Short Communication

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Detection of *Aedes* (*Stegomyia*) *aegypti* (Diptera: Culicidae) Populations in Southern Alabama Following a 26-yr Absence and Public Perceptions of the Threat of Zika Virus

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Abstract

With the establishment of Zika virus in the Americas, an accurate understanding of the geographic range of its primary vector, *Aedes* (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae), is vital to assessing transmission risk. In an article published in June 2016, Hahn and colleagues compiled county-level records in the United States for the presence of *Ae. aegypti* and *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) reported between January 1995 and March 2016. Despite ecological suitability for both mosquito species along the Gulf Coast, *Ae. aegypti* was not reported in Alabama during the time interval, a result consistent with research suggesting that interactions between these two species often result in displacement of *Ae. aegypti*. Herein, we report the detection of *Ae. aegypti* populations in Mobile, Alabama, after a 26-yr absence and present findings on human perceptions of Zika virus relevant to transmission. It is unclear whether the specimens (69 out of 1074) represent a recent re-introduction or belong to a previously undetected remnant population. Sequencing of mtDNA from identified *Ae. aegypti* matched closest to a specimen collected in Kerala, India. A survey of residents in the surveillance area suggests high encounter rates with mosquitoes in and around homes. Despite high self-reported knowledge about Zika virus, the survey revealed gaps in knowledge regarding its transmission cycle and relative degrees of vulnerability to serious illness among segments of the human population. These findings highlight the importance of continued surveillance, vector control, and public-health education in Gulf Coast states, as well as the potential threat of *Ae. Aegypti*–transmitted pathogens in southern Alabama.

Key words: Gulf Coast, public health, vector-borne diseases, Zika virus

Aedes (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae), primary mosquito vector of Zika virus, yellow fever, and other flaviviruses historically spread across the globe through sailing vessels (Tabachnick 1991). However, in recent years it has been widely displaced by a competing mosquito vector, *Aedes* (*Stegomyia*) *albopictus* (Skuse), or the 'Asian tiger mosquito' (Juliano 1998). Native to Asia, *Ae. albopictus* has invaded every continent except for Antarctica and continues to expand its range (Benedict et al. 2007, Enserink 2008). This species has at least two known advantages over *Ae. aegypti* in that its larvae tend to be better competitors in resource-limited habitats and that males of the species are capable of satyrization, or mating with congeners such as *Ae. aegypti* and rendering females sterile (Juliano 1998; Juliano and Lounibos 2005; Reiskind and Lounibos 2013; Bargielowski and Lounibos 2016). These factors have likely allowed *Ae. albopictus* to invade and widely extirpate *Ae. aegypti* populations (Lounibos et al. 2016).

The United States Gulf Coast has been identified as the region with the greatest risk for the establishment of Zika and other arboviruses due to humid climate, the presence and abundance of *Ae. aegypti* and *Ae. albopictus*, expanding urban environments, and central hubs of human movement (such as seaports) (Kraemer et al. 2015; Monaghan et al. 2016). There is evidence of *Ae. aegypti* persisting in urban areas along the Gulf Coast in Texas, Louisiana, and Florida, and evidence of mosquito-transmitted Zika virus in Florida and Texas in 2016 and in Florida in 2017; however, Alabama has remained an exception.

Ae. albopictus was first detected in 1987 in Mobile, Alabama; however, by 1991, it had displaced *Ae. aegypti* as the main container-breeding mosquito (Hobbs et al. 1991). Subsequently, a detailed compilation of records from the Centers for Diseases Control and Prevention ArboNET database, VectorMap, published literature, and a survey of mosquito control agencies, university researchers, and state and local health departments noted the prolonged absence of *Ae. aegypti* in the state of Alabama from 1995 to 2016 (Hahn et al. 2016). A recent update noted the presence of *Ae. aegypti* in Madison County in northern Alabama (a new record for that county) (Hahn et al. 2017). To date, *Ae. aegypti* has yet to be recaptured elsewhere in Alabama, although abiotic ecological conditions along the coast in the state are favorable for this vector to thrive, and potential reintroduction through seaports is a possibility. Mobile is an ideal location for *Ae. aegypti* reintroduction or for remnant populations to persist, as it is characterized by heavy maritime traffic, and urban, suburban, rural, and industrial environments that provide a range of ecological conditions. Such heterogeneity may allow *Ae. aegypti* to find habitats where it either escapes from disadvantageous interactions with *Ae. albopictus* or has a competitive upper hand.

To mitigate the threat of Zika virus in the United States, it is crucial to identify 1) remnant or re-emergent populations of *Ae. aegypti* along the Gulf Coast, and 2) socioeconomic, behavioral, and geographic risk factors that may contribute to human cases of Zika. Here, we combine entomological surveillance with human surveys in Mobile, Alabama, to address these needs. We hypothesize that remnant or re-emergent *Ae. aegypti* populations occur near seaports and commercial tire shops. We also explore local knowledge and behavior regarding mosquitoes and Zika virus.

Methods

Study Site

Mobile, Alabama, was chosen as the study location due to characteristics conducive to *Ae. aegypti* introduction and establishment, particularly an active international port and environmental heterogeneity within an urban landscape. Mosquitoes were sampled within each of 12 zip codes near the urban center (Fig. 1). In each zip code, based on visual assessment of structures and habitats that would facilitate *Ae. aegypti* breeding (i.e., tire shops, abandoned buildings, and outdoor sheds), 12 locations were sampled. Sampling of resting adult mosquitoes was conducted using a backpack aspirator (Vazquez-Prokopec et al. 2009), and the number of open containers within a 50-m radius was counted. Sites were aspirated for 20 min.

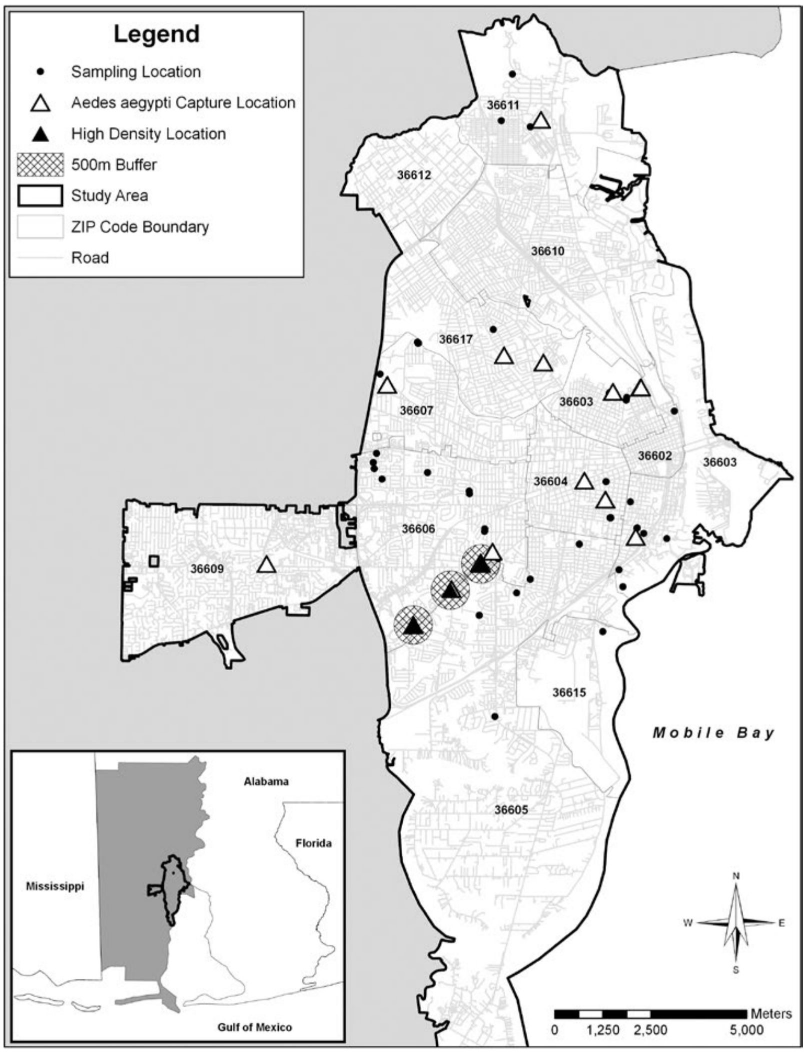

Fig. 1. Map of Mobile mosquito collection sites and zip codes where human surveys were administered. Filled circles represent mosquito collection locations, whereas open triangles represent locations where *Ae. aegypti* was captured and mosquitoes were found in the highest densities.

The container index (CI), or percentage of containers infested with larvae out of the total, was calculated at each location. BG GAT traps were also used, but were not successful capturing *Aedes* spp.; therefore, sampling in this study was limited to aspirated samples. Collections were conducted twice every month from July 2016 to September 2017; however, no sampling was conducted in August of either year due to inclement weather. All mosquitoes were identified morphologically to genus and species (Rueda 2004; Burkett-Cadena 2013), and data deposited into MosquitoNet.

Molecular Identification

DNA was extracted from wings and legs of four mosquitoes collected in 2016 that were morphologically identified as *Ae. aegypti* and used as template in a polymerase chain reaction (PCR) to amplify the mitochondrial gene cytochrome-c oxidase subunit I (COI) (Kumar et al. 2007) with the following primers: Forward-5-GGATTTGGAAATTGATTAGTTCCTT-3, Reverse 5-AAAAATTTTAATTCCAGTTGGAACAGC-3. Following reaction confirmation by agarose-gel electrophoresis, PCR amplicons were purified using the EZNA Cycle Pure Kit (Omega Bio-tek) and outsourced for DNA sequencing (Eurofins Genomics). Returned sequences were searched against the NCBI Nucleotide collection using BLASTn.

Survey

A 50-question survey about human behavior regarding mosquitoes (changing the environment or personal behavior), knowledge of and attitudes toward mosquito-borne diseases (as in Lockaby et al. 2016), Zika virus, and demographics was mailed to a random sample of 1,000 residents in Mobile in July of 2016. The survey followed a design method for best practices in development with an original survey packet and two follow-up notifications (Dillman et al. 2009). Questions were close ended with rating conducted on Likert scales with rating from 1 to 7. Analysis is primarily descriptive with comparisons made using *t*-tests.

Results

A total of 1,074 mosquitoes were captured: 734 *Ae. albopictus,* 271 *Aedes* (*Aedimorphus*) *vexans* (Meigan) or *Aedes* (*Ochlerotatus*) *sollicitans* (Walker), and 69 *Ae. aegypti*. All *Ae. aegypti* were collected from seven zip codes (Table 1) in Mobile in July, September, November 2016, and February–June 2017 (Fig. 2A). The majority of *Ae. aegypti* (59%) were captured in one zip code (36606), where the number of containers was greater than in any other site (over 200 when compared with a maximum of 75). Over the sampling period, CI ranged from 0–37% in that zip code (Fig. 2B). A sequenced COI PCR product from *Ae. aegypti* matched with 99% sequence identity to *Ae. aegypti* via nucleotide BLAST (GenBank accession number: KY514082), confirming the presence of *Ae. aegypti* among the collected samples. The *Ae. aegypti* that was the closest match (GenBank accession number: HM807266.1) was collected in Kerala, India.

There was a mean of 26 open containers within a 50-m radius of the collection sites where *Ae. aegypti* was captured, compared with a mean of four open containers within a 50-m radius of all other collection sites. *Ae. albopictus* density was also higher in the *Ae. aegypti* sites than in other collection locations, suggesting that more plentiful containers may lessen larval competition between species. Sites where *Ae. aegypti* was captured were within close proximity to commercial tire shops within the same zip code. *Ae. albopictus* was captured in all zip codes at all collection sites.

Table 1. Descriptions of sites where *Ae. aegypti* was detected in Mobile, AL in 2016–2017

July-16			
Zip code	**Habitat type**	**Male**	**Female**
36606	tire shop	0	4
September-16			
Zip code	**Habitat type**	**Male**	**Female**
36606	tire shop	0	2
36606	tire shop	0	1
November-16			
Zip code	**Habitat type**	**Male**	**Female**
36606	gas station	0	1
36606	tire shop	0	1
February-17			
Zip code	**Habitat type**	**Male**	**Female**
36603	abandoned house	0	1
36606	tire shop	0	1
March-17			
Zip code	**Habitat type**	**Male**	**Female**
36604	abandoned house	1	2
36604	abandoned house	0	1
36607	gas station	1	
April-17			
Zip code	**Habitat type**	**Male**	**Female**
36603	field w/ tires	2	4
36606	tire shop	4	4
36609	gas station	0	3
36606	tire shop	0	3
36606	tire shop	0	3
May-17			
Zip code	**Habitat type**	**Male**	**Female**
36609	tire shop	0	2
36617	residential	0	1
36606	tire shop	1	1
June-17			
Zip code	**Habitat type**	**Male**	**Female**
36603	field w/ tires	1	1
July-17			
Zip code	**Habitat type**	**Male**	**Female**
36611	residential	1	1
36603	field w/ tires	0	1
36604	abandoned house	0	2
36606	tire pile	1	
36606	tire shop	1	2
36606	tire shop	0	1
September-17			
Zip code	**Habitat type**	**Male**	**Female**
36617	commercial aban	0	1
36603	abandoned house	1	
36606	tire shop	1	2
36606	tire shop	0	5
36606	tire pile	0	2
36603	field w/ tires	1	0

The human survey response rate was 15% yielding 124 usable surveys after undeliverable surveys were deducted. Demographics comparisons from our survey to census figures show that our respondents were evenly distributed by gender but older, and skewed toward higher incomes (Table 2). A large proportion (73%) of respondents reported being bitten by a mosquito near their home during the previous 30 d, and 70% reported a moderate to very high density of mosquitoes near their home. One-fifth (20%) of respondents reported having seen a mosquito in their home during the previous 30 d.

Most frequently used methods by respondents to reduce mosquitoes in the environment were dumping water from containers and

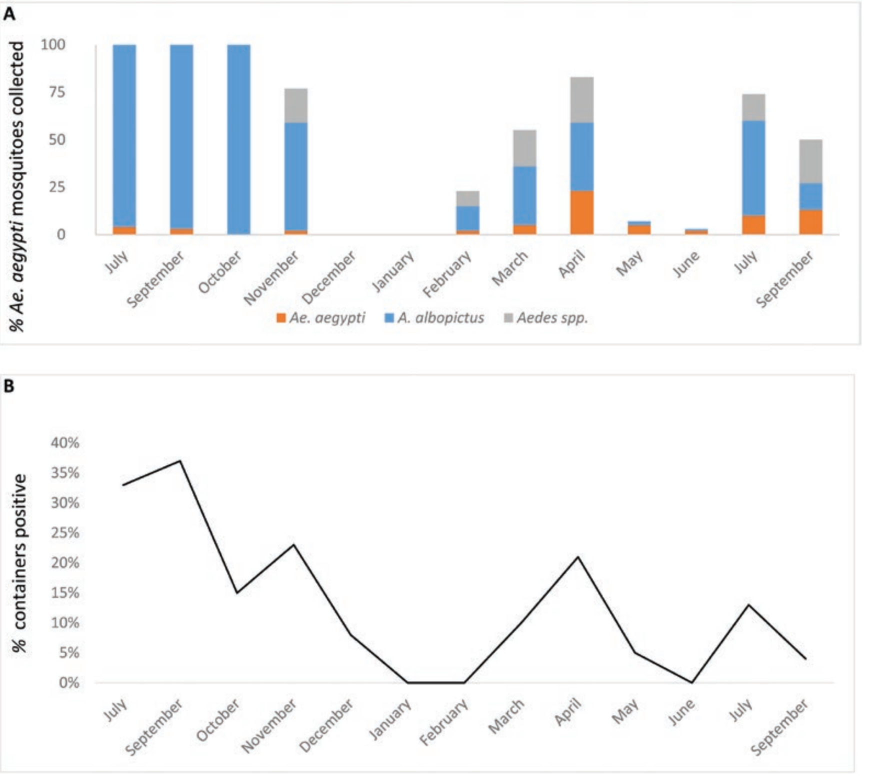

Fig. 2. Mosquitoes captured between July 2016 and September 2017. (A) Percentage of *Ae. aegypti* mosquitoes out of the total number of *Aedes* mosquitoes captured monthly during the study period. (B) Monthly container index (CI) in the zip code where *Ae. aegypti* mosquitoes were most frequently captured.

Table 2. Demographics of survey respondents

Variable	Category	Frequency	Percentage
Age	Mean = 62 (*SD* = 16.09)		
Gender			
	Male	59	47.2
	Female	66	52.8
Education			
	Less than high school	8	6.7
	High school diploma or GED	19	16.0
	Some college	25	21.0
	Associate's degree	13	10.9
	Bachelor's degree	27	22.7
	Graduate or professional degree	24	20.2
	Other	3	2.5
Income			
	Less than $25,000	36	27.5
	$25,000–$49,999	23	21.1
	$50,000–$74,999	23	21.1
	$75,000–$99,999	7	6.4
	$100,000 or more	26	23.9
Ethnicity			
	African American	49	41.5
	Latino	1	.8
	Caucasian	67	56.8
	Other	1	.8

clearing overgrown shrubs and lawn. Nearly two-thirds (64%) of respondents reported removing containers from their yard over the last 30 d. The most frequent behaviors were to avoid areas where mosquitoes are present, minimize time outside, and to wear long pants. One-quarter of the respondents reported using repellant with DEET at least a few times a week. Almost 74% of respondents felt that they could have 'some impact' on mosquitoes around their neighborhood, whereas a smaller 13% of those felt their actions would have a 'significant impact'.

Over half of the respondents felt knowledgeable (39%) to very knowledgeable (12%) about Zika, and similarly over half felt concerned (32%) to extremely concerned (20%) that they or a family member might contract the disease. Many respondents (42%) felt that Zika virus would be extremely serious for their health, whereas another 38% thought that it was at least serious. When asked who they felt was the most vulnerable to Zika, 83% indicated that pregnant women were highly vulnerable and lesser numbers indicated that 'women planning on having a baby soon' (60%) or 'men planning on having a baby soon' (37%) were vulnerable. Large percentages of individuals believed that children (44%), people older than 55 (44%), and immune-compromised people (48%) were highly vulnerable. Respondents primarily received their information about Zika from television (95%), newspaper (49%), radio (32%), word of mouth (20%), and from their health care provider (14%) or a brochure/pamphlet (12%). The most trusted sources were health care providers, television, and newspaper. Furthermore, respondents felt that the local health department (79%), themselves (79%), and the local government (69%) were responsible for mosquito control.

We compared respondents from the zip code with the most *Ae. aegypti* (n = 24) to respondents from the other areas (n = 100) using t-tests. We found no significant differences in behaviors. However, it is noteworthy that the level of concern about themselves or a family member contracting Zika was significantly lower in one zip code where the most *Ae. aegypti* were located (t (120) = 2.653, P = 0.009).

Discussion

Here, we report the first detection of *Ae. aegypti* populations in southern Alabama since 1990 and only the second record in the entire state over the same period. The last statewide survey occurred in 2004–2005 (Qualls and Mullen 2006) when investigators sampled mosquito larvae from May to October in discarded tires in all 67 counties of the state. Tires from 169 sites were sampled resulting in over 13,000 mosquitoes being identified to species. Of these, 70.4% were *Ae. albopictus*, and no *Ae. aegypti* were found. Since then, a number of studies have reported mosquito data from Alabama, but none reported collection of *Ae. aegypti* (Burkett-Cadena et al. 2008; Burkett-Cadena et al. 2011; Estep et al. 2011; Jacob et al. 2011; Burkett-Cadena et al. 2013; Burkett-Cadena et al. 2014).

Ae. aegypti individuals were collected from seven of 12 zip codes sampled, with 59% captured in one specific zip code, suggesting that *Ae. aegypti* populations can be highly focal and missed using traditional surveillance efforts such as the BG sentinel traps and larval collections that did not capture *Ae. aegypti* for 26 yr (Hahn et al. 2016). Additional molecular analyses may reveal unique haplotype patterns and may identify whether these populations represent a reintroduction. Moreover, multilocus genotyping has the potential to reveal the geographic origins of these populations, and phylogenetic clustering of *Ae. aegypti* populations from Mobile when compared with *Ae. aegpyti* populations from neighboring states may suggest whether the detected *Ae. aegypti* suggest a population persistence or introduction from these states.

Our survey results indicate a need for more complete information regarding the human populations at a greatest risk from Zika virus. Respondents are performing a number of useful behavioral and environmental modifications and ranked themselves as personally responsible—along with the local health department and government—for mosquito control. Respondents in the area identified with the most *Ae. aegypti* appear to be the least concerned. Results indicate that having trusted healthcare officials presenting information on television or in the newspaper on risk and best behavioral practices could help improve knowledge of Zika and other arboviruses.

In conclusion, the detection of *Ae. aegypti* populations in southern Alabama highlights the need for continued vector surveillance and control in the Gulf Coast states, and identifies the region as a potential area of risk for transmission of Zika and other *Ae. aegypti*–vectored viruses. This study also highlights the need for specific outreach efforts which include utilizing trusted media sources and disseminating information on best practices for limiting *Ae. aegypti*–human interactions, thus reducing the risk of arbovirus exposure.

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