Vector competence of Virginia mosquitoes for Zika and Cache Valley viruses

Kevin K. Chan¹, Albert J. Auguste¹, Carlyle C. Brewster² and Sally L. Paulson¹∗

Abstract
Background: Vector-borne diseases are a major public health concern and cause significant morbidity and mortality. Zika virus (ZIKV) is the etiologic agent of a massive outbreak in the Americas that originated in Brazil in 2015 and shows a strong association with congenital ZIKV syndrome in newborns. Cache Valley virus (CVV) is a bunyavirus that causes mild to severe illness in humans and ruminants. In this study, we investigated the vector competence of Virginia mosquitoes for ZIKV and CVV to explore their abilities to contribute to potential outbreaks.

Methods: To determine vector competence, mosquitoes were fed a blood meal comprised of defibrinated sheep blood and virus. The presence of midgut or salivary gland barriers to ZIKV infection were determined by intrathoracic inoculation vs oral infection. After 14-days post-exposure, individual mosquitoes were separated into bodies, legs and wings, and saliva expectorant. Virus presence was detected by plaque assay to determine midgut infection, dissemination, and transmission rates.

Results: Transmission rates for Ae. albopictus orally infected (24%) and intrathoracically inoculated (63%) with ZIKV was similar to Ae. aegypti (48% and 71%, respectively). Transmission rates of ZIKV in Ae. japonicus were low, and showed evidence of a midgut infection barrier demonstrated by low midgut infection and dissemination rates from oral infection (3%), but increased transmission rates after intrathoracic inoculation (19%). Aedes triseriatus was unable to transmit ZIKV following oral infection or intrathoracic inoculation. CVV transmission was dose-dependent where mosquitoes fed high titer (ht) virus blood meals developed higher rates of midgut infection, dissemination, and transmission compared to low titer (lt) virus blood meals. CVV was detected in the saliva of Ae. albopictus (ht: 68%, lt: 24%), Ae. triseriatus (ht: 52%, lt: 7%), Ae. japonicus (ht: 22%, lt: 0%) and Ae. aegypti (ht: 10%; lt: 7%). Culex pipiens and Cx. restuans were not competent for ZIKV or CVV.

Conclusions: This laboratory transmission study provided further understanding of potential ZIKV and CVV transmission cycles with Aedes mosquitoes from Virginia. The ability for these mosquitoes to transmit ZIKV and CVV make them a public health concern and suggest targeted control programs by mosquito and vector abatement districts.

Keywords: Zika virus, Cache Valley virus, Vector competence, Aedes japonicus, Aedes albopictus, Aedes triseriatus, Aedes aegypti, Culex pipiens, Culex restuans, Infection barriers

© The Author(s) 2020. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
Zika virus (ZIKV) (family Flaviviridae, genus Flavivirus) is an arthropod-borne virus (arbovirus) of humans and has been linked to congenital malformations and microcephaly in developing fetuses, and Guillain-Barré syndrome in adults [6]. Since its introduction to Brazil in 2015, ZIKV has spread into many new areas within the Americas [7]. ZIKV is transmitted primarily by urban and sylvatic Aedes mosquitoes, with A. aegypti serving as the main vector for human infection outside of Africa [8–10]. This emerging mosquito-borne virus has caused epidemics throughout Africa, Asia, the Pacific Islands and the Americas [11, 12]. Due to the lack of knowledge of ZIKV replication in North American mosquitoes, experimental vector competence studies are necessary to better understand the potential transmission of ZIKV by additional species. Recent studies have shown that some Aedes, Culex and Coquillettidia mosquitoes from temperate regions of North America were not competent for ZIKV [13, 14], but this is a small representation of the species and strain diversity of mosquitoes that are found in the USA.

Cache Valley virus (CVV) (family Peribunyaviridae, genus Orthobunyavirus) is a neuroinvasive arbovirus that is also spread by mosquitoes. Although CVV infection typically causes mild symptoms in humans, fever, meningitis, and encephalitis have been reported [15]. The symptoms of CVV infection are more severe in ruminants, such as sheep or cattle, and include stillbirths, congenital malformations, spontaneous abortions, and death [16]. CVV has a widespread distribution in North America and has been isolated from many species of mosquitoes including Ae. albopictus and Ae. japonicus [17–20]. The principal vector is unknown, but vector competence studies and field isolations have shown that Anopheles quadrivmaculatus and An. punctipennis may play a significant role in the natural transmission cycle [21, 22]. Laboratory transmission studies have also shown that Cx. tarsalis, Ae. taeniorhynchus, Ae. sollicitans and Cq. perturbans are competent vectors of CVV [22–24].

Aedes aegypti and Ae. albopictus are the most important mosquito species responsible for virus transmission to humans in urban environments. Both species are competent vectors for ZIKV, dengue virus (DENV) and yellow fever virus (YFV) [25–27]. The Asian rock pool mosquito, Ae. japonicus, is a relatively new invasive species that can be found in subtropical and temperate regions of the USA. Although Ae. japonicus is not an aggressive human biter, blood meal analysis from field collected mosquitoes have shown high incidences of human blood consumption [28]. Laboratory transmission studies show that Ae. japonicus is a competent vector of WNV, La Crosse virus (LACV), Eastern equine encephalitis virus (EEE) and St. Louis encephalitis virus (SLEV) [29–32]. Aedes triseriatus, the principal vector of LACV, is found extensively throughout eastern USA and parts of Central America [33]. Under laboratory conditions, Ae. triseriatus is a competent vector for WNV, DENV, YFV, EEE and SLE [34]. WNV has been isolated from Culex pipiens and Cx. restuans and both species have been shown to be competent vectors of the virus [35, 36]. Laboratory transmission studies have found that Cx. pipiens is refractory to CVV and ZIKV infections [13, 23, 25, 37, 38].

Between 2015 and 2018, there were more than 5000 imported ZIKV cases in the USA, with over 100 cases in Virginia [39]. Within the continental USA, reports of local transmission by mosquito vectors have occurred in Florida and Texas [40–42]. There have been no human CVV cases reported in Virginia, but the virus has been isolated from field-collected Ae. japonicus within the state [20]. Although CVV has been detected in field mosquitoes, only a few transmission studies have been conducted to determine potential vectors for the virus. With the wide distribution of Ae. albopictus, Ae. japonicus, Ae. triseriatus, Cx. pipiens and Cx. restuans throughout Virginia [43], it is crucial to determine the vector competence of these local mosquito strains. In this study, we investigated the vector competence of Virginia mosquitoes common in urban and suburban environments for ZIKV and CVV to explore their abilities to contribute to potential outbreaks and help inform local mosquito control strategies.

**Methods**

**Mosquito collection and rearing**

All eggs were derived from female mosquitoes collected using gravid traps in forested areas around Blacksburg, VA. After laying eggs, adult mosquitoes were tested for arboviruses by Vero cell plaque assay to ensure the absence of virus in the F1 progeny. A laboratory strain of Ae. aegypti from Vero Beach, FL, was used as our reference vector species and was subsequently tested for CVV vector competence. Mosquitoes were reared in environmental chamber conditions set at 24°C with 75% RH and 16L:8D photoperiod using methods by Jackson et al. [44] to ensure consistent adult size.
Table 1  ZIKV and CVV blood meal titers

| Species             | CVV high blood meal titer (pfu/ml) | CVV low blood meal titer (pfu/ml) | ZIKV blood meal titer (pfu/ml) | ZIKV intrathoracic inoculation titer (pfu/ml) |
|---------------------|-----------------------------------|-----------------------------------|-------------------------------|-----------------------------------------------|
| Aedes albopictus    | $5.25 \times 10^6$                | $2.90 \times 10^3$                | $3.00 \times 10^6$           | $5.25 \times 10^4$                          |
| Aedes aegypti       | $1.98 \times 10^7$                | $6.28 \times 10^3$                | $6.50 \times 10^6$           | $5.25 \times 10^4$                          |
| Aedes japonicus     | $1.99 \times 10^6$                | $4.60 \times 10^3$                | $3.72 \times 10^7$           | $7.50 \times 10^4$                          |
| Aedes triseriatus   | $2.99 \times 10^6$                | $1.40 \times 10^3$                | $4.50 \times 10^7$           | $1.80 \times 10^5$                          |

Cells and virus
African green monkey kidney (Vero) cells (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, Corning, Corning, NY, USA) with 10% fetal bovine serum (FBS), 100 U/ml of penicillin and 100 μg/ml of streptomycin, and maintained at 37 °C with 5% CO2.

The Asian lineage of ZIKV, PRVABC59 (GenBank: KU501215) and CVV strain 4B (GenBank: KX583998) was used in this study. PRVABC59 was isolated from the serum sample of a patient traveling from Puerto Rico in 2015. CVV4B was isolated from field-caught Ae. japonicus during a 2015 field study in Blacksburg, VA. Both viruses were maintained through passage on Vero cells and stored at −80 °C. Infected blood meals consisted of 1 ml of virus mixed with 9 ml of defribinated sheep blood (Colorado Serum Company, Denver, CO, USA).

Mosquito infection
For oral infection, 1-week-old female mosquitoes were starved 24 h before blood-feeding and provided cotton balls soaked with deionized water. Approximately 40–50 female mosquitoes were placed into 1-gallon cages covered with a mesh screen top. The mosquitoes were offered infected blood meals contained in a glass water-jacketed membrane feeder attached to a circulating 37 °C water bath. Pig intestine sausage casing was used as the membrane. After a 2-h feeding period, fully engorged females were anesthetized on ice and transferred to a new 1-liter cage. A 0.5 ml sample of the infected blood was removed after the feeding period and stored at −80 °C for later virus titer. Parenteral infection was done by intrathoracic inoculation of week-old females that had never taken a blood meal with 0.2 µl of virus [45]. Table 1 shows the titers of virus infected blood meals and virus inoculum. Infected mosquitoes were maintained at 24 °C with 75% RH and 16L:8D photoperiod and provided 10% sucrose solution for sustenance.

Saliva extraction
After 14-DPE, female mosquitoes were removed from cages and immobilized by chilling on ice. Saliva was extracted by inserting the proboscis into a capillary tube filled with a 1:1 mixture of 10% sucrose and fetal bovine serum (FBS) [46]. The mosquitoes were given 30 min to feed and salivate. The saliva, legs and wings and body were placed into separate microcentrifuge tubes with DMEM and stored at −80 °C until virus testing.

Virus detection
Mosquito bodies, leg and wing and saliva samples were homogenized with metal pellets in 2 ml of Vero media on a vortex mixer and then clarified by centrifugation at 1500 × g for two min. Supernatants were tested for infection using Vero cell plaque assay following the methods of Barker et al. [47]. If virus was recovered from the body but not the legs and wings, the mosquito was classified as having a non-disseminated infection; if virus was detected in the wings and legs, the mosquito was classified as having a disseminated infection; mosquitoes with virus in saliva were classified as transmitting. Infection rate was determined as the percentage of the orally infected mosquitoes positive for virus in the body. Dissemination rate was determined as the percentage of orally infected mosquitoes that were positive for virus in the legs and wings, regardless of infection status. Transmission rate was determined as the percentage of orally infected mosquitoes that had virus in the salivary expectorant, regardless of infection status. Infectious blood meals were thawed at room temperature, diluted with a series of 10-fold serial dilutions and tested for virus concentration using plaque assay.

Statistical analysis
A Chi-square test was used to compare mean infection, dissemination and transmission rates among mosquito species followed by Fisher’s exact tests for pairwise comparisons [48]. GraphPad Prism 6.0 (La Jolla, CA, USA) was used for all statistical analysis. All statistical analyses were carried out at a significance level of $\alpha = 0.05$.

Results
Vector competence for ZIKV following oral infection
There was a significant difference among infection ($\chi^2 = 58.73, df = 5, P < 0.05$), dissemination ($\chi^2 = 71.21, df = 5$, $P < 0.05$), and transmission ($\chi^2 = 46.57, df = 5$, $P < 0.05$) rates among mosquito species.
Transmission of ZIKV following parenteral infection

Parenteral infection by intrathoracic inoculation resulted in significantly higher rates of transmission compared to oral infection in Aedes albopictus (63% parenteral vs 24% oral) (Fisher’s exact test, $P = 0.0080$, OR: 0.1875, 95% CI: 0.0566–0.6209) and Aedes japonicus (19% parenteral vs 3% oral) (Fisher’s exact test, $P = 0.0119$, 95% CI: 0.0202–0.7088) (Table 3). Mode of infection had no effect on transmission by Aedes aegypti (71% parenteral vs 48% oral) (Fisher’s exact test, $P = 0.1395$, OR: 0.3693, 95% CI: 0.1079–1.2630). No virus was detected in the saliva of Aedes triseriatus from either orally or parenterally infected groups (Table 3).

Vector competence to CVV

When fed a high titer (ht) virus blood meal, Aedes albopictus and Aedes triseriatus showed significantly higher rates of infection ($\chi^2 = 127.5$, $df = 5$, $P < 0.0001$), dissemination ($\chi^2 = 107.8$, $df = 5$, $P < 0.0001$) and transmission ($\chi^2 = 88.08$, $df = 5$, $P < 0.0001$) than Aedes japonicus or Aedes aegypti (Table 4). However, when fed low titer (lt) blood meals, there were no differences among rates for any of the species ($\chi^2 = 5.61$, $df = 3$, $P > 0.05$) (Table 4). Aedes albopictus was the most susceptible to CVV oral infection (ht: 100%, lt: 24%) and had the highest rate of dissemination (ht: 85%, lt: 24%) and transmission (ht: 68%, lt: 24%). Aedes triseriatus was also susceptible to CVV infection (ht: 72%, lt: 15%), dissemination (ht: 69%, lt: 11%) and transmission (ht: 52%, lt: 7%). None of the Aedes japonicus fed a low titer blood meal developed midgut infections. For Aedes aegypti, CVV was able to establish midgut infections (ht: 48%, lt: 11%), cause a disseminated infection (ht: 25%, lt: 11%) and transmit virus (ht: 10%; lt: 7%). Figure 2 shows significant differences in infection, dissemination and transmission between high and low titer blood meals for Aedes albopictus, Aedes triseriatus and Aedes japonicus. Virus titer resulted in significant differences in infection for Aedes aegypti but not dissemination or transmission rates. Neither of the Culex species was able to transmit CVV. No Cx. restuans became infected and only one Cx. ppienis was positive for infection and dissemination (Table 2).

Table 2  Vector competence of ZIKV and CVV in Culex ppienis and Cx. restuans

| Mosquito species | Sample size (n) | Mean titer (pfu/ml) | Mean non-disseminated infection (%) | Mean disseminated infection (%) | Mean transmitting (%) |
|------------------|----------------|--------------------|------------------------------------|-------------------------------|-----------------------|
| ZIKV             |                |                    |                                    |                               |                       |
| Cx. ppienis      | 30             | $3.00 \times 10^7$ | 0                                  | 0                             | 0                     |
| Cx. restuans     | 28             | $5.25 \times 10^6$ | 0                                  | 0                             | 0                     |
| CVV              |                |                    |                                    |                               |                       |
| Cx. ppienis      | 67             | $1.12 \times 10^8$ | 1                                  | 1                             | 0                     |
| Cx. restuans     | 30             | $7.70 \times 10^7$ | 0                                  | 0                             | 0                     |
Assessing the vector competence of local mosquitoes for imported and emerging viruses is critical for public health officials to anticipate patterns of arbovirus transmission, determine the relative roles of the different species for virus amplification and spread, and to select appropriate control responses. This study aimed to determine the risk of local ZIKV transmission and the emergence potential of CVV in Virginia by evaluating the vector competence of the most common mosquito species found in urban and suburban habitats.

A meta-analysis by McKenzie et al. [49] suggested that the vector competence of \textit{Ae. albopictus} for Zika virus varied among geographically disparate populations. We found that the vector competence of a Virginia strain of \textit{Ae. albopictus} was equivalent to that of a Florida strain of \textit{Ae. aegypti}. \textit{Culex} mosquitoes were found to be refractory to ZIKV infection. Other studies have also observed similar results, suggesting that it is unlikely this group plays a role in ZIKV transmission [13, 37, 50, 51]. We also found that \textit{Ae. japonicus} from Virginia was capable of transmitting ZIKV, but at a much lower rate compared to \textit{Ae. aegypti} and \textit{Ae. albopictus}. A study by Aliota et al. [13] showed that laboratory strains of \textit{Ae. triseriatus} were able to become infected with ZIKV PRVABC59, the same strain that we used, but no dissemination or transmission resulted. Our study showed similar results working with an F1 generation of field-caught \textit{Ae. triseriatus} where only midgut infections resulted from oral exposure.

Upon ingesting an infectious blood meal, the virus must surmount several tissue barriers associated with the midgut and salivary glands [52]. We assessed the presence of tissue barriers by intrathoracic inoculation of ZIKV. Injecting virus directly into the hemolymph bypasses the midgut and permits the virus to reach and infect the salivary glands. We detected infectious virus in salivary expectorant of \textit{Ae. japonicus}, but not \textit{Ae. triseriatus}, which indicated the presence of salivary gland

### Table 3 Transmission rate of \textit{Aedes japonicus} and \textit{Ae. triseriatus} intrathoracically inoculated with ZIKV

| Mosquito species | Infection method | Sample size (n) | Virus titer (pfu/ml) | Transmission (%) |
|------------------|------------------|----------------|---------------------|-----------------|
| \textit{Ae. albopictus} | Intrathoracic | 19 | $5.25 \times 10^{4}$ | 63$^a$ |
| | Oral | 37 | $3.00 \times 10^{6}$ | 24$^b$ |
| \textit{Ae. aegypti} | Intrathoracic | 21 | $5.25 \times 10^{4}$ | 71$^a$ |
| | Oral | 25 | $6.50 \times 10^{6}$ | 48$^a$ |
| \textit{Ae. japonicus} | Intrathoracic | 21 | $7.50 \times 10^{4}$ | 19$^a$ |
| | Oral | 73 | $3.72 \times 10^{7}$ | 3$^b$ |
| \textit{Ae. triseriatus} | Intrathoracic | 23 | $1.80 \times 10^{5}$ | 0$^a$ |
| | Oral | 28 | $4.50 \times 10^{7}$ | 0$^a$ |

**Notes:** Differing letters denote significance of transmission rates of the same species after oral or intrathoracic infection by two-tailed Fischer’s exact test, $\alpha = 0.05$

### Table 4 Vector competence for CVV 4B by \textit{Aedes albopictus}, \textit{Ae. triseriatus}, \textit{Ae. japonicus} and \textit{Ae. aegypti} presented as mean % infected, disseminated and transmitting

| Species | Sample size (n) | Mean blood meal titer (pfu/ml) | % infected | % disseminated | % transmitting |
|---------|----------------|-------------------------------|------------|---------------|---------------|
| \textit{High titer} | | | | | |
| \textit{Ae. albopictus} | 34 | $5.25 \times 10^{6}$ | 100$^a$ | 85$^a$ | 68$^a$ |
| \textit{Ae. triseriatus} | 29 | $2.99 \times 10^{6}$ | 72$^a$ | 69$^a$ | 52$^a$ |
| \textit{Ae. japonicus} | 74 | $1.99 \times 10^{6}$ | 41$^b$ | 38$^b$ | 28$^b$ |
| \textit{Ae. aegypti} | 52 | $1.98 \times 10^{7}$ | 48$^a$ | 25$^b$ | 10$^b$ |
| \textit{Low titer} | | | | | |
| \textit{Ae. albopictus} | 21 | $2.90 \times 10^{3}$ | 24$^a$ | 24$^a$ | 24$^a$ |
| \textit{Ae. triseriatus} | 55 | $1.40 \times 10^{3}$ | 15$^a$ | 11$^a$ | 7$^a$ |
| \textit{Ae. japonicus} | 21 | $4.60 \times 10^{3}$ | 0$^a$ | 0$^a$ | 0$^a$ |
| \textit{Ae. aegypti} | 44 | $6.28 \times 10^{3}$ | 11$^a$ | 11$^a$ | 7$^a$ |

**Notes:** Different letters denote significance between different species within respective categories of high or low titer blood meals by two-tailed Fischer’s exact test, $\alpha = 0.05$
barriers. Although transmission for intrathoracically inoculated *Ae. japonicus* was significantly higher than orally infected mosquitoes, the rates were low. The low midgut infection and transmission rates lead us to believe that there are potential midgut and salivary gland barriers that limit *Ae. japonicus* and prevent *Ae. triseriatus* from ZIKV transmission. Although *Ae. albopictus* was capable of ZIKV transmission after oral infection, intrathoracic inoculation significantly increased its transmission rates, indicating the presence of a midgut barrier. Studies of virus and vector systems have shown that these barriers play an important role during the extrinsic incubation period and may limit the ability of the virus to infect the mosquito for successful transmission [53, 54]. In addition, gut microbiota and immune pathways may also be involved when the virus enters the midgut [55–57]. It has been hypothesized that midgut and salivary gland barriers are responsible for the geographical variation in vector competence seen in *Ae. aegypti* and *Ae. albopictus* for ZIKV [58–60].

Although *Ae. japonicus* was capable of ZIKV transmission, it is not an aggressive human biter and predominantly inhabits forested areas, which limits its role in ZIKV transmission. Surprisingly, *Ae. triseriatus*, *Cx. pipiens* and *Cx. restuans* were not competent for ZIKV even though they are competent vectors of other flaviviruses, such as WNV or SLEV [61–63]. *Aedes albopictus*, on the other hand, was highly competent for ZIKV and exhibits aggressive, anthropophilic behaviour. The likelihood for this species to contribute to ZIKV transmission in Virginia is much higher compared to other *Aedes* mosquitoes in this region.

This study also found that *Ae. albopictus*, *Ae. triseriatus*, *Ae. japonicus* and *Ae. aegypti* were susceptible to CVV infection and capable of virus transmission. The combination of high vector competence, previous isolations from the field, and anthropophilic behavior suggests that *Ae. albopictus* could play a major role in CVV transmission in endemic areas [64, 65]. *Aedes triseriatus* and *Ae. japonicus* were also competent for CVV and blood meal analysis has shown that all three species feed on deer, the primary amplifying vertebrate host for CVV [28, 66–69]. We used high and low CVV blood meal titers that bracketed the range of titers found in experimentally infected deer [22] and showed that *Ae. albopictus*, *Ae. triseriatus* and *Ae. aegypti* were susceptible to CVV infection and subsequently transmitted virus even when exposed to low titer blood meals. There is currently no
There are many knowledge gaps in CVV dynamics, especially our understanding of its natural cycle of competent vectors and susceptible amplifying hosts. In addition, CVV infections are often misdiagnosed for other flu-like illness, which presents itself as a challenge for accurate reporting to local or state health departments. In contrast, the ZIKV outbreak in 2015 sparked high demand for all areas of research to understand and control the virus. Although cases have dropped significantly in the USA, ZIKV is still present in parts of Africa, Asia and South America, and may remain indefinitely [11, 80].

Conclusions
Our studies show that a species that has not been tested for ZIKV vector competency, *Ae. japonicus*, was able to transmit the virus, but at a low rate. *Aedes japonicus*, however, was competent for CVV transmission. *Aedes albopictus*, the most widespread anthropophilic mosquito in Virginia, was competent for both ZIKV and CVV. *Aedes aegypti* was competent for both viruses, but its inability to overwinter in colder climates reduces this species’ likelihood of ZIKV transmission in Virginia. With the abundance of highly competent mosquito species, there may be greater concern for increased CVV transmission in temperate regions of the USA.

Abbreviations
ZIKV: Zika virus; CVV: Cache Valley virus; WNV: West Nile virus; LACV: La Crosse virus; DENV: dengue virus; DPE: days post-exposure; lt: low titer; ht: high titer.

Acknowledgements
We thank Andrea Bertke from the Virginia-Maryland College of Veterinary Medicine for providing ZIKV PRVABC59 and Fan Yang for his help on isolating the virus; DENV: dengue virus; PPE: days post-exposure; lt: low titer; ht: high titer.

Authors’ contributions
KC and SLP designed the initial project. KC conducted laboratory experiments. KC and SLP analyzed the results, interpreted data and drafted the manuscript. All authors read and approved the final manuscript.

Funding
This study was supported by a grant from Graduate Research Development Programme at Virginia Tech.

Availability of data and materials
Data supporting the conclusions of this article are included within the article. The datasets used for the present study are available from the corresponding author upon request.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.
References
1. Roehrig JT. West Nile virus in the United States—a historical perspective. Viruses. 2013;5:3088–108.
2. Juliano SA, Lounibos LP. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol Lett. 2005;8:558–74.
3. Cunze S, Koch LK, Kochmann J, Klimpf S. Aedes albopictus and Aedes japonicus—two invasive mosquito species with different temperature niches in Europe. Parasit Vectors. 2016;9:573.
4. Kaufman MG, Fonseca DM. Invasion biology of Aedes japonicus japonicus (Diptera: Culicidae). Annu Rev Entomol. 2014;59:31–49.
5. Mitchell CJ. Vector competence of North and South American strains of Aedes albopictus for certain arboviruses: a review. J Am Mosq Control Assoc. 1991;7:464–51.
6. Krauer F, Riesen M, Revei L, Oldado OT, Martinez-Vega R, Prago TV, et al. Zika virus infection as a cause of congenital brain abnormalities and Guillain-Barré syndrome: systematic review. PLoS Med. 2017;14:e1002023.
7. Zanluca C, de Melo VCA, Mosimann ALP, dos Santos GIV, dos Santos CND, Luz K. First report of autochthonous transmission of Zika virus in Brazil. Mem Inst Oswaldo Cruz. 2015;110:569–72.
8. Aylott T, Campos RM, Brasil P, Morone FC, Cámara DCP, Meira GLS, et al. Early evidence for Zika virus circulation among Aedes aegypti mosquitoes, Rio de Janeiro, Brazil. Emerg Infect Dis. 2017;23:1411–2.
9. Ayres CFJ. Identification of Zika virus vectors and implications for control. Lancet Infect Dis. 2016;16:278–9.
10. Marchette NJ, Garcia R, Rudnick A. Isolation of Zika virus from Aedes aegypti mosquitoes in New York State. J Med Entomol. 2006;43:1004–9.
11. Armstrong PM, Andreadis TG, Anderson JF. Emergence of a new Bunyaviridae: Orthobunyavirus in the northeastern United States, 1997–2012. Vector Borne Zoonotic Dis. 2014;14:763–73.
12. Blackmore CG, Blackmore MS, Grimmstad PR. Role of Anopheles quadrimaculatus and Coquillettidia perturbans (Diptera: Culicidae) in the transmission cycle of Cache Valley virus (Bunyaviridae: Bunyavirus) in the midwest, USA. J Med Entomol. 1998;35:660–4.
13. Ayers VB, Huang YS, Lyons AC, Park SL, Higgs S, Dunlop JI, et al. Culex tarsalis is a competent vector species for Cache Valley virus. Parasit Vectors. 2018;11:519.
14. Yuill TM, Thompson PH. Cache Valley virus in the Del Mar Va Peninsula. IV. Biological transmission of the virus by Aedes sollicitans and Aedes taeniorhynchus. Am J Trop Med Hyg. 1970;19:513–9.
15. Liu Z, Zhou T, Lai Z, Zhang Z, Jia Z, Zhou G, et al. Competence of Aedes aegypti, Ae. albopictus, and Culex quinquefasciatus mosquitoes as Zika virus vectors. China. Emerg Infect Dis. 2017;23:1085–91.
16. Miller BR, Mitchell CJ, Ballinger ME. Replication, tissue tropisms and transmission of yellow fever virus in Aedes albopictus. Trans R Soc Trop Med Hyg. 1989;83:252–5.
17. Whitehorn J, Kien DT, Nguyen NM, Nguyen HL, Kyrillos PP, Carrington LB, et al. Comparative susceptibility of Aedes albopictus and Aedes aegypti to dengue virus infection after feeding on blood of viremic humans: implications for public health. J Infect Dis. 2015;212:1182–90.
18. Molaei G, Farajollahi A, Scott JI, Gaugler R, Andreadis TG. Human blood-feeding by the recently introduced mosquito, Aedes japonicus japonicus, and public health implications. J Am Mosq Control Assoc. 2009;25:210–4.
19. Sardelis MR, Turell MJ. Ochlerotatus j. japonicus in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. J Am Mosq Control Assoc. 2001;17:137–41.
20. Sardelis MR, Dohm DJ, Pagac B, Andre RG, Turell MJ. Experimental transmission of eastern equine encephalitis virus by Ochlerotatus j. japonicus (Diptera: Culicidae). J Med Entomol. 2002;39:948–40.
21. Sardelis MR, Turell MJ, Andre RG. Laboratory transmission of La Crosse virus by Ochlerotatus j. japonicus (Diptera: Culicidae). J Med Entomol. 2002;39:9635–9.
22. Sardelis MR, Turell MJ, Andre RG. Experimental transmission of St. Louis encephalitis virus by Ochlerotatus j. japonicus. J Am Mosq Control Assoc. 2003;19:159–62.
23. Sánchez-Trinidad A, Ordoñez-Sánchez F, Valdes-Perezgas MT, Sánchez-Ramos FJ, Zavortink TJ, Cortés-Guzmán AJ, et al. Geographical distribution of the Aedes triseriatus Group (Diptera: Culicidae) in Mexico. J Vector Ecol. 2014;39:134–2.
24. European Centre for Disease Prevention and Control (ECDC). Aedes triseriatus—factsheet for experts, 2014. https://ecdc.europa.eu/en/disea se-vectors/factsheets/aedes-triseriatus. Accessed 3 Mar 2014.
25. Liu Z, Zhou T, Lai Z, Zhang Z, Jia Z, Zhou G, et al. Competence of Aedes aegypti, Ae. albopictus, and Culex quinquefasciatus mosquitoes as Zika virus vectors. China. Emerg Infect Dis. 2017;23:1085–91.
26. Miller BR, Mitchell CJ, Ballinger ME. Replication, tissue tropisms and transmission of yellow fever virus in Aedes albopictus. Trans R Soc Trop Med Hyg. 1989;83:252–5.
27. Whitehorn J, Kien DT, Nguyen NM, Nguyen HL, Kyrillos PP, Carrington LB, et al. Comparative susceptibility of Aedes albopictus and Aedes aegypti to dengue virus infection after feeding on blood of viremic humans: implications for public health. J Infect Dis. 2015;212:1182–90.
