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A SURVEY FOR *AEDES AEGYPTI* IN DELAWARE AND THE VIRUS-POSITIVE POOL RATES OF *AEDES ALBOPICTUS* AND *AEDES TRISERIATUS* FOR WEST NILE AND ZIKA VIRUSES

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ABSTRACT. The introduction of Zika virus to the USA in 2015 engendered heightened interest in its known vectors. *Aedes aegypti* is the primary vector, with *Ae. albopictus* considered a potential secondary vector, together with several other possible marginal vectors. In Delaware, *Ae. aegypti* has been collected rarely, but no breeding populations were detected during past intensive statewide surveillance efforts. However, there is an abundance of *Ae. albopictus* statewide. Both species are container breeders and are peri-domestic—increasing the risk for virus transmission to humans. From July through September 2017, Delaware Mosquito Control conducted surveillance in 16 container-breeding hot spots to search for *Ae. aegypti*, and also ascertain the virus-positive pool rates of *Ae. albopictus* and *Ae. triseriatus* for West Nile virus (WNV) and Zika virus (ZIKV). The survey concluded that there were no known breeding populations of *Ae. aegypti* in Delaware, and no WNV- or ZIKV-positive pools were detected among pools of mosquitoes of the aforementioned species.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, *Aedes triseriatus*, West Nile virus, Zika virus

INTRODUCTION

In 2016, Zika virus (ZIKV) became a nationally notifiable condition in the USA (CDC 2016). From 2016 to 2018, 5,684 total Zika cases were reported, including 5,398 travel-related cases, 231 mosquito-related cases, and 55 other transmission types, such as sexual (CDC 2018a). Significantly, the peak of mosquito-related cases (224) occurred in 2016, with only 7 in 2017, and none in 2018. To date, all mosquito-related cases were reported from Texas or Florida. In Delaware, there were 17 travel-related cases in 2016, with none occurring since then (CDC 2018a). *Aedes aegypti* (L.) is most likely to transmit ZIKV, while *Ae. albopictus* (Skuse) is a potential vector (ECDC 2017a, 2017b).

Because *Ae. aegypti* and *Ae. albopictus* are both peri-domestic species, living conditions play a major role in their human vector status (Moreno-Madrinan and Turell 2017). Important factors include human feeding preference, container prevalence, and human exposure time to mosquito biting. These factors indicated that open access to homes permits *Ae. aegypti* and *Ae. albopictus*, which prefer human blood, to obtain more blood meals, though *Ae. aegypti* is far more anthropophilic than *Ae. albopictus* (Reiter et al. 2006). High abundance of *Ae. albopictus*, as was reported by Delaware Mosquito Control Section staff while fielding mosquito complaints, led to intensified studies of *Ae. albopictus* as a potential vector species. However, the general observations of the Mosquito Control Section field staff indicated that most homes have air conditioning and/or screened windows, likely diminishing the potential risk for mosquito-borne diseases.

Aedes aegypti, *Ae. albopictus*, and *Ae. triseriatus* (Say) are all theoretically capable of transmitting West Nile virus (WNV), based on laboratory studies by Turell et al. (2005). The 1st human infection of WNV in Delaware occurred in 2002, and WNV remained in circulation in Delaware through 2018 (CDC 2018b). In 2018, WNV was found in 10 humans, this being the highest case total in Delaware since 2003 (CDC 2018b). While each species is capable of transmission, their host-feeding patterns limit WNV potential transmission to humans. *Aedes aegypti* feed almost entirely on humans and other mammals, while *Ae. triseriatus* feed mostly on nonhuman mammals, and *Ae. albopictus* mainly feed on humans and other mammals (Burkot and Defollart 1982, Nasci 1985, Gingrich and Williams 2005, Ponlawat and Harrington 2005, Faraji et al. 2014). Both *Ae. triseriatus* and *Ae. albopictus* occasionally feed on wild birds, but only a small proportion of avian species produce sufficient WNV viremia to promote viral infections in mosquitoes (Pérez-Ramírez et al. 2014), limiting their human vector potential compared to *Culex pipiens* L.

Nevertheless, the potential introduction of *Ae. aegypti* to Delaware is a major concern for mosquito control entities. The primary concern with *Ae. aegypti* is its ability to carry multiple arboviruses, including ZIKV, dengue virus (DENV), yellow fever virus, and chikungunya virus (CHIKV) (ECDC 2017b). Despite no proven indigenous human transmission of ZIKV in Delaware, it and neighboring states continue to show low levels of travel-related ZIKV (Delaware Division of Public Health,

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unpublished data 2018), underscoring the need to continue surveillance for *Ae. aegypti*.

Aedes aegypti has been discovered twice in Delaware since Delaware Mosquito Control was formed in the early 1970s, with single specimens twice collected in Seaford in June 2012, and in Milford Neck in June 2015 (Gingrich, unpublished data). The discoveries were deemed incidental travel-related introductions, because intensive surveillance at these sites detected no larval breeding *Ae. aegypti* or additional adult specimens. The closest known established population of *Ae. aegypti* exists year-round underground in the Capitol Hill neighborhood of Washington, DC, while isolated single specimens were collected in nearby Alexandria and Fairfax County (Virginia) and Prince George's County in Maryland annually since 2011 (Gaines, personal communication).

The establishment of invasive *Ae. aegypti* in Delaware largely depends on competition with *Ae. albopictus*. Before *Ae. albopictus* became established in the USA, *Ae. aegypti* commonly bred in artificial containers in Florida because its preferred urban breeding sites were scarce. As *Ae. albopictus* spread, it mostly replaced *Ae. aegypti* in artificial containers (Braks et al. 2003). In Delaware, largely lacking highly urbanized areas except Wilmington, surveillance shifted to other sites where *Ae. aegypti* might breed. Indeed, in Wilmington during 2016, our study was conducted across the city, using oviposition traps to survey 30 sites for *Ae. aegypti*, with none found (Gingrich and Brinson 2017) among thousands of *Ae. albopictus* detected. After 2016, our focus shifted to habitats deemed favorable to both species.

Aedes albopictus was first discovered in Delaware in 1987 (Moore et al. 1988). It has since spread across the state and recently become a concern due to its potential to spread ZIKV in addition to CHIKV, DENV, and WNV (ECDC 2017b). West Nile virus antibodies are commonly detected in sentinel chickens in Delaware each year (Delaware Division of Public Health, unpublished data 2018). While *Ae. albopictus* is judged a weak vector of WNV, it feeds minimally on wild birds (Turell et al. 2001). Still, having found *Ae. albopictus* infected in Montgomery County, PA, in 2000 (Holick et al. 2002), and more recently in Virginia (Gaines, personal communication, 2017) and New Jersey (Reed, personal communication, 2017), highlight its possible infection with WNV in nature. Absent an established *Ae. aegypti* population in Delaware (its earlier presence was inferred based on periodic yellow fever outbreaks in nearby ports such as Philadelphia and Baltimore from the 1700s to the 1820s [Eisen and Moore 2013]), our emphasis shifted to *Ae. albopictus* and its possible infection with ZIKV, while still being vigilant for *Ae. aegypti*. In the greater Washington, DC area, with a climate similar to Delaware, extensive testing was performed on *Ae. albopictus* for ZIKV. Out of approximately 66,000 *Ae. albopictus* tested in northern Virginia during 2017, one-third of which were collected near areas with active ZIKV cases, none were positive for ZIKV (Gaines, personal communication).

MATERIALS AND METHODS

Sixteen surveillance sites were chosen across the state with data from Delaware's Mosquito Control Section, including personal mosquito distribution knowledge from its biology staff. Eight of the sites were located from Smyrna north to Wilmington, while the other 8 sites selected were located south of Smyrna, extending close to the state line near Seaford, DE (Fig. 1). All sites contained suitable container-breeding habitats and shaded areas for optimal trap collections. Tires, cans, and bottles were by far the most common breeding sites for larvae. The sites were chosen to cover the state geographically, and also to monitor high-risk areas such as ports and other sites where *Ae. aegypti* would be suspected to occur.

Each of the 16 sites across the state of Delaware were monitored by 3 types of traps: modified Centers for Disease Control and Prevention (CDC) Miniature Light Trap (CDC traps; J.W. Hock Co., modified Model 512, Gainesville, FL), Gravid *Aedes* Traps® (GATs; Biogents AG, Regensburg, Germany), and oviposition traps; black ovitraps (generic 12-oz [348-ml] plastic drink cups). The CDC traps were baited with a BG-Lure® (Biogents AG) and CO_2 tanks emitting between 250 and 300 ml/min near the fan intake, which was 18–30 inches (45–75 cm) above the ground. The lights on the traps were removed and timers set to run during daylight hours (see below) in order to target day-biting *Aedes* species. The mosquitoes were trapped in a collection cup and terminated by one-tenth of a Hot Shot Pest Strip® (Spectrum Brands, Earth City, MO) containing dichlorvos. Each site contained 1 or 2 CDC traps, according to their area and estimated importance as possible *Ae. aegypti* sites or West Nile hot spots. The GATs were baited with a red oak leaf–infusion water which was aged at least 2 wk. Oak leaf–infusion water contains substances which are attractive to gravid *Aedes* mosquitoes (Trexler et al. 1998), while the black color of the trap also serves as an attractant (Hoel et al. 2011). The kill method used in the GATs was one-tenth of a Hot Shot Pest Strip, like the CDC trap method. The ovitraps were also baited with the same red oak leaf infusion as the GATs. Brown-color seed paper (northern collection sites) or red-velour coated paper (southern collection sites) were used for the collecting of oviposited eggs. The CDC traps were deployed for 3 successive days/wk, set with timers that activated fans and CO_2 flow during times of high mosquito activity: between 6–8 a.m. and 4–9 p.m. for each day. The GATs were deployed for 3 days/wk, while ovitraps were deployed for 7 days/wk.

Mosquito collections and eggs were identified using binocular stereoscopic microscopes at the

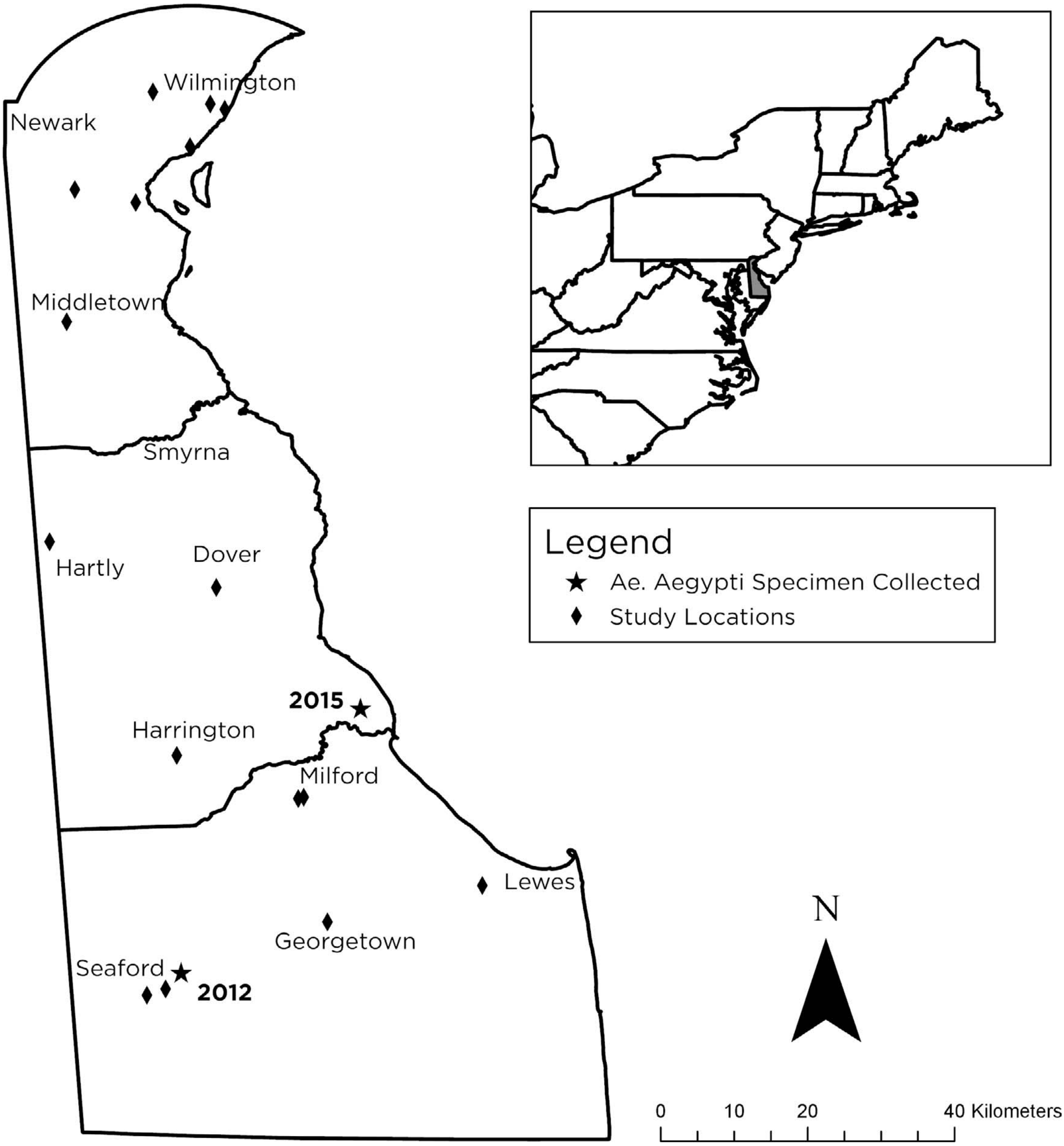

Fig. 1. The map depicts the locations of 16 collection sites for our 2017 study throughout Delaware. Two sites where single specimens of *Aedes aegypti* were collected in 2012 (Seaford) and 2015 (Milford Neck) are also shown (see legend).

Mosquito Control Sections in Glasgow and Milford. Taxonomic keys used were by Harrison et al. (2016). All surfaces in contact with mosquitoes were new from the factory (filter paper) or had been surface-cleaned using 95% ethanol (specifically forceps). On average, 2 pools of 25 *Ae. albopictus* from each site were sent weekly to the Delaware Division of Public Health Laboratory for analysis, with additional pools submitted from sites with large collections or presumed higher probability of infection. The mosquito pools were tested for WNV and ZIKV, using standard quantitative polymerase chain reaction methods (Lanciotti et al. 2008) as performed by our Delaware State Division of Public Health Laboratory in Smyrna, DE.

Eggs laid on seed paper were left to dry in a dark enclosed area at room temperature (25°C) with high relative humidity. The eggs were dried for about 5 days before being submerged in dechlorinated water within rearing chambers (Bioquip, Rancho Dominguez, CA) with about 0.01 g of 3:1 yeast:liver powder mix to stimulate egg hatching. Chambers

Table 1. Numbers of *Aedes albopictus* and *Ae. triseriatus*[1] collected and tested for West Nile virus and Zika virus at 8 sites in northern (North) and 8 sites in southern (South) Delaware during July 5, 2017, to September 25, 2017.

	North sites		South sites	
Date	*Ae. albopictus* (tested)	*Ae. triseriatus* (tested)	*Ae. albopictus* (tested)	*Ae. triseriatus* (tested)
July 5	1,711 (245)	13 (0)	2,366 (295)	13 (0)
July 10	2,138 (316)	3 (0)	3,196 (220)	16 (0)
July 17	2,668 (458)	29 (19)	2,048 (557)	10 (0)
July 24	3,562 (506)	24 (6)	2,504 (656)	12 (0)
July 31	1,093 (583)	20 (36)	1,245 (362)	12 (0)
August 7	2,213 (595)	10 (0)	1,992 (515)	19 (27)
August 14	2,696 (588)	14 (24)	3,066 (588)	38 (15)
August 21	3,323 (600)	15 (0)	3,359 (600)	19 (0)
August 28	1,893 (588)	14 (0)	1,891 (618)	48 (0)
September 4	811 (275)	5 (0)	1,821 (483)	19 (16)
September 11	1,294 (588)	6 (0)	1,714 (525)	28 (46)
September 18	806 (613)	7 (0)	3,081 (525)	63 (53)
September 25	1,899 (618)	19 (0)	2,500 (566)	40 (20)
Totals				
Sum by area, species	26,107 (6,573)	179 (85)	30,783 (6,510)	337 (177)
Sum all	57,406 (13,345)			

[1] Pools of *Ae. triseriatus* were held up to 2 wk to attain pool sizes of 15–25 mosquitoes.

were provided with this same mixture every Monday, Wednesday, and Friday for the 1st 2 wk and Monday and Friday for wk 3–4. Emerged mosquitoes were frozen and identified by just after the end of wk 4 in the northern collections (28 days), or until the completion of the entire study (southern collections). The non-*Aedes* larvae were discarded. Proportions of emerged adult mosquitoes were calculated separately from northern and southern areas.

RESULTS

Our study concluded there were no evident breeding populations of *Ae. aegypti* in the state of Delaware during 2017. The survey included rigorous monitoring of the state across 16 locations with multiple trap types, covering the state geographically as well as targeting likely introduction sites such as ports. In addition to the monitoring through our study, the Delaware Mosquito Control Section routinely monitors 24 sites with New Jersey Light Traps. Moreover, Dover Air Force Base has a mosquito control unit which monitors for introduction of nonindigenous species and human mosquito-borne pathogens. Monitoring across the state has not acquired specimens of *Ae. aegypti* in 2017 or 2016 with routine and special monitoring. Having found no specimens of this species among 57,407 day-biting mosquitoes trapped, it appears highly unlikely that there is currently a year-round breeding population of *Ae. aegypti* in Delaware.

In the samples of *Ae. albopictus* and *Ae. triseriatus* sent to the Delaware Division of Public Health Laboratory, no RNA of ZIKV or WNV was detected. There were 13,213 *Ae. albopictus* and 262 *Ae. triseriatus* tested, out of 57,407 trapped combined (513 were *Ae. triseriatus*—see Table 1). During the survey, gravid females were targeted with GATs to increase the likelihood of capturing a positive mosquito. From a period of June 30 to August 31, GAT collections were monitored for gravidity. Of 658 *Ae. albopictus* trapped, 2.4% were found to be gravid (defined as containing eggs in ovaries during dissection), while 96.1% of 76 *Ae. triseriatus* trapped were gravid (see Table 2). For both species, few males were found in GATs, with 7.8% of *Ae. albopictus* and 4.0% of *Ae. triseriatus* being males. There were also no detections of either virus in females of either species, gravid or otherwise.

Table 2. Gravid rates in gravid *Aedes* traps for *Aedes albopictus* and *Ae. triseriatus*[1] from July 20, 2017, to August 25, 2017.

	Ae. albopictus			*Ae. triseriatus*		
Location	Total[2]	Male	Gravid female	Total	Male	Gravid female
Middletown	52	0	1	1	0	1
Hartley	27	0	1	4	0	4
Delaware City	28	3	0	0	0	0
Glasgow	68	2	4	12	0	12
New Castle	149	2	5	12	1	11
Port of Wilmington	202	31	2	0	0	0
Elsmere	46	5	2	47	2	45
Terminal Avenue	86	8	1	0	0	0
Total	658	51	16	76	3	73
Percentage of total		7.8	2.4		4.0	96.1

[1] Pools of *Ae. triseriatus* were held up to 2 wk to attain pool sizes of 15–25 mosquitoes.

[2] Nongravid females accounted for 89.8% (*Ae. albopictus*) and 0.0% (*Ae. triseriatus*) of total trap counts.

The following data include 8 northern sites only with collections from July 3 through July 31: There were 7,601 eggs collected by oviposition traps over the 8 sites, 1,106 of which had emerged (14.6%). The low emergence rate can be attributed to the density of larvae in the rearing chambers with the short period of time in which they were monitored; i.e., 3–4 wk. Of those that hatched, the dominant species was *Ae. albopictus* (93.1%), with a much smaller proportion of *Ae. triseriatus* (2.8%) and *Ae. japonicus japonicus* (Theobald) (4.1%). *Aedes albopictus* were usually among the first to hatch, with any *Ae. triseriatus* and *Ae. j. japonicus* hatching several days later.

DISCUSSION

The likelihood of encountering a population of *Ae. aegypti* in its cool geographic range largely depends on recent weather patterns. The winters of 2017 and 2016 in Delaware were among the warmest on record in the past 121 years. The winter of 2017 ranked 4th warmest, while 2016 ranked 3rd (NCEI 2017). This may contribute to the recent establishment of the species in nearby Washington, DC. If *Ae. aegypti* were introduced to Delaware in the past 3 years the weather may have enhanced their chance of survival. Meanwhile, the highly competitive *Ae. albopictus* thrived throughout the state, likely influenced by the combination of its greater cold-hardiness and mild winters.

Although only 13,345 of 57,406 mosquitoes trapped (23.2%) were analyzed because of cost considerations, it is still significant that no mosquitoes tested positive for either WNV or ZIKV. We feel the null detection of viruses tested for strongly suggests, subject to the limitations of sample size, that these 3 species were not high-risk vectors of WNV or ZIKV in 2017.

Eisen et al. (2014) found that the success of *Ae. aegypti* overwintering also depends on exposure to weather, especially rain and snow events in addition to freezing and thawing. This likely contributes to the success of *Ae. aegypti* in urban areas, where they may seek refuge in open basements or underground utilities which shield them from weather. While the 2016 Wilmington study did not find any occurrences of *Ae. aegypti* within these habitats, there remain potential refuges in homes, sheds, and workshops across the state, especially those that are unexposed to freezing temperatures. This study aimed to determine presence/absence of the species by surveying a very extensive geographic range of Delaware.

The current study utilized the well-known CDC trap and ovitrap (generic) as well as the newer GATs for detection. While some earlier studies did not have great success collecting *Ae. albopictus* in CDC traps, the modifications made for this study proved to be highly effective in targeting this species. The black-colored ovitraps containing oak leaf–infusion water also proved effective in surveying for container-inhabiting species, with all sites resulting in oviposition week over week from July to September. The less proven GATs did not have the same success as the CDC and oviposition traps. In addition to the low number of mosquitoes collected, very few *Ae. albopictus* in the trap were gravid, making the trap ineffective for monitoring the infection rate of the species in Delaware. However, the GATs yielded high gravidity with *Ae. triseriatus*, with nearly all mosquitoes collected being gravid. This result may perhaps be attributed to its resemblance to a tree hole, the preferred habitat of *Ae. triseriatus*, by employing a small, dark-colored opening, leading to a larger cavity of oak leaf–infused water.

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