses such as West Nile and St. Louis encephalitis viruses (e.g., Kilpatrick et al. 2008; Richards et al. 2009, 2010). To date, no studies have evaluated the vector competence of Florida mosquitoes for DENV-1 and how environmental conditions such as temperature may affect the dynamics of virus transmission.

Current research focuses on assessing vector competence for the likely mosquito vectors of this pathogen, *Ae. aegypti* and *Ae. albopictus* that are sometimes found to coexist in similar habitats in Florida (O'Meara et al. 1995, Braks et al. 2003, Britch et al. 2008). Vaccination campaigns are one of the most effective strategies to control viral disease (World Health Organization [WHO] 2011). Dengue vaccines are in development but there is currently no vaccine available to the public (e.g., Durbin and Whitehead 2011). This study evaluated the vector competence of three Florida mosquito populations for DENV-1 recently isolated in Florida. This baseline knowledge is expected to inform mosquito control and public health agencies and provide the ability to rapidly assess statewide vector populations for their capacity to transmit DENV.

Materials and Methods

Mosquitoes and Virus. Mosquitoes were reared under standard conditions. Larvae of *Ae. aegypti* were collected from Key West (KW) and Stock Island (SI) in Monroe County Florida. *Ae. albopictus* larvae were collected from Vero Beach (VB) in Indian River County, FL. To initiate the experiment, F_2 generation eggs of both species were hatched in enamel rearing pans ($24 \times 36 \times 5$ cm) with 1.0 liters of tap water and 200 mg larval food (1:1 mixture of brewer's yeast: lactalbumin). Larvae that eclosed after 24 h were then pooled by species and colony, and aliquoted to enamel pans with 1.0 liters of tap water and 200 mg larval food (ca. 200 larvae/pan). Similar amounts of supplemental larval food were given to larvae every other day for the next 4 d. Pupae were transferred to 250 ml plastic cups containing ≈175 ml water, and adults were allowed to emerge in square cages (33 cm^3) and provided 20% sucrose ad libitum.

We used DENV-1 (isolate BOL-KW010), originally isolated from a human infected in Key West, FL, in 2010 (Florida Department of Health) and passaged three times in African green monkey kidney (Vero) cells. Virus cultures were propagated according to standard procedures used in our laboratory and described elsewhere (Richards et al. 2007).

Mosquito Infection. Mosquitoes were held in cages with 80% humidity in a photoperiod of 14:10 (L:D) h cycle. *Ae. albopictus* were held at an extrinsic incubation temperature (EIT) of 28°C, while *Ae. aegypti* were held at 28 or 30°C. Respective EITs were chosen based on Monroe County temperatures observed during the focal outbreak in 2009: June and October (average temperature), July through September (average temperature) (http://cdo.ncdc.noaa.gov/).

Vector competence assays were conducted using our previously established methods (Richards et al. 2007, 2009) and freshly propagated DENV was mixed with defibrinated bovine blood (Hemostat, Dixon, CA) before mosquito feeding. Samples of the bloodmeal were stored at −80°C until further processing to determine bloodmeal titer. Mosquitoes (8–12 d old) were fed for 45 min on pledgets soaked with warmed (35°C) DENV-infected blood.

Mosquito Processing. Fully engorged mosquitoes were processed as described by Richards et al. (2010). Briefly, at the end of the 14 d incubation period (IP), live mosquitoes were anesthetized with cold, legs and wings removed and then mosquitoes were allowed to expectorate saliva into capillary tubes filled with immersion oil for 30 min using methods previously described (Anderson et al. 2010). After the salivation period, bodies and saliva samples were also transferred to separate tubes categorized by mosquito code containing 0.9 ml of BA-1 diluent, and all were stored at −80°C until further processing.

Virus Assay. Samples were homogenized and centrifuged as described previously (Richards et al. 2010). Nucleic acids were extracted using the MagNA Pure LC System and Total Nucleic Acid Isolation Kit (Roche, Mannheim, Germany). The amount of viral RNA (plaque forming unit equivalents; PFUeq) in each sample was determined using quantitative real-time Taqman reverse transcriptase polymerase chain reaction (qRT-PCR) with the LightCycler 480 Instrument (Roche) and the Superscript III One-Step qRT-PCR kit (Invitrogen, Carlsbad, CA) using a previously established program (Richards et al. 2007).

The infection rate was the percentage of all mosquitoes tested having infected bodies. The dissemination rate was the percentage of mosquitoes with infected bodies that also had infected legs. The transmission rate was the percentage of mosquitoes with infected bodies that also had infected saliva.

Statistical Analysis. Analysis of variance (ANOVA) was used to evaluate differences in titers of body and leg tissues between species, geographic colonies, incubation periods, and EITs. If significant differences were observed, then a Duncan test was used to determine differences in the means. Fisher exact tests were used to evaluate differences in rates of infection, dissemination, and transmission between species and geographic colonies at different EITs. Only two mosquitoes exhibited saliva infection so this variable was not analyzed further.

Table 1. The mean titers (Log_{10} PFUeq DENV/ml ± SE) and rates of infection (% with DENV-positive bodies), dissemination (% infected with DENV-positive legs), and transmission (% infected with DENV-positive saliva) for *Ae. aegypti* (SI and KW) and *Ae. albopictus* (VB) fed a DENV-infected blood meal (strain BOL-KW10 at a dose of 3.7 Log_{10} PFUeq DENV/ml) and held at 28 or 30°C for 14 d

No. tested	Extrinsic incubation temp	Geographic colony location	No. abdomen infection (%)	No. leg infection (%)	No. saliva infection (%)	Abdomen titer[a]	Leg titer[a]	Saliva titer[a]
Ae. aegypti								
19	28	SI	17 (89)	17 (100)	0	3.1 ± 0.1^{a}	2.3 ± 0.1^{ab}	—
4	30	SI	3 (75)	1 (33)	0	2.9 ± 0.2^{A}	2.2 ± 0.01^{A}	—
4	28	KW	3 (75)	3 (100)	1 (33)	3.1 ± 0.1^{a}	2.5 ± 0.1^{a}	1.0
5	30	KW	4 (80)	4 (100)	0	3.1 ± 0.3^{A}	2.3 ± 0.2^{A}	—
Ae. albopictus								
16	28	VB	13 (81)	12 (92)	1 (8)	3.2 ± 0.2^{a}	1.6 ± 0.2^{b}	1.6

[a] Titers for treatments groups with the same letter in the same column are not significantly different between geographic colony location and species (lowercase: 28°C, uppercase: 30°C).

Results and Discussion

Mosquitoes were fed a dose of 3.7 Log_{10} PFUeq DENV/ml. Rates of infection, dissemination, and transmission are shown in Table 1. At 28°C, no significant differences in infection or dissemination rates were observed between *Ae. aegypti* (KW or SI) and *Ae. albopictus* (VB) (infection: $P = 0.549$; dissemination: $P = 0.391$). At 30°C, there were no significant differences in infection or dissemination rates between the different geographic colonies of *Ae. aegypti* tested (infection: $P = 1.00$; dissemination: $P = 0.206$).

Virus titers of bodies, legs, and saliva are shown in Table 1. Results of ANOVA of titers between species and geographic origin of colonies are shown in Table 2. At 28°C, no differences were observed in body titers between species or geographic colonies. Conversely, at 28°C, leg titers were significantly higher in *Ae. aegypti* (KW) compared with *Ae. albopictus* (VB). At 30°C, no significant differences were observed in body or leg titers between *Ae. aegypti* geographic colonies. Only *Ae. aegypti* (KW) and *Ae. albopictus* (VB) transmitted DENV-1 in their saliva. This shows that these colonies exhibit similar barriers to midgut infection, midgut escape, salivary gland infection, and salivary gland escape under the experimental conditions used here. In addition, the lack of correspondence between rates of dissemination and transmission indicates that there is a barrier preventing virus presence in saliva even after successful dissemination from the midgut. Additional experiments are planned to increase the sample size of these vector competence assays as large sample sizes are important to differentiate measures of vector competence (Richards et al. 2009, 2010).

Chen et al. (1993) orally delivered a DENV-1 (Taiwanese strain) dose of 6.7 Log_{10} PFU/ml (after diluting 1:1 virus:blood) to *Ae. albopictus* and *Ae. aegypti.* The same study showed that 3% of blood fed *Ae. albopictus* and 50% of *Ae. aegypti* transmitted DENV after a 14 d IP at 32°C. Mosquitoes exhibited a salivary gland infection and/or escape barrier, regardless of the higher dose, higher EIT, and different virus and mosquito strains used by Chen et al. (1993). Another study fed *Ae. albopictus* DENV-1 (Brazilian strain SPH 194762; 6.8 Log_{10} $TCID_{50}$/ml) and showed an approximate 6% infection and 0–22% dissemination rate (dissemination rate calculated based on infection rate) after an IP of 14 d at 26°C (Fernandez et al. 2004). The aforementioned study delivered a higher dose than the current study, yet again demonstrates barriers to infection and dissemination. However, our study shows higher infection (81%) and dissemination (92%) rates in *Ae. albopictus* at 28°C compared with the study by Fernandez et al. (2004) where mosquitoes were incubated at 26°C. Boromisa et al. (1987) showed differential vector competence of different geographic colonies of *Ae. albopictus* for DENV-1 isolated in Fiji, showing that vector-virus interactions may differ between colonies. Others have shown complex vector-virus interactions that are affected by biological and environmental variables for other vector-virus systems (e.g., Richards et al. 2009, 2010). These types of interactions should be evaluated for the strain of DENV-1 found in Florida. Caution should be taken when making predictions about field populations based on laboratory experiments as complex interac-

Table 2. ANOVA showing differences in the mean titers (Log_{10} PFUeq DENV/ml) of bodies and legs between species (*Ae. aegypti* and *Ae. albopictus*) and geographic location of colonies in Florida (SI, KW, and VB)

Variable	df (numerator, denominator)	*F*	*P*[a]	df (numerator, denominator)	*F*	*P*
Body titer		28°C			30°C	
Species	1, 31	0.59	0.446	—	—	—
Geographic location	2, 30	0.29	0.752	1, 4	0.35	0.579
Legtiter						
Species	1, 30	10.41	**0.003**	—	—	—
Geographic location	2, 29	5.22	**0.012**	1, 4	0.23	0.658

[a] Significant *P* values are in bold.

tions affecting vector competence are not fully understood.

International travel may contribute to the spread of DENV (Weaver and Reisen 2010). There have been several reports of travelers importing DENV into the United States, including Florida (e.g., CDC 1998, 1999, 2006, 2007; FDOH 1995, 2009, 2010, 2011; Gill et al. 2000) and we have shown that Florida populations of both *Ae. albopictus* and *Ae. aegypti* are competent vectors of DENV. The DENV-1 strain that emerged in Florida in 2009 and 2010 is similar to a Nicaraguan strain (Graham et al. 2011), hence surveillance should continue to monitor imported cases. Mosquito control and public health agencies must prepare for continued DENV outbreaks in Florida. The ability to identify competent vectors of DENV in advance of an outbreak enables mosquito control operators to target control measures in advance of epidemics. This study is the first to report the vector competence of Florida mosquitoes, *Ae. aegypti* and *Ae. albopictus*, for DENV-1 that recently emerged in Florida. Future larger-scale studies will address potential geographic differences in vector competence and focus on statewide risk assessment strategies for reemerging viruses such as DENV in Florida.

Acknowledgments

We thank Lillian Stark at the Florida Department of Health Bureau of Laboratories for supplying the DENV isolate. We appreciate the comments of two anonymous reviewers that improved the manuscript. This research was supported by the Florida Department of Agriculture and Consumer Services grant 016603.

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Received 30 December 2011; accepted 5 April 2012.