Isolations of Cache Valley Virus From *Aedes albopictus* (Diptera: Culicidae) in New Jersey and Evaluation of Its Role as a Regional Arbovirus Vector

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ABSTRACT The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an invasive species and a major pest problem in urban and suburban locales in New Jersey. To assess its potential role as an arbovirus vector, we sampled *Ae. albopictus* from two New Jersey counties over a 3-yr period and estimated the prevalence of virus infection by Vero cell culture and reverse transcription-polymerase chain reaction assays. Three virus isolates were obtained from 34,567 field-collected *Ae. albopictus*, and all were identified as Cache Valley virus by molecular methods. *Ae. albopictus* ($N = 3{,}138$), collected in Mercer County from late July through early September 2011, also were retested for West Nile virus (WNV) by reverse transcription-polymerase chain reaction, and all were negative. These results corroborate previous findings showing that *Ae. albopictus* may occasionally acquire Cache Valley virus, a deer-associated arbovirus, in nature. In contrast, we did not detect WNV infection in *Ae. albopictus* despite concurrent WNV amplification in this region.

KEY WORDS *Aedes albopictus*, arbovirus, Cache Valley virus, West Nile virus, mosquito

The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), is a highly invasive species that has spread from East Asia to Europe, Africa, the Middle East, and the Americas over the past few decades (Benedict et al. 2007). This mosquito uses artificial containers as larval habitat and most likely expanded its range by inhabiting tires and other water-holding containers that are shipped worldwide (Hawley et al. 1987, Estrada–Franco and Craig 1995). In the United States, an infestation of *Ae. albopictus* was first discovered in Houston, TX, in 1985 (Sprenger and Wuithiranyagool 1986) and since then, this species has become established throughout the eastern half of the country from Texas to Illinois and east from Florida to southern New York (Benedict et al. 2007, Rochlin et al. 2013).

A. albopictus is an aggressive human-biter that was first detected in New Jersey during 1995 (Crans et al. 1996), but was not considered a major pest or public health concern in the state until the mid-2000s (Farajollahi and Nelder 2009). The species currently occurs in all 21 counties within New Jersey, and has expanded further north into neighboring New York City, and Long Island, NY (Rochlin et al. 2013). *Ae. albopictus* could serve as an arbovirus vector in this region because of its opportunistic feeding patterns, close association with humans, and laboratory vector competence for multiple arboviruses (Estrada-Franco and Craig 1995, Gratz 2004, Paupy et al. 2009, Egizi et al. 2013). Several arboviruses including eastern equine encephalitis virus (EEEV), Cache Valley virus (CVV), La Crosse virus (LACV), and West Nile virus (WNV) have been isolated from *Ae. albopictus* collected from central and southeastern United States (Mitchell et al. 1992, 1998; Gerhardt et al. 2001; Dennett et al. 2007), whereas in the Northeast only WNV has been detected in *Ae. albopictus* using polymerase chain reaction (PCR) based methods (Holick et al. 2002, Farajollahi and Nelder 2009). Nevertheless, northeastern populations have not been systematically surveyed for the presence of other arboviruses by cell culture methods. Accordingly, we estimated the prevalence of viral infection in *Ae. albopictus* from New Jersey to evaluate its status as a regional vector of arboviruses. Host-seeking females were collected in Mercer and Monmouth counties from 2009 to 2011 and screened for a diversity of arboviruses by Vero cell culture assay.

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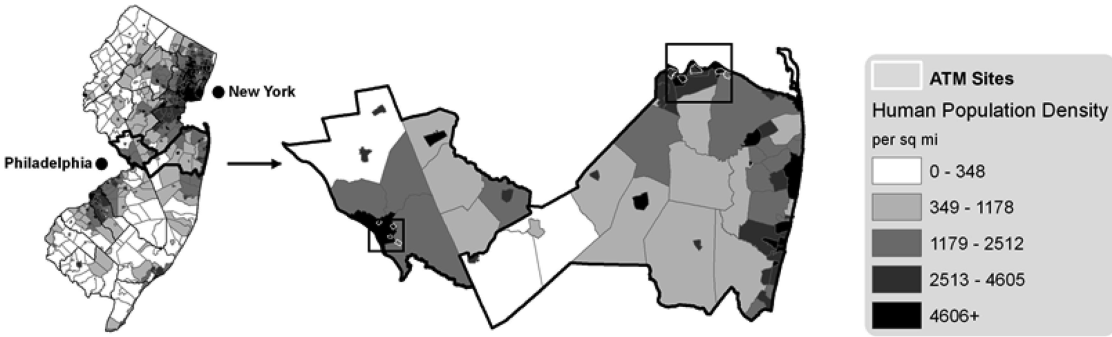

Fig. 1. Asian tiger mosquito trapping locations in Mercer and Monmouth Counties, NJ, during 2009–2011.

Materials and Methods

Mosquito Collections. Sampling sites were selected in Mercer (40° 2,223′ N, 74° 7,296′ W) and Monmouth (40° 4,181′ N, 74° 1,782′ W) Counties based on previously described protocols (Unlu et al. 2011, Fonseca et al. 2013). Mercer County study sites were primarily urban and occupied by single-family residences and two-story residential homes (average parcel size 199.5 ± 18.3 m^2), whereas Monmouth County sites were primarily suburban with single-family residences and multiple family dwellings (average parcel size 571.1 ± 31.2 m^2). In Mercer County, two sampling sites were selected in the City of Trenton, and one site was selected in Hamilton Township (Fig. 1). In Monmouth County, the study sites were located along the Raritan Bay shore region in the municipalities of Aberdeen Township, Union Beach Borough, and Middletown Township (Fig. 1). Mosquito sampling was conducted using BioGents Sentinel (BGS) traps (BioGents AG, Regensburg, Germany) deployed weekly from May through November of each year. Traps were placed in shaded habitats within residential parcels and operated for 24 h using the BG-lure (BioGents AG, Regensburg, Germany) containing ammonia, caproic acid, and lactic acid to capture host-seeking *Ae. albopictus.* Trapping effort remained consistent between the years and ≈94–118 BGS (37–51 in Mercer and 57–67 in Monmouth) traps were deployed weekly in both counties during the sampling period. In the field, collection nets were placed on dry ice immediately, and contents transferred into 500-ml plastic widemouth jars (Uline, Pleasant Prairie, WI) for transport back to the laboratory on dry ice. Species identification and enumeration was conducted under a dissecting microscope on a chill table or dry ice, and mosquito pools containing ≤50 individuals were placed in 2-ml microcentrifuge tubes for storage at −80°C.

Virus Detection. Mosquito pools were processed for virus isolation by adding 1 ml of PBS-G (phosphate buffered saline, 30% heat-inactivated rabbit serum, 0.5% gelatin, and 1× antibiotic or antimycotic) and a copper BB to each tube. Mosquito pools were homogenized using the MM300 Mixer Mill (Retsch Laboratory, Hann, Germany) set for 4 min at 25 cycles/s. Mosquito homogenates were spun for 6 min at 7,000 rpm in a refrigerated microcentrifuge, and 100 μl of the supernatant were inoculated into Vero cell cultures (clone E6) growing in minimal essential media, 5% fetal bovine serum, and antibiotics. Vero cells were maintained at 37°C and 5% CO_2 and examined daily for cytopathic effect from days 3–7 postinoculation. RNA was extracted from infected cell supernatants using the viral RNA kit (Qiagen, Valencia, CA) and initially tested for WNV and EEEV by real-time reverse transcription-polymerase chain reaction (RT-PCR) assays (Lanciotti et al. 2000, Lambert et al. 2003). Virus isolates were then identified by RT-PCR using primers BUNS+new and BUNS−new that target the conserved terminal ends of the S-segment of the *Orthobunyavirus* genus as previously described (Armstrong and Andreadis 2006). The M segment of CVV was amplified by RT-PCR using primers M14C and M4510R (Armstrong and Andreadis 2006), and a portion of the L segment (≈630 bp) was amplified using primers BUNL15C (CGGCCAGTAGTGTACTCCTA) and CVLrev (TCATCCATACACCATGGTGCTGT). Amplification products were purified using the PCR purification kit (Qiagen) and commercially sequenced (Keck Center, New Haven, CT). Edited nucleotide sequences were deposited in GenBank (accession numbers KF296339-KF296343) and compared with those available on GenBank using the Blastn search algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Mosquitoes collected in Mercer County from 27 July 2011 through 9 September 2011 were also rescreened for WNV by RT-PCR in addition to cell culture assay. RNA was extracted from mosquito pools containing ≥7 mosquitoes per pool by using the viral RNA kit (Qiagen) and tested for WNV by realtime RT-PCR (Lanciotti et al. 2000). Virus infection rates per 1,000 mosquitoes and 95% confidence limits (CL) were calculated using PoolInfRate add-in for Microsoft Excel (Biggerstaff 2006).

Results

To estimate the prevalence of viral infection in *Ae. albopictus,* we screened a total of 5,530 pools consisting of 34,567 mosquitoes collected in New Jersey over a 3-yr period by Vero cell culture assay (Table 1). Cytopathic virus was isolated from three pools of *Ae. albopictus* collected on 15 and 22 September 2010 from

Table 1. CVV isolated from *Ae. albopictus* collected in New Jersey

Location	Year	No. mosq. pools	No. mosq.	No. virus isolations	Infection rate/1,000 mosq. (95% CI)
Mercer county	2009	703	4,660	0	
	2010	712	3,052	1	0.33 (0–0.97)
	2011	1,098	7,590	0	
Monmouth county	2009	711	3,503	0	
	2010	1,087	6,902	2	0.29 (0–0.69)
	2011	1,219	8,860	0	
Total		5,530	34,567	3	

Monmouth County and on 22 August 2010 from Mercer County. Virus isolates were negative for WNV and EEEV by real-time RT-PCR but generated amplification products of the appropriate size (≈950 bp) when screened by RT-PCR using generic primers targeting the S-segment of bunyaviruses. Nucleotide sequencing of amplification products revealed that all three virus isolations had identical or nearly identical sequences and were identified as CVV based on a 97–99% nucleotide sequence identity to CVV sequences available on GenBank. The CVV isolate from Mercer County was further characterized by sequencing a 2,025-nucleotide portion of the M segment and 585 nucleotides of the L segment. These sequences most closely matched CVV on GenBank and all three segments (S, M, and L) were most similar (99% nucleotide sequence identity) to a CVV isolate from a human case in upstate New York in 2011 (Nugyen et al. 2013). In Mercer County, the CVV minimum field infection rate (MFIR) per 1,000 mosquitoes was 0.33 (95% CI, 0–0.97) during 2010 and 1.01 (95% CI, 0–2.98) for 992 (149 pools) *Ae. albopictus* sampled during August of 2010. In Monmouth County, the MFIR was 0.29 (95% CI, 0–0.69) during 2010 and 1.39 (95% CI, 0–3.33) for 1434 (228 pools) *Ae. albopictus* collected during September of 2010. No other arboviruses were detected from any of the *Ae. albopictus* pools tested.

To assess the validity and sensitivity of our cell culture assay, we rescreened a cohort of 3,138 (205 pools) *Ae. albopictus* for WNV infection by RT-PCR. Mosquitoes were collected in Mercer County during a period of WNV amplification from late July through early September of 2011. None of these samples were positive for WNV by real-time RT-PCR, confirming prior results by cell culture assay.

Discussion

In the current study, we tested >34,000 *Ae. albopictus* from New Jersey over a 3-yr period to evaluate its importance as a regional arbovirus vector. Despite an intensive sampling effort during a period of WNV amplification, only three virus isolations were recovered from this species during 2010 (one from Mercer County and two from Monmouth County). All of the isolates were identified as CVV, and the overall infection rates were <1 per 1,000 mosquitoes tested (Table 1). These results are somewhat similar to mosquito trapping and testing data from the New Jersey vector surveillance program (http://vectorbio.rutgers.edu/reports/vector/index.php) that uses only PCR-based assays for detection of WNV (Farajollahi et al. 2005). This program detected WNV infection in two pools of *Ae. albopictus* ($N = 2{,}512$, 493 pools, MFIR/1,000 = 0.80) versus 138 pools of *Culex* species ($N = 24{,}408$, 1,223 pools, MFIR/1,000 = 5.65) from mosquitoes collected in Mercer and Monmouth Counties during the same time period (2009–2011) of this study. Together, these findings indicate that *Ae. albopictus* has a limited contribution as an arbovirus vector in this region of the United States.

CVV previously was isolated from a pool of *Ae. albopictus* collected from a tire dump in Illinois in 1995 (Mitchell et al. 1998). Our isolations of CVV from three pools of *Ae. albopictus* indicate that this infection was not an isolated incident. CVV is maintained in a cycle involving large ungulates (primarily deer) and mosquitoes (Blackmore and Grimstad 1998), and infects a diversity of mammalophilic mosquito species but is most commonly isolated from *Anopheles* species in the eastern United States (Calisher et al. 1986). CVV infection in *Ae. albopictus* is not surprising, given that this mosquito feeds mainly on mammals including deer (Savage et al. 1993, Richards et al. 2006, Egizi et al. 2013). CVV has been associated with three cases of human disease, including one fatality in North Carolina (Sexton et al. 1997), and was responsible for epizootics causing fetal death and congenital defects in sheep (Edwards 1994). Nevertheless, there is no evidence implicating *Ae. albopictus* as the responsible vector for any episode of human or animal disease. The ability of *Ae. albopictus* to acquire and transmit CVV has not been measured experimentally in the laboratory, so its vector competence remains unknown.

In northeastern United States, EEEV and WNV are the two most important arboviral pathogens, yet we did not detect either of these viruses in this study. Both viruses are maintained in an enzootic cycle involving ornithophilic mosquitoes (*Culex* or *Culiseta* species) and passerine bird hosts, whereas other mosquito species that feed more opportunistically on both avian and mammalian hosts could function as bridge vectors from birds to humans. Previous studies indicated that *Ae. albopictus* could potentially fulfill this role because this species is a highly competent vector for EEEV and WNV (Turell et al. 1994, 2001) and an aggressive human biter that occasionally acquires bloodmeals from birds (Savage et al. 1993, Richards et al. 2006). Furthermore, EEEV and WNV have been occasionally isolated from or detected in field-collected females, suggesting that this species may serve as a bridge vector when certain conditions prevail (Mitchell et al. 1992, Holick et al. 2002, Kutz et al. 2003, Cupp et al.

2007, Dennett et al. 2007, Farajollahi and Nelder 2009). However, an analysis of the blood-feeding habits of *Ae. albopictus* in urban Mercer County and suburban Monmouth County during 2008–2011 failed to detect the presence of avian-derived bloodmeals in *Ae. albopictus* (n = 140) but instead documented an almost exclusive preference for mammalian hosts including dogs, cats, and especially humans (Egizi et al. 2013). These and our findings suggest that in this region of the northeastern United States, with a high human population density, *Ae. albopictus* is not significantly involved in the transmission of either of these two arboviruses.

One possible limitation of the current study concerns the use of BGS traps to collect *Ae. albopictus* for testing by cell culture methods. These traps were used because they are highly effective in trapping diurnal, container-inhabiting mosquitoes such as *Ae. albopictus* (Farajollahi et al. 2009). Despite this advantage over conventional mosquito traps, BGS trap-collected mosquitoes are primarily nulliparous (thus lower infection rates vs. gravid traps) and have a higher tendency for desiccation and mortality during the trapping period before sample collection. This raises concerns about losing viable virus from field-collected mosquitoes that are killed in the trap. Turell et al. (2002) showed significant declines in infectious virus titer after maintaining WNV-infected mosquito pools at room temperature for 48 h, but holding temperature or period (up to 2 wk) did not affect detection of viral RNA by PCR-based assays. Although the condition of field-collected mosquitoes was not always optimal, we retested >3,000 mosquitoes by RT-PCR to determine whether some WNV infections went undetected by cell culture. None of these mosquitoes, collected during the peak of WNV amplification in Mercer County in 2011, were positive by RT-PCR, reconfirming results based on cell culture.

We conclude that *Ae. albopictus* may occasionally acquire arboviral infections in New Jersey, but its importance as a vector remains limited in this region. This mosquito has a more prominent role in tropical regions of the world where it serves as a vector of dengue virus (DENV) and chikungunya virus (CHIKV) (Gratz 2004, Paupy et al. 2009). Both of these viruses are maintained in a human–mosquito transmission cycle and therefore, require the involvement of a highly anthropophilic mosquito vector to perpetuate. The threat of disease outbreaks caused by these exotic arboviruses remains a distinct possibility in New Jersey, given its high human population density and proximity to major ports of entry for international travelers in the Northeast. The potential for such an outbreak will also depend on the degree of human–mosquito contact, which must be sufficiently frequent to ensure virus amplification. These conditions are typically associated with economically developing and impoverished regions in the tropics but may also occur in unexpected locations as illustrated by recent outbreaks of CHIKV in France and Italy (Rezza et al. 2007, Grandadam et al. 2011) and DENV in France, Croatia, and Hawaii (Effler et al. 2005, Schaffner et al. 2013), that were driven by *Ae. albopictus.* A previous study found that >60% of *Ae. albopictus* from New Jersey had acquired bloodmeals from human hosts (Egizi et al. 2013). This suggests that local populations feed frequently on humans and if competent could support autochthonous transmission of CHIKV and DENV if successfully introduced into the northeastern United States.

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References Cited

Armstrong, P. M., and T. G. Andreadis. 2006. A new genetic variant of La Crosse virus (Bunyaviridae) isolated from New England. Am. J. Trop. Med. Hyg. 75: 491–496.

Benedict, M. Q., R. S. Levine, W. A. Hawley, and L. P. Lounibos. 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus.* Vector Borne Zoonotic Dis. 7: 76–85.

Biggerstaff, B. J. 2006. PooledInfRate, version 4.0: a Microsoft Excel add-in to compute prevalence estimates from pooled samples. (http://www.cdc.gov/westnile/resourcepages/mosqSurvSoft.html).

Blackmore, C. G., and P. R. Grimstad. 1998. Cache Valley and Potosi viruses (Bunyaviridae) in white-tailed deer (*Odocoileus virginianus*): experimental infections and antibody prevalence in natural populations. Am. J. Trop. Med. Hyg. 59: 704–709.

Calisher, C. H., D. B. Francy, G. C. Smith, D. J. Muth, J. S. Lazuick, N. Karabatsos, W. L. Jakob, and R. G. McLean. 1986. Distribution of Bunyamwera serogroup viruses in North America, 1956–1984. Am. J. Trop. Med. Hyg. 35: 429–443.

Crans, W. J., M. S. Chomsky, D. Guthrie, and A. Acquaviva. 1996. First record of *Aedes albopictus* from New Jersey. J. Am. Mosq. Control Assoc. 12: 307–309.

Cupp, E. W., H. K. Hassan, X. Yue, W. K. Oldland, B. M. Lilley, and T. R. Unnasch. 2007. West Nile virus infection in mosquitoes in the mid-south USA, 2002–2005. J. Med. Entomol. 44: 117–125.

Dennett, J. A., A. Bala, T. Wuithiranyagool, Y. Randle, C. B. Sargent, H. Guzman, M. Siirin, H. K. Hassan, M. Reyna-Nava, T. R. Unnasch, et al. 2007. Associations between two mosquito populations and West Nile virus in Harris County, Texas, 2003–06. J. Am. Mosq. Control Assoc. 23: 264–275.

Edwards, J. F. 1994. Cache Valley virus. Vet. Clin. North Am. Food Anim. Pract. 10: 515–524.

Effler, P. V., L. Pang, P. Kitsutani, V. Vorndam, M. Nakata, T. Ayers, J. Elm, T. Tom, P. Reiter, J. G. Rigau-Perez, et al. 2005. Dengue fever, Hawaii, 2001–2002. Emerg. Infect Dis. 11: 742–749.

Egizi, A., S. P. Healy, and D. M. Fonseca. 2013. Rapid blood meal scoring in anthropophilic *Aedes albopictus* and application of PCR blocking to avoid pseudogenes. Infect Genet. Evol. 16C: 122–128.

Estrada–Franco, J. G., and G. B. Craig. 1995. Biology, disease relationships, and control of *Aedes albopictus.* Pan American Health Organization, Technical Publication No. 42. PAHO, Washington, DC.

Farajollahi, A., and M. P. Nelder. 2009. Changes in *Aedes albopictus* (Diptera: Culicidae) populations in New Jersey and implications for arbovirus transmission. J. Med. Entomol. 46: 1220–1224.

Farajollahi, A., W. J. Crans, P. Bryant, B. Wolf, K. L. Burkhalter, M. S. Godsey, S. E. Aspen, and R. S. Nasci. 2005. Detection of West Nile viral RNA from an overwintering pool of *Culex pipiens pipiens* (Diptera: Culicidae) in New Jersey, 2003. J. Med. Entomol. 42: 490–494.

Farajollahi, A., B. Kesavaraju, D. C. Price, G. M. Williams, S. P. Healy, R. Gaugler, and M. P. Nelder. 2009. Field efficacy of BG-Sentinel and industry-standard traps for *Aedes albopictus* (Diptera: Culicidae) and West Nile virus surveillance. J. Med. Entomol. 46: 919–925.

Fonseca, D. M., I. Unlu, T. Crepeau, A. Farajollahi, S. P. Healy, K. Bartlett–Healy, D. Strickman, R. Gaugler, G. Hamilton, D. Kline, and G. G. Clark. 2013. Area-wide management of *Aedes albopictus:* II. Gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. Pest. Manag. Sci. (in press).

Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus.* Emerg. Infect Dis. 7: 807–811.

Grandadam, M., V. Caro, S. Plumet, J. M. Thiberge, Y. Souares, A. B. Failloux, H. J. Tolou, M. Budelot, D. Cosserat, I. Leparc–Goffart, et al. 2011. Chikungunya virus, southeastern France. Emerg. Infect Dis. 17: 910–913.

Gratz, N. G. 2004. Critical review of the vector status of *Aedes albopictus.* Med. Vet. Entomol. 18: 215–227.

Hawley, W. A., P. Reiter, R. S. Copeland, C. B. Pumpuni, and G. B. Craig, Jr. 1987. *Aedes albopictus* in North America: probable introduction in used tires from Northern Asia. Science 236: 1114–1116.

Holick, J., A. Kyle, W. Ferraro, R. R. Delaney, and M. Iwaseczko. 2002. Discovery of *Aedes albopictus* infected with West Nile virus in southeastern Pennsylvania. J. Am. Mosq. Control Assoc. 18: 131.

Kutz, F. W., T. G. Wade, and B. B. Pagac. 2003. A geospatial study of the potential of two exotic species of mosquitoes to impact the epidemiology of West Nile virus in Maryland. J. Am. Mosq. Control Assoc. 19: 190–198.

Lambert, A. J., D. A. Martin, and R. S. Lanciotti. 2003. Detection of North American eastern and western equine encephalitis viruses by nucleic acid amplification assays. J. Clin. Microbiol. 41: 379–385.

Lanciotti, R. S., A. J. Kerst, R. S. Nasci, M. S. Godsey, C. J. Mitchell, H. M. Savage, N. Komar, N. A. Panella, B. C. Allen, K. E. Volpe, et al. 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. J. Clin. Microbiol. 38: 4066–4071.

Mitchell, C. J., M. L. Niebylski, G. C. Smith, N. Karabatsos, D. Martin, J. P. Mutebi, G. B. Craig, Jr., and M. J. Mahler. 1992. Isolation of eastern equine encephalitis virus from *Aedes albopictus* in Florida. Science 257: 526–527.

Mitchell, C. J., L. D. Haramis, N. Karabatsos, G. C. Smith, and V. J. Starwalt. 1998. Isolation of La Crosse, Cache Valley, and Potosi viruses from *Aedes* mosquitoes (Diptera: Culicidae) collected at used-tire sites in Illinois during 1994–1995. J. Med. Entomol. 35: 573–577.

Nugyen, N. L., G. Zhao, R. Hull, M. A. Shelly, S. J. Wong, G. Wu, K. St. George, D. Wang, and M. A. Menegus. 2013. Cache Valley virus in a patient diagnosed with aseptic meningitis. J. Clin. Microbiol. 51: 1966–1969.

Paupy, C., H. Delatte, L. Bagny, V. Corbel, and D. Fontenille. 2009. *Aedes albopictus,* an arbovirus vector: from the darkness to the light. Microbes Infect. 11: 1177–1185.

Rezza, G., L. Nicoletti, R. Angelini, R. Romi, A. C. Finarelli, M. Panning, P. Cordioli, C. Fortuna, S. Boros, F. Magurano, et al. 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370: 1840–1846.

Richards, S. L., L. Ponnusamy, T. R. Unnasch, H. K. Hassan, and C. S. Apperson. 2006. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in relation to availability of human and domestic animals in suburban landscapes of central North Carolina. J. Med. Entomol. 43: 543–551.

Rochlin, I., D. V. Ninivaggi, M. L. Hutchinson, and A. Farajollahi. 2013. Climate change and range expansion of the Asian tiger mosquito (*Aedes albopictus*) in Northeastern USA: implications for Public Health Practitioners. PLoS ONE 8: e60874.

Savage, H. M., M. L. Niebylski, G. C. Smith, C. J. Mitchell, and G. B. Craig, Jr. 1993. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) at a temperate North American site. J. Med. Entomol. 30: 27–34.

Schaffner, F., J. M. Medlock, and W. Van Bortel. 2013. Public health significance of invasive mosquitoes in Europe. Clin. Microbiol. Infect.19: 685–692.

Sexton, D. J., P. E. Rollin, E. B. Breitschwerdt, G. R. Corey, S. A. Myers, M. R. Dumais, M. D. Bowen, C. S. Goldsmith, S. R. Zaki, S. T. Nichol, et al. 1997. Life-threatening Cache Valley virus infection. N. Engl. J. Med. 336: 547–549.

Sprenger, D., and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2: 217–219.

Turell, M. J., J. R. Beaman, and G. W. Neely. 1994. Experimental transmission of eastern equine encephalitis virus by strains of *Aedes albopictus* and *A. taeniorhynchus* (Diptera: Culicidae). J. Med. Entomol. 31: 287–290.

Turell, M. J., M. L. O'Guinn, D. J. Dohm, and J. W. Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. J. Med. Entomol. 38: 130–134.

Turell, M. J., A. R. Spring, M. K. Miller, and C. E. Cannon. 2002. Effect of holding conditions on the detection of West Nile viral RNA by reverse transcriptase-polymerase chain reaction from mosquito (Diptera: Culicidae) pools. J. Med. Entomol. 39: 1–3.

Unlu, I., A. Farajollahi, S. P. Healy, T. Crepeau, K. Bartlett–Healy, E. Williges, D. Strickman, G. G. Clark, R. Gaugler, and D. M. Fonseca. 2011. Area-wide management of *Aedes albopictus:* choice of study sites based on geospatial characteristics, socioeconomic factors and mosquito populations. Pest Manag. Sci. 67: 965–974.

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