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Dengue Outbreaks in Abidjan: Seroprevalence and Cocirculating of Three Serotypes in 2017

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Abstract

Dengue fever is a major public health problem in the world, because it is especially endemic in the tropical and subtropical areas. Arbovirus infection is less well documented in African countries. We aimed to assess the distribution of patients in the dengue epidemic and the seroprevalence of different serotypes of the circulating dengue virus. A retrospective study included analyses of human blood samples sent to the National Reference Laboratory for diagnosis during dengue infection outbreak. Samples were screened by IgM capture ELISA (MAC-ELISA) or by RT-PCR. Of the 2,849 serum samples from suspected dengue cases, 2,297 (80.6%) were from Abidjan. The seroprevalence of dengue was (15.1%) during this epidemic. The seroprevalence of dengue virus serotypes in cocirculation was predominated by DENV-2 with 189 cases (6.6%), followed by DENV-3 77 cases (2.7%), and DENV-1 14 cases (0.5%). The seroprevalence in children was 8.7% compared to 19.0% in adults. The age group of 16 to less than 45 years accounted for 54.0% of total positive cases. In addition, positive peak was observed in July (28.3%) and Abidjan East was the most affected locality. The increasing trend of serotypes of the dengue virus cocirculation suggests that Abidjan is becoming a hyperendemic state from an endemic one.

Keywords: dengue virus, outbreaks, surveillance, National Reference Laboratory, epidemiology, Abidjan

Background

Among arboviruses, dengue fever is a major public health problem in the world, especially in the tropical and subtropical areas.[1] As proof, several studies have reported on its distribution.[2–5] They show that more than 3.97 billion people in 128 countries are currently exposed to this infection.[6] Dengue viruses (genus *Flavivirus*, family *Flaviviridae*) are mosquito borne and the principal vector (*Aedes aegypti*) is a day-biting domestic mosquito of public importance that breeds in stagnant water.[3,7] Dengue illnesses are caused by any of the five serologically related viruses designated as dengue virus (DENV), DENV-1-5.[8–10] Infection with any one of these serotypes mostly causes a mild, self-limiting febrile illness (classical dengue fever), although a few cases develop severe life-threatening, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). However, this classification can be summarized as dengue with warning signs versus severe dengue. Severe dengue known as DHF or DSS has become a major public health problem.[11] The estimated number of 50–100 million infections per year, results in 250,000–500,000 cases of DHF and 25,000–50,000 deaths each year.[1,12] The routine laboratory diagnosis of dengue virus infection is primarily achieved by serodiagnosis, detection of IgM/IgG antibodies, and/or molecular detection by the demonstration of viral ribonucleic acid (RNA) by reverse transcription–polymerase chain reaction (RT-PCR).[13–15]

Arbovirus infections were less well documented in coastal African countries.[16,17] Dengue seroprevalence rates reported were lower than those in Asia or America.[18] From Bhatt et al., the burden of dengue fever in Africa may be similar to that of the Americas.[19] Brady et al. has published a summary of evidence of dengue fever in West Africa.[2] That was the case for countries experiencing recurrent epidemics of dengue fever of various serotypes. In Burkina Faso, serotype 3 has been detected in 2003, 2004, 2007, 2013 and 2016.[20] In Guinea 2007, a study reported several arbovirus infections.[21] In Mali, a study found a seroprevalence of 93% anti-dengue IgG and serotype 1 and 3 circulation by Sang R.C.[1,16] Serotype 3 was responsible for the epidemics that occurred simultaneously in 2009 in Senegal and for the first time in Cape Verde.[22,23]

Dengue fever is endemic in Ivory Coast. However, since 2008, we have had epidemic peaks of dengue fever, with cocirculation of yellow fever and dengue fever.[24] In 2010, an epidemic of dengue fever syndromes was reported in Ivory Coast.[25] Unfortunately, every year sporadic cases of dengue fever are detected. That was the case in 2015, where three cases were confirmed, and in 2016, six cases were detected (Unpublished). The year 2017 was marked by the biggest epidemic in Ivory Coast. Ivory Coast and other West African countries are increasingly equipped with national reference laboratories and qualified personnel. These countries are now able to detect epidemics through their epidemiological surveillance system. These different tools have better documented the circulation of all dengue serotypes in West Africa.[16,17,26–32] The role of support to the health system is meaningful and reflected in the information and alerts provided to health authorities during the epidemic outbreak.

Unfortunately, in this context, little is known about the distribution of the profile of patients affected by the dengue virus during the Abidjan epidemics in Ivory Coast and especially on the serotypes of the virus circulating in that country. The objective of this study was to draw up a map of the distribution of the patient population of this dengue epidemic and the seroprevalence of different serotypes of the dengue virus circulating in Abidjan.

Materials and Methods

Study Setting and Design

This retrospective study included analyses of human blood samples sent to the National Reference Laboratory (NRL) of the Epidemic Virus Department of the Institut Pasteur de Côte d'Ivoire (IPCI) between January to December 2017 for diagnosis during the dengue infection outbreak in Ivory Coast.

Since its creation in 1972, the IPCI through the Epidemic Virus Department had missions in addition to research, to ensure thorough collection of the virologic component of the epidemiological surveillance of arbovirus and viral hemorrhagic fevers through the NRL. The NRL for arboviruses and viral hemorrhagic fevers participated in the management of epidemics by confirming suspected cases: diagnosis (serological and molecular biology: typing and genomic sequencing), then virus identification by virus isolation and tissue culture (if possible) and finally by validating new diagnostic techniques.

The arboviruses and hemorrhagic fever virus unit dedicated the study of viruses transmissible to humans by arthropods, *Flaviviridae* (yellow fever virus, dengue virus), *Alphaviridae* (Chikungunya virus), and *Arenaviridae* (Lassa virus), for the alert and investigation of epidemics and the seroprevalence study.

Sample Collection and Processing

Blood samples were collected from patients with fever presenting to outpatient departments of 83 health districts in the country. These blood samples were packed with ice to the laboratory of virus transmitted by vectors in the Epidemic Virus Department for detection of dengue viruses. The clinical basis for diagnosing the patients as having dengue virus infection was based on World Health Organization (WHO) definitions.[23] In the NRL of hemorrhagic viral fevers, samples were received in triple packs. Red cap sampling tubes were used for collection of whole blood. They were all accompanied by a standard survey form for collecting epidemiological and biological information from the patient. Three aliquots were prepared on each sample after centrifuging. One aliquot was transferred to the biobank for long-term conservation in the liquid nitrogen. The other two remained in the laboratory: one in a dedicated freezer at -70°C and the other was screened for the presence of dengue specific IgM antibodies by IgM capture ELISA (MAC-ELISA) or by RT-PCR, using a commercial kit, following the manufacturer's protocol.

Serological Tests

The dengue tests were performed in accordance with the WHO diagnostic guidelines for Africa.[33] These tests have been made available to the NRL for viral hemorrhogic fevers by WHO through the Institut Pasteur de Dakar (IPD). The sera diagnosed as positive are then sent to the WHO Collaborating Center for Arboviruses and Hemorrhagic Fever Viruses (CRORA) at IPD for confirmation.

All samples received during the outbreak period were tested by IgM antibody capture ELISA. Transparent clear Flat-Bottom Immuno-nonsterile 96-well Plates (Thermo Scientific™) were sensitized with an anti-IgM capture antibody and incubated overnight at 4°C (Kirkegaard and Perry Laboratories, with Affinity Purified Antibody to Human IgM (μ), F(ab')). Samples were diluted to 1:100 and tested in duplicate. A mixture of Pasteur institute of Dakar 1-4 DENV antigen was diluted and incubated for one hour at 37°C. A 6B6C-1 peroxidase-labelled antibody (IgM/IgG conjugate peroxidase fraction of anti-body foam) specific to the flavivirus group was added. After 10 minutes' incubation in a darkroom at room temperature with a 3.3' and 5.5'-tetramethylbenzidine (SIGMA) substrate, the plate was read at 620 nm. The samples were interpreted by dividing the mean optical density (OD) of the samples by the mean OD of the negative control (positive/negative ratio). 2.0 values were considered positive.

Polymerase Chain Reaction Detection

This study selected the manual viral RNA extraction system commonly available in public health laboratories. For the manual viral RNA extraction from serum specimens, the silica-based filter column systems QIAamp Viral RNA Mini kit (Qiagen) was selected. After elution the extraction product was stored at -70°C or used for RT-PCR immediately.

To investigate the presence of dengue virus in patients, the Trioplex real-time RT-PCR Assay Primer and Probe Set (CDC; catalog #KT0166) developed by the CDC was used, but with some modifications regarding the enzyme kit.

Briefly, the reaction mix for the one step RT-PCR was performed in total of 25 μL including: 12.50 μL of 2X RT-PCR Buffer (AgPath-ID™ One-Step RT-PCR Reagents-Applied Biosystems™), 9.50 μL of nuclease-free water, 1.25 μL of forward and reverse primers, 0.5 μL of TaqMan® probes, 0.25 μL of 25X RT-PCR Enzyme Mix and 1 μL extracted RNA.

The cycling profile performed by StepOnePlus™ Real Time PCR system (Applied Biosystems™) involved reverse transcription at 50°C for 10 min and initial denaturation at 95°C for 15 min followed by 40 cycles with 95°C for 15 min and 1 min at 60°C. The fluorescence acquisition was made in the cycling step.

The dengue positive sera by Trioplex real-time RT-PCR were re-run in another PCR to determine the type. Dengue typing was performed by real-time RT-PCR using primers and probes. The reaction mix contained 12.5 μL of 2X reaction buffer (Ambion Kit), 8.25 μL of miliQ 1 water, 25 μL of each primer (FP, RP at 10 μM), 0.5 μL of the probe (P at 10 μM) and 0.25 μL of enzyme. The amplification of the viral genome was carried out with a 7500 PCR machine (Applied Biosystems) under the following conditions: Reverse transcription: 5°C, 5 min, initial denaturation: 95°C, 15 min: (45 cycles) 95°C, 5 sec, 60°C, and 15 sec. Positive controls were RNA from Dengue positive strains and negative controls were performed by RNase-free water in each amplification cycle.

The positive dengue case was defined as the one with positive RT-PCR or the ELISA positive for dengue IgM. The algorithm shown in Figure 1 allows better understanding of which tests were employed.

Statistical Analysis

Descriptive statistics for the study population characteristics and laboratory findings were performed by using EPI-Info 6 version 3.3.2. The seroprevalences of dengue serotype (DENV-1, DENV-2, DENV-3 and DENV-4) were expressed for the entire study group. Qualitative variables were characterized by numbers and percentages. Quantitative variables were described by mean and standard deviation. The chi-square test was applied in the analysis of the qualitative variables to establish a link, and *p*-values below 0.05 were considered statistically significant.

Results

A total of 2,849 samples were received for cases of suspected dengue infection and screened. There were no deaths in that outbreak. Of all the serum samples, 552 (19.4%) were from the interior of the country versus 2,297 (80.6%) from the economic capital of Ivory Coast (Abidjan).

At the national level, table 1 presented the overall seroprevalence of dengue (15.1%) among the suspected population during this epidemic. Our data showed that the seroprevalence of different dengue virus serotypes in cocirculation was predominated by DENV-2 with 189 cases (6.6%); followed by DENV-3 with 77 cases (2.7%), and DENV-1 14 cases (0.5%).

Table 1. Distribution of diagnostic and seroprevalence of dengue cases during the 2017 outbreak

Diagnostic dengue	n (%)
Negative	2,420 (84.9)
Positive	429 (15.1)
DENV-1	14 (0.5)
DENV-2	189 (6.6)
DENV-3	77 (2.7)
DENV IgM positive	149 (5.2)
Total	**2,849 (100)**

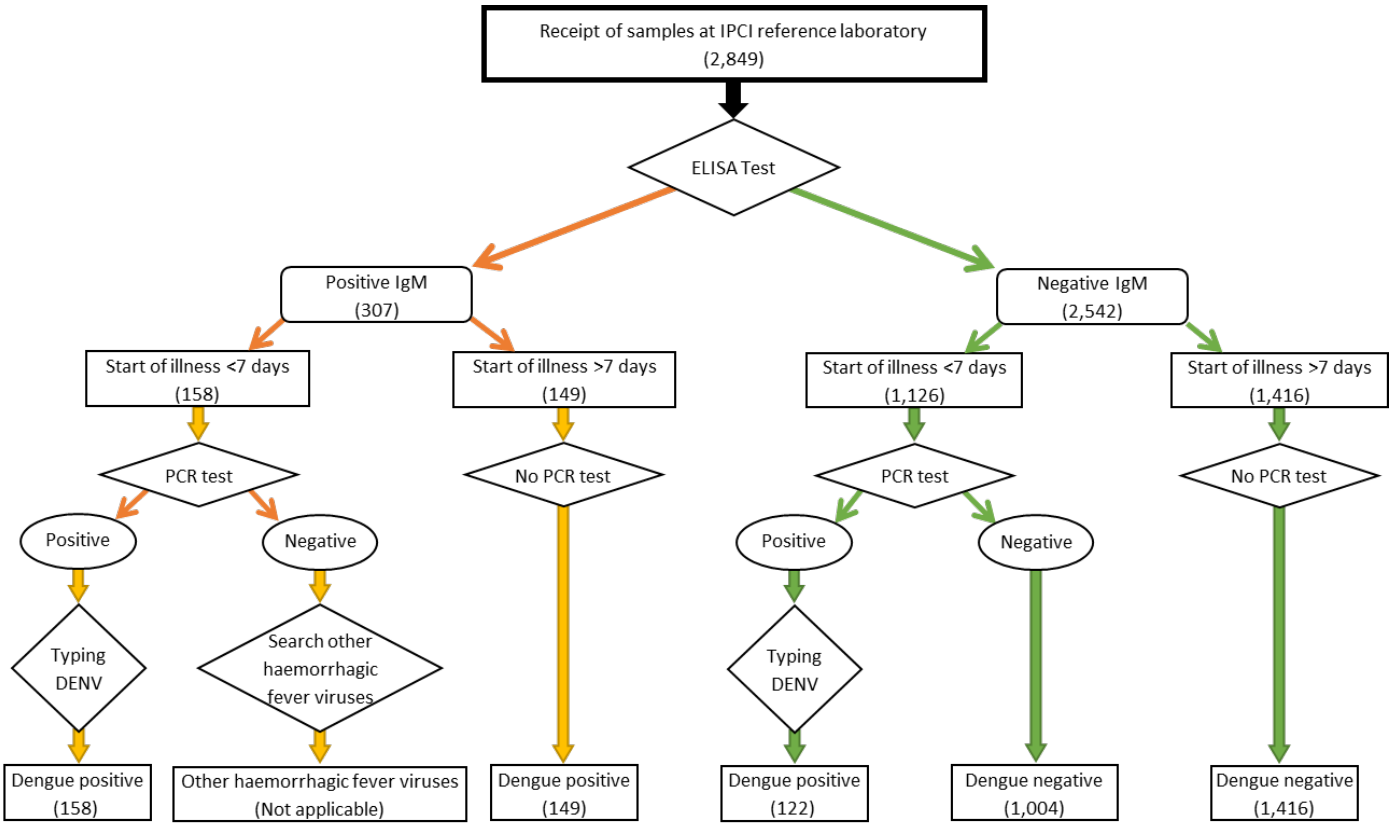

Figure 1. Algorithm for the analysis of suspected dengue patient samples at the IPCI reference laboratory

Table 2 showed the socio-demographic distribution of suspected dengue cases during the outbreaks and that only gender was not associated with the dengue diagnoses. Males were 1,519 (53.3%). The male to female ratio was 1.1:1. The mean and standard deviation of population age was 36.1±19.0 years. The seroprevalence in children (0-16 years) was 8.7% compared to 19.0% in adults. The largest number of positive samples was from the age group of 16 to less than 45 years, which accounted for 54.0% of total positive cases.

Table 2. Socio-demographic distribution of dengue cases during the 2017 outbreak among 2,849 suspected dengue cases

	Diagnostic of dengue cases			*p*-value
	Negative n (%)	**Positive n (%)**	**Total tested n (%)**	
Gender (n=2,849)				0.2598
Male	1,301 (85.6)	218 (14.4)	1,519 (53.3)	
Female	1,119 (84.1)	211 (15.9)	1,330 (46.7)	
Age group (years) (n=2,699*)				<0.001
<16	868 (91.3)	83 (8.7)	951 (35.2)	
16 to <30	528 (81.5)	120 (18.5)	648 (24.0)	
30 to <45	517 (83.3)	104 (16.7)	621 (23.0)	
45 to <60	255 (75.9)	81 (24.1)	336 (12.5)	
≥60	116 (81.1)	27 (18.9)	143 (5.3)	
Place of residence (n=2,849)				<0.001
Country Interior	552 (100.0)	0 (0.0)	552 (19.4)	
Abidjan (capital)	1,868 (81.3)	429 (18.7)	2,297 (80.6)	
Abidjan North (abobo; anyama)	71 (95.9)	3 (4.1)	74 (3.2)	
Abidjan Center (adjame-plateau)	27 (84.4)	5 (15.6)	32 (1.4)	
Abidjan East (cocody-bingerville)	1,591 (79.7)	405 (20.3)	1,996 (86.9)	
Abidjan South (grand-bassam; koumassi; marcory, treichville)	146 (92.4)	12 (7.6)	158 (6.9)	
Abidjan West (yopougon, songon)	33 (89.2)	4 (10.8)	37 (1.6)	

*Missing data at the age level

In Abidjan among the 2,297 suspect cases the seroprevalence was 18.7% (429/2,297). The geographical repartition of seroprevalence of positive cases reported showed that this prevalence was distributed as follows: 20.3% (405/2,297) was seroprevalence for Abidjan East and followed by Abidjan Center, Abidjan West, Abidjan South and Abidjan North (Table 2). The seroprevalence of different dengue virus serotypes in the cocirculation in the capital was 0.6% (14/2,297) for DENV-1; 8.2% (189/2,297) for DENV-2; and 3.4% (77/2,297) for DENV-3.

The distribution of dengue patients by time of year in figure 2 showed that the prevalence increased rapidly from 0.9% in March to 5.2% in April; 23.6% in May and 27.6% in June. The peak was observed in July at 28.3% with a rapid decline in August.

Table 3. Distribution of dengue virus serotypes by socio-demographic characteristics during the 2017 dengue outbreak

	DENV-1 n (%)	DENV-2 n (%)	DENV-3 n (%)	PCR positive n (%)	IgM positive n (%)	Total positive n (%)
Gender (n=429)						
Male	8 (5.9)	86 (63.7)	41 (30.4)	135 (61.9)	83 (38.1)	218 (50.8)
Female	6 (4.1)	103 (71.0)	36 (24.8)	145 (68.7)	66 (31.3)	211 (49.2)
Age (years) (n=415)						
<16	0 (0.0)	39 (72.2)	15 (27.8)	54 (65.1)	29 (34.9)	83 (20.0)
16 to <30	5 (5.4)	64 (69.6)	23 (25.0)	92 (76.7)	28 (23.3)	120 (28.9)
30 to <45	8 (11.6)	42 (60.9)	19 (27.5)	69 (66.3)	35 (33.7)	104 (25.1)
45 to <60	0 (0.0)	30 (69.8)	13 (30.2)	43 (53.1)	38 (46.9)	81 (19.5)
≥60	1 (7.1)	10 (71.4)	3 (21.4)	14 (51.9)	13 (48.1)	27 (6.5)
Period (n=429)						
March	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	0 (0.0)	1 (0.2)
April	0 (0.0)	3 (60.0)	2 (40.0)	5 (83.3)	1 (16.7)	6 (1.4)
May	1 (0.7)	30 (75.0)	9 (22.5)	40 (57.1)	30 (42.9)	70 (16.3)
June	7 (5.2)	62 (72.9)	16 (18.8)	85 (66.4)	43 (33.6)	128 (29.8)
July	6 (4.4)	91 (62.3)	49 (33.6)	146 (66.4)	74 (33.6)	220 (51.3)
August	0 (0.0)	3 (100)	0 (0.0)	3 (75.0)	1 (25.0)	4 (0.9)

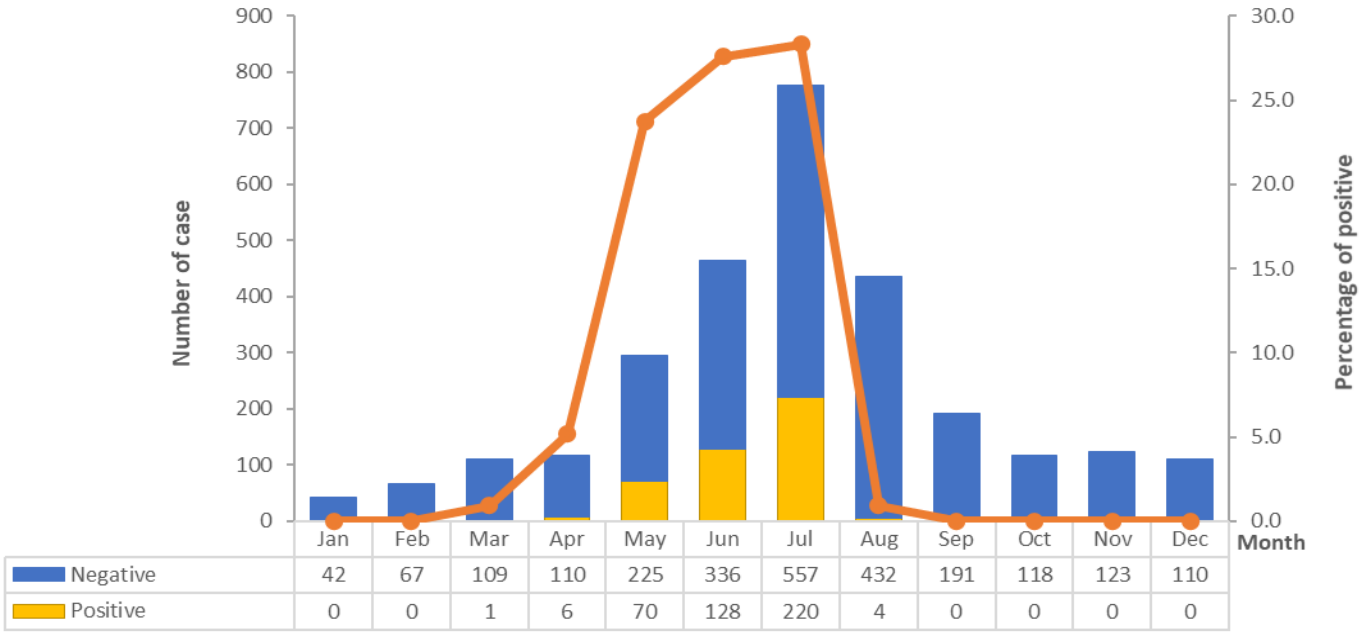

Figure 2. Seasonal distribution of diagnosed dengue cases during the 2017 outbreak

Discussion

To the best of our knowledge, this was the first study to employ socio demographical-based and laboratory reporting data to investigate the dengue outbreak in Ivory Coast country. Our study found that the corresponding rate for dengue seroprevalence was 15.1% and 3 serotypes of dengue viruses cocirculate in the country, which were consistent with previous studies in West African countries.[1,34-36] With reports of dengue outbreaks in other parts of Africa and importation of dengue into Europe from West Africa.[37,38] It is noteworthy that the incidence of dengue in this part of West Africa is very high. During the studied outbreak, the seroprevalence appeared high even if it remained lower than those found in other studies. Gupta in India found 52.3%, Gregianini et al. in Brazil obtained a prevalence of 29.3% and Onoja et al. in Nigeria revealed 23.4%.[5,35,39] The seroprevalence that we found could be explained by the location of the epidemic in Abidjan, but there were also logistical issues related to the management of reagents and laboratory input.

This study found no difference in seroprevalence of dengue virus infection between men and women in the period analyzed. Other authors have made the same observation.[38] Dengue affects humans of all age groups worldwide, even though in some parts of the world it is mainly the pediatric public health problem.[8,12,40] Our study also showed that a fare proportion of dengue fever cases were children. Studies in Latin America where current reports of adolescents with recent dengue infections have been established lends credence to the conclusions of our current study.[19,41-43] The mean of age of our participants was close to that of Yeh 45.6±21.2 years.[44] Older individuals (those ≥20 years of age) in this study is an indication that they are more commonly affected. Therefore, the active population of our studied country is seriously threatened.

Larger proportions of serologically positive cases were observed among adults.[45] This has confirmed the economic importance of dengue fever in developing countries.[3] The difference between numbers of serologically positive cases among adult and pediatric groups was significant (p <0.05). Studies have shown that during dengue outbreaks, several serotypes cocirculate with a predominant serotype.[8,34,35,39,46] This finding was confirmed by our study in which the DENV-1 to 3 serotypes cocirculated with the predominance of DENV-2. RT-PCR offers accuracy and speed along with serotype specific diagnosis of various circulating dengue viruses and information about cocirculation of different subtypes.[7] The high seroprevalence of IgM positive (undetermined serotype) could be explained by the delay in the consultation of patients and their therapeutic course. This result is exceeding the viraemia period, which is seven days.

More dengue virus exposure occurred during the rainy season.[34,35,47] This is due to abundant vegetation cover that is available in tropical rain forests region and the high relative humidity.[35] This present study found monthly peaks in dengue seroprevalence during the rainy season from April to July. Positive correlation was found between dengue outbreak and rainfall pattern from increase in number of breeding sites.[48] Our observation was similar to that made by Li in his study in Selangor, Malaysia. Dengue in the tropical rain forest region has increased due to uncontrolled urbanization, and lack of effective and sustainable vector control programs.[49]

In Nigeria, in the study by Onoja et al., it was found that transmission of the dengue virus in several urban areas indicated that it is maintained in both urban and human populations.[35] A Taiwanese study also reported that 74.1% of patients affected by the dengue epidemic lived in urban areas.[40] Many of these areas are forested, with stagnant water and indiscriminate hollow containers, throwing garbage everywhere, providing excellent breeding sites for increased vector activity. Mosquito-DENV-Human cycle has been reportedly found in nearly all urban and peri-urban environments throughout the tropics.[50] All these assertions were corroborated in our study. The Trioplex real-time RT-PCR tests enabled a differential diagnosis of Zika and Chikungunya simultaneously.[51] This test whose performance has been demonstrated by Santiago et al. allows simultaneous detection of dengue (DENV), Chikungunya virus (CHIKV) and Zika (ZIKV) on the same well plate in RT-PCR multiplex.[52] The genome screens for dengue virus types 1–4 by fluorogenic RT-PCR using the technique described by Ito et al.[53]

There are some limitations in this study. First, this was a retrospective study and without predictive assessment that was conducted using reported data. Second, the limitation that concerns most clinical studies on dengue fever is that for molecular analysis based on PCR, which gives high specificity of viral serotype and sensitivity, the sample must be collected within 5 days of onset of symptoms. However, dengue symptoms can be mild, so most samples are collected after this time window, thus requiring other assays to diagnose the case that do not allow identification of DENV serotype. Third, the limitation found in the present study was the lack of information in the

epidemiological records about the symptoms, co-morbidities and chronic diseases presented by the patients, and information about previous vaccination against yellow fever was missing.

Conclusion

This study has revealed that DENV-2 has dominated this outbreak even though all three serotypes were found to be cocirculating as detected by RT-PCR. The increasing trend of cocirculation dengue virus serotypes suggests that Abidjan is becoming a hyperendemic state from an endemic one. The study has highlighted a high percentage of concurrent infections by different dengue virus serotypes for the first time from Ivory Coast. It is unrealistic to consider dengue fever as one of the less serious acute febrile diseases (like malaria). This consideration requires early detection and appropriate treatment of cases before they progress to the severe form. To do this, regular communication campaigns are essential to help prevent dengue and other arboviruses.

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Ethical

Since the diagnostic samples were received during an outbreak, no prior ethical clearance was required. However, the patient information was de-linked from sample information to protect the privacy of the patients.

Competing Interests

The author(s) declare that they have no competing interests.

Authors' Contributions

SY carried out all the RT-PCR experiments, RNA extraction, helped in careful collection of case history and analyzed them. DKM and SY were responsible for collection and storage of clinical samples and carried out the clinical sample processing and drafted and reviewed the manuscript. AVE and KH supervised the Virology diagnostic laboratory. DKM and SY conceived the study and all the research was carried out in the Virology Diagnostic Laboratory. DM reviewed the manuscript. All authors read and approved the final manuscript.

Suggested Citation

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