Vector Status Of Aedes Albopictus In Connecticut: Analyses Of Invasion Pattern, Geographic Distribution, And Disease Risk

Meredith Bagger
mbagger15@gmail.com

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Vector Status of *Aedes albopictus* in Connecticut: Analyses of Invasion Pattern, Geographic Distribution, and Disease Risk

Meredith Bagger

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Public Health in the Department of Epidemiology of Microbial Diseases

Yale School of Public Health

2023

Primary Advisor: Philip M. Armstrong, ScD

Secondary Advisor: Andrea Gloria-Soria, PhD
Abstract

Introduction: *Aedes albopictus* is a highly successful invasive mosquito. Its presence and expansion into Connecticut have implications for human and animal health. The factors limiting *Ae. albopictus* spread and risk by the pathogens it carries have not been previously examined.

Methods: This study leveraged data from the Connecticut Mosquito and Arbovirus Surveillance Program during 2006-2022 and a project designed to characterize mosquito-borne filarial parasite species in Connecticut from 2020-2021. We examined correlations between mosquito abundance and winter temperature as well as land use. We described the arboviruses detected in *Ae. albopictus* collections and calculated the risk of *Ae. albopictus* transmitting *Dirofilaria immitis* to mammals.

Results: *Ae. albopictus* has successfully invaded Fairfield and New Haven counties and spread northward into Hartford County in a 16-year period. Mosquito abundance was significantly correlated with cumulative winter days below 0°C (*r* = -0.73, -0.91 to -0.30) and percentage of developed land use (*r* = 0.73, 0.52-0.86). Only 6 *Ae. albopictus* pools (0.03%) tested positive for arboviruses, including Cache Valley virus, Potosi virus, and West Nile virus. However, *Ae. albopictus* had the highest entomological risk to transmit *D. immitis* among 17 species tested.

Conclusion: Current risk of *Ae. albopictus* to transmit arboviruses in Connecticut is low, in contrast to its likely important role in *D. immitis* transmission. Continued surveillance of *Ae. albopictus* is necessary to monitor its geographic distribution, abundance, and vector status. Future research could model changes in *Ae. albopictus* expansion and potential to transmit diseases due to climate change, urbanization, and globalization.
Acknowledgements

First and foremost, I would like to thank my thesis advisors, Dr. Phil Armstrong and Dr. Andrea Gloria-Soria. Your feedback, mentorship, and expertise were invaluable during my thesis and degree program. Thank you both for the opportunities to participate in your research and the surveillance program at the Connecticut Agricultural Experiment Station, which laid the foundation for this project. I am also grateful to researchers at CAES, including but not limited to John Shepard, Tanya Petruff, and Mike Misencik, who inspired me and provided a significant portion of the data used in this project. I would also like to show my appreciation for my professors, classmates, and the Yale School of Public Health community. Lastly, I would like to thank my friends and family for their endless encouragement and moral support.
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Introduction

*Aedes albopictus* is an invasive mosquito species first detected in Connecticut in 2006 that can transmit many infectious diseases to humans and other animals.\(^1\) It is one of the most successful invasive species in the world due to its temperature tolerance, desiccation-resistant eggs, ability to breed in artificial containers, and aggressive and opportunistic feeding behaviors.\(^2\) Human activity is responsible for the spread of this mosquito from its native range. *Ae. albopictus* was brought to the United States in the 1980s as a result of the used tire trade and has been expanding its range ever since.\(^3\) Southern New England, including Connecticut, represents the thermal limit of this mosquito in the United States.\(^1\) The introduction and expanding range of *Ae. albopictus* poses a significant public health risk because this species can carry and transmit many pathogens.

*Ae. albopictus* has the ability to transmit at least 22 viruses and a few species of filarial parasites.\(^4\) This has been shown by testing field caught mosquitoes, experimentally by infecting mosquito laboratory colonies, and historically through records of outbreaks. The viruses previously isolated in Connecticut that could potentially be spread by *Ae. albopictus* include Eastern Equine Encephalitis virus (EEE), West Nile virus (WNV), Jamestown Canyon virus (JCV), Potosi virus (PTV), La Crosse virus (LACV), and Cache Valley virus (CVV).\(^5,6\) These viruses can cause illness in humans that ranges in severity from asymptomatic infection, febrile illness, severe neurological disease, and to death.\(^7\) Infections with these viruses are rare in humans because they do not play a role in their natural transmission cycle.\(^8\) Among the parasites found in Connecticut that could potentially be spread by *Ae. albopictus* include species from the genus *Dirofilaria*.\(^5,9\) *Dirofilaria* are roundworms that infect mammals and can cause canine heartworm
and pulmonary dirofilariasis in humans.\textsuperscript{10} \textit{D. immitis} is the species most commonly associated with canine and human cases of dirofilariasis in the United States.\textsuperscript{10}

A One Health approach is necessary to evaluate \textit{Ae. albopictus} as a vector of public health concern because the pathogens transmitted by this species are zoonotic and influenced by their vector species, host species, and shared environments. One Health is described as an approach to achieve optimal health outcomes by recognizing the interconnectedness of humans, animals, and their shared environments.\textsuperscript{11} The transmission and development cycles for these pathogens require stages in both mosquitoes and animals such as birds, deer, dogs, and rodents.\textsuperscript{12} Mosquitoes are considered the vector species and animals are the reservoir species. Disease can occur in humans, companion animals, and livestock when spillover outside the natural transmission cycle occurs. Humans and other animals that are not part of the natural transmission cycle are dead-end hosts. Spillover occurs when there are interactions between the vector species, reservoir species, and dead-end hosts. These interactions are facilitated by human activity and factors of their shared environment.\textsuperscript{11} Human populations are expanding and coming into contact more frequently with vector species and reservoir species. As their shared environment changes, the risk of diseases spread by \textit{Ae. albopictus} could also be changing.

Previous research has demonstrated and forecasted changes in the geographic range and abundance of \textit{Ae. albopictus} populations as a result of climate change and urbanization. However, there are gaps in the literature because existing studies were conducted at broader scales. \textit{Ae. albopictus} expanding range and geographic distribution in Connecticut were previously described in 2017 but has not been examined since.\textsuperscript{1} By the end of the 21\textsuperscript{st} century,
*Ae. albopictus* is expected to expand its range north to include the entire state of Connecticut and beyond. Factors expected to contribute to this expanding range include warmer winter temperatures and deforestation for urbanization. Warmer winter temperatures in northern locations allow *Ae. albopictus* eggs to overwinter in areas further north. Urbanization allows this species to expand its range as this mosquito lays eggs in artificial water containers often found in peridomestic environments but not in undeveloped land. Increased temperatures, humidity, and rainfall, consequence of climate change, create optimal habitats that can support larger populations of *Ae. albopictus* and have been associated with an increase in abundance of *Aedes* mosquito species. The relationships between overall mosquito abundance and winter temperature and land cover have previously been estimated in Connecticut. The relationships between *Ae. albopictus* abundance and winter temperature and land cover were previously examined in the New York metropolitan area. However, these relationships were never estimated at the species level for *Ae. albopictus* in all regions of Connecticut. The public health risk by *Ae. albopictus* in Connecticut for human pathogens and for other mammals has not been previously estimated.

This study investigated the status of *Ae. albopictus* as a vector of public health concern. The specific objectives were to 1: describe the invasion pattern and history of *Ae. albopictus* in Connecticut and its relationship with winter temperature, 2: describe the current geographic distribution of *Ae. albopictus* in Connecticut and its relationship with land use patterns, and 3: describe the detection of arboviruses and *D. immitis* in *Ae. albopictus* from Connecticut and estimate the entomological risk indexes of pathogens of public health concern.
Methods

Mosquito Collection and Testing

Adult female mosquitoes were collected as part of the Connecticut Mosquito and Arbovirus Surveillance Program run by the Connecticut Agricultural Experiment Station. This program started in 1997 with 36 trapping sites selected to monitor EEE activity. In 2000 and 2001, in response to the WNV outbreak, the program was expanded to 73 and then 91 trapping locations. These 91 locations were consistently used with more trapping locations added on a seasonal basis to increase coverage and to evaluate EEE and WNV transmission risk. Since 2020, traps were deployed at 108 locations throughout the state. Traps were set on a rotating basis every 10 days and at higher frequency in the event of an arbovirus detection. At all trapping locations CO₂-baited CDC light traps and gravid mosquito traps were used. BG sentinel traps were used only at select locations. Mosquitoes were identified at the species level and consolidated into pools of 50 or fewer individuals based on date, trap type, species, and trapping location.

All mosquito pools collected as part of the Connecticut Mosquito and Arbovirus Surveillance Program were processed and tested for virus presence by passage in tissue culture using Vero cells, followed by virus identification using RT-PCR based assays.¹⁷

The filarial parasite screening study was conducted at the Connecticut Agricultural Experiment station to characterize the parasite species found in Connecticut and identify potential mosquito vector species. Mosquito pools for this study were prepared and tested for the presence of the canine heartworm parasite *Dirofilaria immitis* using RT-PCR and PCR based assays.¹⁸⁻²⁰ Parasite species was confirmed by Sanger sequencing of the C01 and 16S gene
fragment amplified products. Mosquito pools for the *D. immitis* screen were selected for testing based on the following criteria: negative for virus testing, mosquito species known to feed on mammals, an average of 20 individuals per pool, and located in Fairfield and New London counties. The 17 mosquito species selected included: *Ae. albopictus, Ae. cinereus, Ae. vexans, An. punctipennis, An. quadrimaculatus, Cq. perturbans, Cx. pipiens, Cx. restuans, Cx. salinarius, Cs. melanura, Oc. canadensis, Oc. japonicus, Oc. taeniorhynchus, Oc. thibaulti, Oc. triseriatus, Ps. ferox, and Ur. sapphirina*. On average a random sample of 20 pools for each species were selected for the 2020 collection year and an average of 60 pools per species for the 2021 collection year.

**The invasion pattern of *Aedes albopictus* and its relationship with winter temperature**

We examined the presence, number of individuals, and abundance of *Ae. albopictus* by geographic location and year. First, we mapped locations and the number of individuals where *Ae. albopictus* was collected each year (Figure 1). We also plotted mean annual abundance stratified by county over time (Figure 2). Mosquito abundance was calculated from the mosquito collection data as the number of *Ae. albopictus* collected per trap night.

Mosquito traps are available with different designs and methodologies. We evaluated the most effective method for collecting *Ae. albopictus* by comparing mean number of *Ae. albopictus* collected per trap night across the three trap types: CO₂-baited CDC light traps, gravid mosquito traps, and BG sentinel traps. Our analysis only included years and locations where all three trap types were in operation. This covered the years 2017-2022 and 15 trapping locations. The mean number of *Ae. albopictus* collected per trap night for each trap type was compared using Kruskal-Wallis one-way analysis of variance (ANOVA). Then the Pairwise Wilcox
post-test was completed to determine which pair of traps were significantly different from each other.

We examined correlations of temperature variables and mosquito abundance to describe the relationship between *Ae. albopictus* invasion and winter temperature. We obtained temperature data from the National Oceanic and Atmospheric Association National Centers for Environmental Information (NOAA-NCEI). The weather stations selected included: New Haven Tweed Airport, Hartford Bradley International Airport, Bridgeport Success Hill, and Igor Sikorsky Memorial Airport. Mean winter temperature was calculated by averaging mean monthly temperatures for December, January, and February across the weather stations for each year. We correlated mean winter temperature with annual mean *Ae. albopictus* abundance using Pearson’s correlation coefficient. Cumulative winter days below 0°C was calculated by adding days below 0°C for the months of December, January, and February and then averaging across the weather stations for each year. We correlated cumulative winter days below 0°C with annual mean *Ae. albopictus* abundance using Pearson’s correlation coefficient. Mosquito abundance was calculated as number of individuals collected per trap night averaged by year.

**Geographic distribution of *Aedes albopictus* and its relationship with land use patterns**

We obtained the most recent land cover classification data (2015) from the University of Connecticut Center for Land Use Education and Research (CLEAR). This dataset classifies every 30 square meters of land in Connecticut into 12 discrete categories: developed, turf and grass, other grass, agricultural field, deciduous forest, coniferous forest, water, non-forested wetland, forested wetlands, tidal wetland, barren, and utility. We drew 1 Km buffer around the
mosquito trapping locations in ArcGIS Pro and clipped the land cover dataset by this buffer and calculated the percentage of land in the buffer classified as developed for each location. Using Spearman’s rank correlation coefficient, we determined the correlation between the percentage of developed land and the most recent mosquito abundance data from 2022. Mosquito abundance was calculated as the number of individuals collected per trap night averaged by trapping location. We mapped presence and absence of *Ae. albopictus* at each trapping location onto a map of land cover classification (Figure 3).

To further describe the current geographic distribution of *Ae. albopictus* and its relationship with land use patterns, we mapped *Ae. albopictus* trapping locations onto a map of human population density by town (Figure 4). The most recent population density data (2021) was obtained from the Connecticut Department of Health. To calculate population density, we divided total population size per town by the town area. We examined the correlation of human population density by town and the most recent mosquito abundance data from 2022 using Spearman’s rank correlation coefficient. Mosquito abundance was calculated as number of individuals collected per trap night averaged by town.

**Arbovirus and *Dirofilaria* infection in *Aedes albopictus* and entomological risk indexes**

We obtained arbovirus infection information from the Connecticut Mosquito and Arbovirus Surveillance Program and summarized the results for *Ae. albopictus* together with results from the *Dirofilaria* screening study in Table 1. We calculated the entomological risk index of *D. immitis* for *Ae. albopictus* and other mosquito species that tested positive for the parasite including *Ae. cinereus*, *An. punctipennis*, *An. quadrimaculatus*, *Oc. canadensis*, *Oc. japonicus*, *Oc. taeniorhynchus*, *Oc. thibaulti*, *Oc. triseriatus*, and *Ps. ferox* (Table 2). We adapted
the entomological risk index formula from the formula developed by Kilpatrik et al. Entomological risk index is an estimate of the risk or probability a species of mosquito will infect a mammal with *D. immitis* and is calculated by: 

$$ERI = A \times P \times F_m$$

where A is mosquito abundance, P is the infection prevalence, and $F_m$ is the fraction of blood meals taken from mammals. We calculated mosquito abundance (A) as the mean number of individuals of a particular species collected per trap night. We calculated infection prevalence (P) as the maximum likelihood estimate (MLE). MLE is the infection rate of *D. immitis*, an estimate of the number of infected mosquitoes per 1000 tested. We calculated MLE using the PooledInfRate R software package developed by the Centers for Disease Control and Prevention. The fraction of blood meals taken from mammals ($F_m$) was obtained from host-feeding pattern literature. In Figure 5 using a bar plot, we visually compared the entomological risk indexes of *D. immitis* for mosquito species tested. We calculated percent risk for each species in Table 2 by dividing the entomological risk index for a given species by the sum of entomological risk for all species.

**Results**

**The invasion pattern of *Aedes albopictus* and its relationship with winter temperature**

The first *Ae. albopictus* individual was an adult female trapped in West Haven, Connecticut in 2006. Since 2010 this species has been collected consistently each year. To date 15,368 individuals have been collected in Connecticut at 51 trapping locations, 41 towns, and 7 counties. As depicted in Figures 1 and 2, *Ae. albopictus* was introduced in Fairfield and New Haven counties and then spread up the Southern coast of Connecticut and northward into Hartford County. There was a steady increase in cumulative numbers of individuals and
abundance every year from 2010-2018, with a large increase observed in 2016, particularly in Fairfield County. Between 2019-2022 there was an overall decline in number of individuals collected, a decline in abundance in Fairfield County, and an increase in abundance in the other counties. Trapping effort was increased in 2016 when a location in Bridgeport was added that routinely collects *Ae. albopictus*. From all *Ae. albopictus* ever collected in Connecticut, 37% were trapped at this Bridgeport site, the highest percentage of any site. Trapping effort was also increased in 2017 with the consistent use of BG sentinel traps at 15 trapping locations.

![Figure 1. Series of Connecticut maps showing the geographic locations and number of adult female *Aedes albopictus* individuals collected during 2006-2022. Black circles indicate the location of collections and are scaled by number of individuals collected.](image)

We compared the mean number of individuals per trap night for the BG sentinel trap (mean=0.40/ trap night), gravid mosquito trap (mean=0.08/ trap night), and CO₂-baited CDC light trap (mean=0.31/ trap night) to evaluate their effectiveness at collecting *Ae. albopictus*. Pairwise comparison determined significant differences between all trap types (Kruskal-Wallis test).
ANOVA p-value < 0.001), with BG sentinel traps being the most successful at trapping *Ae. albopictus*.

We evaluated the relationship of mosquito abundance with winter temperature because it is one of the major limitations of *Ae. albopictus* invasion and spread. The cumulative number of winter days below 0°C each year ranged from 57 to 83. The mean winter temperature each year ranged from -2.38°C to 2.75°C. During 2010-2022, the year 2016 had the warmest winter measured in both cumulative days below 0°C and mean temperature. We found a significant negative correlation of mean annual abundance and cumulative winter days below 0°C ($r = -0.73, -0.91$ to $-0.30$) (p=0.005). We also found a small significant positive correlation between mean annual abundance and mean winter temperature ($r = 0.58, 0.04-0.86$) (p=0.04).

**Geographic distribution of *Aedes albopictus* and its relationship with land use patterns**

We examined the relationship of mosquito abundance and land use patterns because habitat type and availability likely influence *Ae. albopictus* invasion and expansion. As depicted in Figure 3, the majority of trapping locations where *Ae. albopictus* was collected are classified as developed land. The percent land cover classified as developed for the locations where *Ae. albopictus* was collected ranged from 9%-96%. The trapping location in Bridgeport had the
highest percentage of developed land cover. We found a significant positive correlation between percentage of developed land cover and mean *Ae. albopictus* abundance by trapping location ($r = 0.73, 0.52-0.86$) ($p<0.001$).

![Figure 3. Map of Connecticut land cover classification from 2015 and mosquito trapping locations. Every 30 square miles of land was classified into 12 discrete categories and assigned a unique color. Black circles indicate locations where *Aedes albopictus* has been collected and white circles indicate locations where *Aedes albopictus* has never been collected.](image)

We also examined the relationship between mosquito abundance and human population density because *Ae. albopictus* is peridomestic. Developed land cover, as examined previously, does not always imply locations of human habitats but land disturbed by humans.

As depicted in Figure 4, the majority of trapping locations where *Ae. albopictus* was collected are found in Connecticut towns with high human population density. The town human population density ranged from 178 persons per sq mile to 9,271 persons per sq mile, with Bridgeport having the highest human population density. We found a weak significant positive
correlation between human population density by town and mean *Ae. albopictus* abundance by town (*r* = 0.49, 0.13-0.74) (*p*=0.01).

![Map of Connecticut population density (persons per square mile) in 2021 by town and mosquito trapping locations. Towns are colored by a gradient with darker colors indicating higher population density. Black circles indicate locations where *Aedes albopictus* has been collected and white circles indicate locations where *Aedes albopictus* has never been collected.](image)

**Arbovirus and *Dirofilaria* infection in *Aedes albopictus* and entomological risk indexes**

*Ae. albopictus* has the ability to transmit many pathogens of public health concern to mammals including humans. We examined the virus testing results from the Connecticut Mosquito and Arbovirus Surveillance Program to determine the arbovirus species carried by *Ae. albopictus* in Connecticut. Between 2006-2022, 6 pools of *Ae. albopictus*

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Year</th>
<th>Town</th>
<th>No. Positive Sites</th>
<th>No. Positive Pools</th>
</tr>
</thead>
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<tr>
<td>D. immitis</td>
<td>2020, 2021</td>
<td>Bridgeport</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>CVV</td>
<td>2014</td>
<td>Stratford</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>PTV</td>
<td>2017</td>
<td>West Haven</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>WNV</td>
<td>2016, 2018</td>
<td>Bridgeport</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

*Note: CVV = Cache Valley Virus, PTV = Potosi Virus, WNV = West Nile Virus*

*Table 1. Pathogens detected in collections of Aedes albopictus in Connecticut. All collections of Aedes albopictus were tested for virus identification during years 2006-2022. Select collections of Aedes albopictus were tested for D. immitis presence during years 2020-2021.*
(0.03%) tested positive for arboviruses. As depicted in Table 1, *Ae. albopictus* tested positive for Cache Valley virus (CVV), Potosi virus (PTV), and West Nile virus (WNV). CVV was isolated twice at one trapping location in Stratford in 2014. PTV was isolated twice at one trapping location in West Haven in 2017. WNV was isolated twice at one trapping location in Bridgeport, once in 2016 and once in 2018.

We also examined the *D. immitis* testing results from the filarial parasite screening study conducted between 2020-2021. As shown in Table 1, we identified *D. immitis* in 12 pools of *Ae. albopictus* (14%) at one trapping location in Bridgeport. In 2020, 7 pools (13%) tested positive for *D. immitis*. In 2021, 5 pools (17%) tested positive for *D. immitis*. However, *Ae. albopictus* was not the only mosquito species that tested positive for *D. immitis* in Connecticut.

We calculated the entomological risk index of *D. immitis* for mosquito species known to feed on mammals in Connecticut (Table 2). As seen in Figure 5, *Ae. albopictus* had the largest entomological risk index, which was almost double the risk of the species with second largest. *Ae. albopictus* accounted for ~40% of the risk of dirofilariaisis in Connecticut during 2020-2021. In addition, *Ae. albopictus* had the largest infection rate or maximum likelihood estimate compared to other species.

Table 2. Summary of *D. immitis* testing and risk in select pools of mammal feeding mosquito species in Connecticut during years 2020-2021.
Discussion

This study examined the status of the invasive mosquito *Ae. albopictus* as a vector of public health concern in Connecticut. First, we described its invasion pattern and its relationship with winter temperature. Second, we described its current geographic distribution and its relationship with land use. Lastly, we described the pathogens identified and estimated their entomological risk indexes.

*Ae. albopictus* invaded Connecticut from the southwest and spread northward, with population size growing each season. Mean annual abundance declined after 2018 but its geographic distribution widened. This trend suggests that the population is stabilizing and becoming permanent. The population genetics of Northeastern *Ae. albopictus* are in agreement with this trend and genetic evidence supports the presence of established overwintering populations.25

In this study the most successful trap type at collecting *Ae. albopictus* was the BG sentinel trap when compared to the CO2-baited CDC light trap and gravid mosquito trap. BG sentinel traps were specifically designed to capture this species of mosquito and our results support its effectiveness.26 This finding agrees with other studies that evaluated trap...
performance at collecting *Ae. albopictus* in locations with longer established permanent populations.\(^{17,18}\)

We found a relationship between mosquito abundance and winter temperature, consistent with previous research establishing winter temperature as one of the major limiting factors in the northern range expansion of *Ae. albopictus*.\(^{1,20,30}\) Mean winter temperature was identified as the strongest environmental predictor of *Ae. albopictus* range in the US.\(^{13}\) Mean winter temperature is also routinely used to calculate thermal limits of *Ae. albopictus*.\(^{31}\) However, in our study, we found a weak positive correlation between abundance and mean winter temperature. Mean winter temperature may not accurately represent winter temperature because it masks extreme temperature events. We found a strong correlation between cumulative winter days below 0°C and mosquito abundance. As there are more winter days below freezing, the subsequent summer has fewer *Ae. albopictus*. Cumulative winter days below 0°C are a better measure of winter temperature because it takes into account extreme temperature events. Warmer winters and extreme temperature events are expected to increase in frequency as a result of climate change, which could continue to support larger *Ae. albopictus* populations.\(^{32}\)

We also found a relationship between mosquito abundance and developed land use, which is consistent with previous research.\(^{13,24}\) *Ae. albopictus* is a container breeding species that prefers peridomestic habitats and its movement is associated with human activity.\(^2\) In this study, we identified a strong positive correlation between mosquito abundance and percentage of developed land cover. The greater the percentage of developed land cover, the larger abundance of *Ae. albopictus*. Developed land provides ample breeding sites and preferred
blood meal sources, such as humans and domestic animals, for *Ae. albopictus*. Developed land implies deforestation for urbanization, which has increased over time.\textsuperscript{13} Also, we identified a weak positive correlation between mosquito abundance and human population density. Therefore, the greater the human population density, the larger abundance of *Ae. albopictus*. This is consistent with other studies that found a difference in mosquito abundance comparing urban and suburban sites with rural sites.\textsuperscript{33} The available human population density data we used is aggregated by town, compared to the land use cover data which is specific to the 1 Km area surrounding the trap. The difference in resolution may explain the differences in our ability to detect correlations. Urbanization and human population density are both expected to increase because of globalization and exponential human population growth, which will continue to favor habitats for *Ae. albopictus*, promoting larger populations and range expansion.\textsuperscript{32}

In this study CVV, PTV, and WNV were isolated from *Ae. albopictus*, indicating it could play a role as an arbovirus vector in Connecticut. Virus isolations from *Ae. albopictus* are rare in Connecticut: only 6 pools tested positive for any virus out of 3,525 pools tested (0.03%). CVV was also detected in *Ae. albopictus* populations in the surrounding states New York and New Jersey.\textsuperscript{26, 27} WNV was isolated from *Ae. albopictus* populations in the nearby states New Jersey and Pennsylvania.\textsuperscript{36, 37} Prior research has demonstrated North American populations of *Ae. albopictus* are competent vectors for WNV.\textsuperscript{38} Beyond the three viruses previously isolated, populations of *Ae. albopictus* from Connecticut have been shown to be susceptible to and transmit Zika, Dengue, and Chikungunya viruses in laboratory settings.\textsuperscript{39} Therefore, *Ae. albopictus* in Connecticut could be a potential vector for these three viruses and under optimal
conditions a travel-associated case of one of these viruses could lead to an outbreak. Travel-associated cases of arboviruses may become more frequent in Connecticut and neighboring states with ever increasing globalization. However, the few numbers of positive pools and sporadic isolations suggest its current role as an arbovirus vector in Connecticut is limited. Continued trapping and testing of *Ae. albopictus* in Connecticut is warranted to monitor its role and prevent transmission of arboviruses of public health concern.

We determined *Ae. albopictus* contributed the highest entomological risk of *D. immitis* among the mammal feeding mosquito species in Connecticut meaning it has the highest risk of infecting a mammal with *D. immitis* compared to the other species. In addition, *Ae. albopictus* had the largest infection rate or maximum likelihood estimate compared to other species. Therefore, *Ae. albopictus* is the most likely mosquito species to acquire *D. immitis* in Connecticut. Other states such as Oklahoma, Florida, and Georgia have also identified *Ae. albopictus* as a primary vector of *D. immitis*.18,40,41

*D. immitis* is the pathogen that causes canine heartworm and human pulmonary dirofilariasis. The number of canine heartworm cases in Connecticut has increased during the last decade.42 This trend could in part be explained by the successful invasion and expansion of *Ae. albopictus* in Connecticut. Prevalence of canine heartworm could continue on its trajectory and worsen over time in part due to *Ae. albopictus*, but also because dog ownership in the US is increasing with an estimated pet dog population in 2020 of 83.7 million.43 Dogs are companion animals and provide services so their health is a function of public health. Dogs improve human health by acting as service dogs, provide opportunities for exercise, and improve mental health by reducing depression, anxiety, and loneliness.44–47 In addition, humans can develop
Pulmonary dirofilariasis and 6 human cases have been reported in Connecticut as of 2005. Pulmonary dirofilariasis is not life threatening to humans and is often asymptomatic but can lead to unnecessary invasive procedures to exclude more serious differential diagnoses such as lung cancer. Continued monitoring of *Ae. albopictus* populations in Connecticut is necessary to estimate the risk of *D. immitis* infection to dogs and humans.

This study had a few limitations. The first limitation is the temperature data obtained from NOAA-NCEI weather stations had missing data. However, averaging the temperature data across the 4 weather stations reduces the impact of missing data and the overall trend in temperature across the weather stations was similar. A second limitation is the most recent land use data from CLEAR was updated in 2015. Data from 8 years ago could be considered outdated, although it is unlikely that land use changed significantly in that time. A third limitation is the correlation analyses do not take into account potential confounders and mediators that could be distorting the true relationship between mosquito abundance and winter temperature. Winter temperature might not be sufficient to explain the history of *Ae. albopictus* abundance in Connecticut. As identified in a previous study, potential confounders or mediators of this relationship include other climate variables such as precipitation, humidity, and snow cover. Another limitation is the sample sizes for arboviruses detected were too small to calculate their entomological risk indexes. During the 16-year period, only 6 of *Ae. albopictus* pools (0.03%) were positive for any arboviruses. Finally, the entomological risk indexes calculated for *D. immitis* is spatially and temporally context specific. This study examined entomological risk at a broad scale of over two years throughout two counties in the state of Connecticut. Entomological risk magnitude might differ and other vector species might
be contributing more risk on a more local scale or during different periods of the transmission season.

In conclusion, *Ae. albopictus* has successfully invaded and spread throughout Connecticut in a 16-year period. This invasion and expansion are correlated with winter temperature and developed land use. *Ae. albopictus* should be considered a vector of public health concern in Connecticut because it carries arboviruses and is the mosquito species with the highest entomological risk for *D. immitis*. Its expansion throughout the state and potential to transmit pathogens is expected to continue with ever increasing climate change, urbanization, and globalization. This will present challenges to public health agencies to surveil and prevent diseases spread by *Ae. albopictus*. Future research could aim to project and model these trends. Continued trapping and testing of *Ae. albopictus* in Connecticut is necessary to monitor the geographic distribution, abundance, and vector status of this highly invasive species.
References


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