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Major Article

Molecular and clinical epidemiological surveillance of dengue virus in Paraíba, Northeast Brazil

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Abstract

Introduction: Despite being the most prevalent arboviral disease worldwide, dengue has been neglected lately. However, recent epidemics of arboviruses such as Zika and chikungunya in locations throughout the world have alerted health authorities to these diseases. This study evaluated the incidence pattern of dengue, its clinical characteristics, and co-circulation of serotypes from 2007 to 2015 in Paraiba State, Northeast Brazil. **Methods:** Data on dengue cases from 2007 to 2015 were extracted from clinical reports of the National System for Notifiable Diseases [*Sistema Nacional de Agravos de Notificação* (SINAN)] of Brazil provided by the Paraiba Health Department. Reverse transcription polymerase chain reaction (RT-PCR) assays for dengue serotypes were carried out on plasma samples obtained from patients with suspected dengue. The data were analysed using descriptive statistics.**Results:** According to clinical features, dengue fever [n = 39,083 (70.2%)] and dengue without warning signs [n = 15,365 (27.7%)] were the most common classifications of dengue. On RT-PCR, DENV 1 was the most commonly identified serotype (80.5%) in all years studied. Co-circulation of all four DENV serotypes was observed in 2013 and 2014. Furthermore, we observed an increase in dengue notifications in 2015, possibly due to the rise of Zika and chikungunya. **Conclusions:** Our findings support the hypothesis that co-circulation of the four DENV serotypes may be a reason for the increased prevalence of severe forms of dengue in the years studied. This study may contribute to directing research, health policy, and financial resources toward reducing poorly controlled epidemic diseases.

Keywords: DENV serotypes. Molecular epidemiology. Arboviruses.

INTRODUCTION

Dengue has been an endemic disease in Brazil for many decades[1]; however, an unparalleled epidemic of diverse arboviruses, such as Zika and chikungunya, emerged as a new public health risk in 2015. Even though these arboviruses have some unique characteristics, they also share several clinical features that make them difficult to diagnose[2].

Among Brazilian states, Paraíba has a high prevalence of these arboviruses. Until 2015, there were no notification systems able to distinguish dengue from Zika and chikungunya cases. Currently, all cases are notified as suspected dengue and are only correctly classified after laboratory tests[3]. Molecular surveillance to differentiate these arboviruses is therefore essential and should result in infections being correctly classified and assessment of the effectiveness of public health approaches, especially towards post-infection clinical outcomes.

Dengue virus (DENV) is one of the most common cause of morbidity and mortality[4] and the most prevalent viral hemorrhagic fever in many tropical countries. It infects approximately 100 million people annually, causing epidemics in urban and peri-urban areas[5]. Southeast Asia has a particularly high disease burden; however, in the last two decades, Latin America and the Caribbean have also seen considerable expansion in case numbers[6].

Dengue virus is a mosquito-borne virus of the Flaviviridae family and *Flavivirus* genus[7]. Its major vectors are *Aedes aegypti*, which prefers resting in sheltered dark areas inside houses[8], and *Aedes albopictus*, which is not as well adapted to urban domestic environments, preferring the natural environment[9].

The illness can be caused by any of the four DENV serotypes, DENV 1, DENV 2, DENV 3, and DENV 4[7], and presents as

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various clinical forms with irregular clinical pathogenesis and outcomes. Moreover, some symptoms, including fever, skin rashes, conjunctivitis, and muscle and joint pain, may be easily confused with those of other arboviral infections[10].

In order to categorize the most common manifestations of dengue, the World Health Organization (WHO) developed a classification system, which grouped dengue disease as dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS)[11]. As using this classification in endemic areas was impractical, a new classification was proposed in 2009: dengue without warning signs (DwWS), dengue with warning signs (DWS), and severe dengue (SD). This new classification, based on degrees of severity, facilitates clinical care by determining how intensively patients should be observed and treated[12].

Between 2001 and 2007, nearly 4 million dengue cases were notified in the Americas, over 75% of which were reported in Brazil[13]. Northeast Brazil has one of the highest prevalences of dengue cases, due to favorable socioeconomic and environmental conditions[14].

A cross-sectional serological survey of the epidemic conducted in 2006 in Recife, Pernambuco, a State of Northeast Brazil, showed the overall prevalence of DENV immunoglobulin G to be 80%, demonstrating that the majority of inhabitants had been infected at least once by the DENV[15]. Between 1997 and 2012, 229,922 dengue cases were notified in Paraíba, 33 of which were fatal[16]. In 2014, the majority of cases notified in Paraíba occurred in the Cities of João Pessoa, Campina Grande, Patos, Monteiro, Juazeirinho, Guarabira, Cabedelo, Areia, and Alhandra[17].

Paraíba has a large number of individuals infected with or at risk of DENV infection and other emergent arboviruses such as Zika and chikungunya. Few studies have described the epidemiology of these diseases. Therefore, the aim of this study was to provide the first description of incidence of dengue disease, its seroprevalence, serotype distribution, and other relevant epidemiological data in Paraíba between 2007 and 2015.

METHODS

Study area and population

Paraíba is located in the east of the Northeast region of Brazil, bordering the States of Rio Grande do Norte, Pernambuco, Ceará, and the Atlantic Ocean. According to the 2010 census of the *Instituto Brasileiro de Geografia e Estatística*[18], Paraíba has a territory of 564,697.44km² divided into 223 municipalities and a population of 3,766,528 inhabitants with a population density of 69.32 inhabitants per square kilometer. The capital, João Pessoa, has 723,515 inhabitants, according to the 2010 census, being the eighth most populous city in the Northeast region.

Data collection

In Brazil, dengue is a disease of compulsory notification according to Ordinance no. 104 of the Brazilian Ministry of Health. Thus, medical reports of suspected dengue cases are mandatorily filed with the National System for Notifiable Diseases [*Sistema Nacional de Agravos de Notificação* (SINAN)]. The files of dengue reports from Paraíba from January 2007 to December 2015 were provided to the study group by the Paraíba Health Department.

Eligibility criteria for suspected and confirmed cases

Inclusion criteria for the study were confirmed and suspected cases notified to SINAN; suspected cases included people who had lived in or traveled to endemic dengue areas and, within a 14-day period, developed clinical symptoms, such as fever, with two or more of the following manifestations: nausea, vomiting, skin erythema, generalized body ache, myalgia, arthralgia, petechiae, thrombocytopenia, or a positive tourniquet test.

Confirmed cases were selected for each year based on laboratory confirmation by serology, nonstructural protein 1 (NS1), or polymerase chain reaction (PCR) tests (detection of genotype in samples of acute serum).

During the study period, there was a change in SINAN descriptors, e.g., nomenclature of dengue classifications, and removal of descriptors, e.g., hemorrhagic manifestations, plasma leakage, and plaques.

Data sources

The data on reported dengue cases were collected from private and public health services included in the SINAN. Until 2013, the system adopted the WHO's traditional classification, where cases were classified as dengue fever (DF), dengue hemorrhagic fever (DHF), or dengue shock syndrome (DSS). However, due to difficulties in classifying severe cases, such as those with clinical outcomes that did not completely meet the traditional criteria for DHF, an intermediate classification called dengue with complications [dengue com complicações (DWC)] was adopted in Brazil and is used in SINAN reports.

Although the WHO adopted new terminology for dengue classification in 2009 (DWS, DwWS, and SD), this was only introduced into the Brazilian Health System in 2014. Therefore, in the present study, both WHO systems of nomenclature were used in the data analysis.

Nested reverse transcriptase PCR assay

Dengue ribonucleic acid (RNA) was extracted, if present, from plasma samples obtained from patients with suspected dengue fever. All four DENV serotypes were detected and genotyped using the methodology described by Lanciotti et al.[19]. Reverse transcriptase polymerase chain reaction (RT-PCR) tests were performed using the QIAmp Viral Mini Kit (QIAGEN, Inc., Valencia, USA) at the Central Public Health Laboratory (LACEN) of Pernambuco between 2007 to 2012 and, from 2013 to 2015, at the Molecular Biology of Cancer and Infectious Diseases Laboratory of the Federal University of Rio Grande do Norte.

Statistical analysis

Microsoft Excel® software (Microsoft Corporation, Redmond, WA, USA) was used to enter data and generate all

graphs and tables. Statistical analysis was performed using SPSS® 13.0 statistical software (IBM Corporation, Armonk, NY, USA), and a 5% significance level was used in cross-tabulation chi-square analysis.

Ethical considerations

This study was approved by the Ethics Committee on Human Research of the Federal University of Paraíba (*Universidade Federal da Paraíba* - UFPB) (Certificate of presentation to the ethics committee – CAAE: 36522414.2.0000.5183) and was conducted according to the ethical guidelines of the Declaration of Helsinki. The requirement for informed written consent was waived by the ethics committee because the study was retrospective. The study was carried out in partnership with the Health Department of the State of Paraíba.

RESULTS

Suspected cases, confirmed cases, and incidence

Between January 2007 and December 2015, there were 118,179 reports of suspected dengue cases as follows: 2007, n=12,687 (10.7%); 2008, n=11,179 (9.5%); 2009, n=1,594 (1.4%); 2010 n= 8,611 (7.3%); 2011 n= 16,180 (15.4%); 2012, n=11,440 (9.7%); 2013, n= 17,999 (15.2%); 2014, n= 7,262, (6.1%); and 2015, n=29,227 (24.7%).

In Paraíba, except for a sharp decline in 2009 when only 509 (0.9%) cases were reported and a slight decrease in 2014 [n=3,620 (6.5%)], the number of dengue cases was consistent in 2007 [n=5,131 (9.3%)], 2008 [n=4,423 (7.6%)], and 2010 [n=4,405 (7.9%)]. A considerable increase in confirmed cases was seen in 2011, 2012, and 2013, with 8,667 (15.6%), 6,869 (12.4%), and 8,640 (15.6%) cases, respectively. Interestingly, in 2015 [n=13,416 (24.2%)], a large increase in cases was observed (**Figure 1A**). The fluctuation in incidence in João Pessoa was similar to that observed in the entire state, except for 2013, which had a lower number of confirmed cases (**Figure 1B**).

The years with the highest incidence in Paraíba were 2015 (337.74/100,000 inhabitants/year) and 2013 (220.72/100,000 inhabitants/year) (**Figure 1A**) and, in João Pessoa, 2012 (515.43/100,000 inhabitants/year) and 2015 (496.69/100,000 inhabitants/year) (**Figure 1B**). An incidence threshold of 90.00/100,000 inhabitants/year was observed, except for 2009, when the incidence rate was 13.50/100,000 inhabitants/year for Paraíba and 16.09/100,000 inhabitants/year for João Pessoa (**Figure 1**).

Clinical features

Of the confirmed cases, 39,083 (70.2%) patients were diagnosed with DF using the following clinical and epidemiological classifications: 15,365 (27.7%) cases of DwWS; 329 (0.6%) cases of DF; 504 (0.9%) cases of DWC; 281 (0.5%) cases of DWS; 26 (0.04%) cases of DSS; and 25 (0.04%) cases of SD. Even though there were low numbers of DWC, DWS, and DHF, a slight increase was observed from 2010. Of all outcomes, *cured* [n=49,969 (90.1%)] was the most frequent in all years.

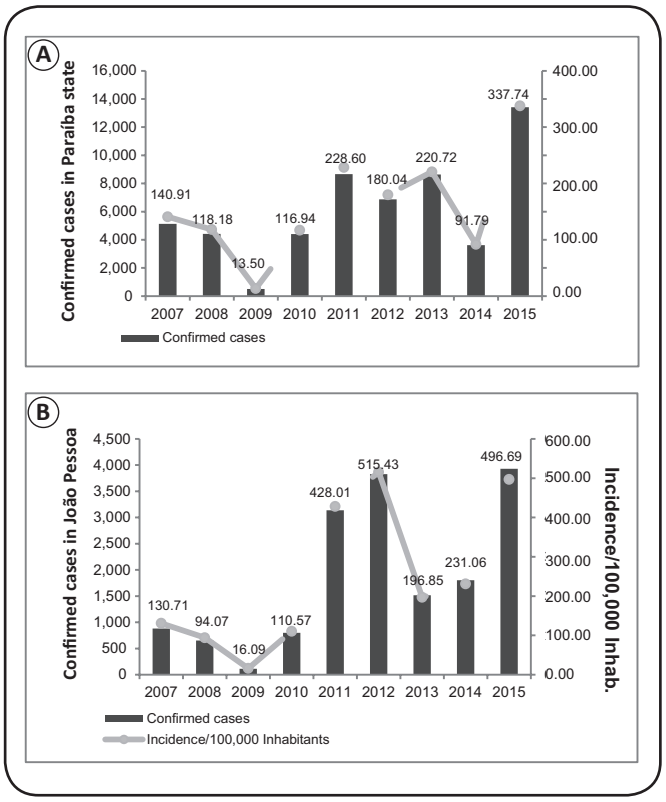

FIGURE 1 - Dengue incidence and confirmed cases from 2007 to 2015. (A) Dengue incidence in Paraíba and confirmed cases from January 2007 to December 2015; (B) Dengue incidence in João Pessoa and confirmed cases from January 2007 to December 2015.

The study population was characterized according to distribution of age and sex (**Figure 2**). The mean age of confirmed cases was 30 years. Females had the highest prevalence among the age groups 20-24 [n=3,230 (33%)], 25-29 [n=3,378 (34.5%)], and 30-34 years [n=3,189 (32.6%)]. Among males, peaks were observed at 15-19 years old [n=2,302 (32.3%)], 20-24 years old [n=2,469 (34.7%)], and 25-29 years old [n=2,348 (33%)]. When considering only sex as a factor, prevalence was highest among females, comprising 58.8% of all cases (p=0.0001).

Hospital admissions

Although the number of admissions to public and private hospitals was only 3.9% (n=2,164) of all confirmed dengue cases, the number of admissions was as high as 81% for some severity classifications in this study (e.g., DWC and DHF). Of the 1,166 patients who presented with severe symptoms, 737 were hospitalized. Hospitalization rates in other classifications were as follows: DWC, 410/504 (81.3%) cases; DWS, 17/281 (6%) cases; DHF, 289/339 (85.3%) cases; DSS, 14/26 (53.8%) cases; and SD, 8/25 (32%) cases. The mean age of confirmed dengue patients treated in hospital was 29 years. There was no significant difference in autochthony of patients admitted to hospital with dengue (**Table 1**).

It is important to emphasize that, of all notified cases of pregnant women with dengue (n=137), only 18 (13.1%) were

TABLE 1

Characteristics of hospital admitted patients with confirmed dengue in Paraíba from 2007 to 2015.

Hospitalization parameters	Yes	No	Total	P value
Mean age	29.30	28.92	29.97	
Pregnancy				0.001
yes	18	119	137	
no	634	3,527	4,161	
Autochthonous				0.0001
yes	1,231	11,840	13,071	
no	933	2,567	3,500	
Classification				<0.0001
DF	1,334	12,128	13,462	
DwWS	92	2,054	2,146	
DHF	289	11	300	
DWC	410	45	455	
DWS	17	151	168	
DSS	13	4	17	
SD	25	3	28	

DF: dengue fever; **DwWS:** dengue without warning signs; **DHF:** dengue hemorrhagic fever; **DWC:** dengue with complications; **DWS:** dengue with warning signs; **DSS**: dengue shock syndrome; **SD:** severe dengue.

referred to hospital (**Table 1**) and one pregnant patient died. Not all patients with dengue made a full recovery: 64 patients died, of whom 50 were admitted to hospital and were categorized as DWC [24 (48%) cases], DWS [1 case (2%)], DHF [12 (24%) cases], DSS [7 (14%) cases], and SD [6 (12%) cases].

Serotype presentation by year, age, and sex

Between 2007 and 2015, RNA sequencing of DENV was performed and confirmed by RT-PCR. The main serotype identified was DENV 1 [n=396 (80.5%)], which was prevalent during the whole study period, followed by DENV 4 [n=67 (13.6%)], DENV 2 [n=17 (3.5%)], and DENV 3 [n=12 (2.4%)]. DENV 1 prevalence persisted throughout the years analyzed as follows: 2007, n=41 (89.1%); 2008, n=77 (90.6%); 2009, n=3 (60%); 2010, n=60 (81.1%); 2011, n=107 (92.2%); 2012, n=31 (77.5%); 2013, n=52 (63.4%); 2014, n=13 (41.9%); and 2015, n=12 (92.3%). Co-circulation of the four serotypes was observed only from 2013; DENV 2 was absent in 2008, 2011, and 2015, and DENV 3 and DENV 4 in 2012 and 2009 (**Figure 3**). The infection was more prevalent among females than males, among all serotypes. The largest difference between the sexes was of DENV 4 (1:1.91), while the seroprevalences of DENV 1 (1:1.73), DENV 2 (1:1.42), and DENV 3 (1:1.4) had disproportional sex ratios.

The relationships between dengue serotypes and age, hospitalization, severity, and outcomes are presented in **Table 2**. The mean age of patients for all serotypes was 30.73 years. Furthermore, the lowest severity forms, DF and DwWS,

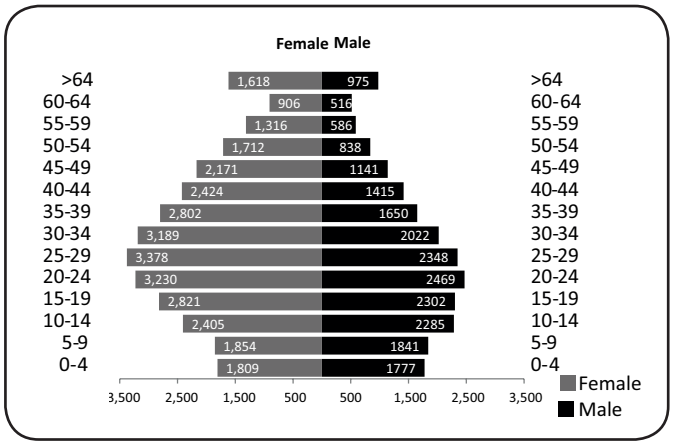

FIGURE 2 - Age and sex distribution of dengue cases in Paraíba, Brazil, from 2007 to 2015. Ignored ages are not included in this graph. (p= 0.0001).

were associated with DENV 1 (n=358), although hospitalization of patients [n=22 (5.5%)] was more likely with this serotype. Although 40 people from our study died from dengue, only three of these patients were serologically classified [DENV 1 (n=2) and DENV 4 (n=1)].

Diagnostic assays

From 2007-2015, among confirmed patients, 18,220 diagnostic assays were performed; the most used assays

TABLE 2

Association of dengue patients' clinical patterns with DENV serotype in Paraíba, from 2007 to 2015.

Parameters/serotype	DENV1	DENV2	DENV3	DENV4	Total	P value
Mean age	29.85	36	29.5	30.15	30.73	
Hospitalization						NS
yes	22	2	2	7	33	
no	87	4	1	24	116	
Classification						<0.0001
DF	349	9	3	32	393	
DwWS	9	1	2	4	16	
DHF	1	2	2	2	7	
DWC	1	0	1	3	5	
DWS	1	0	0	0	1	
DSS	2	0	0	0	2	
SD	0	0	0	1	1	
Deaths for dengue	2	0	0	1	3	NS

DENV: dengue virus; **DF:** dengue fever; **DwWS:** dengue without warning signs; **DHF:** dengue hemorrhagic fever; **DWC:** dengue with complications; **DWS:** dengue with warning signs; **DSS**: dengue shock syndrome; **SD:** severe dengue; **NS**: not significant.

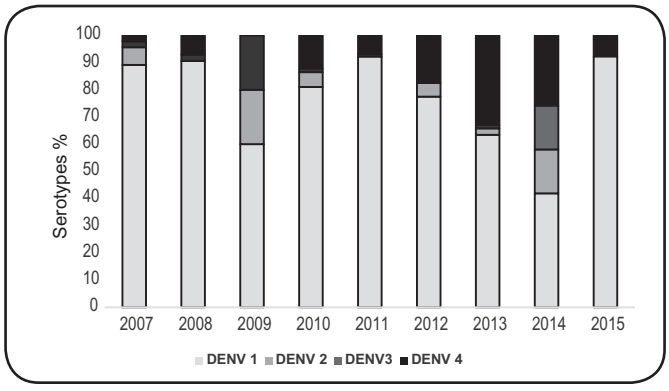

FIGURE 3 - Seroprevalence rates per year of dengue confirmed cases in Paraíba from 2007 to 2015. DENV: dengue virus.

in all years were serology tests [15,873 (87.1%) assays], followed by viral isolation [532 (2.9%) assays], NS1 tests [529 (2.9%) assays], histopathology tests [472 (2.6%) assays], immunohistochemistry [453 (2.5%) assays], and RT-PCR tests [361 (2%) assays]. Numbers of diagnostic tests performed in 2009 reflected the profile of confirmed cases in that the lowest total number of tests was performed of all years[n=201 (91.1%)]. However, in terms of relative numbers of tests (performed tests/confirmed cases), the lowest rates were in 2011 (3,804/8,667) and 2014 (868/3,620). The NS1 test was first introduced in 2010 when only one test was performed, although this number increased in subsequent years: 2011 (n=163), 2012 (n=91), 2013 (n=115), 2014 (n= 55), and 2015 (n= 104).

Symptoms associated with severity

During the study, patients with confirmed disease presented with various hemorrhagic manifestations (n=599). Research files revealed only one patient presenting with hemorrhagic manifestations in 2014 and 2015. Another clinical sign associated with dengue severity is plasma leakage (n=703), which was observed from 2007 to 2013 in patients, respectively.

DISCUSSION

The main aim of this study was to evaluate the status of dengue in Paraiba, Northeast Brazil. This analysis is important as there is huge concern in Paraíba about other emergent arboviruses, such as Zika and chikungunya, and their clinical similarities to dengue[20]. Peaks in incidence of these diseases correspond with peaks of confirmed dengue cases, with the highest numbers in 2015, 2011, and 2013 in Paraíba. These peaks are probably associated with the life cycle of the disease vector, *Aedes aegypti*[21]. Researchers have demonstrated that the prevalence of confirmed cases occurred in months with the highest pluviometer readings and appearance of vectors[21-23], as well as surges in urbanization and increases in population density, factors that also favor the development of *Aedes aegypti*.[24]

Interestingly, a huge increase in suspected and confirmed dengue cases was observed in 2015, overlapping with the appearance of Zika and its repercussions[20]. The appearance

of newborns with microcephaly due to infection with Zika[20], especially in Paraíba, may be a possible explanation for this increase in notifications, demonstrating that anxiety generated from an epidemic may be responsible for a drastic change in disease scenery. Despite the increase in notifications in 2015, cases were still rarely confirmed by molecular diagnostic techniques, probably due to low government investment, demonstrating the challenges in identifying circulating serotypes in Paraíba[25,26].

Most of the confirmed cases were classified as DF (70.2%) and DwWS (27.7%), a prevalence profile similar to that in other states of Northeast Brazil[27]. Although there were low numbers of DCC, DWS, and DHF, there was a slight increase in the proportion of these cases from 2010. The increase in severe dengue cases may have been associated with the reintroduction of a different serotype[28].

Most of the cases in this study were cured. In 2007, there was no registered death from dengue, and in 2009, there was only one death. These data may be a consequence of larger numbers of DwWS and DF in Paraíba, and low rates of DCC, DWS, DHF, DSS, and SD, which are commonly associated with high mortality, as observed in Recife-PE[29].

Prevalence of dengue was higher in females [31,635 (58.8%)] than males [22,165 (41.2%)], corroborating the results found in Recife-PE, Teresina-PI, and São Luis-MA[29,30]. Authors commonly attribute this to the higher tendency of women to seek hospital treatment[31], thereby increasing the number of notified cases among women, and, consequently, increasing the number of confirmed female cases. Other studies in dengue epidemiology have demonstrated no significant difference in numbers of male and female patients[22].

There was increased prevalence in the 15-34 year old age group (n=21,759). This data is in accordance with other Brazilian studies that have shown that the largest number of cases occurs in adult age groups[32]. Nevertheless, in the present study, peaks of dengue in the elderly (>64) of both sexes were observed. The mean age (30 years) of confirmed dengue cases in this study was lower than that found in Rio de Janeiro (32.8 years), evidencing the high number of cases in younger aged groups in our study[32].

In other studies, there was higher mortality among elderly patients, which may be related to a high frequency of comorbidities. However, data on comorbidities are not included in the SINAN, and can only be obtained through investigation of fatal dengue cases[33-36].

In this study, 2,164 (3.9%) patients with confirmed dengue were admitted to public and private hospitals. The number of hospital admissions was as high as 81% for some severity classifications in this study. Trends in hospitalization rates reflect severe dengue cases[13], as cases characteristic of DCC, DWS, DHF, DSS, and SD sometimes require hospital admission and are frequently fatal[37].

Despite pregnancy being associated with higher disease severity[38], only a small number of pregnant women in our study were referred to hospital. This may explain why only one case was fatal. The possibility of dengue was not considered during medical assessment in a case report of two parturients with dengue, who exhibited signs and symptoms and lived in an endemic area[39], even though several complications, such as vaginal bleeding, maternal death, prematurity, and spontaneous abortion, are associated with dengue during pregnancy[40]. These complications reflect the need for precise clinical management of potential health risks during pregnancy. In order to confirm dengue cases, Brazilian public and private facilities perform various tests, besides using the WHO clinical classifications. A total of 18,220 tests were performed on patients suspected of having dengue. Of these, 15,873 (87.1%) cases were serologically confirmed, while RT-PCR tests were used in only two cases. Until 2008, Brazil diagnosed most dengue cases using clinical and epidemiological criteria, as isolation and identification of DENV by PCR was rare[13]. Viral RNA detection assays provide a sensitive and rapid diagnosis. However, this approach requires specialized equipment and technicians, which are limitations in developing countries due to costs[41], thus explaining the prevalence of the immunoglobulin M antibody capture technique.

The introduction of DENV to previously unaffected populations has the potential to produce explosive epidemics. The introduction of a new serotype can cause a new epidemic, since each serotype produces only permanent immunity to itself[1,2], and the sequence of introduction and co-circulation of specific serotypes has been implicated in the increase of clinically severe cases[6,42].

Thus, serotype distribution explains disease patterns, with specific serotype predominance determining changes in the epidemiological profile of dengue. Our analysis revealed that the co-circulation of all four serotypes in 2013 and 2014 may be explained by the large number of cases in 2011 and 2012. Despite the emergence and re-emergence of DENV 2, DENV 3, and DENV 4 throughout the years of the study, DENV 1 remained dominant in the State of Paraíba.

An epidemiological survey in Brazil, comprising a series of DENV isolations from 2000 to 2008, was performed by the Brazilian Ministry of Health. At the beginning of the decade, DENV 1 had the highest incidence, then DENV 3 became predominant from 2003, and DENV 2 from 2007[13]. Geographical distributions of serotypes from different states, such as Pernambuco, Goiânia, and Ceará[43] revealed a similar trend to national trends. Our epidemiological results, however, did not corroborate this evolution of the DENV serotype, even though they revealed diverse co-circulation: DENV 1 remained the most predominant DENV serotype in Paraíba from 2007 to 2015.

Although DENV 1 is associated with the least severe disease, we found a strong correlation between DENV 1 with a severe outcome and hospitalization compared with other serotypes. Yung et al.[44] found that more severe cases were associated with DENV 1 in Singapore. However, other reports suggest a more significant correlation between greater disease severity and DENV 2[45]. These differences may be attributable to variations in plasma viral RNA levels among serotypes. Nevertheless, we

were unable to fully determine this in our study since data on serotypes was absent in many confirmed cases.

This study was the first to evaluate characteristics of dengue and problems with notification of the disease in Paraíba, Northeast Brazil. Our findings support the hypothesis that co-circulation of the four DENV serotypes may a reason for the increase in severe forms of dengue. Accurate estimates of dengue cases are important, not only to determine risk factors associated with severity, but also to inform policy decisions, and thereby identify research priorities and increase dengue awareness in all levels of society. This study may help prioritize research, health policy, and financial resources toward reducing this poorly controlled disease and other emergent arboviruses.

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Conflict of interests

The authors declare that are no conflict of interests.

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