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The reintroduction of DENV-2 in 2011 in Panama and subsequent outbreak characteristic

Yamilka Diaz[a], **Julio Cisneros**[a], **Hilda Guzman**[b], **Paola Cordoba**[a], **Jean-Paul Carrera**[a], **Brechla Moreno**[a], **Rubing Chen**[b], **Juan Castillo Mewa**[e], **Lourdes Garcia**[g], **Lizbeth Cerezo**[g], **Amelia Travassos da Rosa**[b], **Nathan D. Gundacker**[h], **Blas Armién**[f,i], **Scott C. Weaver**[b,c,d], **Nikos Vasilakis**[b,c,d], **Sandra López-Vergès**[#a,*], and **Robert Tesh**[#*,b]

[a]Department of Research in Virology and Biotechnology Department, Gorgas Memorial Institute of Health Studies, Panama City, Panama, Justo Arosemena avenue and 35st street, Zip Code 0816-02593;

[b]Department of Pathology and Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, Texas, United States of America, 301 University Boulevard Galveston, TX 77555-0609;

[c]Center for Tropical Diseases and [4]Institute for Human Infections and Immunity, University of Texas Medical Branch, Galveston, Texas, United States of America, 301 University Boulevard Galveston, TX 77555-0609;

[d]Department of Microbiology and Immunology, University of Texas Medical Branch, Galveston, Texas, United States of America, 301 University Boulevard Galveston, TX 77555-0609;

[e]Department of Research in Genetics and Proteomics, Gorgas Memorial Institute of Health Studies, Panama City, Panama, Justo Arosemena avenue and 35st street, Zip Code 0816-02593;

[f]Department of Research in Zoonotic and emergent diseases, Gorgas Memorial Institute of Health Studies, Panama City, Panama, Justo Arosemena avenue and 35st street, Zip Code 0816-02593;

[g]Epidemiology Department, Ministry of Health of Panama, Panama City, Panama, Ancon, Gorgas street, building 265;

[h]University of Alabama at Birmingham, Birmingham, Alabama, United States of America, Birminghan AL 35294;

[i]Research Direction, Universidad Interamericana de Panama, Panama City, Panama, Ricardo J. Alfaro avenue.

[*]Corresponding authors: Robert Tesh MD., Department of Pathology and Center for Biodefense and Emerging Infectious Diseases, University of Texas Medical Branch 301 University Boulevard Galveston, TX 77555-0609. rbtesh@comcast.net and Sandra López Vergès PhD. Department of Research in Virology and Biotechnology, Gorgas Memorial Institute for Health Studies, Justo Arosemena avenue and 35st street, Zip Code 0816-02593. slopez@gorgas.gob.pa.

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[#] These authors contributed equally to this work.

Abstract

The circulation of the South-east Asian/American (AS/AM) dengue 2 virus (DENV-2) genotype in the Americas has been associated with a high rate of severe disease. From 1993, the year DENV was reintroduced in Panama, until 2011 there were 29 dengue-associated deaths, 17 of which occurred in 2011, the most severe outbreak with a case fatality rate (CFR) of 44% (17 deaths out of 38 severe dengue cases). During this outbreak DENV-2 was reintroduced into the country, whereas over the prior five years DENV-1 and −3 were predominant. Herein, we describe the 2011 Panama outbreak and genetically characterize the Panamanian DENV-2 strains, which were associated with severe dengue disease in Panama. Our results suggest that the DENV-2 isolates from this outbreak belonged to the AS/AM genotype sub-clade 2BI and were genetically close to viruses described in the outbreaks in Nicaragua, Honduras, Guatemala and Mexico from 2006–2011. Sub-clade 2BI has previously been associated with severe disease in Nicaragua during outbreaks from 2005–2007.

Graphical abstract

During 2011, DENV-2 South-east Asian/American genotype, sub-clade 2BI was introduced in Panama, after 5 years without detection of DENV-2. This sub-clade, has been previously associated with severe disease in others Central American countries.

Keywords

DENV-2; outbreak; phylogenetics; epidemiology; case fatality rate

1 Introduction

Dengue viruses (DENV) are the most common mosquito-borne arbovirus in the world with approximately 390 million cases annually[1]. It is an enormous world wide economic burden[1] costing the Americas 1.9–2.2 billion dollars annually[2]. DENV belong to the genus *Flavivirus*, family *Flaviviridae*[3] and there are currently four recognized serotypes (DENV-1–4). It is transmitted by the mosquitoes *Aedes aegypti* and *Ae. albopictus* as primary and secondary vectors respectively[4]. Clinical disease caused by DENV is classified as dengue, dengue with warning signs and severe dengue base on the patients clinical presentation[4]. The underlying mechanisms for progressing to severe disease have not been fully elucidated. However these are likely influenced by both specific host and viral factors that act in concert[5]. Severe disease has been associated with: secondary infection with a different serotype[6–8], host genetic susceptibility[9], patient co-morbidities[10,11], specific DENV serotypes and genotypes[8,12–14], viral load[15] and the immune response induced by the DENV-NS1 protein[16–19].

Phylogenetic and molecular analyses have revealed extensive genetic diversity among DENVs, leading to the recognition of different genotypes within each serotype[20,21]. DENV-2 has 5 genotypes (Asian I, Asian II, Cosmopolitan, American and South-east Asian/American). The initial introduction of DENV-2 in Americas was caused by American

genotype, then in 1975, it was replaced by the Asian/American (AS/AM) genotype in the Caribbean, with dissemination to Central and South America in the 1980–90s[22]. Its reintroduction in Nicaragua, Peru and Brazil since 2000's has been associated with more severe outbreaks[23–25].

Recent analysis of DENV-2 strains from Nicaragua, Honduras and Guatemala showed that the AS/AM circulating in Central America is divided into 2 clades; clade 2 is further divided in 2 sub-clades: 2A and 2B[26]. Sub-clade 2B has been implicated in severe cases in patients with primary exposure to DENV-1 in Nicaragua[27].

Dengue was responsible for outbreaks in Panama in 1904[28], 1912[29] and 1941–1942[30]. In 1958 Panama was declared *Ae. aegypti*-free after an intense vector control campaign. Panama was re-infested by *Aedes aegypti* in 1985[31], and in 1993, a limited outbreak of dengue occurred in Panama City, confirming the return of autochthonous circulation of DENV-2[31,32]. Since then, the four DENV serotypes have circulated in Panama and continue to be a substantial public health burden[33].

In Panama, during the first half of 2011, DENV-1 and DENV-3 comprised the majority of circulating DENV. However, during the second half of the year, DENV-2 was detected and became the predominant circulating serotype and a corresponding increase in dengue deaths was observed. DENV-2 previously circulated in Panama in 1993–1994 and 1999 to 2004 (www.paho.org) and each reintroduction was associated with outbreaks but none were apparently associated with an increase in mortality. A retrospective analysis of data available through the National Dengue Epidemiologic Surveillance System was performed to characterize the 2011 DENV-2 outbreak in Panama. Herein, we report the results of the outbreak investigation as well as the genetic analysis of the viral strains detected during the outbreak.

2 Materials and Methods

2.1 Ethical considerations

The permit to study arboviral outbreaks was approved by The National Committee of Bioethics in Panama, IRB number: 0277/CBI/ICGES/15. Patient information was de-identified in order to protect the confidentially of individuals involved.

2.2 Case definition

The Dengue case definition used during 2011 Dengue outbreak in Panama, was based on the 1997-World Health Organization (WHO) classification system which includes: Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS)[4]. The epidemiological link criterion was defined as a case in close contact with a confirmed case within 30 days of symptoms onset.

2.3 Dengue surveillance program and Epidemiological Data Collection

Using the WHO's Dengue case definition[4,34] the Ministry of Health of Panama (MINSA) and Gorgas Memorial Institute of Health Studies (ICGES) established the National Dengue Surveillance program in 1988 across the country. Around 10% of dengue-suspected cases

from the country are sent to the ICGES laboratory for confirmation and serotype characterization, whereas most of the cases are laboratory confirmed by enzyme-linked immunosorbent assay (ELISA) of Dengue IgM/secondary IgG and NS1 (PANBIO) detection in local health care institutions. All Dengue cases seen in both public and private health care institutions are reported to National Department of Epidemiology (NDE), as regulated by the executive order N°268 from 2001. The dengue surveillance database was obtained from the National Department of Epidemiology of Ministry of Health and demographic information was available from the National Institute of Statistics and Census.

2.4 Epidemiological and Statistical methods

Epidemiological and demographic variables were analyzed using Epi-Info 7, Microsoft Excel and STATA SE software, version13.1. Dengue incidence, mortality and case fatality rate (CFR) were estimated using data from dengue surveillance database and the estimated population at July 1st of 2011 using demographic information of the General Comptroller of the Republic of Panama. To calculate the endemic channel percentiles, the epidemiological data from previous years (2004–2010) were used; this included 2 epidemic years (2005 and 2009), which increases the superior limit of the endemic channel.

2.5 Correlation of dengue cases and vector index

A systematic entomological monitoring system is in place throughout the year. Entomological data are collected from cluster surveys to establish the *Ae. aegypti* and *Ae. albopictus* infestation rates, using the survey of 100% of houses in a block. The number of containers with larvae per household was quantified. Positive houses were reported if at least one recipient has one larva. The larvae Breteau (BI) was estimated with the number of positive containers per 100 houses investigated. While the house indices (HI) index was estimated by the following equation: $HI = \frac{number\ of\ infected\ houses}{number\ of\ inspected\ houses} \times 100$ Using the HI index, MINSA classified the Dengue transmission risk as >4: high, 4 to 2: moderate and <2: low risk[35] . Entomologic surveillance database was obtained from the Vector National Department of the Ministry of Health. This was used to analyze the relationship between vector infestation rates and dengue cases during 2011 using Spearman's Rho correlation analysis with STATA SE software v13.1.

2.6 Laboratory Assays

2.6.1 Molecular Testing—Acute sera samples were sent to ICGES for serotype and genotype surveillance through the Dengue virus surveillance program. RNA was extracted using QIAamp RNA Viral Extraction Kit (Qiagen, Germany) and DENV viral RNA and serotype-specificity was detected using qRT-PCR specific protocol[36]. Positive samples were used for viral isolation.

2.6.2 Serologic Testing—Serum samples from DHF suspected cases with ≥ 5 days from onset of symptoms were tested using NS1 and DENV-IgG Indirect ELISA (PANBIO). A total of 10% of qRT-PCR dengue negative samples were also tested for antibodies against flaviviruses and alphaviruses known to circulate in Panama with the use of hemagglutination-inhibition assays[37].

2.6.3 Viral Isolation—Viral isolation was attempted by inoculation of Vero cells (African green monkey-ATCC CCL-81. USA), with acute sera or 10% tissue homogenates (spleen and liver) from acute DENV or fatal cases respectively, and daily observation for cytopathic effects (CPE) was done. When CPE were evident, viral RNA was extracted using Quiagen and tested again using DENV-specific qRT-PCR[36].

2.6.4 E gene and complete genome sequencing—Twenty-seven isolates of DENV-2 were obtained from acute sera. Six DENV-2 isolates that circulated in different regions of the country were randomly selected for analysis. Viral RNA was extracted and RT-PCR was performed to obtain 5 overlapping fragments for the complete viral envelope (E protein) gene using the Titan Kit (Roche) with specific primers described previously[38]. Amplicons were purified with the QIAquick PCR purification kit (Qiagen) and sequences were obtained using an Applied Biosystems 410 Genetic Analyzer (Foster City, CA), following the manufacturer's protocols. Only one complete genome of the strain 250330 was obtained using overlapping primers derived from the reference strain DENV2–16881 (primers available upon request).

2.7 Phylogenetic analysis

The six Panamanian 2011 DENV-2 E sequences (1485 nt) were aligned with 1,965 sequences of DENV-2[39] from the Genbank library and from Virus Pathogen Database and Analysis Resource (ViPR) using SEAVIEW 4.0 software (Gouy M et al, Mol Biol Evol 2010). This alignment was used to do a Neighbor Joining (NJ) Tree using a heuristic search with Nearest Neighbor Interchange (NNI) operation using the GTR+G+I model showing that Panama 2011 DENV-2 belong to South East Asian/American (AS/AM) genotype (data not shown). The closer 63 sequences to the five Panamanian 2011 E sequences (Genbank numbers KY977455, KY977456, KY977457, KY977458, KY977459), selected from the DENV-2 AS/AM genotype, were used to construct a maximum likelihood (ML) tree under GTR+G+I model with midpoint root for clarification. The statistical significance of the tree topology was evaluated by bootstrap resampling of the sequences 1000 times. Phylogenetic and molecular evolutionary analyses were conducted using PAUP* version 4.0,10b (Swofford DL. PAUP*: Phylogenetic Analysis using Parsimony (*and Other Methods) Sinauer Assoc Sunderland, Massachusetts. 2002;1–142) and MEGA7 * Kamura, Stecher and Tamura 2015).

Amino acid sequences of complete E gene of the 6 Panamanian 2011 DENV-2 and the complete ORF of KY977454 isolate were analyzed and compared to the closest isolates from 2B and 2A DENV-2 subclades using MEGA7.

3 Results

3.1 Outbreak Description

A total of 3,850 dengue cases were reported in 2011. An increase of dengue cases that surpassed the upper quartile of the endemic channel was observed during epidemiological week 26 (June) through week 36 (September), when 62.2% of the cases occurred (Figure 1). The total incidence rate of dengue in the country was 103.4 per 100,000 inhabitants. The

majority of cases occurred in Bocas del Toro, San Miguelito and Panama City areas with an incidence rate of 269.8 per 100,000, 279.9 per 100,000 and 114.4 per 100,000 inhabitants respectively (Table 1, Figure 2). Persons 0–14 years old had an incidence of dengue of 69.9 per 100,000 inhabitants, 15–29 was 114.6 per 100,000 inhabitants, 30–44 was 121.8 per 100,000 inhabitants, 45–59 was 102.4 per 100,000 and over 60 was 109.2 per 100,000 (Table 2). An univariate analysis comparing dengue cases by sex and age range revealed no statistical difference.

From the 3,850 dengue cases there were 38 cases of DHF (0.99%). Of these, there were 17 fatal cases across the country (Supplementary Table 1 and Figure 2). Two fatal cases occurred in the 0–14 years of range, 4 in those 15–29, 3 in those 30–44, 5 in those 45–59, and 3 in those over 60 (Table 2). The mortality of dengue cases during this year was 0.46% (17 death/3,723,821 total inhabitants) per 100,000 inhabitants, whereas CFR of deaths to DHF were 44.7% (17 death/38 DHF cases). The CFR of DHF was high (>60%) in children (0–14 years old), and those more than 45 years old (Table 2). Fatal cases affected more males than females, with 64.7% males and 35.3% females respectively.

Of total dengue cases 3,129 (81.3%) were confirmed by laboratory tests: 2,843 (90.9)% by ELISA or rapid antibody test detecting IgM, NS1, IgG-indirect or tissue culture at local health institutions or ICGES, 286 (9.1%) by qRT-PCR at ICGES; and 721 (18.7%) cases were confirmed by epidemiological link. In 2011, as part of the national dengue surveillance program, ICGES received 1,197 acute sera and 18 homogenized tissue samples (liver and spleen), of which only 6 had paired acute sera. Of the acute sera samples 23.9% (286/1197) were positive for DENV by qRT-PCR and/or viral isolation. Of these 286 confirmed cases, 197 were identified by qRT-PCR as DENV-2, 59 as DENV-1 and 30 as DENV-3. Of the 18 tissue samples received from suspected dengue cases, one was positive for Varicella Zoster virus by RT-PCR and viral isolation from spleen and liver and was negative for dengue. From the subsequent 17 fatal cases due to dengue, only one was diagnosed by epidemiological link, 10 were diagnosed by serological methods (IgM/IgG Dengue ELISA or NS1 ELISA), and 6 were diagnosed by qRT-PCR (Supplementary Table 1). Of these 6 cases, 5 were identified as DENV-2 and one as DENV-3. Viral isolation was attempted for all fatal cases; however, it was unsuccessful due to multiple breaks in the cold chain. For this study, from the seventeen fatal cases, only seven had corresponding clinical data which was available for analysis. This clinical data was similar to that previously described in the literature[40–42] (Supplementary Table 1).

Around 10% of dengue negative acute samples (97/911 negative samples), were tested for other arboviruses, which have circulated in Panama. Alphaviruses (VEEV and EEEV) and flaviviruses (Yellow fever virus: YFV, Ilheus virus: ILHV, West Nile virus:WNV and Saint Louis virus:SLEV) was tested using hemaglutination inhibition test (HI). HI activity was detected in 53 samples. Of these samples, 14 were positive for antibodies against DENV (10 for DENV-2, two for DENV-1 and two for DENV-3) without cross-reactivity with any of the other tested arboviruses. Twelve samples presented an HI pattern for secondary response against Dengue, five samples showed titles for other flaviviruses, 2 for flavivirus and alphavirus and 20 sera showed cross-reactivity between different flaviviruses (Supplementary Table 2).

3.2 *Aedes* vector surveillance

A moderate increase of the *Aedes* infestation index (*Ae. aegypti* and *Ae. albopictus*) occurred during epidemiological week 22, four weeks before Dengue clinical cases started to rise (Figure 3). This increase in *Aedes* circulation reached its highest point at week 30, and slowly decreased to a low risk index of <2 during week 35. The decrease in *Aedes* infestation rate is followed by a decrease in dengue reported cases, 5 weeks later. A correlation analysis of rho= 0.69 ($p<0.001$) between incidence rate and *Aedes* index infestation by week showed a moderate relationship between *Aedes* infestation and the incidence of dengue during the 2011 outbreak.

3.3 Phylogenetic analysis

Six complete E gene sequences and one complete genome of the Panama DENV-2 isolates from the 2011 outbreak were aligned with representative sequences of all DENV-2 genotypes. Phylogenetic analysis of DENV-2 E gene alignment using NJ and ML methods created phylogenetic trees with identical topologies showing that Panama DENV-2 isolates clustered together and originated from the AS/AM lineage similar to previously reported DENV-2 isolates found in the Americas since 1979[22] (Figure 4). Using the same classification as Añez G *et al*[26], the Panamanian isolates cluster in the AS/AM genotype clade 2 sub-clade 2b.I. This is confirmed by the full genome analysis with Panamanian strain 250330, KY977454 (Figure 4).

The alignment between the six Panamanian DENV-2 2011 E gene sequences with the fourteen closest sequences from DENV-2 (five from Mexico, 2011; two from Guatemala, 2007 and 2009; one from Honduras 2007; five from Nicaragua, one from 2005, three from 2006, one from 2007; and one from Puerto Rico 2013) was done to look for specific mutations. Among the six Panamanian sequences from DENV-2 2011, there were only 8 nucleotide changes and all of them were synonymous. Among the Panamanian and the other analyzed sequences, 11 nucleotide differences. Only one of them was non-synonymous and resulted in an A50T amino acid substitution in PA-2011 and PR-2013 strains (Figure 4).

The amino acid sequence of the complete polyprotein of Panama DENV-2 2011 strain was analyzed. The Panama DENV-2 sequence had the two expected mutations specific of the clade 2b (compared to clade 2a): serine S in residue 245 from NS4B and lysine K in residue 94 from NS1[26] (Figure 4). It was confirmed that it had some of the mutations specific of 2b.I sub-clade like the M492 from E, L279 from NS1, K200 from NS5 and T290 from NS5. There is one mutation that substituted H249Y in NS3 in the PA-2011 as well as MX-2011, which is not found in the subclade 2b.I. Finally, there was one amino change, V71I from NS2A, specific to PA-2011 compared to the other available complete genome of Dengue 2b.I.

4 Discussion

During 2011, Panama experienced a dengue outbreak associated with the re-introduction of DENV-2. This study describes the characteristics of this outbreak and analyzes for the first time the genetic variation of DENV-2 strains that circulated in the country in 2011. Although

the circulation of DENV-2 was detected in April, the majority of cases occurred from July to September. Similar to Vargas et al[43], the *Aedes* infestation index (HI) was predictive of an increase in dengue cases with a preceding interval of six weeks. Our analysis showed a moderate correlation between the *Aedes* infestation index and when dengue cases surpassed the third quartile of the endemic channel. This suggests that the *Aedes* infestation index (HI) could be predictive of human dengue transmission. Other studies showed *Aedes* adult index and pupae index are better predictors of human dengue transmission[44] however for this specific outbreak larvae index were enough.

Before 2011, the major dengue outbreak reported in Panama occurred in 2009 with a total of 7,459 Dengue cases. During this year the dengue mortality rate was 0.19 per 100,000 inhabitants. The CFR of DHF was 15%, (7 deaths / 46 DHF cases). During this outbreak DENV-1 and DENV-3 were the circulating serotypes. In 2010, the Americas experienced a large dengue outbreak with some countries having >200 cases per 100,000 inhabitants[23]. During this time DENV-2 was present throughout endemic countries in the Americas except in the USA, Panama and several Caribbean islands. Panama reported a decrease of cases in 2010, with a total of 2,002 cases, 3 DHF and no fatalities. At that time DENV-1 and DENV-3 were still co-circulating.

The Panama 2011 outbreak had a dengue mortality rate of 0.46 per 100,000 inhabitants and a case fatality rate of 44.7%, both the highest since the 1993 reemergence of dengue. When compared to other countries with a predominance of DENV-2 during the 2010 outbreak Panama had the highest CFR. In Colombia, the CFR was 2.3% (217 deaths/9,482 DHF) and in Honduras it was 2.54% (83 deaths/3,266 severe)[23]. However the number of DHF/total dengue cases is 1% in Panama, much lower than the 6% and 4.9% in Colombia and Honduras respectively. During 2011, the mortality rate in Panama was eighth in the Americas, just below Bolivia (0.47%) and above Brazil (0.24%). This could be due to improved healthcare infrastructure, less virulent DENV strain, errors in epidemiologic data collection or an underreporting of DHF cases. In addition, differential detection of severe dengue cases due to the recent change in the clinical dengue classification systems could have artificially decreased DHF cases and thus increased the Panama CFR.

Severe dengue and death is associated with host health status, genetic and previous immunity as well as viral features[24]. The presence of co-infections also plays a role in the development of severe dengue infections. Martins VDCA, et al[41] did not find any difference between dengue serotype and severity of the disease during an outbreak with all four serotypes; however the severity of the disease was higher in patients infected with multiple serotypes when compared to those mono-infected. We analyzed 1,197 acute dengue cases, and no co-infection was detected by viral isolation or qRTPCR. The role of dengue serotype and genotype in the development of severity is controversial, however recent studies have shown that some specific clinical manifestations could be overrepresented by a specific serotype even in primary infections[8,14]. Our study reports that the Panamanian DENV-2 strain, circulating during 2011 outbreak, belongs to Asian/American genotype (AS/AM). The introduction of this genotype has been linked with outbreaks with high mortality rates[41,45,46] in Peru[47], Nicaragua[27] and Brasil[48]. Our phylogenetic analysis clustered Panama 2011 DENV-2 strains in clade 2 sub-clade 2b.I using previous classification of

DENV-2 AS/AM circulating in Central America[26], closely related with strains from Mexico 2011 and Guatemala 2009. OhAinle et al[27] reported the dominance of sub clade 2b after 2005/06 epidemic seasons in Nicaragua, explained by its higher median relative fitness than clade 1 and the higher viremia compared with clades 1 and 2A. It was observed in this study that DENV-3 immunity was associated with more severe disease among clade DENV-2 2b infections, while the DENV-2 clade I was more virulent in children immune to DENV-1[27]. The high CFR during Panama 2011 outbreak could be explained by an introduction of this DENV2 sub-clade 2b, when DENV-1 and DENV-3 were co-circulating, considering this DENV-2 clade characteristics and its association with outbreaks in several countries of the region. The observed mutations in the 2011 Panamanian strains not previously described (A50T from E protein, V71I from NS2A, H249Y from NS3) need to be studied to determine if they are implicated in viral fitness or immune responses. Indeed A50 is one of the residues from the C chain of the E protein that interacts with a potent DENV antibody[49], thus the A50T substitution could play a role in antibody recognition or other immune responses.

This study has several limitations. Epidemiological data analyzed was obtained from the dengue surveillance program, which does not contain variables such as symptoms, comorbidities, immunologic disorders, laboratory findings or travel history which all of which may have affected outcomes. In addition, acute serum was not always available and the cold chain for tissue samples was insufficient. This may have resulted in the inability to culture and/or detect dengue serotypes in all cases. We were not able to determine if severe or fatal DENV-2 infection were secondary; a cohort study in Panama would be necessary to characterize the dynamic interaction of circulating serotypes and the effect on dengue clinical manifestations.

This descriptive study of the 2011 dengue outbreak in Panama suggests that the introduction of DENV-2 AS/AM genotype subclade 2b.I contributed to the increase in cases and the high case fatality rate of deaths over DHF cases. The recent introduction of dengue vaccines make understanding severe dengue more important as these vaccines may promote severe disease in certain subpopulations[50]. An improved understanding of dengue and its dynamic circulation are required for successful public health policies and vaccination programs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- During the outbreak, a moderate correlation between the *Aedes* infestation index and dengue cases was observed when cases surpassed the third quartile of the endemic cannel.
- During 2011, the introduction of DENV-2 AS/AM genotype subclade 2b.I after five years without DENV-2 circulation might have contributed to the increase in cases and the high case fatality rate of deaths over DHF cases.
- Two mutations in the E gene and NS2A 2011 Panamanian strains have not been described previously.

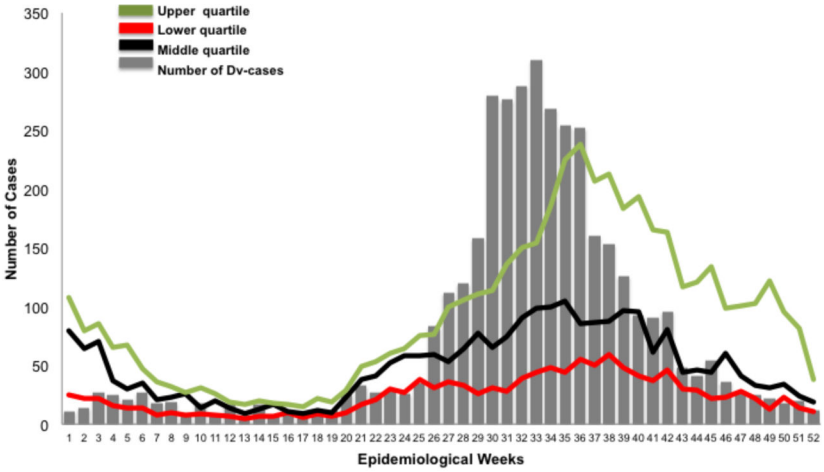

Figure 1. Endemic channel of Dengue cases during 2011 outbreak.

In grey bars, number of dengue cases per epidemiological week: lines show the endemic channel with green representing the upper quartile, red the lower quartile, black the middle quartile. Since epidemiological week 26, an increase of cases is shown; which is maintained for 11 weeks, with a sustained increase during epidemiological weeks 30 to 33. The endemic channel was constructed using 7 years of data, which included two Dengue epidemic years 2005 and 2009.

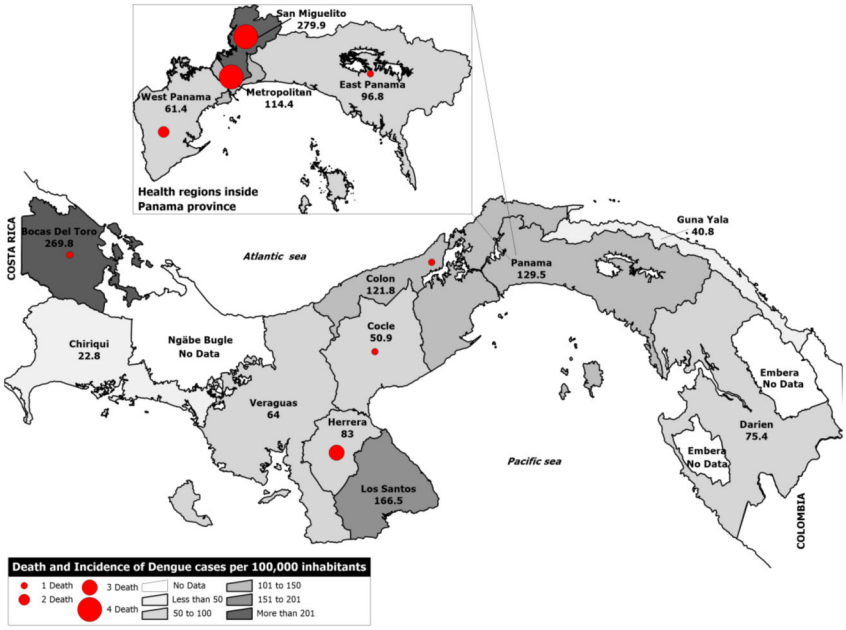

Figure 2. Map of dengue cases in Panama during 2011.

Map shows, using a grey color scale, the incidence of dengue cases with red dots corresponding to the number of fatal cases per province or indigenous reservation (Guna Yala, Embera, Ngäbe Bugle). No cases were reported from other indigenous reservations besides the Guna Yala.

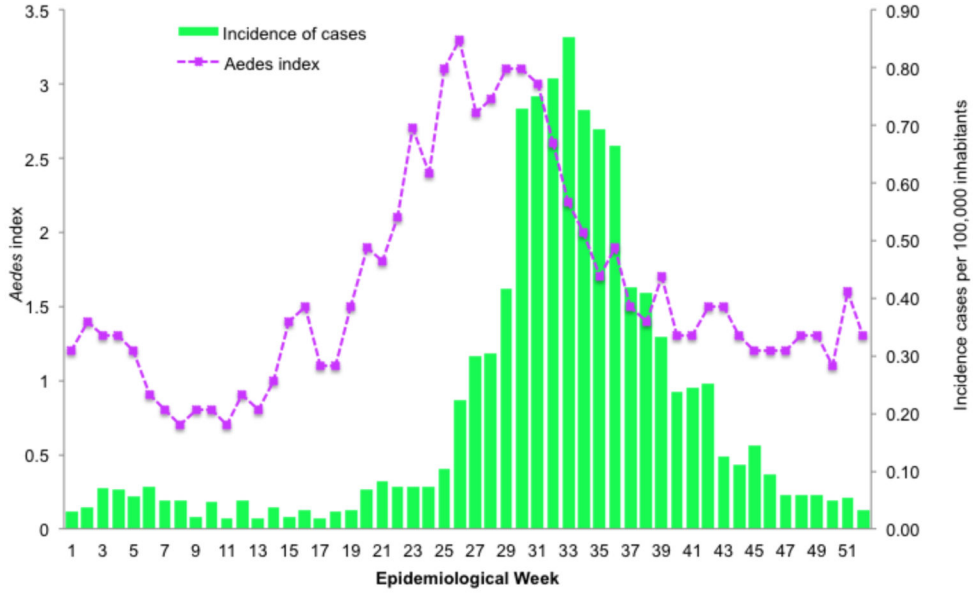

Figure 3. Incidence of dengue cases and *Ae*. infestation index during 2011.

In green the incidence of dengue cases per 100,000 inhabitants and in purple the *Aedes* infestation index per epidemiological week. The *Aedes* infestation index are classified as <2=low risk; 2–4=medium risk; >4=high risk.

Figure 4. Panamanian Dengue virus type 2 Phylogeny.

Maximum likelihood tree (MLT) of DENV-2 based on complete E gene nucleotide sequences of South-east Asian American genotype from GenBank (n=60) and Panamanian strains (n=8). MLT was constructed using MEGA 7. Bootstrap values (1000 replications) above 70 were showed along the branch. The tree is mid-point rooted. Red branches represent clade 2B-I. Red spots represents Panamanian DENV-2 strains. The two Panamanian DENV-2 strains in red correspond to the isolates from the fatal cases.

Table 1.

Dengue confirmed cases by laboratory and epidemiological link, and total incidence rate by province during 2011.

Province	Percentage of Dengue cases confirmed by epidemiological link %(n)	Percentage of Dengue confirmed cases by Laboratory tests %(n)	Total of Dengue cases	Incidence rate* (x10^5 inhabitants, July 1st, 2011)
Total	**18.7 (721)**	**81.3 (3129)**	**3850**	**103.4**
Bocas del Toro	45.3 (170)	54.7 (205)	375	269.8
Code	27.8 (35)	72.2 (91)	126	50.9
Colon	0.6 (2)	99.4 (314)	316	121.8
Chiriqui	1 (1)	99 (99)	100	22.8
Darien	35.9 (14)	64.1 (25)	39	75.4
Herrera	0 (0)	100 (97)	97	83.0
Los Santos	12.1 (19)	87.9 (138)	157	166.5
Panama city	24.5 (286)	75.5 (879)	1165	114.4
West Panama	45.3 (139)	54.7 (168)	307	61.4
East Panama	2 (1)	98% (48)	49	96.8
San Miguelito	5.3 (50)	94.7 (899)	949	279.9
Verag lias	0 (0)	100% (154)	154	64.0
Guna Yala	25 (4)	75 (12)	16	40.8
Embera	0 (0)	0 (0)	0	0
Ngäbe-Bugle	0 (0)	0% (0)	0	0

Table 2.

Incidence, Mortality and Case Fatality Rate of Dengue during 2011.

Age group (yr)	*Incidence of Dengue **(cases/ population)	Mortality rate of Dengue **(deaths / total population)	(%) CFR of DHF (deaths/DHF cases)
0–14	69.9 (746/1,071,235)	0.19 (2/1,071,235)	66.7 (2/3)
15–29	114.6 (1072/935,364)	0.43 (4/935,364)	30.8 (4/13)
30–44	121.8 (984/807,909)	0.37 (3/807,909)	33.3 (3/9)
45–59	102.4 (552/539,286)	0.93 (5/539,286)	62.5 (5/8)
>60	109.2 (404/370,027)	0.81 (3/370,027)	60 (3/5)
Total	103.4 (3850/3,723,821)	0.46 (17/3,723,821)	44.7 (17/38)

* Incidence calculated per 100 000 inhabitants

** Total population estimated to July 1st of 2011.