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Spatiotemporal distribution and molecular characterization of circulating dengue virus serotypes/genotypes in Senegal from 2019 to 2023

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Abstract: Dengue virus is becoming a major public health threat worldwide principally in Africa. From 2016 to 2020, 23 outbreaks were reported in Africa principally in West Africa. In Senegal, dengue outbreak are reported yearly since 2017 ; data about the circulating serotypes and their spatial and temporal distribution were limited to 2017 – 2018 period. Herein we describes up to date molecular surveillance of circulating DENV serotypes in Senegal between 2019 to 2023 and their temporal and spatial distribution around the country. For this purpose collected suspected DENV infections samples were subjected to dengue detection and serotyping using RT-qPCR methods. Positive samples were used for temporal and spatial mapping. Subset of DENV+ samples were then sequenced and subjected to phylogenetic analysis. Results show a co-circulation of three DENV serotypes with an overall predominance of DENV-3. In term of abundance DENV-3 is followed by DENV-1 with scarce cases of DENV-2 from February 2019 to February 2022. Interestingly, data shows extinctinction of both serotype 1 and serotype 2 and the only circulation of DENV-3 from March 2022 to July 2023. At the genotype level, analysis shows that sequenced strains belong to same genotype as previously described : Senegalese DENV-1 strains belong to genotype V, DENV-2 strains to cosmopolitan genotype and DENV-3 strains to Genotype III. Interestingly, newly obtained DENV 1-3 sequences clustered in different clades within genotypes. This co-circulation of strains belonging to different clades could have effect on virus epidemiology and transmission dynamic. Overall our results highlight DENV serotypes replacement by DENV-3 accompanied by wider geographic distribution in Senegalese regions. All mentioned results highlight the importance of virus genomic surveillance and call for further viral fitness studies using both in vitro and in vivo model as well as in depth phylogeographic study to uncover the virus dispersal patterns across the country.

Keywords: DENV; Serotypes; Senegal; Spatio-temporal distribution ; Genotypes

1. Introduction

Dengue fever (DF) is recognized as the most widespread arboviral disease globally (1). Its transmission occurs when infected mosquito vectors from the *Aedes* genus bite humans (2). Astonishingly, over one-third of the world's population is at risk of DENV infection (3). Many factors including climate changes , increasing travel and trades and urbanization (2) participate to the disease spread worldwide. According to WHO estimates approximately 3.6 billion people worldwide are

at risk of dengue infections (4), and reported cases range from 50 to 100 million annually (1).Tragically, the disease results in an estimated 10,000 deaths each year (3). Infection with dengue virus (DENV), the etiological agent of DF, can manifest in various clinical forms, ranging from a self-limited disease known as DF to the life-threatening severe dengue (5).

DF is prevalent in tropical and subtropical areas ; the virus epidemiology is well known in Asia and America (6).

In Africa the virus was thought to be rare for long but detection from returning travelers (7,8) and recently reported outbreaks highlight the virus circulation in the continent (9,10). Due to the lack of sufficient diagnostic tools and effective surveillance, the true burden of DENV infection is likely to be underestimated (8,11).

In Senegal the first dengue case was reported in 1970 and was mainly dominated by the occurrence of sylvatic cycle up to years 2000s (12). The first urban dengue epidemic took place in 2009 and was caused by DENV-3 ; following years yearly outbreak affecting different region of the countries (13–15) and linked to different serotypes were noticed (13,16,17). Despite the recurrent reports few studies assessed the virus diversity in Senegal exist in the literature (16).

DENV belonging to the *Flavivirus* genus within the *Flaviviridae* family [Kraemer, 2015]. DENV is enveloped and possess a single-stranded RNA genome with positive polarity, approximately 10.6 kilobases in length. The DENV genomic RNA encodes three structural proteins namely: capsid (C), pre-membrane /membrane (prM), and envelope (E) proteins, along with seven nonstructural (NS) proteins: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 [Chambers, 1990].

Antigenically DENVs are categorized into four distinct serotypes, namely DENV-1, DENV-2, DENV-3, and DENV-4, each inducing only limited cross-protection immunity (18). Serotypes share 65-70 % in amino acid sequence (19). Furthermore, each serotype is subsequently subdivided into various genotypes which follow a marked geographic distribution (18). Within each serotypes genotypes are defined by group of virus sharing less than 6% nucleotide divergence (19).Antigenic differences among serotypes and genotypes play a crucial role in dengue epidemiology and pose challenges for vaccine development and disease control strategies (19,20).

Therefore, it is essential to engage in monitoring strains that are prevalent in a specific region. This surveillance will inform the choice of suitable prophylactic and preventive actions (10). Additionally, diverse DENV serotypes or genotypes have been observed to elicit distinct immune responses. This variance influences their capacity to infect particular target cells , more severe manifestations of dengue or infect mosquitoes (21–23).

Although reports of dengue circulation in Africa exist, there are only a few studies that have examined the genetic make up of the prevalent strains, focusing on both the serotype and/or genotype levels (24–26).

Dengue strains in Africa remain poorly characterized with African sequences representing < 1% of global sequences data (Phillipe Selhorst., 2023). Existing sequences data are mainly obtained during outbreak periods (Burkina, Senegal) or returning travelers (8). In contrast many studies in Asia (27–30) and America (31) were focused on the genetic diversity of circulating DENV strains.

Due to the unprecedented and growing numbers of confirmed DENV cases in Senegal (*Multifoci*) and the co-circulation of different viral serotypes (29) continuous monitoring of circulating virus variants (serotypes/genotypes) and genomic surveillance of viral strains appears to be pivotal to anticipate worsen situations. Herein to get insight about the circulating DENV serotypes/genotypes and understand their spatial and temporal distribution across the Senegal we combine epidemiology, RT-qPCR and genome sequencing to uncover the virus genetic diversity in from January 2019 to March 2023.

2. Materials and Methods

2.1. The 2.1 Febrile illnesses surveillance system

The increasing threat of emerging pathogens to public health requires a reliable surveillance system to control their spread. In Senegal, the Institut Pasteur de Dakar partnered with the Senegalese

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Ministry of Health and the WHO country office to implement a nationwide Syndromic Sentinel Surveillance System called the 4S network in 2011 (32). Initially, the surveillance was limited to virologic surveillance of Influenza-like Illness (ILI). In 2015, it was expanded to include a wider range of pathogens associated with public health priority syndromes such as malaria, dengue-like syndromes, and diarrheal syndromes. This syndromic approach enables the early detection of unexpected and/or unusual occurrences of specific symptoms to monitor the evolution of the diseases under surveillance, investigate outbreaks, and implement appropriate response actions. The network has up to 20 sentinel sites distributed across the 14 administrative regions of Senegal, selected based on the WHO-recommended attributes (33). The DENV suspected samples tested during this study were collected throughout the 4S network system.

2.2. Samples shipping to WHOCC

At a weekly basis suspected samples collected from sentinel sites were shipped with clinical and demographic forms at the virology department at the Institut Pasteur de Dakar. At IPD samples were subjected to molecular screening for the detection of 07 medically important arboviruses including Dengue, Zika, Yellow Fever, Chikungunya, Rift valley fever, West Nile and Crimee Congo Hemorrhagic fever virus.

2.3. RNA extraction

Blood sample from suspected dengue cases were subjected to centrifugation at 2000 rpm for 5 mn to obtain sera which were harvested and aliquoted on 2ml cryotubes for immediate use and further biobanking. RNA extraction was performed using 140 µl of sera using Qiagen viral RNA mini kit according to the manufactures' recommendation. RNA was eluted to a final volume of 60µl and conserved to - 80 until further use.

2.4. RT-qPCR DENV detection

DENV RNA presence on extracted RNA was assessed using RT-qPCR with sets of primers targeting 3'-UTR region of all dengue serotypes (34). Reaction were performed on CFX machine (14); following temperature profile were used during reaction : 50°C – 10mn, 40 cycles of 95° C – 1mn ; 95° C – 15 secondes and 95 ° C – 30 secondes. All sample with a Ct values below the fixed cut of value of 32 were considered as DENV+.

2.5. DENV serotyping

Serotypes of DENV+ samples were assessed by RT-qPCR according to a protocol previously described by Dieng and colleagues (35). Briefly CDC dengue typing kit (36) were used according to the manufactures recommendations. The system allow the simultaneous detection of DENV serotypes from 5µl of input RNA. Each of DENV serotypes can be read in different dye channels.

2.6. cDNA synthesis and amplicons generation

To maximize yield and genome coverage, a subset of DENV+ RNA samples with Ct values < 32 was chosen for sequencing. For selected samples cDNA synthesis was carried out using the Luna Script RT SuperMix (5X) from New England Biolab, Ipswich, MA, USA. In brief, 8µl of RNA was mixed with 2µl of master mix, pipetted up and down up to ten times, and briefly centrifuged. The mixture was then incubated at 25°C for 2 minutes, 55°C for 20 minutes, and 95°C for 2 minutes, and finally placed directly on ice until further use. Then according to the serotype a specific whole genome multiplex PCR was conducted in order to amplify the entire coding region of DENV using two primer pools (1 & 2) in separated tubes. Reactions conditions were previously described by Dieng and colleagues (37). Amplification success was checked at the end of the reaction by agarose gel based electrophoresis.

2.7. Library preparation and Sequencing

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Obtained amplicons were purified using 1X Ampure XP Beads (Beckman Coulter Inc.), and cleaned-up concentrations of each PCR product were measured using a Qubit dsDNA HS Assay kit (Thermo Fisher Scientific) on a Qubit fluorimeter (Thermo Fisher Scientific). Targeted whole-genome sequencing of DENV 1-3 was undertaken for each sample; equal concentrations of pool 1 and pool 2 amplicons were pooled per sample before library preparation using Illumina DNA preparation kit Nextera DNA flex (Illumina Inc.) according to manufacturer's recommendations; whole-genome sequencing was performed with paired-end reads using Illumina MiSeq reagent kit V3 (300 cycles) on an Illumina MiSeq instrument. Consensus sequences of around 10 Kb (corresponding to the full CDS) were generated by de-novo assembling using Genome Detective (https://www.genomedetective.com/app/ accessed on February 15, 2023).

2.8. Datasets construction and phylogenetic analysis

DENV serotypes/genotypes/lineages identification was performed using dual procedures:

i) by using the genome detective dengue typing tool and ii) by using a maximum likelihood (ML) phylogenetic analysis to put newly sequenced DENV strains in a global context and explore the relationship with others available global sequences.

For this purpose we retrieved from US National Institutes of Health National Institute of Allergy and Infectious Diseases Virus Pathogen Database and Analysis Resource (http://www.viprbrc.org) representative sequences of described dengue genotypes for each serotype. Downloaded dataset for each serotype (DENV-1, n = 202; DENV-2, n = 257 , DENV-3, n = 133) containing all genomes from Africa and ≈10% of the remaining genomes. Newly generated sequences were aligned with downloaded datasets , full details of used sequences can be found in (Table S1). Multiple sequence alignment was performed using MAFFT (ref) and then manually curated to remove artefacts with AliView (38). Maximum Likelihood (ML) trees were generated using IQ-TREE (39), under appropriate models which were inferred as best fit models for DENV 1-3 by ModelFinder application implemented in IQ-TREE software ((40). Tree topologies robustness was determined using 1000 replicates. Tree visualization was performed using Figtree (http://tree.bio.ed.ac.uk) and R software (41).

2.8. Temporal trend and Spatial mapping of detected serotypes

According the RT-qPCR serotyping results temporal and spatial mapping of detected DENV serotypes were performed using epidemiological week of sampling and informations related to latitude and longitude of region where sentinels sites from which samples were collected. Temporal trends was represented using barplot, spatial distribution was represented by a maps made using maplots package within R ; pie chart representing the proportion of each detected serotypes at a given region were represented.

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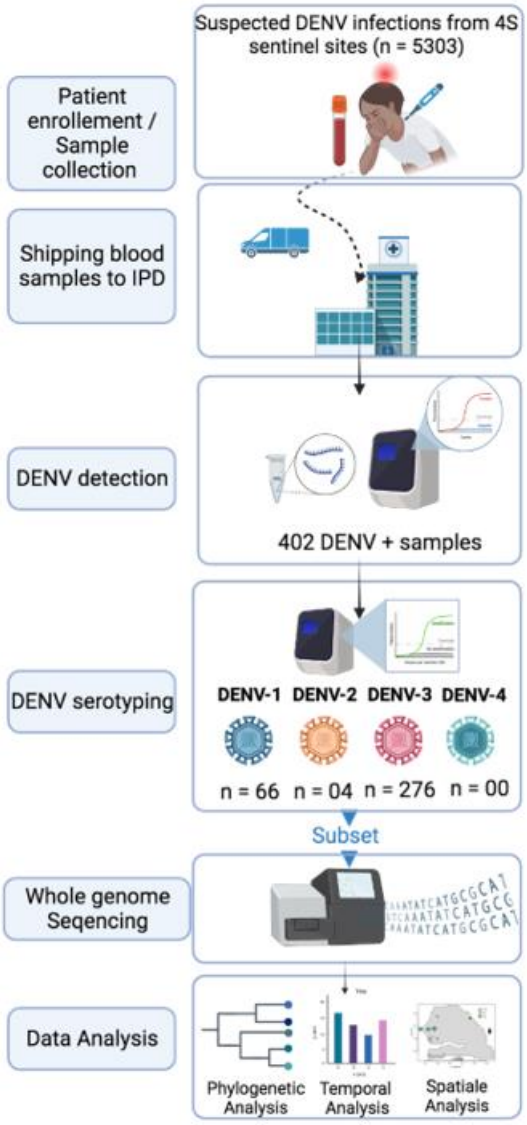

Figure 0. Summary of used workflow during this study

3. Results

From January 2019 to February 2023 5303 suspected arboviral cases were collected from 4S sentinel sites and shipped to WHOCC for arboviruses and haemorrhagic fever viruses where they are subjected to molecular testing for the detection of DENV RNA ; 402 among them where panDENV positives by RT-qPCR.

According to the region of provenance RT-qPCR DENV+ samples were collected from twelve out of fourteen administrative regions of Senegal. In term of occurrence the highest number of confirmed DENV+ cases were recorded in Matam (n = 166), Saint louis (n = 64), Dakar (n = 60) , Thies (n = 39), Kaffrine (n = 29) and Kaolack (n = 16). Remaining regions including Diourbel, Fatick, Kedougou, Kolda, Louga, Tambacounda and Ziguinchor all recorded less than ten confirmed DENV+ cases (Figure 1 and Table S1).

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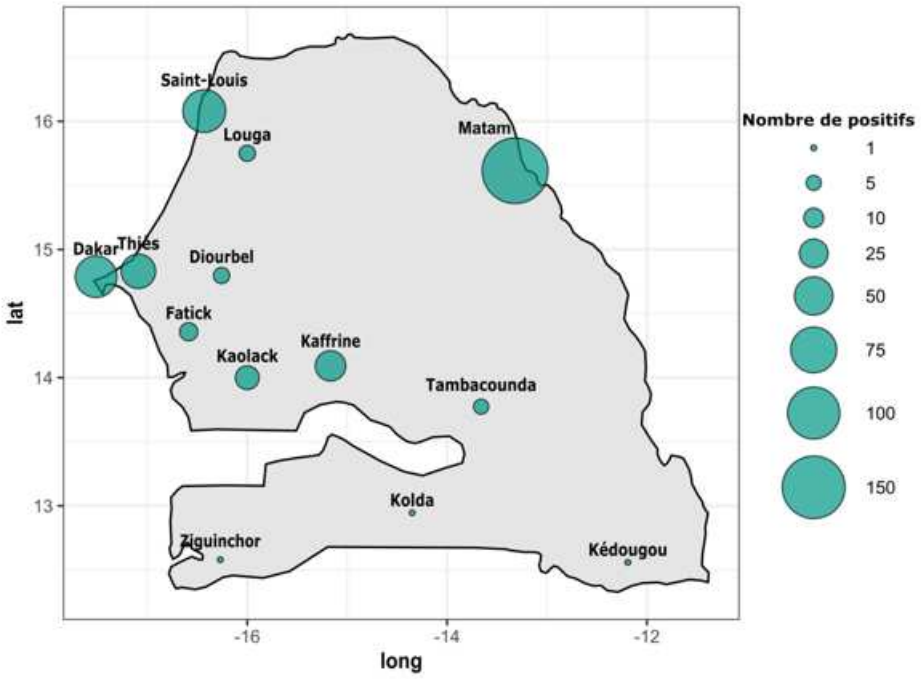

Figure 1. Map showing the spatial repartition of DENV RT-qPCR positives samples collected between January 2019 to March 2023. The size of the dot is proportional to the number of recorded dengue positives case on each administrative region of Senegal.

According to the year of collection the table summarise the number of suspected and confirmed DENV+ samples according to the year of collection. Briefly, the highest number of confirmed dengue cases was obtained in 2022, the same year we recorded the highest confirmed DENV+ cases (n = 216). The lowest number of suspected (n = 109) and confirmed cases (n = 15) were obtained during the year 2023.

Table 1. Repartition of suspected and confirmed DENV cases according to the year of sampling.

Year of collection	Number of suspected cases	Number of confirmed DENV cases
2019	890	19
2020	862	20
2021	1353	131
2022	2089	216
2023	109	15

Serotyping results by RT-qPCR of 346 out of 402 DENV+ samples shows the circulation of three DENV serotypes.

In term of occurrence DENV-3 is the most prevalent serotype (n = 276) followed by DENV-1 (n = 66) ; the less prevalent serotype among screened DENV+ samples was DENV-2 (n = 04) (Table S2). Interestingly temporal distribution of circulating serotype during study period show that between February 2019 to March 2022 the three serotypes co-circulated after March 2022 DENV 1-2 were no longer detected and only DENV-3 circulated till March 2023 (Figure 2 and Table S3 & Table S4).

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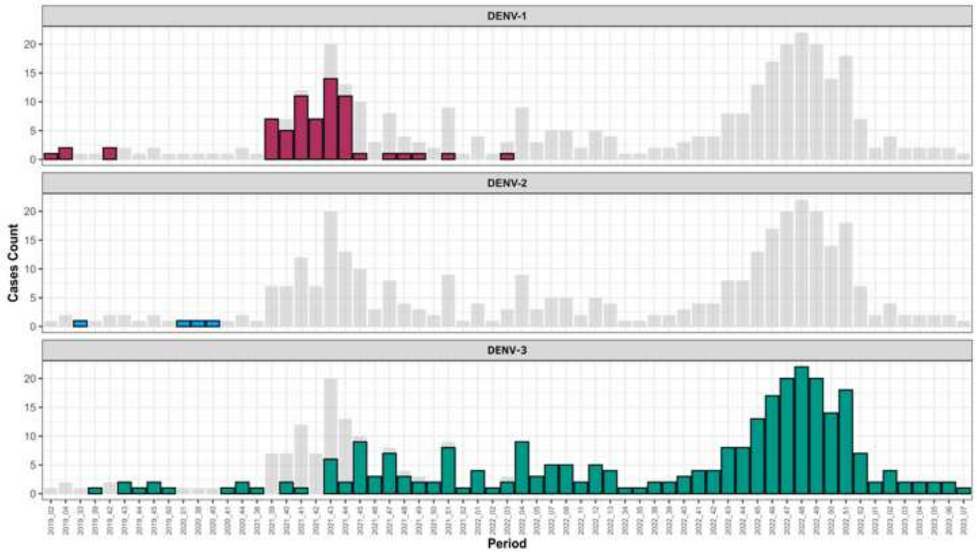

Figure 2. Pattern of DENV serotypes circulation in Senegal through the 4S network from 2019 to early 2023. Numbers of positive were represented on a weekly basis ; serotypes are coloured as follow DENV-1 in red, DENV-2 in blue and DENV-3 in green. Grey background represent the total of serotyped samples per weeks.

Geographically the most widely distributed serotype was DENV-3 found in ten out of ten regions were serotypes samples were retrieved ; the highest number of DENV+ cases associated to this serotype was found in Matam (n = 151), Dakar (n = 44) and Thies (n = 32). DENV-1 was detected in four regions including Saint-Louis (n = 53), Dakar (n = 04, Matam (n = 04),), Louga (n = 03), Kaffrine (n = 01) and Thies (n = 01). Finally DENV-2 is the less detected and spreaded serotype with the detection of only 04 cases in Kaffrine (n = 2), Kaolack (n = 1) and Thies (n = 01) (Figure 2 ; Table S2)

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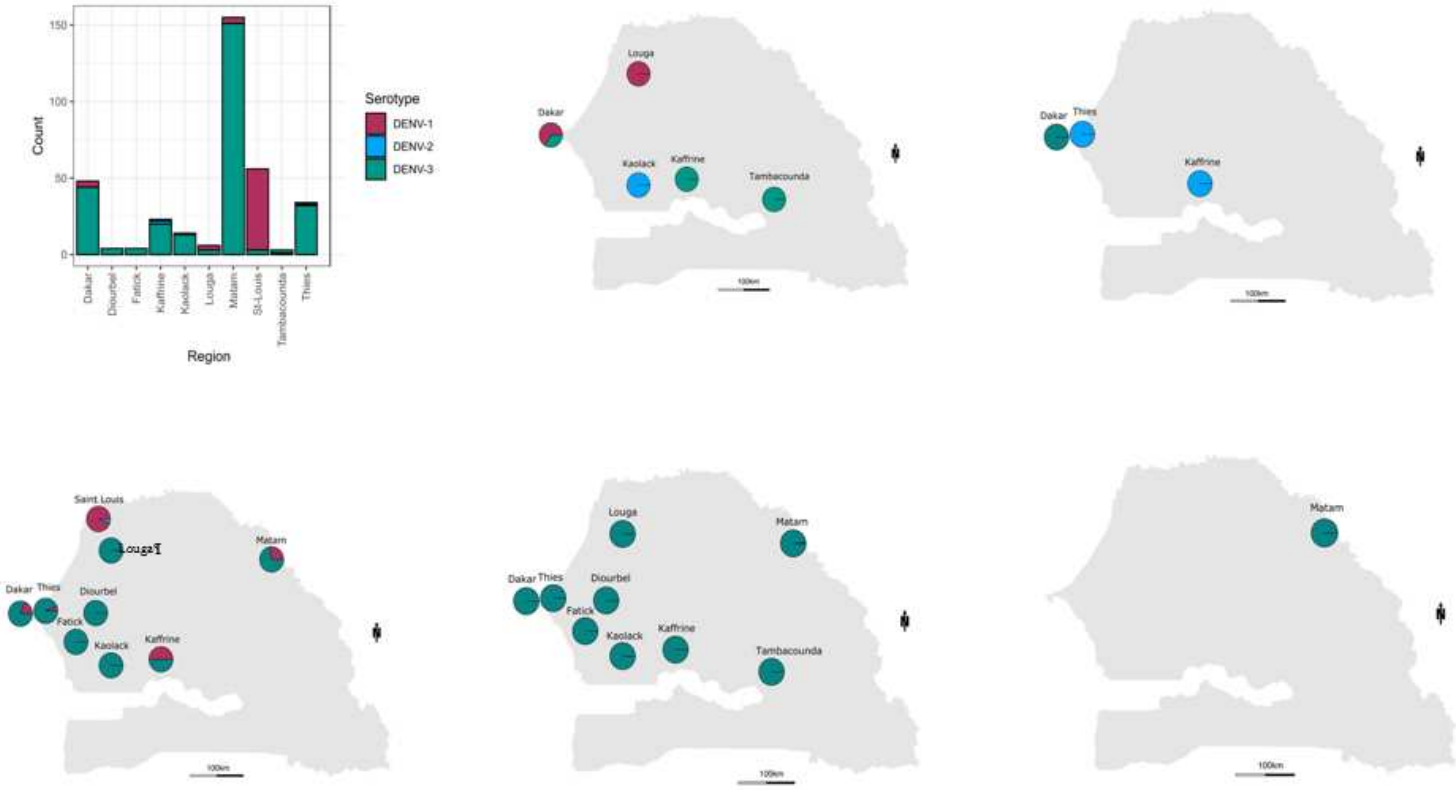

Figure 2. : (A) Barplot showing the number of detected serotypes per region , **(B to F)** Maps showing the spatial repartition of detected dengue serotypes from 2019 to early 2023 (up to week 07 of year 2023). Pie charts for each sampling region display the proportion of serotyped samples by RT-qPCR. DENV-1, DENV-2,DENV3 are colored respectively in red, blue and green. The size of the circle is not proportional to the number of cases. A summary of serotyped sample numbers and results for each monitoring region can be found in Table S1.

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DENV typing tool results (Table S7) classify all DENV-1 sequences into genotype V (Figure 3), DENV-2 into cosmopolitan genotype (Figure 4) and finally DENV-3 as genotype III (Figure 5).

Table 2. Summary of sequenced DENV samples during this study.

ID	Virus type	Genotype	Region	Isolate	Sample type	Collection Date
SH 377553	DENV-1	Genotype V	Saint-Louis	Human	Serum	29-10-2021
SH 377552	DENV-1	Genotype V	Saint-Louis	Human	Serum	29-10-2021
SH 377551	DENV-1	Genotype V	Saint-Louis	Human	Serum	28-10-2021
SH 377538	DENV-1	Genotype V	Saint-Louis	Human	Serum	01-11-2021
SH 377555	DENV-1	Genotype V	Saint-Louis	Human	Serum	29-10-2021
SH 377554	DENV-1	Genotype V	Saint-Louis	Human	Serum	29-10-2021
SH 322838	DENV-1	Genotype V	Dakar	Human	Serum	21-10-2019
SH 322765	DENV-1	Genotype V	Dakar	Human	Serum	15-10-2021
SH 318479	DENV-1	Genotype V	Louga	Human	Serum	24-01-2019
SH 318478	DENV-1	Genotype V	Louga	Human	Serum	24-01-2019
SH 318267	DENV-1	Genotype V	Louga	Human	Serum	10-01-2019
SH 326097	DENV-2	Genotype II	Kaffrine	Human	Serum	30-09-2020
SH 323592	DENV-2	Genotype II	Thies	Human	Serum	05-01-2020
SH 377545	DENV-3	Genotype III	Saint-Louis	Human	Serum	27-10-2021
SH 377540	DENV-3	Genotype III	Saint-Louis	Human	Serum	26-10-2021
SH 377524	DENV-3	Genotype III	Thies	Human	Serum	28-10-2021
SH 377522	DENV-3	Genotype III	Thies	Human	Serum	28-10-2021
SH 392244	DENV-3	Genotype III	NA	Human	Serum	NA
SH 402404	DENV-3	Genotype III	Fatick	Human	Serum	22-08-2022
SH 392518	DENV-3	Genotype III	Tambacounda	Human	Serum	19-03-2022
SH 392408	DENV-3	Genotype III	Matam	Human	Serum	15-03-2022
SH 392406	DENV-3	Genotype III	Matam	Human	Serum	15-03-2022
SH 392265	DENV-3	Genotype III	Matam	Human	Serum	25-02-2022
SH 392263	DENV-3	Genotype III	Matam	Human	Serum	25-02-2022
SH 392260	DENV-3	Genotype III	Matam	Human	Serum	23-02-2022
SH 392169	DENV-3	Genotype III	Matam	Human	Serum	22-02-2022
SH 392168	DENV-3	Genotype III	Matam	Human	Serum	21-02-2022
SH 330006	DENV-3	Genotype III	Dakar	Human	Serum	03-11-2020
SH 330004	DENV-3	Genotype III	Dakar	Human	Serum	03-11-2020
SH 329067	DENV-3	Genotype III	NA	Human	Serum	NA
SH 327002	DENV-3	Genotype III	Dakar	Human	Serum	12-10-2020
SH 392572	DENV-3	Genotype III	Matam	Human	Serum	21-03-2022
SH 392571	DENV-3	Genotype III	Matam	Human	Serum	22-03-2022
SH 323342	DENV-3	Genotype III	Kaffrine	Human	Serum	10-12-2019
SH 322872	DENV-3	Genotype III	Kaffrine	Human	Serum	28-10-2019

All this assignment were confirmed by phylogenetic analysis (Figure 1, Figure 2 and Figure 3). Additionnally, phylogenetic analysis shows that in comparison to limited previously available full genome sequences sequences from Senegal DENV-1 and DENV-3 sequences clustered in different clades (hereafter namely as Novel Clades (2019 - 2023) for DENV-1 and Novel Clade III (2020 - 2022)) ; DENV-2 sequences are closely related to DENV-2 cosmopolitan detected in West Africa.

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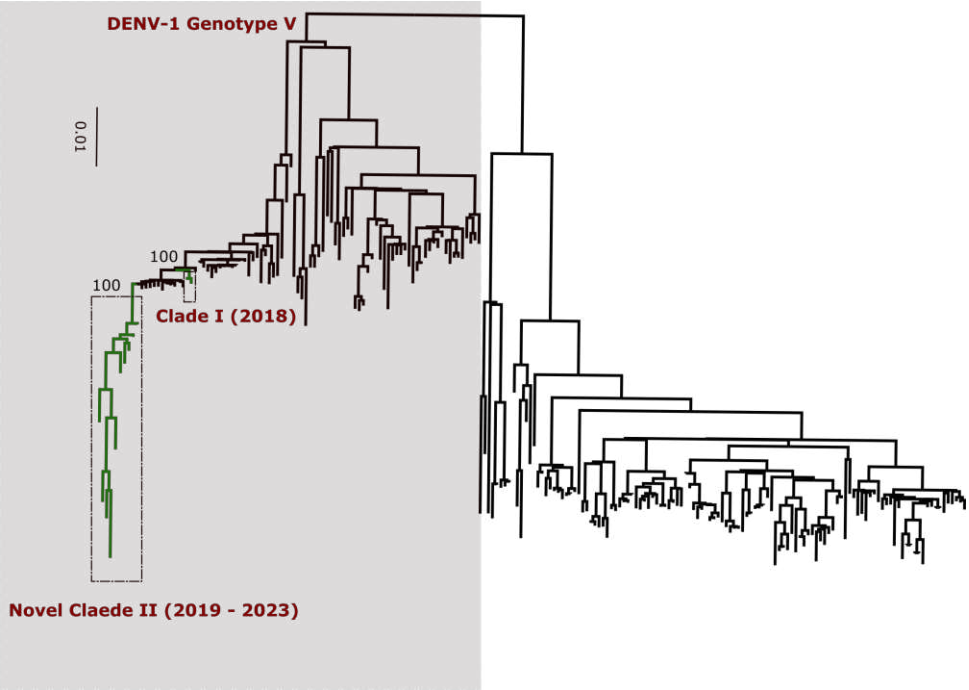

Figure 3. Maximum Likelihood (ML) tree of DENV-1 genotype V in Senegal from January 2019 to March 2023. The analysis was based on 11 nearly complete genome of DENV-1 generated during this study in addition to n = 202 available sequences retrieved from VIPR database. The tree is midpoint rooted and the scale bar represent the number of nucleotide substitution per site (s/s).

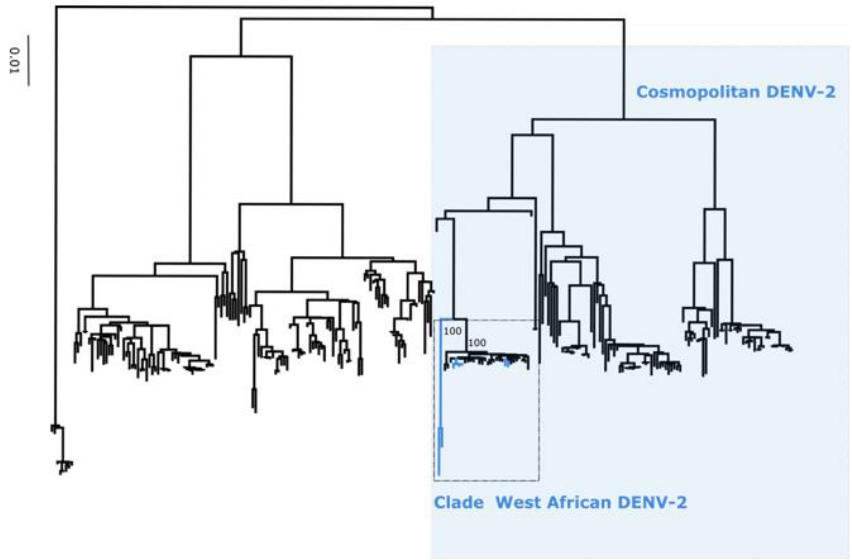

Figure 4. Maximum Likelihood (ML) tree of DENV-2 genotype cosmopolitan in Senegal from January 2019 to March 2023. The analysis was based on 02 nearly complete genome of DENV-2 generated during this study in addition to n = 257 available sequences retrieved from VIPR database. The tree is midpoint rooted and the scale bar represent the number of nucleotide substitution per site (s/s).

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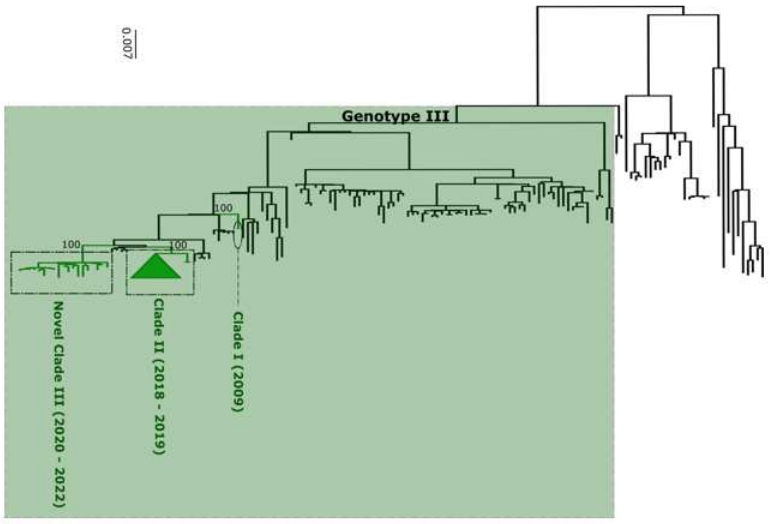

Figure 5. Maximum Likelihood (ML) tree of DENV-3 genotype III in Senegal from January 2019 to March 2023. The analysis was based on 21 nearly complete genome of DENV-3 generated during this study in addition to n = 133 available sequences retrieved from VIPR database. The tree is midpoint rooted and the scale bar represent the number of nucleotide substitution per site (s/s).

4. Discussion

Senegal is a West African country with a reliable and efficient syndromic surveillance system as exemplified by previous early detection of epidemic prone disease and subsequent organization of appropriate response (13,42,43).

This system allowed the notification of many DENV outbreak in Senegal (13,14,17) ; despite the recurrent occurrence of dengue epidemics and/or sporadic cases studies focusing on the circulating serotypes/genotypes and their associated spatial and temporal distribution are limited (16). This present study aimed to address this concern by investigating the circulating dengue variants in Senegal between 2019 to 2023 through the syndromic sentinel surveillance network of Senegal (4S network). To the best of our knowledge this study represent the first multiyear countrywide study focusing on temporal and spatial distribution of dengue virus serotype/genotype and viral genetic diversity using full genome sequences.

Among collected suspected dengue samples (n = 5303), 402 were DENV RNA positive samples (Figure 0). Interestingly the confirmed cases were distributed around twelve out of fourteen administrative regions of Senegal vs seven regions during 2017 -2018 study (Multifoci), with a DENV RNA positivity rate of 7.58 %. The highest number of dengue positive cases were recorded in Matam region with 166 cases followed by Saint-Louis (n = 64), Dakar (n = 60), Kaffrine (n = 29) ; others regions where dengue was detected recorded a number of cases below twenty (Table S1). Interestingly, the Matam regions was never been associated to any dengue outbreaks or recurrent cases notifications in the past supporting studies highlighting that the introduction of new groups of viruses to populations lacking prior exposure (serological naivety) has the potential to trigger unprecedented outbreaks and can be linked to more severe manifestations of dengue. (27,44).

Compared to previous Senegalese study (Multifoci) the highest number of suspected DENV samples were enrolled thus probably lead to the highest number of confirmed cases recorded during the present study. In contrast a study on genetic diversity of dengue virus in Bangkok yield a highest prevalence of DENV positivity (25.09 %) compared to our study (29) while a DENV RNA prevalence of 38.24 % was obtained during single year study in India (45). This discrepancy is probably do the fact that compared to Senegal dengue is highly endemic in Thailand and India. Indeed Bangkok , the capital city of Thailand, is located in the center of the country and serve as a transportation hub and

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dengue is known to circulate there since 1950s (46,47). In India studies report that DENV is reported every year and the size, severity, duration of outbreaks are increasing (45,48).

All together findings highlight the rapid spread of arboviral disease between neighboring regions thanks travel and trades activities (49,50). It is well known that frequent reintroductions of pathogens pose a significant challenge to elimination campaigns, especially in areas experiencing substantial regional and international travel. This is because humans serve as the reservoir host for both epidemic dengue and chikungunya (51). None DENV+ RNA sample was recorded in Sedhiou region in Southern Senegal. This is probably due to the fact that the sentinel site in this region was implemented recently and issues on proper samples transportation du IPD were noticed (Samba Sagn personnal communication). According to the year of collection the highest number of confirmed DENV cases was noticed in 2022 ; the trends follow the number of collected samples during the same year which is higher compared to other years (Table 1). This is probably due to the fact that between 2019 to 2021 most of the surveillance effort were focused on the covid-19 pandemic.

For assignment of DENV serotype/genotype the only nationwide dengue spatial mapping study based on partial CprM gene used limited number of samples collected between 2017 to 2018. To get more insight and up to date genetic diversity of circulating dengue strains at the serotype/genotype levels using RT-qPCR we serotyped 347 out of 402 dengue positives samples and generated 34 nearly complete DENV genomes (Table 2). Serotyping using RT-qPCR shows that detected dengue virus strains in Senegal during the study period belong to DENV 1-3 ; the most represented serotype was DENV-3 (n = 276), followed by DENV=1 (n = 66) and finally DENV-2 (n = 04). None of the DENV+ samples was linked to DENV-4 (Table S2). This finding corroborate those obtained by Dieng and colleagues which showed the co-circulation of DENV 1-3 in Senegal between 2017 to 2018 (16).

Any marked spatial distribution pattern of serotype was observed compared to previous study in Senegal (16). In contrast a study performed in India show a regional diversity of DENV serotypes (45).

But based on observations DENV-3 was the dominant serotype in Dakar, Thies, Fatick, Diourbel, Kaolack, Kaffrine, Tambacounda and Matam. DENV-1 was dominant serotype in Saint-Louis. DENV-2 and DENV-3 were co-dominant in Louga (Figure 2 A ; Table S2). Multiple serotype infections were more prominent in samples tested in Thies and Kaffrine where DENV 1-3 were noticed. In Africa limited countries as Gabon, Burkina Faso reported the co-circulation of at least three DENV serotypes (10,52). This is probably linked to the fact that dengue surveillance and awareness is lacking in the continent (8). Limited availability of data about DENV circulating serotype/genotypes is mainly to the limited availability of national research institutions in many areas (48).

In contrast studies in South America reveals the co-circulation in high frequency of dengue viruses serotypes (53). In another hand the hyperendemic behaviour of DENV virus epidemiology is well known and documented in Asian countries as India (27,28,54) and in China, Malaysia , Thailand (29).

Interestingly temporal trends of serotype's circulation shows that from the third week of year 2022 DENV-1 and DENV-2 serotypes were no longer circulating among confirmed DENV cases but only DENV-3 was noticed (Figure 2). This serotype shift was associated with a widespread and increased frequency of cases related to this serotype. Findings corroborate those of Suzuki and Colleagues in a study performed in Japan (55). The fact that only DENV-3 was detected in Senegal up to may be due to an increased viral fitness of this serotype compared to DENV 1-2 (56). In is well know that different virus serotypes can be associated to different phenotypic traits (57). For instance, a research conducted in Colombia examined the replicative capability of DENV within C6/36 mosquito cells and populations of A. aegypti. This was done using a distinct viral strain for each DENV serotype, revealing varying degrees of fitness among the serotypes (57). Beside intra-serotypic genetic diversification other parameters as cross-protective immunity between serotypes may explain observed DENV serotype replacement phenomenon (58). All up mentioned hypothesis about differential viral fitness should be confirmed by in vitro and in vivo studies since vector-driven

selection may contributed to viral replacement phenomenon as described previously in New Caledonia (23).

Performed phylogenetic analysis as well as genotyping using genomedetective dengue typing tools show that the genotype diversity of detected DENV serotypes was relatively low during our study. Indeed, each of all detected serotype consisted of a single genotype ; DENV-1 belong to genotype V, DENV-2 to genotype cosmopolitan and finally DENV-3 to genotype III. This trends is comparable of genotypic dengue virus make up found in Africa (25). However the genetic diversity of Senegalese DENV strains was more pronounced within each genotype. Indeed in each genotype of characterized DENV sequences fall into different clades ; with the exception of DENV-2 sequences which falls into one clade composed by virus detected in West Africa as previously described by Dieng and Colleagues (37). DENV-1 sequences were distributed in two separate clade namely clade I 2018 composed by viruses sampled during 2018 outbreak in Thies regions (15) and Clade 2019 – 2022 including principally strains associated to outbreak in Rosso in 2021 (17) in addition to sporadic cases collected in late 2019 and beginning of year 2022. The same trend was observed for DENV-3 with the observations of the occurrence circulation of viruses belonging to two clades : Clade II 2018 -2019 shared with strains circulating in Thies 2018 (15), in Senegal 2019 in addition to a newly identified Clade III 2020 – 2022 which is closely related to virus sampled in Burkina Faso 2017 and Ethiopia 2019. The occurrence of viral strains belonging to different clusters is a hallmark of different origin of transmission and call for in depth phylogeographic studies to elucidate origin and dispersal patterns.

In general, the observed presence of various strains from different serotypes/genotypes/clades could potentially account for the consistent reports of dengue outbreaks within the country since 2017. Collectively, these findings underscore the vital importance of maintaining an ongoing genomic surveillance of DENV in Senegal. The data generated through this surveillance can be utilized to support public health laboratories in monitoring the diversity of the virus, which is essential for implementing effective control measures.

5. Conclusions

In summary, it is essential to maintain ongoing monitoring of the circulating DENV serotypes/genotypes in Senegal. This ongoing surveillance will provide crucial information to guide proactive and well-informed public health interventions. By remaining vigilant and adaptable in the face of viral variants, we can effectively navigate and respond to emerging waves of infections, minimizing their impact and safeguarding the health of the population. Consistent genomic surveillance, coupled with real-time data analysis, offers invaluable insights into the evolutionary dynamics of the virus. This, in turn, aids in making informed decisions for public health responses. Comprehending the patterns of circulation of these DENV variants contributes to a comprehensive understanding of the virus's current status at a local/regional context. Such understanding enables authorities to implement appropriate measures, including refining testing strategies and enhancing contact tracing efforts. These measures effectively mitigate the impact of new infection waves and prevent rapid spread within communities.

Supplementary Materials: Table S1: Number of DENV positives samples recorded per regions from 2019 to 2023 through the 4S network ; Table S2 : Summary of the number of serotyped samples at each monitoring region ; Table S3: Reparation of detected DENV RNA positive samples per Year_Week from 2019 to 2023 ; Table S4: Reparation of detected DENV serotypes per Year_Week from 2019 to 2023

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