Dengue in the campus community of an overseas American university: A cross-sectional study

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Abstract
Introduction: After a large outbreak of dengue virus (DENV) serotype-3 in Saint Kitts and Nevis (SKN) in 2008, we performed a cross-sectional study to determine the prevalence of anti-DENV immunoglobulin G (IgG) antibodies in expatriate and local persons affiliated with an American veterinary school there.

Methodology: This campus community comprised mostly expatriate students and faculty and Kittitian administrative staff. In 2009, a stratified random sample of students, faculty and staff was invited to complete an electronic survey to assess risk factors for DENV and provide blood for testing for anti-DENV IgG antibodies by an enzyme-linked immunosorbent assay. IgG-positive specimens were also tested by a 90% plaque reduction neutralization test (PRNT90) to determine immunoreactivity to DENV (1-4) serotypes and West Nile virus. Risk factors for anti-DENV IgG seropositivity were determined using simple and adjusted logistic regression.

Results: Of the 118 participants, the overall prevalence of DENV IgG antibodies was 44.1% (95% confidence interval [CI]: 35.1-53.0%), ranging from 30.1% in students, 100.0% in staff and 57.9% in faculty (p < 0.001). Duration of residence in St. Kitts was the only variable significantly associated with seropositivity on multiple logistic regression (adjusted odds ratio [95% CI]: 1.21 [1.07-1.37]). The serotype of DENV was determined in 11 persons: DENV-1 (n = 4), DENV-2 (n = 3), and DENV-3 (n = 4).

Conclusions: Expatriate students and faculty moving to St. Kitts from non-endemic areas were at high risk of DENV infection. There is a need for increased emphasis on pre-travel mosquito-borne virus prevention education for persons moving to St. Kitts to study and work.

Key words: Dengue fever; Saint Kitts and Nevis; travellers.


(Received 12 July 2018 – Accepted 21 January 2019)

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Introduction

Dengue fever (DF), the most common arboviral disease in the world, is caused by infection with any of the four dengue virus serotypes (DENV-1, -2, -3 and -4). The majority of DENV infected individuals are asymptomatic [1-3]; however, the spectrum of clinical illness includes non-severe dengue, with or without warning signs, and severe dengue [4]. While there is a dengue vaccine registered for use in people aged 9-45 years living in endemic areas, its use is not yet widespread and there is no recommendation for travellers at this time [5]. Therefore, the key means of DENV prevention is through avoidance of bites from the mosquito vector. The principal DENV vector, the Aedes aegypti mosquito, is common throughout the tropics and subtropics. Over one-half of the world’s population is estimated to be at risk of DENV infection, with an estimated 390 million dengue cases occurring worldwide, annually [6].

The Federation of Saint Kitts and Nevis (SKN), an independent twin-island state of the Caribbean, is located roughly 300 km to the southeast of Puerto Rico. St. Kitts, with an area of 168 km², is the larger island and home to the majority of the estimated 50,000 residents of the Federation. This island hosts an American Veterinary Medical Association-accredited veterinary school and the vast majority of its students are from North America and are required to spend at least 2.25 years in St. Kitts. The university employs mostly Kittitian administrative staff and North American faculty; in most cases, persons on faculty live in St. Kitts for longer durations than students.
Passive surveillance for dengue is conducted by the Health Information Unit (HIU) of the Ministry of Health (MOH) of SKN and the Caribbean Public Health Agency (CARPHA) in Port of Spain, Trinidad and Tobago. Most cases are reported by public health clinics and the two public hospitals in the country. Dengue in SKN is seasonal, with more cases usually reported during the rainy season (June-December) of each year. There are periodic outbreaks and, prior to 2009, the two most recent occurred in 2001 and 2008 [7,8]. The outbreak serotype during 2008 was DENV-3 [9] but, on separate occasions, all four serotypes have been found in SKN [http://www.who.int/denguenet]. During 2008, 100 suspected dengue cases were reported to the HIU. Several members of the veterinary school campus community sought care for febrile, dengue-like illness during this outbreak, but many of them did not have diagnostic testing.

Dengue has been endemic in the Caribbean for several years, but there is little prevalence data from SKN, and even less from expatriate persons resident in this country. This campus community provided an opportunity to determine the prevalence of dengue antibodies in a study population of both expatriate and local residents of SKN, less than a year after the outbreak of 2008. The primary objective of this study was to determine the prevalence of anti-DENV immunoglobulin G (IgG) antibodies among the campus community of this veterinary school.

Methodology

Ethics statement

This study was approved by the Institutional Review Board of the Ross University School of Medicine (Dominica, West Indies) and by the Ethical Board of the MOH. Informed consent was obtained from all participants.

Study design and sample size

A cross-sectional study was performed between June-August 2009 on a stratified random sample of the campus community using a serological survey and a self-administered online questionnaire to assess risk factors for DF. The study population was defined as all students, staff and faculty affiliated with the school; the sampling frame comprised all enrolled students and employed staff and faculty that had been residents of SKN for at least nine months (since September 2008). The duration of residence restriction was to ensure that all persons had been living in SKN for at least one dengue season. The sampling frame was derived from the school’s emergency contact roster; there were 717 individuals meeting the inclusion criteria as of May 2009. A stratified random sample was selected, with the strata defined by occupation (student, staff, or faculty), and with proportions allocated to the sample reflecting those of the campus community (68% students, 23% staff and 9% faculty). Assuming a DENV antibody prevalence of 70%, precision of 10%, type-I error rate of 5%, power of 80% and with a 20% inflation for refusals, the target sample size was set at 240.

Participant recruitment

On a weekly basis, roughly 40 of the randomly selected individuals were contacted by e-mail and invited to participate in the study. Those that did not respond by the end of the week were reminded (one additional e-mail and two phone-calls), as necessary, over the following two weeks. Participants provided consent through the online questionnaire and before having their blood drawn. In addition, to have their serum specimens undergo testing using a 90% plaque reduction neutralization test (PRNT90), participants had to sign an additional consent form. All study participants were given a voucher for a free drink at the campus coffee shop.

Data collection

Study participation required completion of an online questionnaire (Survey Monkey, Palo Alto, California, USA) and a visit to the campus nursing services office for a 5 mL blood draw. The questionnaire was pilot tested with local and expatriate members of the campus and the general community, and contained items measuring demographic and household characteristics, travel history and clinical history (including self-report of febrile illness of at least one-day duration from September to December 2008). Participants were sent a link to the online questionnaire by email; however, they were also given the option to complete a hardcopy questionnaire.

Laboratory testing

Whole blood was allowed to clot and serum separated by centrifugation (Thermo Scientific, Waltham, Massachusetts, USA) at room temperature at the Diagnostic Research Laboratory (DRL) of the veterinary school. The serum was stored at -20°C until tested by an enzyme-linked immunosorbent assay (ELISA) for anti-DENV IgG [10] at the DRL. The serum was titrated using four-fold dilutions starting at 1:40 and ending with 1:655 360. Seropositivity was defined as an optical density (OD) > 0.15 in any of the 7 dilutions, and the final titer was determined by the last
dilution with an OD > 0.15. Positive specimens of persons who consented were tested using a PRNT<sub>90</sub> to confirm the specificity of anti-DENV IgG and immunoreactivity to DENV (1-4) serotypes or West Nile virus (WNV) [11]. The samples were titrated using two-fold dilutions starting with 1:40 and ending with a dilution of 1:640. This testing was performed at the Dengue Branch of the Centers for Disease Control and Prevention in San Juan, Puerto Rico.

Data analysis
Analyses were performed using SPSS version 17.0 (IBM, Armonk, New York, USA) and SAS version 9.2 (SAS Institute, Cary, North Carolina, USA). The frequencies of participants' demographic and clinical characteristics were determined. To assess any differences between students, staff and faculty, the Pearson’s chi-square and student’s t-tests were used. The primary outcome for bivariate and multivariable analysis was seropositivity (yes vs. no), and these analyses were performed using the Pearson’s chi-square test, Fisher’s exact test, student’s t-test, Kruskal-Wallis test and binary logistic regression. All variables that were significantly associated with seropositivity on bivariate analysis were considered for inclusion in the final multivariable model, which was determined using stepwise selection to obtain a parsimonious model. Adjusted odds ratios (aOR) and 95% confidence intervals (CIs) were determined. All tests for significance were performed at a type I error rate of 5%. Secondly, a descriptive analysis was performed to determine the occupations, areas of residence and travel histories of cases of recently acquired DENV infection, which was defined as follows: living in SKN no earlier than January 1, 2008 (for a single dengue season), born in a non-endemic country, no self-report of DF prior to 2008, and seropositive.

Results
Description of participants
Of the 240 individuals randomly selected to participate in the study, 135 (56.3%) consented and completed the questionnaire. There was a significant difference in participation rate when stratified by occupation [students (59.1%), staff (38.2%) and faculty (87.0%) (p < 0.001)], and the proportions of these groups in the sample varied from those of the study population (p = 0.003). Of the 135 persons who completed the questionnaire, 118 (87.4%) provided blood samples. There was no significant difference in age, gender or ethnicity between those who did or did not provide a blood sample (data not shown).

The median age of these 118 participants was 27 years (range: 21-71 years); most were female (67.8%), white (77.3%), and born outside a dengue-endemic country (81.4%). Twenty-eight participants (23.7%) reported experiencing a febrile illness of at least 1 day duration from September to December 2008, and 5.9% reported having DENV infection during that dengue season, born in a non-endemic country prior to living in SKN (87.4%) provided blood samples. There was no significant difference in age, gender or ethnicity between those who did or did not provide a blood sample (data not shown).

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Table 1. Characteristics of study participants from the campus community<sup>1</sup> stratified by occupation (St. Kitts, 2009) (n = 118)<sup>2</sup>.

<table>
<thead>
<tr>
<th></th>
<th>Students (n = 83)</th>
<th>Staff (n = 16)</th>
<th>Faculty (n = 19)</th>
<th>p-value&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>25.0</td>
<td>33.0</td>
<td>46.0</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Median duration of residence in St. Kitts (years)</td>
<td>1.6</td>
<td>30.5</td>
<td>6.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Female (%)</td>
<td>77.1</td>
<td>62.5</td>
<td>31.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Black (%)</td>
<td>0.0</td>
<td>93.8</td>
<td>15.8</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Born in Saint Kitts and Nevis (SKN) (%)</td>
<td>0.0</td>
<td>87.5</td>
<td>0.0</td>
<td>0.004*</td>
</tr>
<tr>
<td>Born in a dengue-endemic country (%)</td>
<td>2.4</td>
<td>93.8</td>
<td>26.3</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Travel history to a dengue-endemic country prior to living in SKN (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>60.2</td>
<td>0.0</td>
<td>68.4</td>
<td>0.823</td>
</tr>
<tr>
<td>Ever vaccinated for Yellow Fever (%)</td>
<td>8.4</td>
<td>6.3</td>
<td>42.1</td>
<td>0.058</td>
</tr>
<tr>
<td>Ever vaccinated for Japanese Encephalitis (%)</td>
<td>1.2</td>
<td>0.0</td>
<td>10.5</td>
<td>0.775</td>
</tr>
<tr>
<td>Had dengue fever previously (before 2008) (%)</td>
<td>1.2</td>
<td>12.5</td>
<td>21.1</td>
<td>0.005*</td>
</tr>
<tr>
<td>Had a febrile illness&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>24.1</td>
<td>31.3</td>
<td>15.8</td>
<td>0.709</td>
</tr>
<tr>
<td>Had screened windows at residence&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>97.8</td>
<td>57.1</td>
<td>95.0</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Daily use of air-conditioning at residence&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>66.7</td>
<td>4.8</td>
<td>40.0</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Stored water for household use&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>20.4</td>
<td>33.3</td>
<td>20.0</td>
<td>0.257</td>
</tr>
<tr>
<td>Bitten by mosquitoes at home&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>89.2</td>
<td>90.5</td>
<td>80.0</td>
<td>0.833</td>
</tr>
<tr>
<td>Bitten by mosquitoes on the porch&lt;sup&gt;4&lt;/sup&gt; (%)</td>
<td>91.4</td>
<td>62.5</td>
<td>69.2</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

<sup>1</sup>Defined as all enrolled students and employed faculty and staff of the veterinary school, as of September 2008; <sup>2</sup>Only the participants who did the questionnaire and the blood draw are considered in this analysis; <sup>3</sup>Only asked of individuals born in a non-endemic country; <sup>4</sup>These questions were asked about the period September-December 2008 to assess risk factors for dengue infection during that dengue season; <sup>*</sup>Based on Pearson’s chi-square (categorical variables) or student’s t- (continuous variables) tests; <sup>*</sup>p < 0.05.
and 6.6 years respectively ($p < 0.001$). Further comparison of the students, staff and faculty included in the study is given in Table 1.

**Prevalence of dengue antibodies and associated risk factors**

The overall prevalence of anti-dengue IgG antibodies among the 118 participants was 44.1% (95% CI: 35.1–53.0%), and was 30.1% in students, 100.0% in staff and 57.9% in faculty ($p < 0.001$). Among students, despite most commonly reporting daily usage of air-conditioning at their residence during the last dengue season, they were significantly more likely to report being bitten by mosquitoes while on their porches (Table 1).

Age, duration of residence, ethnicity and birth in a dengue endemic country were all significantly associated with seropositivity (Table 2) and were considered in the final model. Due to strong collinearity between being born in SKN and being born in a dengue-endemic country, only the latter was considered in the final model. Additionally, occupation (student vs. staff vs. faculty) was not included because all staff members were positive. However, this variable was strongly correlated with other variables considered for inclusion in the model. On multivariable analysis, the only factor significantly associated with seropositivity was duration of residence in SKN (aOR 1.21, 95% CI: 1.07–1.37).

**Table 2.** Bivariate associations with IgG antibody to dengue virus, defined by anti-DENV IgG enzyme-linked immunosorbent assay (ELISA)\(^1\), among members of the campus community\(^2\) (St. Kitts, 2009) (n = 118)\(^3\).

<table>
<thead>
<tr>
<th></th>
<th>Seropositive (n = 52)</th>
<th>Seronegative (n = 66)</th>
<th>p-value(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, years)</td>
<td>30.0</td>
<td>25.0</td>
<td>0.003*</td>
</tr>
<tr>
<td>Duration of residence in St. Kitts and Nevis (SKN) (median, years)</td>
<td>2.2</td>
<td>1.8</td>
<td>0.023*</td>
</tr>
<tr>
<td>Female (%)</td>
<td>41.3</td>
<td>58.8</td>
<td>0.430</td>
</tr>
<tr>
<td>Black (%)</td>
<td>94.4</td>
<td>5.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Occupation - Student (%)</td>
<td>30.1</td>
<td>69.9</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>- Staff (%)</td>
<td>100.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>- Faculty (%)</td>
<td>57.9</td>
<td>42.1</td>
<td></td>
</tr>
<tr>
<td>Born in SKN (%)</td>
<td>100.0</td>
<td>0.0</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Born in a dengue-endemic country (%)</td>
<td>86.4</td>
<td>13.6</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Travel history to a dengue-endemic country prior to living in SKN (%)</td>
<td>36.5</td>
<td>63.5</td>
<td>0.481</td>
</tr>
<tr>
<td>Ever vaccinated for Yellow Fever (%)</td>
<td>62.5</td>
<td>37.5</td>
<td>0.179</td>
</tr>
<tr>
<td>Ever vaccinated for Japanese Encephalitis (%)</td>
<td>100.0</td>
<td>0.0</td>
<td>0.075</td>
</tr>
<tr>
<td>Had a febrile illness from September-December 2008 (%)</td>
<td>46.4</td>
<td>53.6</td>
<td>0.952</td>
</tr>
<tr>
<td>Had dengue fever previously (before 2008) (%)</td>
<td>71.4</td>
<td>28.6</td>
<td>0.238</td>
</tr>
</tbody>
</table>

\(^1\) A positive result was defined as an optical density (OD) > 0.15 in any of the 7 dilutions of the ELISA; \(^2\) All participants are from the campus community, which is defined as all enrolled students and employed faculty and staff of the veterinary school, as of September 2008; \(^3\) Defined as a positive result [optical density (OD) > 0.15 in any of the 7 dilutions] on the anti-dengue IgG enzyme-linked immunosorbent assay, in a person who was from a non-endemic country, did not report having dengue before 2008, and had lived in St. Kitts no earlier than January 1, 2008 (one dengue season). There were 20 persons with recent infection, 19 of which provided details on their residence; \(^4\) Based on unadjusted logistic regression analysis; \(^*p < 0.01\).
Recently acquired dengue infections

Based on previously described criteria, out of the 118 participants, there were 20 (16.9%) persons with evidence of recent infection. Of these 20 persons, 19 provided details on their town of residence during September-December 2008, the most common of which was West Farm (n = 9, 47.4%) (Figure 1). When compared to faculty (10.5%) and staff (0.0%), more students (21.7%) had evidence of recent infection. Among the individuals with evidence of recent infection, only 1 person reported travel to a dengue-endemic country or territory (St. Thomas, United States Virgin Islands) during the period September-December 2008 (not shown). Four (20%) of the recently infected individuals reported having a febrile illness of at least 1-day duration during September to December 2008.

Infecting DENV serotypes

Of the 52 seropositive participants, 48 agreed to additional testing by PRNT_{90}. Immunity to all four DENV serotypes was found; no samples demonstrated immunity to WNV infection. A PRNT_{90} pattern consistent with a primary DENV infection was found in 11 persons: four with infection by DENV-1, three with DENV-2 and four with DENV-3. Among these 11 individuals, six were born outside dengue endemic countries and had spent a median (range) of two years (1.4-10.4 years) living in SKN. The 2008 outbreak serotype was DENV-3, and two of the DENV-3 infected persons met the criteria for a recent infection; neither had travelled outside SKN during September to December 2008. One reported a febrile illness with headache, retro-orbital pain, petechial rash and a loss of appetite, while the other reported no febrile illness during that period.

Of the remaining 37 persons, 10 had a PRNT_{90} pattern consistent with a secondary DENV infection and the infecting DENV serotypes could not be determined, while 27 did not have detectable neutralizing antibodies to DENV or WNV. All secondary infections were detected in individuals born in the Caribbean; none reported being vaccinated for Japanese encephalitis virus and only one had been vaccinated for Yellow Fever virus.

Discussion

In this study, more than two out of every five members of this campus community in SKN had evidence of a prior dengue infection. This finding is particularly striking as the sample was mostly comprised of people from non-endemic areas and the overall median duration of residence in SKN was less than two years. The likelihood of being seropositive increased with duration of residence of SKN; this finding is intuitive and emphasises the need for ongoing mosquito avoidance practices for both local and expatriate residents of SKN. Additionally, only 20% of people with recent DENV infection had a febrile illness during the 2008 outbreak; this is consistent with previous studies demonstrating a low rate of symptomatic infections [12-14].

This study has a number of strengths as it assessed anti-DENV seropositivity in a sample of people from both endemic and non-endemic areas living in the Caribbean. It also employed robust microbiological techniques to define seropositivity and to rule out cross-reactivity with other flaviviruses. As the start date for sampling was later than the usual duration of the anti-DENV IgM antibody response, we did not assess IgM seropositivity, a more accurate indication of recent infection, so we used a proxy measure instead. Furthermore, we did not examine associations between factors that mitigate exposure to mosquito vectors in the last dengue season (e.g., using insect repellent, screened windows and doors, air-conditioner use) and seropositivity, as we did not know the exact time of dengue infection.

We conducted this study during the year following a dengue outbreak in SKN, which may have resulted in the high prevalence of anti-DENV IgG antibodies among persons from non-endemic countries. The results of this study can only be generalized to the campus community, and not the general population of SKN. However, despite employing probability sampling, the high proportion of refusals compromised the generalizability of our findings. The researchers did not recruit volunteers or perform replacement sampling to avoid introducing selection bias. Participation may have been biased towards persons who had, or had heard of, dengue before the study, but only one of every four participants of this study had reported a febrile illness during the 2008 outbreak period.

All individuals born in SKN were seropositive; this is consistent with another seroprevalence study which found that, amongst a sample of up to 50 pregnant women in SKN, all were seropositive for anti-DENV IgG antibodies [15]. To our knowledge, there are no other seroprevalence studies for DENV or other flaviviruses in SKN. When considering long-term travellers to DENV-endemic regions, the seroprevalence found in our study is higher than that of previous studies, but those studies were performed in different study populations. Amongst Israeli travellers who spent at least three months in the tropics, 7%
seroconverted during travel while less than 3% had evidence of an increase in IgG antibodies [16]. The findings of other studies of long-term non-vacationing travellers, such as aid-workers [17–19], may be more comparable to our study. In one instance, German aid workers had only a 6.4% prevalence of DENV infection, despite having spent an average of 37.7 months in tropical areas of Asia and Africa [17]. More recently, as many as 19.5% of German travellers to tropical and subtropical countries were found to have anti-dengue IgG antibodies [20].

Almost one-third of students in this study was seropositive. As most students are from the United States, given the high frequency of travel to the continental United States during semester breaks, increased awareness of the risk of dengue and other arboviruses is essential to preventing reintroductions of these viruses while in their primary place of residence. Many would argue that the concerns over the emergence of chikungunya and Zika and the reintroduction of dengue to the continental United States [21] have been substantiated, given the recent cases of autochthonous transmission in Florida and Texas [22–29].

**Conclusion**

This study presented a unique opportunity to evaluate DENV seroprevalence in a sample of mostly expatriate, long-term residents of a dengue endemic small-island state in the Caribbean. A future research study should explore the incidence of DENV or other arboviruses in new members of this campus community by taking baseline serum specimens on arrival then follow-up specimens when leaving SKN. Studies such as this can provide valuable information for calculating the force of DENV infection and to assess the risk of dengue in residents and visitors to these countries [30]. We found that people who moved to St. Kitts to study or work at this veterinary school were at a high risk of DENV infection over a very short period of time, which limits the window of opportunity for dengue prevention in this population. As no vaccine is currently recommended for use in travellers, an increased emphasis on pre-travel arboviral prevention education is vital.

**Acknowledgements**

The authors acknowledge the support of Dr. Patrick Martin, the Chief Medical Officer of the MOH of SKN. They also acknowledge the guidance of Drs. Samuel Rawlins (Emeritus Entomologist with the Caribbean Epidemiology Centre), Fermin Arguello and Hal Margolis (Dengue Branch), and the assistance provided by the research assistants Kevin Lura, Jennifer Peterson and Keith Mihansky of Ross University School of Veterinary Medicine (RUSVM). They also thank Sarah Quesnel (CARPHA) for her advice on statistical analysis. The PRNT10 testing was supported by Aixa Sanchez-Crespo at the Dengue Branch.

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**Conflict of interests:** No conflict of interests is declared.