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Dengue outbreak in Martin County, Florida in 2013

Abstract

Following a 2013 outbreak of dengue serotype 1 in Martin County, Florida, plasma samples from individuals with suspected dengue illness were tested for serotype-specific neutralization of dengue viruses 1-4. Titers and seroprevalence in these suspected cases from Florida were compared to those from (1) healthy donors from the U.S. and (2) dengue-suspected and dengue-confirmed samples collected from dengue-endemic regions during outbreaks in Puerto Rico in 2010 and Brazil in 2014. By dengue plaque reduction neutralization titer assays, we confirmed that four of the nine suspected individuals from Martin County Florida had been exposed to dengue virus serotype 1, and that these were primary dengue viral infections. Furthermore, primary and secondary dengue viral infections were observed in the Brazilian cohort, and a high incidence of secondary infections was observed in the Puerto Rican samples. Routine serotype-specific surveillance may be needed to continue monitoring establishment of dengue serotype 1 and introduction of other serotypes in Florida.

Keywords: Dengue, Serosurveillance, PRNT, Emerging diseases, Dengue endemic

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Abbreviations: DENV, Dengue Virus; PRNT, Plaque Reduction Neutralization Titer; RT-PCR, Reverse Transcription Polymerase Chain Reaction; ELISA, Enzyme Linked Immunosorbent Assay

Introduction

Dengue virus (DENV) is a positive-sense, single-stranded RNA virus that is transmitted by Aedes aegypti and Aedes albopictus mosquitoes. Greater than 100 million cases of dengue fever occur annually and over 2 billion people live in areas at risk for dengue transmission. There are four phylogenetically and serologically distinct DENV, which are capable of human-to-mosquito-to-human transmission.[1,2] Primary DENV infection is often subclinical or causing mild to moderate symptoms including; fever, headache, muscle and joint pain. Secondary, heterotypic DENV infection can result in more severe disease, termed dengue hemorrhagic fever, which can be fatal without medical intervention.

In the 1940's, large outbreaks of autochthonous dengue were documented in the continental United States (U.S.), but subsequent mosquito eradication efforts limited local DENV transmission.[2] The majority of recent dengue cases in the US are associated with recent travel to endemic regions and are self-limiting.[1,2] Since 1999, however, clusters of autochthonous DENV outbreaks have emerged in the continental U.S. and Hawaii.[3-5] In 2009-2010, an outbreak of locally acquired serotype 1 dengue (DENV-1) cases occurred in Key West (Monroe County), with additional cases in Broward and Miami-Dade Counties.[3-6] In August 2013, the latest dengue outbreak in Florida occurred in Martin County (Rio/Jensen Beach) and resulted in persistent transmission.[7] The Department of Health identified 28 individuals that were infected with DENV. Twenty-four of these individuals were symptomatic between June-September and 6 were hospitalized. There were 4 asymptomatic, but laboratory-confirmed DENV positive individuals. The strains isolated in the Martin County outbreak originated from a DENV-1 circulating in the Caribbean from South American origins, while the 2010 Key West outbreak strain was most closely related to Central American viruses.[8]

These two viruses are distinct and did not result from a single DENV-1 introduction into Florida with subsequent local transmission. In order to determine how widespread the DENV outbreak was during the summer of 2013, we performed a survey of residents in Martin/St. Lucie counties of Florida. Serum samples were collected from 70 residents ranging from 23-82 years of age and tested for antibodies against all 4 serotypes of DENV. Samples were tested for anti-dengue E antibodies by ELISA and dengue serotype-specific plaque reduction neutralization titer (PRNT) assays to confirm dengue infection and to determine the serotype of the infecting dengue virus, and to determine if the subjects had been previously exposed to other dengue serotypes. We also received 9 clinical samples from nine individuals suspected of dengue infection as identified by the local Martin Health Systems hospital. The Florida Department of Health laboratory performed dengue-specific RT-PCR and IgM antibody diagnostic tests on samples collected from these 9 individuals. All these results were compared and reported here.

Methods

Study samples

Plasma samples were collected from a total of 70 Martin and St Lucie County Florida residents between August-December, 2013 with consent (IRB FLU-002 and DEN-001). Blood samples were collected from symptomatic patients that presented at the local Martin Health System Hospital in Fort Pierce, Florida. Blood samples were collected by the attending physician's staff and were sent to the Florida Department of Health (FDH) for diagnostic testing. Acute phase samples (1-5 days after onset of symptoms) were tested at the Public Health Department in Jacksonville, FL using the DENV-1-4 real-time RT-PCR assay for detection of dengue virus nucleic acid (protocol by Centers for Disease Control and Prevention).[9] If samples were collected from the patient more than five days after onset of symptoms or if the RT-PCR assay was negative, the IgM antibody test was performed. Patients who have IgM antibodies to dengue in their serum are classified as having recent probable dengue infection.[10]

The Florida Health Department Laboratory uses the IgM antibody capture ELISA (MAC-ELISA) testing procedure. Briefly, human IgM in the patient's sample is captured on a microtiter plate using anti-human IgM antibody. Dengue virus envelope protein is then added,

and is captured by plate-bound, dengue-specific IgM. After incubation and washing steps, an enzyme-labeled, dengue-specific monoclonal antibody is added for detection. After washing, substrate is added and absorbance is measured. For comparison, we also obtained samples from blood donors that were previously collected by the American Red Cross in Puerto Rico, coinciding with a 2010 DENV outbreak. Dengue infection was also confirmed in these Puerto Rican samples by CDC DENV-1-4 real-time RT-PCR assay[11] and anti-DENV envelope IgM capture ELISA. Additional samples were obtained from dengue-suspected cases during a 2014 outbreak in Brazil.

Dengue serotype-specific IgG ELISA

High-binding ELISA plates (Costar, Corning, NY) were coated overnight at 4˚C with 100µl/well serotype-specific recombinant DENV envelope protein (Virostat, Portland ME) diluted to 2µg/mL in PBS pH 7.5 containing 8µg/mL bovine serum albumin (BSA) (Equitec-Bio, Kerrville, TX) in a humidified chamber. Control plates were coated with 10µg/mL BSA in PBS pH 7.5. Plates were blocked with ELISA blocking buffer (PBS containing 5% BSA, 2% bovine gelatin and 0.05% Tween 20) for 90min at 37˚C. Plasma samples diluted 1:500 in ELISA blocking buffer were added in duplicate to plates coated with individual serotype-specific recombinant DENV envelope protein or BSA alone and incubated overnight at 4˚C. Plates were washed five times with PBS.

Horseradish peroxidase conjugated goat anti-human IgG Fc secondary antibody (Southern Biotech, Birmingham, AL, USA) diluted 1:2000 in blocking buffer was added and plates were incubated for 60 min at 37˚C. Following additional PBS washes, ABTS substrate (Amresco, Solon, OH) was added and plates were incubated for 30min at 37˚C. Colorimetric conversion was terminated by addition of 5% SDS (50µl/well) and optical density was measured at 414nm. For individual samples, the mean OD414 from control BSA coated wells was subtracted from the mean OD414 from DENV envelope protein coated wells. The cut-off for seropositivity was defined as a serotype-specific E protein OD414 that was greater than background plus five times the standard deviation of blank wells. Samples were scored as seropositive if reactivity repeated in 2 or more independent experiments.

Plaque reduction neutralization titer assay

Plasma samples were tested by PRNT assay in Vero cells cultured to confluence in 96-well plate. The plasma samples were serially-diluted in DMEM supplemented with supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, and 2.5% fetal bovine serum (2.5% FBS-DMEM) and incubated with 50 plaque-forming units of DENV for 30 min at 37ºC. Prototype viruses for each serotype were used: DENV-1 (Hawaii), DENV-2 (NGC), DENV-3 (H87), and DENV-4 (H241). Vero culture media were replaced with these virus-antibody solutions. After 90 min at 37ºC, the virus-antibody solutions were removed and the cells were overlaid with 1% methylcellulose in 2.5% FBS-DMEM. The plates were incubated for 2-3 days at 37ºC and 5% CO2, and fixed with 1:1 acetone to methanol (v/v). DENV plaques were developed via an indirect immunostaining method employing DENV-specific antibodies 9.F.10 pan-dengue antibody (Santa Cruz Biotechnology, Dallas, TX), pan-dengue antibody (ACRIS, San Diego, CA), NR-15515anti-DENV3 (BEI Resources, Manassas, VA), and NR-15536 anti-DENV4 (BEI Resources), along with a peroxidase-based detection kit (Vector Laboratories, Burlingame, CA). Along with a peroxidase-based detection kit. Results are reported as the PRNT with 90% or greater reduction in DENV plaques (PRNT90), and 1:20 was considered a positive titer.

Results

Study population in Florida

In July-August 2013, an outbreak of dengue was confirmed in Martin and St. Lucie Counties in Florida.[7] The goal of this study was to determine how widespread this outbreak of dengue virus was in these two counties; therefore, serum samples were collected anonymously from 61 persons that ranged in age from 23 to 82 years of age (Table 1). Overall, 3.3% of serum samples collected from healthy people were IgG positive for the DENV-1 (Figure 1), but were less than 2% positive for DENV-2 or DENV-4. Sixteen percent of samples from healthy individuals were positive for DENV-3. This is in contrast to samples collected from 9 subjects, ages 26-73 that presented in the hospital and were symptomatic for dengue virus infection (Table 1). Forty-four percent (4 out of 9) had IgG antibodies to DENV-1 and two of the patients had antibodies to DENV-3, but none of the patients were positive to DENV-2 or DENV-4. Two of the 9 patients also tested positive for IgM antibodies, one of which was also anti-DENV-1 IgG positive. These two individuals were the only patients to also have a positive RT-PCR test for detection of DENV-1 viral RNA (Table 2).

Table 1 Demographics by Cohort

Cohort	Gender	Age (Years)
Martin Symptomatic (FL, USA)	Male (n=6) Female (n=3)	26-73
Martin/St Lucie Counties (FL, U.S.)	Male (n=15) Female (n=46)	23-82
Puerto Rico	Male (n=22) Female (n=3)	21-72
Brazil	n/a	n/a

Table 2 Martin, FL 2013: Clinical Diagnostics and DENV Neutralization titers

IgG by Serotype							DV1		DV2		DV3		DV4	
Donor	IgM	DV1	DV2	DV3	DV4	RT-PCR	PRNT90	Neutralize	PRNT90	Neutralize	PRNT90	Neutralize	PRNT90	Neutralize
DEN-101	-	+[b]	-	-	-	n.d.	320	Y	80	Y	80	Y	2D	Y
DEN-102	-	-	-	-	-	-	0	N	0	N	0	N	0	N
DEN-1 D3	-	-	-	-	-	-	0	N	0	N	0	N	0	N
DEN-104	-	-	-	-	-	DV1+	320	Y	320	Y	80	Y	80	Y
DEN-105	+	+[b]	-	+	-	DV1+	320	Y	50	Y	200	Y	20	Y
DEN-106	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
DEN-107	-	+[b]	-	+	-	n.d.	320	Y	20	Y	2D	Y	20	Y
DEN-108	+[a]	+	-	-	-	-	0	N	0	N	0	N	10	N
DEN-109	-	-	-	-	-	n.d.	0	N	0	N	0	N	0	N

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Table Continued...

IgG by Serotype							DV1		DV2		DV3		DV4	
Donor	**IgM**	**DV1**	**DV2**	**DV3**	**DV4**	**RT-PCR**	**PRNT90**	**Neutralize**	**PRNT90**	**Neutralize**	**PRNT90**	**Neutralize**	**PRNT90**	**Neutralize**
Healthy Control 101	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 102	n.d.	-	-	-	-	n.d	0	N	0	N	0	N	0	N
Healthy Control 103	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 104	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 105	n.d.	-	-	+	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 108	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 110	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 113	n.d	-	-	-	-	n.d	0	N	0	N	0	N	0	N
Healthy Control 115	n.d.	-	-	+	-	n.d.	0	N	0	N	0	N	10	N
healthy Control 118	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 120	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N
Healthy Control 123	n.d.	-	-	-	-	n.d.	0	N	0	N	0	N	0	N

[a]Retest of the sample 3 weeks later yielded negative results for IgM, IgG, and RT-PCR.

[b]Samples exhibited DENV-1-specific IgG titer ≥ 5X cut-off for seropositivity.

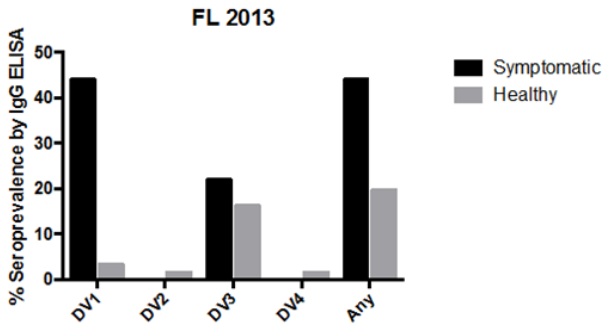

Figure 1 Dengue virus seroprevalance in Florida by IgG ELISA.

Dengue serotype-specific IgG responses in symptomatic and healthy samples from Florida were detected by ELISA.

To confirm these results, we tested the symptomatic and healthy cohorts for neutralizing antibodies to viruses representing each DENV serotype (Table 2). Overall, serum samples from the healthy population of Florida lacked antibody neutralization activity to any of the 4 DENV (Table 2). However, 4 symptomatic patients (DEN-101, DEN-104, DEN-105, and DEN-107) had neutralizing antibodies to each of the DENV serotypes. All 4 of these patients had 1:320 PRNT90 to DENV-1, with titers ranging between 1:20 to 1:320 to DENV-2,-3, and -4. Of note, patient DEN-104 was not scored positive for anti-DENV-1 IgG, but was positive on the basis of anti-DENV-1 IgM ELISA, RT-PCR, and PRNT 90. Furthermore, patient DEN-108 exhibited IgG+ reactivity with DENV-1 by ELISA, but was negative for PRNT90 against all DENV serotypes.

Study populations from Puerto Rico and Brazil

In order to determine if the results in Florida were indicative of populations where dengue is prevalent or where dengue does not circulate, we examined the seroprevalence from serum samples collected from San Juan, Puerto Rico and Sao Paolo, Brazil (Tables 3 & 4). From the Puerto Rican 2010 cohort, subjects with positive IgM and RT-PCR results were considered confirmed cases (Table 3). There were two time-points for 7 individuals and one time-point for all others. Seropositive rates for this cohort ranged between 72-84% against the 4 DENV serotypes and the PRNT90 titers ranged from 1:20-1:20,480. Even though some of the plasma samples were negative for anti-DENV IgM antibodies or DENV negative by RT-PCR, they still had positive PRNT90 titers that ranged between 1:55-1:195. This indicated that these latter donors had previous exposures to DENV, prior to the 2010 outbreak in Puerto Rico. In contrast, samples that were confirmed as DENV positive by IgM and RT-PCR analyses had significantly higher PRNT90 titers (ranging between 1:5741-1:6506).

Samples from several time-points (2-30 days post-index) were collected per individual in the Brazilian cohort representing acute infection to convalescence. Plasma samples collected between 2-6 days had low PRNT90 (1:20-1:80) for all DENV serotypes (Table 4), with a few exceptions. For example, sample collected from patient DENMS106 or DENMS203 had high titers (1:5,120-1:81,920) against all 4 DENV serotypes. Ninety-three percent (15/16) of Brazilian subjects had a peak PRNT90 between days 10-30 post-index.

Discussion

The 2013 dengue outbreak in Martin and St. Lucie Counties in Florida was not widespread. We chose two different strategies to assess dengue antibodies, the IgG ELISAs and neutralization assays.[10-12] The standard ELISA was designed to detect anti-E antibodies in the plasma from collected blood samples. The PRNT assay measures the ability of antibodies in polyclonal plasma to neutralize virus infection in vitro and is the most serologically virus-specific test for

flaviviruses[12] and serotype-specific test for DENV. Although PRNT with 50% reduction in the number of DENV plaques (PRNT50) is acceptable for serotyping, we report the PRNT with 90% or greater reduction in DENV plaques (PRNT90). The PRNT90 is more useful for epidemiological or diagnostic studies in dengue endemic locations, because there is decreased background serum cross-reactivity and the PRNT90 offers less variability than the PRNT50. While the threshold for seroprotection is not known, many use a seropositivity threshold of 1:10; we used a slightly more stringent threshold for a positive titer of 1:20.[12]

Table 3 Puerto Rico 2010: Clinical Diagnostics and DEW Neutralization Assays

Donor	Days post index	Symptom	confirmed positive	DV1		DV2		DV3		DV4	
				PRNT90	Neutralize	PRNT90	Neutralize	PRNT90	Neutralize	PRNT90	Neutralize
1	9	As	N	20	Y	80	Y	20	Y	20	Y
2	11	As	N	0	N	0	N	0	N	0	N
3	13	As	N	320	Y	320	Y	80	Y	20	Y
4	14	As	N	10	N	80	Y	10	N	50	Y
5	18	As	N	0	N	0	N	0	N	0	N
6	12	As	N	320	Y	0	N	0	N	0	N
7	12	As	N	800	Y	50	Y	n.d.	n.d.	50	Y
8	13	As	N	20	Y	50	Y	320	Y	20	Y
8	17	As	N	20	Y	80	Y	20	Y	170	Y
9	13	As	N	320	Y	200	Y	80	Y	20	Y
10	7	As	Y	320	Y	20	Y	20	Y	20	Y
11	20	As	Y	1280	Y	3200	Y	3200	Y	20480	Y
12	21	As	Y	1280	Y	12800	Y	5120	Y	3200	Y
13	15	As	Y	0	N	0	N	0	N	50	Y
13	21	As	Y	0	N	0	N	0	N	80	Y
14	5	As	Y	20480	Y	20480	Y	3200	Y	80	Y
14	21	As	Y	3200	Y	5120	Y	2720	Y	320	Y
15	20	As	Y	320	Y	0	N	20	Y	10	N
16	9	As	Y	20480	Y	5120	Y	5120	Y	1280	Y
16	15	As	Y	5120	Y	26240	Y	1280	Y	800	Y
17	8	As	Y	1280	Y	0	Y	20	Y	n.d.	n.d.
17	15	As	Y	320	Y	15	N	50	Y	10	N
18	13	As	Y	20480	Y	80	Y	20	Y	20	Y
19	13	S	Y	12800	Y	20480	Y	n.d.	n.d.	n.d.	n.d.
20	19	S	Y	80	Y	0	N	0	N	0	N
21	19	S	Y	1280	Y	5120	Y	5120	Y	10880	Y
22	20	S	Y	1280	Y	3200	Y	12800	Y	20480	Y
23	19	S	Y	1280	Y	1280	Y	51200	Y	51200	Y
24	15	S	Y	20480	Y	5120	Y	20480	Y	5120	Y
24	21	S	Y	3200	Y	5120	Y	1280	Y	1280	Y
25	7	S	Y	5120	Y	12800	Y	3200	Y	5120	Y
25	21	S	Y	800	Y	3200	Y	5120	Y	3200	Y

Table 4 Sao Paulo, Brazil 2014: DENV Neutralization Titers

Donor	Day	PRNT90	DV1 Neutralize	PRNT90	DV2 Neutralize	PRNT90	DV3 Neutralize	PRNT90	DV4 Neutralize
DENMS101	10	120	Y	0	N	0	N	10	N
DENMS 101	30	240	Y	U	N	20	Y	0	N
DENMS102	6	0	N	0	N	0	N	0	N
DENMS 102	30	80	Y	0	N	10	N	0	N
DENMS105	6	40	Y	0	Y	50	Y	80	Y
DENMS105	10	160	Y	20	Y	20	Y	80	Y
DENMS105	14	120	Y	40	Y	20	Y	0	Y
DENMS 105	30	240	Y	20	Y	0	N	0	N
DENMS 106	4	20480	Y	7680	Y	81920	Y	5120	Y
DENMS106	6	20480	Y	5120	Y	81920	Y	20480	Y
DENMS 106	10	20480	Y	10240	Y	81920	Y	5120	Y
DENMS106	14	20480	Y	5120	Y	12800	Y	5120	Y
DENMS109	6	0	N	0	N	0	N	0	N
DENMS 109	10	640	Y	30	Y	200	Y	20	Y
DENMS111	6	320	Y	0	N	0	N	0	N
DENMS111	10	160	Y	20	Y	20	Y	0	Y
DENMS 111	14	120	Y	20	Y	50	Y	40	Y
DENMS 111	30	640	Y	10	N	50	Y	0	N
DENMS116	4	0	N	0	N	0	N	0	N
DENMS 116	6	960	Y	200	Y	200	Y	20	Y
DENMS 116	10	2640	Y	320	Y	800	Y	RO	Y

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Table Continued...

Donor	Day	PRNT90	DV1 Neutralize	PRNT90	DV2 Neutralize	PRNT90	DV3 Neutralize	PRNT90	DV4 Neutralize
DENMS116	14	800	Y	200	Y	800	Y	80	Y
DENMS116	30	15360	Y	180	Y	320	Y	170	Y
DENMS 118	14	5120	Y	100	Y	80	Y	80	Y
DENMS118	30	200	Y	20	Y	80	Y	20	Y
DENMS 120	2	0	N	0	N	0	N	0	N
DENMS 120	10	60	Y	10	N	0	N	0	N
DENMS120	14	360	Y	0	Y	0	N	20	Y
DENMS 120	30	20480	Y	0	N	0	N	20	Y
DENMS 128	2	20	Y	0	N	0	N	0	N
DENMS128	17	200	Y	0	N	0	N	20	Y
DENMS132	4	0	N	0	N	0	N	0	N
DENMS132	10	0	N	0	N	0	N	0	N
DENMS139	14	1320	Y	90	Y	80	Y	50	Y
DENMS 139	30	200	Y	60	Y	20	Y	20	Y
DENMS140	4	20	Y	0	N	0	N	0	N
DENMS140	10	100	Y	0	N	0	N	0	N
DENMS141	14	80	Y	0	N	20	Y	20	Y
DENMS141	17	320	Y	10	Y	50	Y	20	Y
DENMS201	2	480	Y	160	Y	1280	Y	1280	Y
DENMS201	6	960	Y	240	Y	320	Y	1280	Y
DENMS201	10	960	Y	120	Y	1280	Y	12S0	Y
DENMS201	30	320	Y	80	Y	1280	Y	SOO	Y
DENMS203	6	20480	Y	20480	Y	81920	Y	81920	Y

While RT-PCR and IgM antibody capture ELISA are important tools in the diagnosis of DENV, their sensitivity is limited to the first 5 days of symptoms, even though IgM titers may remain elevated 2-3 months after illness.[10] Since there was an absence of complete and definitive dengue diagnostic results for seven of the nine symptomatic cases of dengue presented to the Martin Health System, we first performed serotype-specific IgG ELISA (Table 1). The advantage of using IgG ELISA is that it is a rapid and high-throughput test for diagnosis. However, because IgG ELISA lacks specificity in differentiating infections caused by viruses in the flavivirus serocomplex, we also performed PRNT assays. Of the nine symptomatic cases, four had PRNT antibodies and they were positive by at least one other diagnostic assay (RT-PCR, IgM ELISA, IgG ELISA). One sample from the symptomatic cohort and two samples from the healthy cohort that were positive for DENV by IgG ELISA were negative by PRNT assay, indicating possible infection with another flavivirus. This underscores the importance of validating anti-DENV ELISA results with more than one diagnostic assay, particularly the PRNT assay.

The 2013 DENV outbreak in Florida was attributed to introduction of DENV-1,[8] as our PRNT results also confirmed. The infected individuals were previously naive to DENV, as supported by overall low titers and low cross-reactivity to other serotypes.[12] Cross-protection to other serotypes within a short-lived, 8-week window is expected, and this cross-protection wanes between 4-9 months post-infection.[13] Thus, we would expect a reduction in PRNT90 for DENV serotypes 2-4 after 4 months in these samples. We next compared the serotype-specific activity in these samples from Florida to plasma samples from people living in dengue-endemic regions.

To determine if the serosurveillence results we observed in Florida in 2013 were indicative of a low dengue prevalent location or a dengue-prevalent region, we compared our results to two locations with many years of active dengue infections. For people living in Brazil, DENV-1 and -4 were the infecting serotypes in 2014,[14] and the majority of our PRNT positive subjects had the most robust titers to DENV-1, although DENMS201 also had stronger titers against DENV-3 and DENV-4. Robust neutralization responses against more than 1 serotype suggest previous infection with other serotypes. This would be expected in dengue-endemic countries, such as Brazil, that recently recorded circulation of strains from all four serotypes.[15]

In contrast to Florida and Brazil, PRNT90 in DENV-confirmed Puerto Rican samples were high across multiple serotypes, although the infecting serotypes were reported to be DENV-1 and -4.[11] These results are consistent with a secondary exposure to DENV.[16] In these areas, high DENV neutralizing titers are routinely observed to exposure and non-exposure serotypes. Thus, upon a future establishment of DENV-1, as well as the introduction of other serotypes into Florida, we would have expected to observe higher PRNT90 titers than in 2013 and more cross-reactivity between dengue viruses.

Over the past decade there has been isolated outbreaks of dengue virus infections in people living in south Florida,[3-7] but as we demonstrate in this report, outbreaks of dengue virus are isolated and self- limiting, therefore, confirming again that dengue virus has yet to establish/re-establish itself in the United States. No DENV cases were reported in 2014 or 2015 in Florida. However, with increasing temperatures, and especially in the absence of surveillance or active measures, DENV and other tropical diseases may become established, since Florida is a gateway location to other mainland states. As primary DENV infections tend to be asymptomatic or mild, routine serosurveillance for DENV may be essential in monitoring for the establishment of dengue viruses in Florida and other regions of the United States.

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Conflicts of interest

None.

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