

# A cross sectional serologic and epidemiological study of dengue virus infection in north central area of Trinidad and Tobago.

## ABSTRACT

**Aims:** This study was carried out to determine the observed serological and significant epidemiological risk factors for dengue fever infection in a cross-section of the population in Trinidad and Tobago.

**Study design:** This was an **observational** cross sectional study.

**Place and Duration of Study:** The study was carried out in the department of Paraclinical Sciences of the University of the West Indies, St. Augustine Campus, Trinidad and Tobago, over a period of 10 months, October 2016 to July 2017.

**Materials and Methods:** Over 450 individuals from a cross section of the population residing in the northern part of Trinidad Island were surveyed. These included individuals suspected of having dengue fever that presented to the health care facilities with complaints of fever along with some other **symptoms suggestive of dengue viral illness**. There was no age, gender or ethnic bias. Standardized questionnaire was used to obtain epidemiological data. Blood samples taken from consented participants were analyzed using rapid immune chromatographic tests (ICTs) – Panbio, SD Bioline and Enzyme Linked Immunosorbent Assays (ELISA). The samples were also tested for baseline blood parameters **such as** platelets and haemoglobin. The epidemiological data was analyzed using SPSS version 21.

**Results:** Analysis of 380 individuals who fulfilled study criteria revealed that there were no demographic characteristics (age, gender, locality, etc.) that showed statistical significance with having a dengue infection. Retro-orbital pain, headaches and respiratory symptoms (e.g., cough, cold) showed differences that were significant with those having a dengue infection. No statistical significance was found in any comorbidity (diabetes, hypertension and asthma) factors considered and patients with dengue infections. Evaluation of platelet counts showed that only 5.4% samples had abnormal range, **while 80% those that tested positive were not significant either**. Monitoring of platelet levels is still very important, but it showed that it is not an indicator of worsening dengue because 95.3% of the positive cases were within normal levels.

**Conclusions:** Except for nonspecific symptoms observed among patients suspected of dengue fever, there were no other significant factors that were exclusive in identifying dengue infection among the subjects studied. **Platelet monitoring may not be the only parameter to use in determining deteriorating dengue patients. Vector eradication activities should be intensified with other efforts such as education program.**

**Keywords:** Dengue fever, ELISA, Epidemiology, Serology, Panbio, Trinidad and Tobago.

## 1. INTRODUCTION

Dengue is a global public health problem and in the last decade has increased substantially due to human travel and changing suitability for the mosquito vector<sup>1, 2, 3</sup>. Dengue is endemic in more than 100 countries with an estimated 50 – 100 million infections annually<sup>4, 5</sup>. Dengue fever is an acute manifestation of an arthropod borne viral infection belonging to the *Flaviviridae* family. The dengue virus is transmitted by female mosquito *Aedes aegypti*. Four serotypes of the virus are known to exist DEN-1-4<sup>6</sup>, and a recently documented fifth serotype appears to have been emerged<sup>7</sup>. Classic dengue fever is usually self-limiting, especially in children. Dengue infection characterized by sudden onset of headache, retro-orbital pain, high fever, joint pain and rash. More serious manifestations dengue virus infection includes the dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).<sup>2</sup> Dengue haemorrhagic fever is associated with re-infection, characterized by the defects in homeostasis and plasma leakage into interstitial spaces associated with increased levels of vasoactive cytokines<sup>8</sup>. This leads to life threatening shock (DSS) in some cases.

27 The severe syndromes occur in patients with passively acquired or pre-existing, non-neutralizing,  
28 heterologous antibody caused by a previous infection with a different serotype of the virus<sup>9</sup>. The  
29 antibodies from the previous infection bind to the new infecting serotype and facilitate viral entry via  
30 Fc-receptor binding cells, so the number of antigen-presenting cells is increased at secondary  
31 infection.<sup>8</sup> In 2016, there was a recorded 1,801 probable cases alone in Trinidad and Tobago out of  
32 the total 9,993 probable cases in the non-Latin (English, French and Dutch) Caribbean<sup>10</sup>. This is a  
33 significant decrease in the number of reported probable cases when compared to 2014; with 5,157  
34 probable dengue cases. As was noted in a prospective sero-epidemiological study from Trinidad and  
35 Tobago, many dengue infections do not produce severe symptoms and the number of reported cases  
36 underestimates the actual prevalence of dengue in the population<sup>11, 12</sup>.  
37 The aim of this study was to serologically confirm the frequency of dengue virus infection and  
38 determine epidemiological risk factors associated with dengue infections among patients suspected of  
39 having dengue fever and attending health care facilities in the north central region of Trinidad and  
40 Tobago.

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## 42 2. MATERIAL AND METHODS

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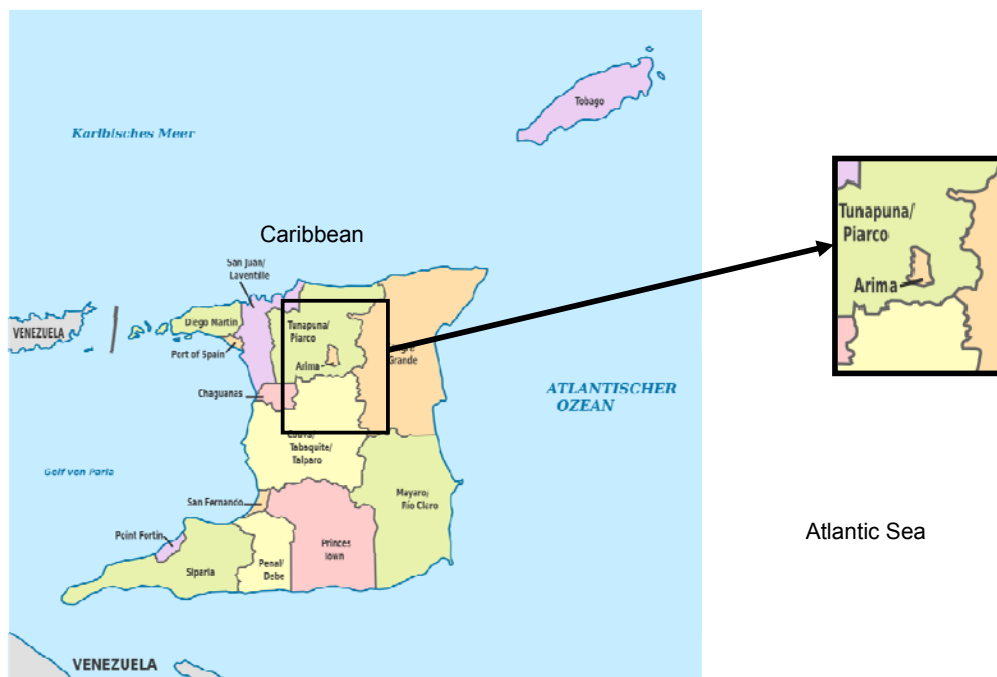
### 44 2.1 Study design, sites and population

45 This was an observational cross sectional study conducted during the period of October 2016 – July  
46 2017, among patients with suspected dengue infection. The study was carried out at two health care  
47 facilities of the North Central Regional Health Authority (NCRHA) in Trinidad of the twin Island,  
48 Trinidad and Tobago with catchment areas as indicated in the figure below (Figure 1). This area has a  
49 high population density in the country and most dengue cases in the past were localized to this  
50 region.<sup>13</sup> This region was chosen as the area of study so as to reassess the current burden of dengue  
51 virus infection. This study was carried out among patients who presented to these health care facilities  
52 with suspected dengue infection. Suspected dengue infection is characterized by fever along with the  
53 following symptoms - anorexia, rash, aches and pains, vomiting and nausea, abdominal pains and  
54 warning signs include positive tourniquet test, leukopenia, thrombocytopenia (platelet count  $<150 \times 10^9/L$ ),  
55 abdominal tenderness, clinical evidence of plasma leakage and/or increase in haematocrit<sup>14</sup>.  
56 The study enlisted voluntary participants who gave written consent and were systematically randomly  
57 selected. Standardized data collection form was used to obtain epidemiological information from all  
58 enrolled participants who were seen and physically examined by a medical personnel in the study.

59

60 **Figure 1. Geographical map of Trinidad and Tobago showing the locality of individuals**  
61 **surveyed for dengue virus fever in Trinidad and Tobago.**

62



63

64 **2.2 Inclusion criteria**

65

66 All patients of all age groups, gender, ethnic groups, social and educational level who presented to  
67 these health facilities with suspected dengue infection symptoms as enumerated above and gave  
68 written consent or assent were included in the study. Any patient who did not meet the previously  
69 mentioned requirements for suspected dengue infection or did not give consent was excluded from  
70 the study.

71

72 **2.3 Collection of Specimen**

73

74 A **standardized** questionnaire was used to obtain patient biodata or information and clinical history.  
75 This was administered by one of the trained investigators to avoid bias and misinterpretation or  
76 misrepresentation of the responses from the participants.

77

78 About 10ml of blood (5ml each in red and purple top tubes) was obtained through venipuncture and  
79 transported to the Department of Paraclinical Sciences, The University of West Indies, St. Augustine  
80 Campus; and Pathology Laboratory at the Eric Williams Medical Sciences Complex for further  
81 analysis. The blood samples were allowed to clot at room temperature, centrifuged and separated as  
82 soon as possible **the same day for the rapid kits (Panbio and SD Bioline)**. They were then stored at 2-  
83 8°C for a maximum of two days or stored frozen at -30°C until complete testing **using the ELISA kits  
that were performed in batches.**

84

85 **2.4 Laboratory Analysis - Complete Blood Count**

86

87 All samples were subjected to a routine complete blood count as part of the routine services offered to  
88 the patients by the health care facilities including platelet counts for each patient.

89

90 **2.5 Rapid Immuno-chromatographic tests (ICTs)**

91

92 The samples collected in the red top tubes were subjected to serological analysis using enzyme  
93 linked immunosorbent assay - ELISA, (Dengue Virus IgM/IgG capture DxSelect ELISA, Focus  
94 Diagnostics, Cypress, PA, USA) for detection of human serum IgM and IgG antibodies in dengue  
95 virus (DV) infections. Rapid immune-chromatographic tests (ICT) kits were used for detection of IgM  
96 and IgG antibodies, and non-structural protein 1 (NS1) antigen; of sera collected and the results were  
97 recorded. **The relative sensitivity and specificity for the Panbio rapid ICT is 96.3% (90.8 -99.0 %) and  
98 95.0% (87.7 – 98.6%), respectively. The sensitivity and specificity of the SD Bioline rapid ICT is  
99 92.8% and 98.4%, respectively. The kits were used within one to three months of procuring them from  
100 the distributors and manufacturers, while their life span (expiration dates) were still within two to three  
101 years.**

102

103 **2.6 Quality Controls**

104 Controls for both the IgM/IgG ELISA kits were provided as follows: detectable controls (human sera),  
105 non-detectable controls (human sera) and cut-off calibrators (human sera). Samples that were  
106 collected from asymptomatic and healthy individuals during the time of the study were used as  
107 controls for both of the rapid ICT tests. Controls were run every time when procedures were carried  
108 out.

109

110 **2.7 Statistical Analysis**

111 Microsoft Excel was used for data entry and data analysis was performed using Statistical Package  
112 for the Social Sciences (SPSS) 23.0 software. Chi-square test and Fisher's exact test were used to  
113 compare categorical variables. The Chi-square was chosen for determination of association between  
114 a tested variable and a positive dengue result. If a relationship existed between any of the variables,  
115 the Chi-square value (p value) would reflect the strength of the association. The Fisher's exact test is  
116 used in place of the Chi-square to measure the same association for smaller sample sizes. In cases  
117 where the frequency counts are fewer than five in a two by two table, the test statistics (p) used is the  
118 Fisher's exact value. A probability value (p) of < 0.05 was considered statistically significant.

119

120 **3. RESULTS**

121

122 **More than 450 individuals were recruited for this study but only 380** of these gave consent, completed  
123 the questionnaire, got evaluated, **had venipuncture and** were included in the final analysis. Patients

124 included were noted to have come from different ethnic groups of people living in this part of the  
125 country. Among the study participants, 38.7% were of mixed ethnicity followed by patients of African  
126 descents, 36.6%. The East Indian and Spanish descents were 22.6% and 1.1 % respectively. Most of  
127 the study participants were females (61.3 %) and the median age of all analyzed individuals in the  
128 study was 26 years (range, 3 years to 87 years) but the prevalent age group surveyed was between  
129 21 – 30 years (Figure 2). The median time between onset of illness and collection of specimens was 3  
130 days (range, 1 to 50 days).

131  
132 As shown in Table 1, the laboratory tests of the blood samples using the ELISA reference for dengue  
133 IgM and IgG, initially classified the analysis as 92.5% positive for dengue and 7.5% non-dengue. Of  
134 those that tested positive for dengue, females were in the majority (60.5%) and 32.6% of all positive  
135 cases were between the ages of 21-30 years old. Based on the clinical history, presentation of fever,  
136 body aches and headache, the blood samples and the subjects were further defined or classified as  
137 acute cases or phase (74.2 %), convalescent cases or phase (18.3 %); and based on immune status,  
138 as primary 5.4 % or secondary, 87.1 %. An acute sample was recorded as one with  $\leq 7$  days post  
139 onset of symptoms while those  $\geq 7$  days post onset of symptoms were recorded as convalescent.

140  
141 Demographics were the first parameters used to determine what would qualify as risk factors in  
142 acquiring a dengue infection. Being of a particular ethnic group had no bearing or significance on  
143 whether the patient tested dengue positive. The majority of the positives (38.4%) were found to be of  
144 'mixed' descent, followed by African descent (37.2%). There was also no association between living in  
145 a particular area and contracting dengue, although most recruits were from the Arima area (Figure 1  
146 above), and there was a high percentage (47.3%) that tested positive there.

147  
148 The statistical analysis in this study revealed that retro-orbital pain, respiratory symptoms (cold,  
149 cough, runny/stuffy nose) and headache had significant association with samples that tested positive  
150 for dengue ( $p < 0.05$ ), Table 1. More than half (53.3 %) of patients surveyed that tested positive for  
151 dengue reported experiencing retro-orbital pain; 88.4 % of dengue- positive patients experienced  
152 headaches while 80.2 % experienced respiratory symptoms (Table 1).

153  
154 Platelet levels of the patients were analyzed and categorized as abnormal ( $\leq 150 \times 10^9/L$ ) and normal  
155 ( $\geq 150 \times 10^9/L - 450 \times 10^9/L$ ). As shown on Table 2, the largest numbers of dengue positives were  
156 found in the age group 21-30, 27.9% in the normal platelet range and 4.7% in the abnormal platelet  
157 range, however, this difference was not statistically significant ( $p = 0.172$ ). The age group 11-20  
158 showed the second highest number of dengue positives with 18.6%. The mean age of those that  
159 tested positive was 29 years old, while the mean platelet counts were 130,000 and 293,000 within the  
160 abnormal and normal range, respectively. Except for the age groups 21 – 30 that recorded abnormal  
161 platelet counts, all the other age groups had no abnormal platelet counts (Table 2).

## 162 163 DISCUSSION

164  
165 The objective of this study was to use serological analysis to determine the frequency of dengue virus  
166 infection and make association between epidemiological risk factors that may exist among the  
167 patients suspected of the infection in a cross section of individuals in Trinidad and Tobago. Results  
168 from studies such as this can assist physicians in making definitive diagnosis of dengue in our locality  
169 since many cases go unnoticed or recorded as acute viral illness (AVIs). While accurate laboratory  
170 diagnosis can be very helpful in confirming the disease, it will also provide key data on the  
171 epidemiology and health burden of dengue, which is very useful for accurate public health  
172 surveillance<sup>15</sup>. Detection of seropositive cases of dengue in this region of study still suggest that  
173 vector control operations that have previously been carried out in this region failed to achieve the  
174 desired target of reducing mosquito densities in the eight counties to below the disease transmission  
175 threshold as previously reported by Chadee et al.<sup>13</sup> These authors had reported two decades ago that  
176 the Trinidad vector control program relied on the chemical approach with the application of  
177 insecticides in artificial containers;<sup>13</sup> and this has continued to date. Perhaps more intensive and  
178 aggressive efforts may turn to health education.

179  
180  
181 Females were noted to be the majority (60.5%) of the dengue cases in our study which is different  
182 from what has been reported in other countries.<sup>16</sup> Adults were more affected in our analysis with ages  
183 21-30 having 32.6% of all positive cases. This again was not in agreement with Anker and Arima that

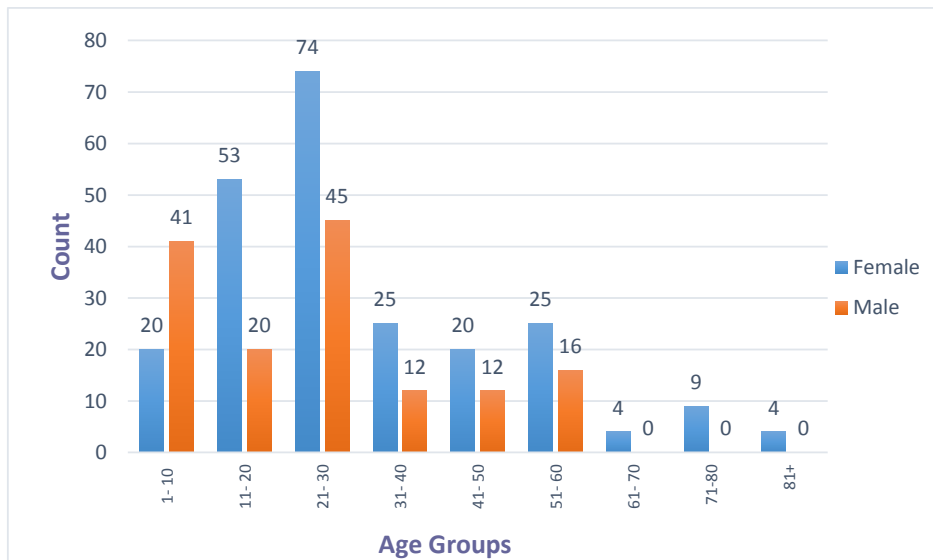
184 reported more of their positive cases occurring more in those over 15 years in the countries they  
185 studied.<sup>16</sup> Anker and Arima attributed the dominance of the males and the age group to cultural and  
186 economic reasons. Female were more perhaps because more took part in the study despite the fact  
187 that participants were systematically selected randomly. Economic differences could not have  
188 influenced our results as reported by others<sup>16</sup> since medical care is free in our country and so all are  
189 afforded the opportunity to seek medical care.

190  
191 In this study, similar number of individuals reported their ethnicity to be either of African descent or  
192 mixed race; and many of these tested positive for dengue virus infections. This was not in agreement  
193 with what was reported by Rojas et al in Colombia that Afro-Colombians population had a significantly  
194 lower risk of getting dengue and its complications, compared with the non-Afro-Colombians  
195 population.<sup>17</sup> Trinidad and Tobago is a cosmopolitan society with several ethnic groups, although the  
196 African and Indian descents dominates in number; but dengue virus infection could not be selective  
197 because all the different groups live together. Also majority of the participants surveyed gave their  
198 location to be Arima area which was also noted to be a significant factor in this study. The high  
199 number of positive results in each of these categories appears to only reflect the majority within the  
200 sampled population.

201  
202 Symptoms were statistically analyzed to determine their associations with a dengue virus infection  
203 although dengue infections may initially be asymptomatic in 50 – 90% of individuals<sup>18</sup>. The significant  
204 ones include retro-orbital pain (eye pain), headaches and respiratory symptoms which are similar to a  
205 previous report<sup>19</sup>. Eye pain is particularly common in dengue infection along with headaches but the  
206 degree to which they are experienced are not quantifiable and so they remain non-specific. Most  
207 patients who tested positive for dengue antibodies also complained of body pains; but this was not  
208 found to be significant. Reporting of having a previous infection of either dengue, chikungunya or zika,  
209 also did not show any differences for those who tested positive. Among the several patients that had  
210 already suffered from a dengue infection, none of them showed signs or symptoms that were more  
211 severe than those who said they never were infected with dengue. As dengue is one of the most  
212 under reported tropical diseases<sup>8</sup>, it is very possible that patients who claimed to have never had  
213 dengue may be unaware of the past diagnoses seeing that symptoms are non-specific and home  
214 remedies are administered by patients themselves until symptoms subside. This way, there is and can  
215 be no accurate monitoring of the actual disease or possible burden of infection.

216  
217  
218 **Figure 2. Age and gender distribution of participants surveyed for dengue virus infections in**  
219 **Trinidad and Tobago.**

220



221  
222  
223

**Table 1. Characteristic features of individuals surveyed for dengue virus infection in the north central regional health authority, Trinidad and Tobago, 2016 – 2017.**

Characteristics	Negative (%)	Positive (%)	p value
<b>Demographics</b>			
Male	8 (28.6)	139 (39.5)	0.702
Female	20 (71.4)	213 (60.5)	0.702
African descent	12 (42.9)	131 (37.2)	1.000
East Indian descent	4 (14.3)	82 (23.3)	1.000
Mixed	12 (42.9)	135 (38.4)	1.000
Spanish	0	4 (1.2)	1.000
<b>Symptoms</b>			
Rash	4 (14.3)	41 (11.6)	1.000
Headache	16 (57.1)	311 (88.4)	0.054
Retro-orbital pain	0	188 (53.5)	<b>0.012*</b>
Body pain	20 (71.4)	274 (77.9)	0.654
Joint pain	4 (14.3)	176 (50)	0.115
Diarrhoea	8 (28.6)	119 (33.7)	1.000
Cough, cold, runny nose	8 (28.6)	282 (80.2)	<b>0.007*</b>
Gum/Nose bleeds	0	33 (9.3)	1.000
<b>Previous infections</b>			
None	28 (100)	254 (72.1)	0.184
Dengue	0	65 (18.6)	0.600
Chikungunya	0	29 (8.1)	1.000
Zika	0	4 (1.2)	1.000
<b>Co-morbidities</b>			
Hypertension	0	17 (4.7)	1.000
Diabetes	0	8 (2.3)	1.000
Diabetes + HTN	0	4 (1.2)	1.000
Asthma	0	37 (10.5)	1.000
Other – Arthritis, PCOS, etc.	4 (14.3)	29 (8.1)	0.479
None	24 (85.7)	254 (72.1)	0.670
<b>Mosquito Conditions</b>			
Many mosquitoes in the area	24 (85.7)	237 (67.4)	0.428
Nets/Screens at home	0	61 (17.4)	0.593
Blocked drains around house	0	70 (19.8)	0.342
Get bitten often	20 (71.4)	193 (54.7)	0.459
No mosquito problems	4 (14.3)	111 (31.4)	0.670

\*p < 0.05 is considered statistically significant. P-values were determined using Chi – square tests. Data are presented as n (%) or median (interquartile range); HTN – hypertension, PCOS – polycystic ovary syndrome

Co-morbidities such as hypertension, diabetes mellitus and asthma are among the non-communicable illnesses that are most prevalent in Trinidad and Tobago<sup>20</sup>. If left unmanaged they can lead to high morbidity and mortality rates. Whether or not either of these had any effects on the prevalence of dengue infection was also investigated. Most of those that were found positive for dengue infection showed no significant associations with having any medical conditions (asthma, diabetes, hypertension), being on any particular medications or having received any vaccines in the last two months prior to being enrolled. However, a study in Asia, attempted to show the association of diabetes mellitus with DHF. The study found that female, Chinese, age group 30-49 years with pre-existing diabetes mellitus or diabetes with hypertension were risk factors of developing DHF during an epidemic while dengue serotype 2 was predominant<sup>21</sup>. As stated above, neither of these characteristics were found to show any significant differences in our current study despite age group (21-30 years), gender (more females than males) or ethnicity (more of mixed ethnic group descents) gave more numbers; and also the fact that 25.5% of the sampled population in this study suffered from comorbidities.

**Table 2. Age distribution of participants for dengue who were ELISA positive categorized by platelet counts.**

Age Groups	Negative ELISA		Positive ELISA	
	Abnormal (%)	Normal (%)	Abnormal (%)	Normal (%)
1 – 10	0 (0)	16(4.3)	0(0)	45(11.8)
11 – 20	0(0)	8(2.1)	0(.0)	65(17.2)
21 – 30	4(1.0)	0(.0)	16(4.3)	98(25.8)
31 – 40	0(.0)	0(.0)	0(.0)	38(9.7)
41 - 50	0(.0)	0(.0)	0(.0)	33(8.6)
51 – 60	0(.0)	0(.0)	0(.0)	41(10.8)
61 – 70	0(.0)	0(.0)	0(.0)	4(1.1)
71 – 80	0(.0)	0(.0)	0(.0)	8(2.2)
81+	0(.0)	0(.0)	0(.0)	4(1.1)
TOTAL	4(1.0)	24(6.4)	16(4.3)	336(88.3)

The Platelet counts were considered as abnormal ( $\leq 150 \times 10^9/L$ ) and normal ( $\geq 150 \times 10^9/L - 450 \times 10^9/L$ )

In our locality where we do not have problem of distinguishing dengue from malaria that produces low platelet counts<sup>22</sup>, hence platelet counts have been one of the most important factors in tracking the progress of dengue infection. Monitoring platelet levels however, should not be the sole criteria to presume dengue infection as many patients in this study tested dengue positive without abnormal platelet counts that is indicative of plasma leakage. In a study by Lovera et al, they investigated platelet count as a risk factor of shock. Using a cut-off of  $< 100 \times 10^9/L$  they found that children who did not develop shock exhibited similar percentage level of thrombocytopenia compared to patients who eventually developed it (47 % vs 49 %). The results were similar when the comparison included patients only with platelet counts  $< 50,000/mL$  (28 % vs 25.6 %).<sup>23</sup> In this present study, the mean platelet count for positive samples in patients 1- 10 years of age was  $295 \times 10^9/L$ . Those with abnormal counts were only found in the 21 – 30 year-old age group and 80% of them tested positive for dengue virus. This adds up to 4.3% of those who tested positive but was not of any significance. None of the patients had platelet levels that were  $< 50 \times 10^9/L$ . Lam et al reported and the WHO guideline states that, daily platelet counts can be used to predict the development of DSS.<sup>24, 25</sup> Also in an extensive review, Leal de Azeredo et al concluded that thrombocytopenia, coagulopathy, and vasculopathy are hematological abnormalities related to platelet and endothelial dysfunction generally observed in severe dengue.<sup>26</sup> We do not have proven explanations why majority of the patients who were suspected of dengue in our study had normal plate counts, but we can only speculate that their platelets were normal because they may have recovered.

The Pan American Health Organization (PAHO) has already issued a release of the number of reported cases of dengue and severe dengue in the Americas by country for epidemiological week 39 (updated October 13, 2017). After week 32 in Trinidad and Tobago the number of probable reported cases were 206, none of which were laboratory confirmed<sup>10</sup>. This is as a result of non-availability of the laboratory facilities because of lack of economic resources. It is however very critical that identification, isolation of the virus or confirmation of the dengue diagnosis be made so that dengue can successfully be managed and differentiated from other viral infections. It is also of utmost importance that all probable cases not only be reported but confirmed, especially if headway is to be made on curbing infection and development/implementation of a vaccine. The first dengue vaccine – the Sanofi CYD-TDV vaccine, has now been licensed by several endemic countries for use in 9-45 years and 9-60 year olds. The vaccine was unusual in that the recommended target population for vaccination was not only defined by age but also by transmission setting as defined by seroprevalence. The WHO has stated their position on the newly developed vaccine (CYD-TDV) saying that countries should consider introduction of the dengue vaccine only in geographic settings where epidemiological data indicate a high burden of disease.<sup>27, 28</sup> The vaccine, also known as Dengvaxia, is a live attenuated (recombinant) tetravalent vaccine that was created to be administered by 3 doses of 0.5ml given at 6-month intervals. We cannot indicate high burden of disease if the

342 epidemiological data being collected is recorded incorrectly or disregarded. Hence, all assumptions  
343 for diagnoses need to be confirmed by the most accurate methods.  
344

#### 345 4. CONCLUSION

346  
347 Despite the limitations of this study that include the small sample size and lack of use of molecular  
348 tests, viral isolation or virus detection using indirect immunofluorescence for confirmation of dengue  
349 virus, the study still detected positive cases of dengue virus infections in the country. Except for  
350 nonspecific symptoms observed among patients suspected of dengue fever, there were no other  
351 significant factors that were exclusive in identifying dengue infection among the subjects studied.  
352 Platelet monitoring may not be the only parameter to use in determining deteriorating dengue  
353 patients. Vector eradication activities in the country may not have been fully effective after all and so  
354 attention may also focus on other areas such as education program.  
355

#### 356 CONSENT

357  
358 Informed consent was also obtained from each of the patients, along with assent from children that  
359 were included in the study. Patients under the age of 18 were considered as children.  
360

#### 361 ETHICAL APPROVAL

362  
363 Ethics approval for this study was obtained from the Campus Ethics Committee of the University of  
364 the West Indies St. Augustine Campus and the North Central Regional Health Authority (NCRHA)  
365 Ethics Committees. The study was carried out in accordance with the ethical standards laid down in  
366 the 1964 declaration of Helsinki.  
367

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