

Short Communication

Higher West Nile Virus Infection in *Aedes albopictus* (Diptera: Culicidae) and *Culex* (Diptera: Culicidae) Mosquitoes From Lower Income Neighborhoods in Urban Baltimore, MD

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Abstract

The temperate United States has experienced increasing incidence of mosquito-borne diseases. Recent studies conducted in Baltimore, MD have demonstrated a negative relationship between abundances of *Aedes albopictus* (Skuse) and *Culex* mosquitoes and mean neighborhood income level, but have not looked at the presence of pathogens. Mosquitoes collected from five socioeconomically variable neighborhoods were tested for infection by West Nile, chikungunya, and Zika viruses in 2015 and 2016, and again from four of the neighborhoods in 2017. Minimum infection rates of pooled samples were compared among neighborhoods for each year, as well as among individual blocks in 2017. West Nile virus was detected in both *Ae. albopictus* and *Culex* pools from all neighborhoods sampled in 2015 and 2017. No infected pools were detected in any year for chikungunya or Zika viruses, and none of the target viruses were detected in 2016. Infection rates were consistently higher for *Culex* than for *Ae. albopictus*. Minimum infection rate was negatively associated with mean neighborhood income for both species in 2015. Although earlier work has shown a positive association between block-level abandonment and mosquito abundance, no association was detected in this study. Still, we demonstrate that viral infection in mosquito pools can differ substantially across adjacent urban neighborhoods that vary by income. Though trap security and accessibility often inform city sampling locations, detecting and managing arboviral risk requires surveillance across neighborhoods that vary in socioeconomics, including lower income areas that may be less accessible and secure but have higher infection rates.

Key words: arbovirus, city, income, mosquito

Transmission of mosquito-borne diseases in the United States has increased in recent decades (Leisnham and Juliano 2012, LaDeau et al. 2015, Moreno-Madriñán and Turell 2018). There have been >50,000 confirmed human cases of West Nile virus in the United States since it was first detected in North America in 1999 (CDC 2019b). Chikungunya virus has been locally transmitted in the continental United States and its territories since 2014 (CDC 2020), and the arrival of Zika virus in 2015 has resulted in >5,000 human cases in the states and >37,000 cases in United States territories (CDC 2019a).

Infection rates of most mosquito-borne viruses are likely underestimated by human case documentation. West Nile virus is asymptomatic in ~80% of infections, leading some researchers to estimate

that there were over one million human West Nile virus cases between 1999 and 2016 (Davis et al. 2006). Infections among individuals in low-income areas are especially likely to be underestimated due to reduced access to quality medical care (Malat et al. 2006). Pathogen surveillance in mosquito populations is, therefore, critical to mitigate the widespread ecological (LaDeau et al. 2008) and human health (Vazquez-Prokopec et al. 2010, Hadler et al. 2014, Osório et al. 2014) impacts of arboviral outbreaks. Even relatively small delays in detection can be detrimental to effective management (Petersen and Hayes 2004, Owen et al. 2006, Vazquez-Prokopec et al. 2010). However, surveillance can be especially difficult in urban landscapes where physical and social variability can lead to spatially heterogeneous vector populations, associated human disease risk,

and the availability of data to estimate either (Kjellstrom et al. 2007, Leisnham and Slaney 2009, LaDeau et al. 2015).

Recent studies in Baltimore, MD, have found higher mean abundances of two potential vector mosquito species, *Aedes albopictus* (Skuse) and *Culex pipiens* L., in lower income neighborhoods (LaDeau et al. 2013, Becker et al. 2014, Little et al. 2017). Within neighborhoods, differences in *Ae. albopictus* densities have been associated with bio-physical conditions at the block scale, wherein blocks with higher infrastructural abandonment and vegetative cover had the greatest mosquito abundances (Little et al. 2017). Adult female *Ae. albopictus* in low-income blocks also appear to be larger (Katz et al. 2019), potentially indicating block- and neighborhood-level variation in vectorial capacity (Nasci 1986, Sumanochitrapon et al. 1998, Juliano et al. 2014). In this study, we test for the presence of West Nile virus, chikungunya, and Zika viruses in *Ae. albopictus* and *Culex* populations and quantify infection rates across these same Baltimore neighborhoods to further investigate how mosquito-borne disease risk varies with socioeconomics in the urban landscape.

Materials and Methods

In 2015 and 2016, we sampled adult mosquitoes from 5 neighborhoods in Baltimore, MD (described in Little et al. 2017). Neighborhoods consisted of row homes and were categorized as below (Franklin Square, Harlem Park), at (Hollins Market, Union Square), or above (Bolton Hill) Baltimore's 2012 median household income of \$41,819. Neighborhoods were located within 2 km of each other, minimizing regional environmental variation. Due to logistical and funding constraints, Bolton Hill was not resampled with the other four neighborhoods in 2017.

BG-Sentinel (BGS) traps (Biogents AG, Germany) baited with CO₂ and a 2.0 ml Octenol Lure (a mammal-derived attractant) were used to trap adult mosquitoes between June and September. Traps were deployed at two locations on each of 2–3 focal blocks per neighborhood (26 total traps deployed for 72 h every 3 wk). Specific trap locations within each block were >50 m apart, shaded, and in a protected but accessible yard. While the BGS trap was designed to target host-seeking *Aedes*, it has been demonstrated to effectively sample blooded specimens of both *Aedes* and *Culex* species in this region (Becker et al. 2014, Faraji et al. 2014). Samples were collected every 24 h and placed on dry ice, at which time a random subset was

sorted into pools (up to 25 individuals) of *Ae. albopictus* or *Culex* females and transferred to an ultracool (–80°C) freezer.

Protocols were consistent across years except that pools were organized by neighborhood in 2015 and 2016, and by individual block within neighborhood in 2017. In total, 85 *Ae. albopictus* and 19 *Culex* pools from 2015, 21 *Ae. albopictus* and 20 *Culex* pools from 2016, and 99 *Ae. albopictus* and 25 *Culex* pools from 2017 were tested for presence of West Nile, chikungunya, and Zika viruses (total individuals across pools in 2015, 2016, 2017: *Ae. albopictus* = 1913, 525, 2315; *Culex* = 310, 428, 355). We extracted viral RNA using QIAGEN Viral RNA Mini Kit (QIAGEN, Germantown, MD) following the manufacturer's protocol. Using primers and probes specific to each virus, we conducted real-time RT-PCR with QIAGEN QuantiTect Probe RT-PCR kits, following the manufacturer's protocol. West Nile primers and probes were obtained from Lanciotti et al. (2000), chikungunya primers and probes were obtained from Pastorino et al. (2005), and Zika primers and probes were obtained from Lanciotti et al. (2008). Positive and negative controls were used for each PCR.

Minimum Infection Rates (MIRs) were calculated using CDC protocols (<https://www.cdc.gov/westnile/resourcepages/mosqsurvsoft.html>). Plots were used to make visual comparisons of MIR among neighborhood income levels because low neighborhood replication prevented meaningful statistical analyses. Correlation between MIR and proportion abandonment among blocks was evaluated using Pearson's tests in RStudio (RStudio Team, 1.3.1073, 2020). We likewise evaluated the association between MIR and numbers of mosquitoes tested to assess sampling effect and found no significant correlation (*Ae. albopictus*: $r = 0.368$, $P = 0.196$; *Culex*: $r = 0.063$, $P = 0.830$) among years.

Results

West Nile virus was detected in both *Ae. albopictus* and *Culex* pools collected from all neighborhoods sampled in 2015 and 2017. West Nile virus was not detected in 2016, and neither chikungunya nor Zika viruses were detected in any year of this study. We detected West Nile virus in 8 (9.4%) *Ae. albopictus* and 8 (42.1%) *Culex* pools in 2015, and in 10 (10.1%) *Ae. albopictus* and 6 (24.0%) *Culex* pools across all neighborhoods sampled in 2017 (Table 1).

West Nile virus infection rates were higher in *Culex* than in *Ae. albopictus* in each neighborhood. Both species demonstrated

Table 1. *Aedes albopictus* and *Culex* pool counts and infection rates by neighborhood, 2015–2017

Neighborhood	Year	No. of pools		No. of positive pools		% Positive pools		MIR	
		<i>Ae. albopictus</i>	<i>Culex</i>	<i>Ae. albopictus</i>	<i>Culex</i>	<i>Ae. albopictus</i>	<i>Culex</i>	<i>Ae. albopictus</i>	<i>Culex</i>
Franklin Square	2015	17	3	0	1	0.0	33.3	0.0	19.2
	2016	4	4	0	0	0.0	0.0	0.0	0.0
	2017	12	4	1	1	8.3	25.0	3.4	40.0
Harlem Park	2015	26	7	5	5	19.2	71.4	8.3	36.2
	2016	5	4	0	0	0.0	0.0	0.0	0.0
	2017	55	10	4	3	7.3	30.0	3.0	14.0
Hollins Market	2015	12	2	0	0	0.0	0.0	0.0	0.0
	2016	4	4	0	0	0.0	0.0	0.0	0.0
	2017	22	6	4	1	18.2	16.7	8.0	10.8
Union Square	2015	12	2	2	1	16.7	50.0	7.2	26.3
	2016	4	4	0	0	0.0	0.0	0.0	0.0
	2017	10	5	1	1	10.0	20.0	5.4	43.5
Bolton Hill	2015	18	5	1	1	5.6	20.0	2.5	14.3
	2016	4	4	0	0	0.0	0.0	0.0	0.0

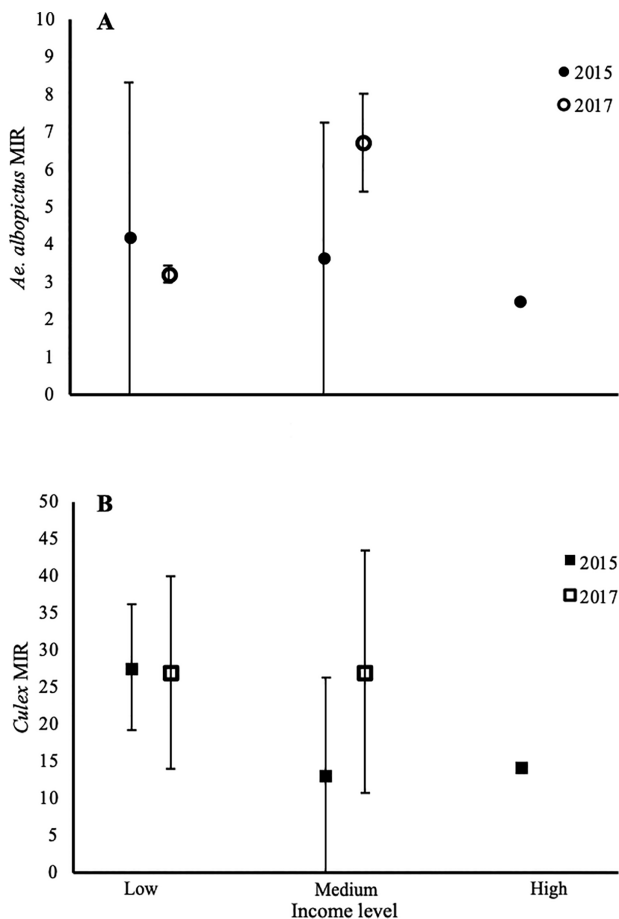


Fig. 1. Mean minimum infection rates (MIRs $\pm$ SE) for *Aedes albopictus* (A) and *Culex* (B) for low- and medium-income neighborhoods in 2015 and 2017, and MIR for a high-income neighborhood in 2015.

a negative relationship with income in 2015, the only year when positive pools were detected at all three neighborhood income levels (Fig. 1). In 2015, mean MIR of each species was nearly two times higher in low- versus high-income neighborhoods. Mean MIR of *Culex* in low-income neighborhoods was similar in 2017 but doubled in medium-income neighborhoods compared to 2015. Medium-income neighborhoods also demonstrated increased mean MIR of *Ae. albopictus* in 2017 compared to 2015, while low-income neighborhoods had a decrease in mean MIR. In 2017, when we separated pools by block, we did not find significant correlations of either *Ae. albopictus* or *Culex* MIRs with proportion abandonment (*Ae. albopictus*: $r = -0.498$, $P = 0.143$; *Culex*: $r = 0.322$, $P = 0.365$; Fig. 2).

Discussion

Both *Ae. albopictus* and *Culex* infection rates exhibited an overall negative relationship with income, just as abundances and female body sizes have in prior studies (LaDeau et al. 2013, Little et al. 2017, Katz et al. 2019). In 2015, mean MIRs were higher for samples collected in low-income relative to high-income neighborhoods. The relationship of MIRs in low- versus medium-income neighborhoods was less clear, with a similar negative association between MIR and income in 2015 but not in 2017, when MIRs of samples from medium-income neighborhoods increased substantially. More

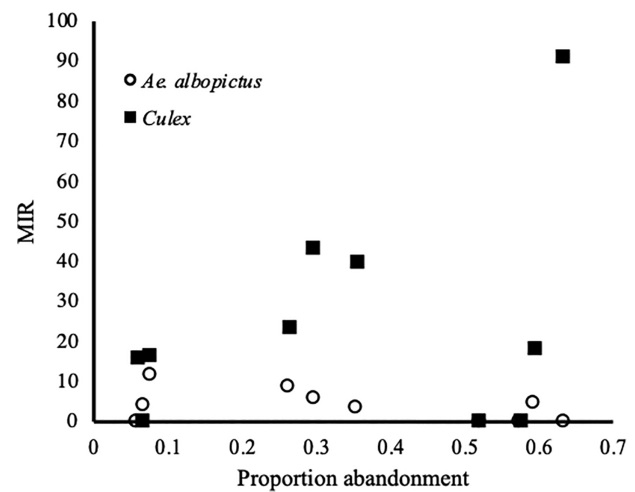


Fig. 2. MIR by proportion abandoned properties among blocks in 2017 for *Aedes albopictus* ($r = -0.498$, $P = 0.143$) and *Culex* ($r = 0.322$, $P = 0.365$).

research is needed to understand the mechanisms that resulted in increased MIRs in medium-income neighborhoods in 2017. It is possible that variable environmental factors influencing developmental habitat quality affected likelihood of infection (Juliano et al. 2014, Shapiro et al. 2016) or that host communities providing blood meals at these sites (Goodman et al. 2018) were somehow altered to facilitate greater infection rates (Kilpatrick et al. 2006, Allan et al. 2009).

Interannual variability in infection rates may also be attributed to differences in weather conditions (Landesman et al. 2007, Ruiz et al. 2010, Wimberly et al. 2014). Higher-than-average summer temperatures in 2016 may have contributed to the absence of positive pools that year. Mean daily temperature in Baltimore in July and August, 2016, was $28.0 \pm 0.4^\circ\text{C}$, compared to $26.1 \pm 0.5^\circ\text{C}$ and $26.6 \pm 2.1^\circ\text{C}$ during those months in 2015 and 2017, respectively (NOAA 2018). Temperatures of 30°C compared to 26°C have been associated with increased mortality and decreased body size of *Ae. albopictus* adults (Alto and Juliano 2001), and temperatures of 28°C compared to 24°C have been associated with decreased adult body size and longevity for some *Culex* species (Ciota et al. 2014). Shorter lifespans reduce the likelihood that female mosquitoes will bite an infected host, and thus shorter-lived females from warmer conditions in 2016 may have contributed to the absence of positive pools that year (Juliano et al. 2014).

West Nile virus MIRs were higher in *Culex* than *Ae. albopictus* in all our study neighborhoods. With only 10 blocks included in the analysis, more data may be necessary to uncover a meaningful correlation between infection rates and abandonment at a finer block scale. *Culex* mosquitoes are the predominant vector for West Nile virus due to their preference for avian hosts (Truempfer and Romero 2007). Blood meal analyses in Baltimore from the same years as our study confirmed that birds were the most frequently detected blood host for *Cx. pipiens* and *Cx. restuans* (Goodman et al. 2018). We found low but consistent infection rates in *Ae. albopictus* despite no evidence of avian hosts in *Ae. albopictus* diets in Goodman et al. (2018). *Aedes albopictus* may be feeding on birds that were not detected by Goodman et al. (2018), or this species could be acquiring West Nile virus infection from small mammals (Padgett et al. 2007). Cats, which contributed 12.4% of *Ae. albopictus* bloodmeals in these neighborhoods (Goodman et al. 2018), may support high enough viremia to infect naïve mosquitoes (Austgen et al. 2004), or

mosquitoes may become infected through cofeeding with viraemic mosquitoes on non-viraemic hosts (Higgs et al. 2005). Thus, *Ae. albopictus*' catholic and aggressive feeding behaviors may bridge West Nile virus to humans.

While documented human West Nile virus cases in the Baltimore Metropolitan Region (MDH 2020) were lower in 2017 ($n = 2$) compared to 2015 ($n = 32$), mosquito infection rates for our neighborhood samples were not reduced in 2017, and even increased in medium-income neighborhoods. Human West Nile virus disease incidence is certainly underestimated, as many human cases of West Nile virus infection are asymptomatic and mild cases in some populations are likely to go undetected (Davis et al. 2006, Malat et al. 2006). It is unclear whether differences in the human case numbers across the years of our study reflect real differences in exposure or unexplained differences in testing and/or severity of disease.

Mosquito surveillance is vital to track the presence of West Nile virus in the environment and should be a basis for understanding local arboviral risk. To our knowledge, this study is the first to document West Nile virus infection in mosquitoes in Baltimore and is one of the first to quantify fine-scale variation in infection rates within a densely populated urban area (Loss et al. 2009, Kilpatrick et al. 2010, Ruiz et al. 2010). Coarse mosquito sampling focused on city parks or other easily accessed sites may miss early and persistent infection in some neighborhoods, especially for mosquito species like *Ae. albopictus* with limited dispersal (Lacroix et al. 2009). Although trap security and accessibility often inform sampling locations, detecting and managing arboviral risk requires surveillance across neighborhoods that vary in socioeconomics, including lower income areas that may be less accessible and secure but have higher infection rates.

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