



A molecular surveillance-guided vector control response to concurrent dengue and West Nile virus outbreaks in a COVID-19 hotspot of Florida

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Summary

Background Simultaneous dengue virus (DENV) and West Nile virus (WNV) outbreaks in Florida, USA, in 2020 resulted in 71 dengue virus serotype 1 and 86 WNV human cases. We hypothesized that we would find a number of DENV-1 positive mosquito pools, and that the distribution of these arbovirus-positive mosquito pools would be associated with those neighborhoods for which imported DENV cases have been recently reported in 2019 and 2020.

Methods We collected and screened *Aedes aegypti*, *Ae. albopictus*, *Anopheles crucians*, *Culex coronator*, *Cx. nigripalpus*, and *Cx. quinquefasciatus* mosquitoes from Miami-Dade County (Florida) for DENV and WNV by rRT-qPCR. Spatial statistical analyses were performed to capture positive mosquito pool distribution in relation to land use, human demography, environmental variables, mosquito trap placement and reported human travel associated DENV cases to guide future mosquito control outbreak responses.

Findings A rapid screen of 7,668 mosquitoes detected four DENV serotype 2 (DENV-2), nine DENV-4 and nine WNV-positive mosquito pools, which enabled swift and targeted abatement of trap sites by mosquito control. As expected, DENV-positive pools were in urban areas; however, we found WNV-positive mosquito pools in agricultural and recreational areas with no historical reports of WNV transmission.

Interpretation These findings demonstrate the importance of proactive arbovirus surveillance in mosquito populations to prevent and control outbreaks, particularly when other illnesses (e.g., COVID-19), which present with similar symptoms, are circulating concurrently. Growing evidence for substantial infection prevalence of dengue in mosquitoes in the absence of local index cases suggests a higher level of dengue endemicity in Florida than previously thought.

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Introduction

As the effects of climate change, increased urbanization, and global travel mount, vector-borne disease burden is increasing. In the United States of America (USA), mosquitoes are the primary vectors of viral pathogens, including dengue viruses (DENV, serotypes 1-4), and

West Nile virus (WNV). Although WNV is endemic in the continental USA, only a handful of states, especially Florida (FL), are at risk of autochthonous DENV transmission.⁵ In southeast FL, Miami-Dade County (FL, USA) (population: 2,701,767), in particular, appears to be an ideal gateway for arbovirus introduction.⁶ Throughout 2020, Miami-Dade County (MDC) was the epicenter of COVID-19 in Florida, and the state experienced in parallel, concurrent human outbreaks of

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Research in context

Evidence before this study

The state of Florida had previously been identified as an arbovirus hotspot within the United States due to climate and habitat suitability for vector species, and the high amount of international travel to and from dengue-endemic countries. Autochthonous dengue cases in Florida have increased since the first documented local case (since 1934) in 2009,^{1,2} alongside cyclical WNV outbreaks. More recently, the first description of silent circulation of DENV (i.e., the presence of DENV in the vector population in the absence of reported local or travel-associated human cases) was reported in Florida.^{3,4}

Added value of this study

This study provides corroborating evidence that DENV may be cyclically entrenched in mosquito populations in Florida and highlights the importance of pre-emptive monitoring of primary arbovirus vectors in Miami-Dade County: *Culex nigripalpus* (for West Nile virus), and *Aedes aegypti* (for dengue virus). Areas of West Nile virus vector positivity were outside of historic areas of West Nile virus concern, suggesting to vector control surveillance strategies, and importantly, enhanced public health messaging.

Implications of all the available evidence

These data suggest a larger, more complicated picture of arbovirus transmission during the 2020 COVID-19, WNV and DENV outbreak in Miami-Dade County (MDC). The presence of DENV serotype 2 and 4 in different mosquito populations in addition to circulating DENV serotype 1 in the human population raises the risk for severe disease resulting from antibody-dependent enhancement, implicating an evolving increase in dengue endemicity in Florida. Given the potential of proactive screening of vector populations in preventing arbovirus outbreaks, and its demonstrated utility in help stemming an ongoing outbreak, independent cost-benefit analyses are needed to determine if existing public health policies in MDC can be overhauled.

DENV and WNV from May–December 2020, resulting in 71 local cases of dengue fever (DENV serotype 1, DENV-1) and 86 cases of WNV.¹ Our overarching hypothesis was that during an ongoing outbreak, active screening of mosquito pools would enable a more targeted mosquito abatement response to those areas where risk of an infectious mosquito bite is high. We hypothesized that we would find a number of DENV-1 positive mosquito pools in Wynwood (the neighborhood in Miami-Dade responsible for previous arbovirus-positive pools during the 2016 Zika epidemic) and the surrounding vicinity, and that the distribution of these

arbovirus-positive mosquito pools would be associated with those neighborhoods for which imported DENV cases have been recently reported in 2019 and 2020. To test these hypotheses, we trapped, and screened mosquitoes from MDC for DENV and WNV during the outbreak period in real-time and used spatial mapping approaches to investigate the contribution of imported DENV hotspots on the distribution of positive pools. Herein, we report the outcome of these vector surveillance and control efforts and utilize spatial mapping approaches to understand factors that may have influenced outbreak dynamics in MDC and can therefore guide mosquito control outbreak response in the future.

Methods

Sample collection and processing

After the Zika virus outbreak in 2016, Miami-Dade Mosquito Control District (MDMCD) established a surveillance grid that was designed to have at least one BG-Sentinel trap (Biogents AG, Regensburg, Germany) per 1 km² in the urbanized areas of the county, as well as CDC light traps along the city limits bordering the Everglades National Park and adjacent green areas. Additional traps were also deployed in points of interest (e.g., tourist areas, shopping malls, botanical gardens, and public outdoor areas) where individuals had increased risk of mosquito exposure. Each of the 309 trap sites was baited with CO₂ (dry ice) and traps are deployed weekly for 24 h for the entire year. MDMCD morphologically identified and sorted adult female mosquitoes by location and created pools of 2–25 mosquitoes of the same species. Mosquito pool samples were kept cold to avoid RNA degradation, shipped on dry ice, and stored at -80°C. In total, 743 samples were created for testing [548 for DENV (pools of *Aedes aegypti* and *Aedes albopictus*, N= 5,079 mosquitoes) and 188 for WNV (pools of *Anopheles crucians*, *Culex coronator*, *Culex nigripalpus*, and *Culex quinquefasciatus*, N= 2,589 mosquitoes)]. Mosquito pool samples that were found to be DENV- or WNV-positive were reported to MDMCD within 48–72 h, which then initiated door-to-door source reduction in the areas where positive mosquito pools were found. Vectobac WGD (Valent Biosciences), a larvicide product containing *Bacillus thuringiensis israelensis* was applied at positive pool sites for four weeks. Adulticide treatment using chlorpyrifos was also conducted (weather permitting) using Mosquitomist (Clarke). Sites were revisited for two weeks following chlorpyrifos treatment to confirm population reduction below the threshold of ≤5 mosquitoes per trap.

RNA extraction

Chilled, sterile 1X phosphate-buffered saline (PBS) and sterile glass beads were added to each sample. Each

1 mL sample was homogenized in a Bullet Blender (speed 8, 5 min) with repeated cooling on ice/cold block. Samples were centrifuged (3,750xg, 3 min) and 140 µL of the homogenate supernatant was added to AVL lysis buffer (560 µL) (Qiagen). RNA was extracted using the QIAmp Viral RNA extraction kit (Qiagen) following the manufacturer's protocols with 2 × 40 µL elution steps. One pool of 5 uninfected, laboratory reared *Ae. aegypti* (Orlando strain) mosquitoes was processed in parallel, serving as a negative extraction control to rule out contamination or spurious amplification.

Real-time RT-PCR virus detection

Sample RNA was either tested for i) WNV (pools of *An. crucians*, *Cx. coronator*, *Cx. nigripalpus*, and *Cx. quinquefasciatus*) or ii) DENV (pools of *Ae. aegypti* and *Ae. albopictus*). Samples designated for DENV screening were first run through a pan-dengue serotype screen, DENVAL (Appendix Table 1), while the remaining samples were screened via a WNV assay (Appendix Table 2), both assays are designed for research, and not diagnostic purposes. Each sample, prepared in a PCR hood with static air, was run as technical duplicates. Each plate included a mosquito extraction control (negative extraction control), a no template control, and either i) a positive WNV control or ii) dengue virus serotype 1, 2, 3, and 4 (DENV-1 through DENV-4) positive controls. All positive RNA controls were obtained from BEI resources diluted 1:10 (Appendix Tables 1, 2 and 5). Analyses used either QuantaBio UltraPlex 1-Step ToughMix (4X) Low-ROX master mix (Appendix Table 3) or SuperScript™ III Platinum™ One-Step qRT-PCR (Appendix Table 4) on a BioRad CFX96 Touch Real-Time PCR Detection System at 50°C for 30 min (for Superscript reactions) or 50°C for 10 min (for QuantaBio reactions), 95°C for 2 min, and 45 cycles of: 95°C for 15 s and 60°C for 45 s. Both assays yielded similar results, which helped overcome supply bottlenecks during the COVID-19 pandemic. Samples that were DENV-positive via the DENVAL assay were analyzed for serotype specificity (Appendix Table 5). Any putative positive or inconclusive (i.e., only one replicate amplified) samples were re-run in confirmatory reactions, adding extra technical replicates and performing additional confirmatory runs as needed to confirm virus positivity. Mosquito homogenates were sent directly to the CDC (via the Florida Department of Health (FLDOH) as per standard protocols) for independent confirmation of positive samples. The inclusion of a pool of lab reared mosquitoes in every extraction and PCR run ruled out contamination during lab handling or spurious amplification of mosquito material (see *Supplementary Information on Contamination troubleshooting*).

Positive and negative sample designation

A sample was considered 'positive' based on positive technical duplicate results in two independent runs

using the WNV assay (Appendix Table 2) and the DENV serotype-specific assay (Appendix Table 5). Samples were considered 'negative' based on no detectable Ct value in either technical duplicate after 45 cycles.

Mapping mosquito samples

To understand the spatial distribution of mosquito pools collected for this effort, we developed maps using ArcMap (v 10.6) at the zip code level, which is the smallest level of resolution obtainable with datasets for descriptive variables of interest in MDC (e.g., de-identified health information). All datasets were either publicly available: MDC Boundaries, population density and land use (2010 US Census, MDC's Open Data Hub), median household income (2010 US Census, Michigan Population Studies), or obtained with permission from personal communications (2018–2020 mosquito prevalence c/o Chalmers Vasquez, MDMCD; 2009–2019 Imported DENV cases c/o Andrea Morrison, FLDOH). We did not have access to zip code level local human case data, nor zip code level data for travel-related cases in 2020. Land-use types were manually concatenated to 11 different primary categories: Agricultural, Cemeteries, Commercial, Educational, Industrial, Marine, Recreational, Residential, Paved, Water, and Vacant. These types were further summed as: 'urban/built' (Commercial, Educational, Industrial, and Paved), 'agricultural/recreational' (Agricultural, Recreational), 'residential' (Residential) and 'other' (Cemeteries, Marine, Water, and Vacant) (Appendix Table 6).

Spatial statistical analysis of vector distribution and spatial visualization of overlapping imported DENV cases and arbovirus hotspots

Spatial descriptive statistics were used to explore the spatial relationship between positivity and overall mosquito sampling. The standard deviation directional distribution and geographic mean center (i.e., measures of spatial dispersion and central tendency, respectively) were computed for DENV- and WNV-positive mosquito pools, for all collected DENV vectors (*Ae. aegypti* and *Ae. albopictus*) and all WNV vectors (*An. crucians*, *Cx. coronator*, *Cx. nigripalpus*, and *Cx. quinquefasciatus*), both individually and together. Kernel density estimation (KDE) with optimal distance bandwidths⁷ was used to visualize the continuous density of DENV- and WNV-positive mosquito pools, as well as traps containing uninfected DENV and WNV vectors. We used local Moran's I, a local indicator of spatial association (LISA),^{8,9} to assess whether arbovirus-positive pools overlapped with areas where there were high concentrations of imported DENV cases previously reported by the FLDOH. Briefly, the local Moran's I statistic is useful in identifying locations within the broader study area with concentrations of high, or low occurrences (i.

Collection Date	Sample ID	Overall sample result	Species	Pool Size
06/09/20	N/A*	WNV positive	<i>Culex quinquefasciatus</i>	18
06/23/20		WNV positive	<i>Culex quinquefasciatus</i>	4
		WNV positive	<i>Culex quinquefasciatus</i>	24
06/30/20		WNV positive	<i>Culex quinquefasciatus</i>	5
07/23/20	0723-30	WNV positive	<i>Culex nigripalpus</i>	25
	0723-31	WNV positive	<i>Culex nigripalpus</i>	25
	0723-39	WNV positive	<i>Culex nigripalpus</i>	25
	0723-44	WNV positive	<i>Culex nigripalpus</i>	25
07/30/20	0730-37	DENV-2 positive	<i>Aedes aegypti</i>	25
	0730-41	DENV-2 positive	<i>Aedes aegypti</i>	25
08/20/20	0820-06	WNV positive	<i>Culex nigripalpus</i>	25
	0820-07	WNV positive	<i>Culex nigripalpus</i>	25
	0820-08	WNV positive	<i>Culex nigripalpus</i>	25
	0820-09	WNV positive	<i>Culex nigripalpus</i>	25
	0820-11	WNV positive	<i>Culex nigripalpus</i>	25
08/28/20	0828-2	DENV-2 positive	<i>Aedes aegypti</i>	23
	0828-10	DENV-2 positive	<i>Aedes aegypti</i>	25
09/17/20	0917-4	DENV-4 positive	<i>Aedes aegypti</i>	13
	0917-8	DENV-4 positive	<i>Aedes aegypti</i>	8
	0917-11	DENV-4 positive	<i>Aedes aegypti</i>	8
	0917-12	DENV-4 positive	<i>Aedes aegypti</i>	4
	0917-13	DENV-4 positive	<i>Aedes aegypti</i>	10
	0917-16	DENV-4 positive	<i>Aedes aegypti</i>	3
	0917-27	DENV-4 positive	<i>Aedes aegypti</i>	6
09/22/20	0922-2	DENV-4 positive	<i>Aedes aegypti</i>	5
09/29/20	0929-07	DENV-4 positive	<i>Aedes aegypti</i>	2

Table 1: Positive mosquito pool results from screening mosquitoes collected in Miami-Dade County (Florida, USA) in 2020. WNV = West Nile virus, DENV-2 = dengue virus serotype 2, DENV-4 = dengue virus serotype 4.

Sample Ct values and positive control Ct values can be found in *Appendix Table 7*.

* Pathogen detection conducted by the CDC.

e., hotspots and cold spots), relative to neighboring locations. Local Moran’s I analyses with inverse distance weighting were performed to detect and identify imported DENV hotspots (i.e., zip codes with elevated DENV prevalence) and locations of arbovirus positive pools were plotted on the resulting hotspot map to determine where positive mosquito pools coincide with high concentrations of DENV cases. All spatial statistical analyses were completed in ArcMap (v 10.6).

Role of the funding source

The funding agencies did not play a role in study design, data collection, analysis, or interpretation, nor in the writing of this manuscript or our decision to submit this manuscript for publication.

Results

Molecular detection of arbovirus-positive mosquito pools

We found that 2.96% of female mosquito pool samples were positive for an arbovirus; four were DENV-2

positive (all CDC-verified), nine were DENV-4 positive (2.37% DENV positivity, DENV-2: 4/548 pools, DENV-4: 9/548 pools), and nine pools were WNV positive (one of which was CDC-verified) (4.79% WNV positivity, 9/188 pools) (Table 1 and Appendix Table 7).

For DENV, we tested 45 *Ae. albopictus* and 510 *Ae. aegypti* mosquito pools, and only *Ae. aegypti* pools were found to be positive for DENV-2 and/or DENV-4 (2.43% positivity).

For WNV, we tested five *An. crucians*, five *Cx. coronator*, 47 *Cx. nigripalpus* and 131 *Cx. quinquefasciatus* pools. Only *Cx. nigripalpus* pools were WNV positive (19.1% positivity).

A spatial-climatic distribution of arbovirus-positive mosquito pools identified exigent and emerging areas of concern

DENV-positive mosquito pools were distributed throughout the county, with most (11/13) positive pools located in central Miami-Dade: downtown Miami (N=3), Wynwood (N=2), and South Miami (N=6) (Figure 1A), residential urban areas with mid-to-high human population density (2,000 – 7,000 people/km²) (Figure 1B). At the zip code level, DENV-positive pools were

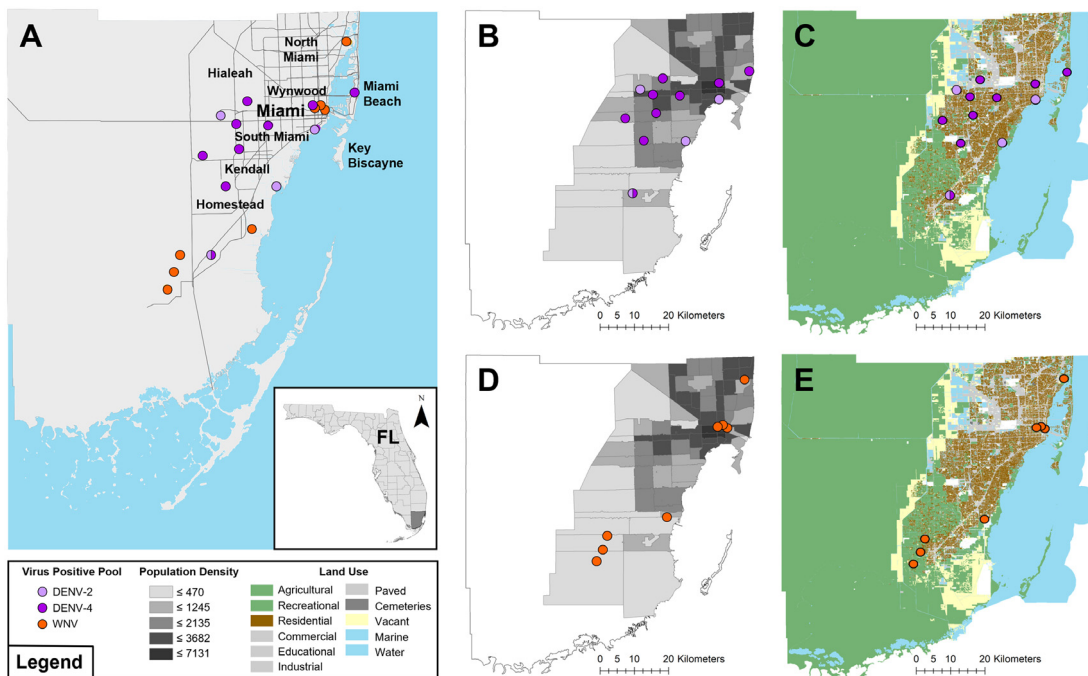


Figure 1. Spatial distribution of arbovirus positive mosquito pools (light purple – DENV-2, dark purple – DENV-4, orange – WNV). A: Positive pool spread throughout Miami-Dade County – DENV positive pools and WNV positive pools. B and D: Positive pool spread overlaid on 2010 Miami-Dade population density (individuals/km²) – DENV positive pools (B), or WNV positive pools (D). C and E: Positive pool spread overlaid on 2010 Miami-Dade land use data – DENV positive pools (C) or WNV positive pools (E).

primarily found in built and residential environments (59.42%) over more rural, (i.e., agricultural, and recreational) areas (14.62%) (Figure 1C). Conversely, the 13 WNV-positive pools (9 identified herein) had three primary infection foci – in central MDC, Wynwood (N=4), high population density (>3000 people/km², urban built environment), in southern MDC, Homestead (N=8), low population density (<470 people/km², mostly agricultural and recreational land), and in North Miami (N=1), mid population density (1,000 – 3,000 people/km² and mixed land use) (Figure 1D–F). WNV-positive pools were primarily from agricultural and recreational areas (69.32%) and were less common in urban built and residential areas (15.98%).

The directional distribution and mean center computations of dengue vector distributions showed that DENV-positive mosquito pools were within the directional distribution of all dengue vector traps (Figure 2A). *Ae. aegypti* prevalence dominated the traps in much of the county and had a much larger directional distribution than *Ae. albopictus*, whose presence was concentrated in southern Miami-Dade County (Figure 2B). Although the directional distribution of all WNV vectors overlapped with the directional distribution of the WNV-positive mosquito pools, the latter extended well beyond the southernmost tip of the WNV vector directional ellipse (Figure 2C). *Culex quinquefasciatus*, *An. crucians* and *Cx. nigripalpus* had distinct

directional distributions in the Miami Beach area, North Miami, and South Miami, respectively (Figure 2D). The distribution of *Cx. coronator* encompassed all three of these zones, lacking any strict boundary (Figure 2D).

The KDE maps show a clear high density of vector trapping efforts across MDC except for the southernmost area (Appendix Figure 1A). The KDE map for DENV-2 positive pools showed high values surrounding each positive pool but low values between pools (Appendix Figure 1B), while the DENV-4 positive KDE map showed connected high values across all positive pools (Appendix Figure 1C). The KDE map for the WNV vector trapping effort showed a high concentration of vectors in the downtown Miami/Miami Beach areas, with areas of mid values dotted throughout the county (Appendix Figure 1E). The WNV-positive pools showed two clear high-value foci, one in Homestead, and one in Miami Beach (Appendix Figure 1F).

DENV-positive mosquito pools were primarily in areas of higher average maximum temperatures, lower average minimum temperatures and higher total precipitation (Appendix Figure 2 A–C). WNV-positive pools displayed the same trend with respect to temperature but were found in areas of lower total precipitation (Appendix Figure 2D–F).

LISA analyses on imported DENV case data showed similar clusters for both the 2009–2019 imported DENV data (Appendix Figure 3A), as well as the 2019

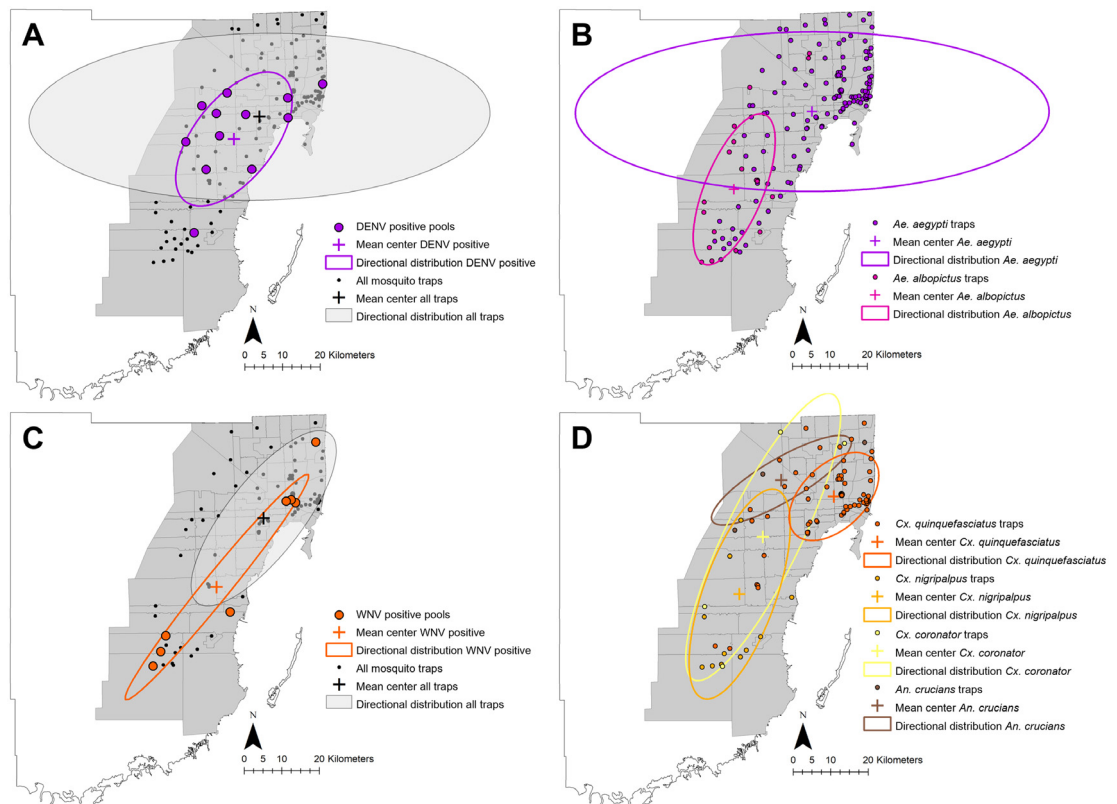


Figure 2. Spatial analysis of A: DENV positive pool distribution (purple) within traps containing DENV vectors (black), B: Distribution of traps containing *Aedes aegypti* (purple) or *Aedes albopictus* (pink), C: WNV positive pool distribution (orange) within traps containing WNV vectors (black), D: Distribution of traps containing *Culex quinquefasciatus* (dark orange), *Culex nigripalpus* (light orange), *Culex coronator* (yellow), or *Anopheles crucians* (brown).

only imported DENV dataset (Appendix Figure 3B). The northwestern area of MDC was a high-high cluster, indicating this area is a hotspot for imported DENV cases. Conversely, the Miami Beach area was a low-low cluster, suggesting a consistently lower-than-average number of imported DENV cases (a cold spot). The low-high outliers for both datasets occurred just below the hotspot in northwest Miami-Dade, while the high-low outlier for the decade-wide dataset was in South Miami, and the 2019 only data had high-low outliers throughout North Miami.

Discussion

Ecological distribution of DENV-positive mosquito pools

Although other dengue vectors (i.e., *Aedes spp.*) are present in MDC,⁶ our data suggest that *Ae. aegypti* is clearly the primary vector of concern. Evidence suggests that Floridian *Ae. aegypti* (Monroe County) and *Ae. albopictus* (Indian River County) are similarly competent for DENV-1.¹⁰ DENV vector competency varies greatly based on DENV serotype and FL *Ae. aegypti* geographic

origin,¹¹ so extrapolations of *Ae. aegypti* and *Ae. albopictus* vector competence across FL may not be appropriate. Our findings could simply be due to *Ae. aegypti* prevalence, as considerably more *Ae. aegypti* were collected than *Ae. albopictus* in traps across the entirety of MDC in 2020, and in former years.⁶

The directional distribution of DENV-positive pools suggests that the current trap spread is more than adequate to reliably detect DENV-positive pools. However, since trap density within the DENV-positive pool distribution area is not as concentrated as in the other *Aedes* positive trap locales, additional traps in the ellipse margins could prove useful. The KDE maps of DENV vectors indicate that while mosquito trapping efforts are generally broad in Miami-Dade County, locations of high concentrations for DENV-positive pools are readily apparent, thus indicating potential targets for increased surveillance activities that may shift with serotype.

Ecological distribution of WNV-positive mosquito pools

All four mosquito species we tested for WNV can transmit WNV¹²; however, we only found nine WNV-positive

Cx. nigripalpus pools, the primary enzootic and epidemic vector of mosquito-borne viruses encephalitides such as WNV, St. Louis encephalitis virus, and eastern equine encephalitis virus throughout southern Florida.¹³ This result is intriguing, considering the overwhelmingly dominant collection of *Cx. quinquefasciatus* throughout the county. Differences in WNV vector positivity may be due to several factors including host preference and vector competence. Previous reports show that *Cx. quinquefasciatus* has a wide range of WNV competency that appears to partition according to spatial and climatic scales and is influenced by virus genetic background.^{10,14,15}

The spatial partitioning of the WNV-positive mosquito pools suggests that the positive pools had a distinct spatial trend within the larger distribution of traps collecting *Culex* and *Anopheles* mosquitoes in MDC. This might indicate that current trap coverage is not adequate to reliably detect WNV-positive mosquito pools, particularly in the southern portion of the county, where trapping density was relatively low and WNV-positive pools were concentrated. As WNV infects a wealth of other non-human animals (i.e., horses, alligators, birds, etc.),¹⁶ controlling the spread of WNV requires additional zoonotic measures including implementing equine WNV vaccines, as well as testing dead birds to understand prevalence and virus hotspots throughout the county.

The WNV-positive mosquito pools were primarily located in areas with lower precipitation, higher maximum temperature, and lower minimum temperature (range of 21-29°C), optimal for multiple *Culex* vectors.¹⁴ As there was no clear overlap between the spatial distribution of WNV vectors and climatic variables, these general climatic conditions are likely ideal for all the WNV vectors we analyzed. The WNV-positive mosquito pools were found primarily in agricultural and recreational areas, which include protected bird sanctuaries, as well as habitats for resident and migrating shorebirds, which are known WNV avian hosts.¹⁶

Epidemiological relevance of DENV-positive mosquito pools

Although local outbreaks have occurred in the state since 2009, the last five years have seen a significant uptick in the number of local DENV cases from zero (2017) to 71 (2020). A rise in cases can also be seen in the imported DENV case numbers (DENV-1 through DENV-4). In 2017, there were only 18 imported cases, 73 in 2018, 395 in 2019, and 41 in 2020.¹ This spike in dengue cases was mirrored in MDC, where local case numbers moved from zero cases in 2017, to one in 2018, fourteen in 2019 and four in 2020, and travel-associated cases progressed from nine in 2017, to thirty-eight in 2018, 226 in 2019 and twenty in 2020. The large spike in imported cases in 2019 may have

introduced other DENV serotypes into the resident mosquito populations, acting as seeding events, which are known drivers of local DENV case incidence.^{17,18} Alternatively, an undetected/unreported traveler(s) may have introduced other serotypes into the Miami-Dade mosquito population in 2020.

To examine whether this could be the case, we analyzed the available zip code-mapped historical (2009–2019) and 2019 imported DENV data using LISA analyses (Appendix Figure 3A and B). The similarity in the decade-long and 2019 LISA analyses suggests that the high number of imported cases in 2019 may have driven the hotspot trends seen in our analyses. However, none of our DENV-positive mosquito pools overlapped with known imported DENV case hotspots, rejecting our hypothesis. This could be due to a lack of zip code level imported DENV data reporting, or alternatively, if mosquitoes bite individuals outside of their listed area of primary residence, such as where individuals spend time outdoors, where they work, or where they socialize, a direct overlap would not be observed. This issue is of important consideration since a human case triggers mosquito abatement at and around the individual's home only.

Local human cases of DENV-1, DENV-2, and DENV-3 have been reported in FL.^{1,2,5,19,20} In 2020, only DENV-1 local cases were reported, and contrary to our hypothesis, we found DENV-2 and DENV-4 positive mosquito pools in the county. 'Silent' DENV circulation, defined as transmission between DENV asymptomatic individuals, as well as DENV maintenance in the vector population despite no reported human infection, likely represents the majority of transmission events.²¹ Finding additional DENV serotypes in the absence of a local human index case is not the first instance of silent DENV circulation in *Ae. aegypti* in the Americas. Previous reports have shown that DENV-4 was found in Manatee County, FL in 2018, and DENV-3 was detected in *Ae. aegypti* in Brazil despite no human index cases.^{3,22} This silent circulation may be due to low but persistent vertical transmission in the mosquito population.^{4,22,23} This disparity could also be due to inherent differences in mosquito vector competence for DENV-1, -2 and -4, as mosquitoes collected in MDC had higher DENV-1 infection and horizontal transmission rates than mosquitoes infected with DENV-2 and -4.¹¹ Although the pathogenicity of the 2020 Miami-Dade human DENV-1 index strain is unknown, since it is the only strain being detected in patients, the strain may be more infectious to, or cause increased disease severity in humans than other circulating dengue strains.

Having multiple concurrent circulating serotypes puts individuals at an increased risk of DENV illness due to complications arising from immune enhancement.²⁴ These risks include dengue fever and severe dengue (dengue hemorrhagic fever and dengue shock syndrome) and can be fatal.²⁴ A woman in her 30's died

from DENV-2 in Miami-Dade in 2019, and subsequent viral analysis suggested that infection occurred after DENV circulation had occurred in the Miami area.²⁵

Epidemiological relevance of WNV-positive mosquito pools

West Nile virus-positive mosquito pools overlapped with the timing of 27 human symptomatic cases (June - August), 33 human asymptomatic blood donors (June - August), as well as 15 WNV-positive birds (June, July), all in MDC.¹ The WNV-positive pools were primarily found in more rural areas with lower median household incomes, and low to medium population density (Figure 1 D–F). Low-income areas have previously been associated with higher WNV prevalence.²⁶

Limitations in confirmatory testing

The CDC independently verified the DENV-2 and WNV positive status of some of our samples but were not able to verify our DENV-4 samples as positive. These differences are likely due to Reference Lab standard operating procedures, which have more conservative thresholds for determining virus positivity.

The perfect storm: arbovirus transmission in a global pandemic

Miami-Dade County was a hotspot of SARS-CoV-2 transmission throughout the pandemic, with the highest case rate and death toll in the state. Unfortunately, due to the non-standardized nature of COVID-19 case reporting throughout Florida (reports range from residence-based, testing locale-based and exposure-based), we could not directly map COVID-19 prevalence alongside mosquito pool positivity. Evidence suggests that concomitant infection of SARS-CoV-2 and dengue result in more severe and fatal outcomes.²⁷

Due to the similar symptoms produced by COVID-19, West Nile, and dengue infection (fever, headache, muscle pain, nausea, vomiting, malaise), symptomatic WNV or DENV infected individuals may have self-isolated assuming they were positive for COVID-19 and never received a correct diagnosis. The asymptomatic and mild disease nature of initial arboviral infection and the high number of asymptomatic WNV-positive individuals identified directly through blood donorship suggests that the proportion of asymptomatic WNV-positive human carriers may be high. Previous reports show that the majority of DENV infected humans are also asymptomatic carriers.²¹ It should be noted that dengue is not currently part of routine blood donor screening in Miami-Dade County.

The large proportion of asymptomatic arbovirus cases highlights the critical need for improved DENV diagnostics, as no dengue rapid diagnostic tests are currently cleared for use in the United States.²⁵ Prevention

and control measures are imperative to prevent future DENV and WNV outbreaks. Ideally, scheduled⁶ vector control efforts involving mosquito pathogen screening of known and predicted²⁸ hotspot vector populations and subsequent targeted applications of insecticides at positive-hotspots are necessary to pre-empt arbovirus transmission especially in the highly conducive environment in MDC. Recognizing the costs inherent for such an approach, the study compels the consideration of independent cost-benefit analyses that can potentially inform public health policies in this region.

Contributors

All authors were involved in project conception and implementation. CV, IU, JM, and WP provided samples and metadata for mosquito testing. HC, JBA, CJS, CW, MF, JAL and RRD completed the diagnostic design and pathogen screening. HC, CAL and SJR created the maps and CAL completed all spatial analyses. HC and RRD wrote the primary manuscript draft, while all authors edited the manuscript. All authors had access to the data and three authors (HC, CAL, JBA) verified the data. The corresponding author (RRD) was responsible for the decision to submit the manuscript for publication.

Data sharing statement

Imported Miami-Dade dengue zip code level human case data can be accessed via a signed data access agreement with the Florida Department of Health. All other data used herein is publicly available. Data generated through this study is contained and available within this manuscript.

Editorial disclaimer

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Declaration of interests

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Supplementary materials

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References

- Morrison A, Rowe D, McDermott C, et al. Florida arbovirus surveillance. 2022 http://www.floridahealth.gov/diseases-and-conditions/mosquito-borne-diseases/_documents/2020-week-53-arbovirus-surveillance-report.pdf.
- Trout A, Baracco G, Rodriguez M, et al. Locally acquired dengue—Key West, Florida, 2009–2010. *Morb Mortal Wkly Rep*. 2010;59:577–581.
- Boyles SM, Mavian CN, Finol E, et al. Under-the-radar dengue virus infections in natural populations of aedes aegypti mosquitoes. *mSphere*. 2020;5. <https://doi.org/10.1128/MSPHERE.00316-20>.
- Ayers JB, Xie X, Coatsworth H, et al. Infection kinetics and transmissibility of a reanimated dengue virus serotype 4 identified originally in wild aedes aegypti from Florida. *Front Microbiol*. 2021;12:734903.
- Rey JR. Dengue in Florida (USA). *Insects*. 2014;5:991–1000. 2014;5:991–1000.
- Wilke ABB, Vasquez C, Medina J, Carvajal A, Petrie W, Beier JC. Community composition and year-round abundance of vector species of mosquitoes make Miami-Dade County, Florida a receptive gateway for arbovirus entry to the United States. *Sci Rep*. 2019;9:8732.
- Fotheringham AS, Brunsdon C, Charlton M. *Quantitative Geography: Perspectives on Spatial Data Analysis*. 6th ed. London: Sage Publications; 2000.
- Anselin L. Local indicators of spatial association-LISA. *Geogr Anal*. 1995;27:93–115.
- Lippi CA, Stewart-Ibarra AM, Romero M, et al. Spatiotemporal tools for emerging and endemic disease hotspots in small areas: an analysis of dengue and chikungunya in Barbados, 2013–2016. *Am J Trop Med Hyg*. 2020;103:149–156.
- Richards SL, Anderson SL, Alto BW. Vector competence of aedes aegypti and aedes albopictus (Diptera: Culicidae) for dengue virus in the Florida Keys. *J Med Entomol*. 2012;49:942–946.
- Stephenson CJ, Coatsworth H, Waits CM, et al. Geographic partitioning of dengue virus transmission risk in Florida. *Viruses*. 2021;13:2232.
- Unlu I, Kramer WL, Roy AF, Foil LD. Detection of West Nile virus RNA in mosquitoes and identification of mosquito blood meals collected at Alligator Farms in Louisiana. *J Med Entomol*. 2010;47:625–633.
- Day JF, Tabachnick WJ, Smartt CT. Factors that influence the transmission of West Nile virus in Florida. *J Med Entomol*. 2015;52:743–754.
- Shocket MS, Verwillow AB, Numazu MG, et al. Transmission of west nile and five other temperate mosquito-borne viruses peaks at temperatures between 23°C and 26°C. *eLife*. 2020;9:1–67.
- Richards SL, Anderson SL, Lord CC. Vector competence of Culex pipiens quinquefasciatus (Diptera: Culicidae) for West Nile virus isolates from Florida. *Trop Med Int Health*. 2014;19:610–617.
- Marra P, Griffing S, Caffrey C, et al. West Nile virus and wildlife. *Bioscience*. 2004;54:393–402.
- Guzzetta G, Marques-Toledo CA, Rosà R, Teixeira M, Merler S. Quantifying the spatial spread of dengue in a non-endemic Brazilian metropolis via transmission chain reconstruction. *Nat Commun*. 2018;9:2837.
- Li N, Feng Y, Vrancken B, et al. Assessing the impact of COVID-19 border restrictions on dengue transmission in Yunnan Province, China: an observational epidemiological and phylogenetic analysis. *Lancet Reg Health West Pac*. 2021;14:100259.
- Radke EG, Gregory CJ, Kintziger KW, et al. Dengue outbreak in Key West, Florida, USA, 2009. *Emerg Infect Dis*. 2012;18:135.
- Teets F, Ramgopal M, Sweeney K, Graham A, Michael S, Isern S. Origin of the dengue virus outbreak in Martin County, Florida, USA 2013. *Virology*. 2014;1–2:2–8.
- Bosch QA, Clapham HE, Lambrechts L, et al. Contributions from the silent majority dominate dengue virus transmission. *PLoS Pathog*. 2018;14:e1006965.
- Ferreira-De-Lima H, Dos P, Andrade S, et al. Silent circulation of dengue virus in Aedes albopictus (Diptera: Culicidae) resulting from natural vertical transmission. *Sci Rep*. 2020;10:3855.
- Stephenson CJ, Coatsworth H, Kang S, Lednicky JA, Dinglasan RR. Transmission potential of Floridian aedes aegypti mosquitoes for dengue virus serotype 4: implications for estimating local dengue risk. *mSphere*. 2021;6. <https://doi.org/10.1128/MSPHERE.00271-21>.
- Gubler DJ. Dengue and dengue hemorrhagic fever. *Clin Microbiol Rev*. 1998;11:480–496.
- Sharp TM, Morris S, Morrison A, et al. Fatal dengue acquired in Florida. *N Engl J Med*. 2021;384:2257–2259.
- Harrigan RJ, Thomassen HA, Buermann W, Cummings RF, Kahn ME. Economic conditions predict prevalence of West Nile virus. *PLoS One*. 2010;5:15437.
- Tsheten T, Clements ACA, Gray DJ, Adhikary RK, Wangdi K. Clinical features and outcomes of COVID-19 and dengue co-infection: a systematic review. *BMC Infect Dis*. 2021;21:1–9.
- Yang B, Borgert BA, Alto BW, et al. Modelling distributions of aedes aegypti and aedes albopictus using climate, host density and interspecies competition. *PLoS Negl Trop Dis*. 2021;15:e0009063.