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Re-emergence of Dengue serotype 3 in the context of a large religious gathering event in 2018 in Touba City, Senegal

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Abstract

Dengue virus (DENV) was detected in Senegal in 1979 for the first time. Since 2017, unprecedented frequent occurrence of DENV outbreaks was noticed yearly. In this context, epidemiological and molecular evolution data are paramount to decipher virus diffusion route. In the current study, a dengue outbreak occurred in Senegal in 2018 in the context of the largest religious gathering with 263 confirmed DENV cases out of 832 collected samples, including 25 life-threatening cases and 2 deaths. It was characterized by a co-circulation of dengue serotypes 1 and 3. Phylogenetic analysis based on the *E* gene revealed that the main detected serotype in Touba city was DENV-3 and belonged to Genotype III. Bayesian phylogeographic analysis was performed and suggested one viral introduction around 2017.07 (95 % HPD = 2016.61 – 2017.57) followed by cryptic circulation before identification of the first case on 01 October 2018. DENV-3 strains are phylogenetically related, with strong phylogenetic links between strains retrieved from Burkina Faso and West African countries. These phylogenetic data substantiate epidemiological data of the DENV-3 origin and spread between African countries and subsequent diffusion after religious mass events. The study also highlighted the usefulness of the Mobile Laboratory during outbreak response allowing rapid diagnosis resulting in improved patient management.

Introduction

Dengue fever (DF) is a growing public health problem globally, and is endemic in more than 125 countries worldwide (1). The infection may be asymptomatic or can result in a wide range of clinical symptoms ranging from a mild fever to severe forms such as dengue haemorrhagic fever [DHF] or dengue shock syndrome [DSS] (2). Dengue virus (DENV) is a mosquito-borne virus transmitted by *Aedes spp* and belongs to the *Flavivirus* genus, in the family *Flaviviridae*. It was first isolated in 1943 in Japan, with 4 well-distinct serotypes (DENV1 to DENV4) (3) Immunity is serotype specific and does not guarantee cross protection to other serotypes. Subsequent infections by a different serotype may increase the risk of developing severe dengue forms (4). Estimates reveal that dengue epidemics have increased around 30 times throughout the last 50 years. Around 10^8 new cases have occurred annually in more than 100 endemic countries, putting more than 40% of the global population at risk (*ca*. 2.5 billion of people). Most of these cases are reported in Asia and Africa, which together bore over 80% of the global burden (5). In Africa, the incidence of DF may be under-estimated, due to similarities of the clinical symptoms of endemic infections such as Malaria (6). The first documented dengue outbreak in the continent occurred in Durban, South Africa in 1927 (7), and subsequently, numerous outbreaks have been reported (8,9).

By 08th October 2018, preliminary biological investigation of suspected DENV cases from Touba City, in the west central Senegal confirmed dengue virus (DENV) circulation (**Figure 1**). This finding led to

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rapid deployment of a multi-disciplinary team for outbreak management with mobilization of a Mobile Biosafety Laboratory (MBS-Lab), equipped to safely handle any type of infectious samples. The MBS-Lab was also equipped with a scalable molecular diagnostic platform, including a fully automated and portable PCR system for a rapid diagnosis (10). Here we report the results of this investigation, laboratory findings from this outbreak.

Material and Methods

Ethical Consideration

The Senegalese National Ethical Committee of the Ministry of Health approved the surveillance protocol as a less than minimal risk research, and written consent forms were not required. Oral consent to participate was obtained from all patients or parents/ guardians of minors included in this study as required by the Senegalese National Ethical Committee of the Ministry of Health. Throughout the study, the database was shared with the Epidemiology Department at the Senegalese Ministry of Health and Prevention for appropriate public health action.

Real time case notification and MBS-Lab deployment

There was notification of increasing cases of fever in the city of Touba through the sentinel surveillance network in Senegal (4S network). On October 8th, 2018, blood samples were collected from suspicious patients and sent to the Department of Virology of the Institut Pasteur de Dakar (IPD) for further investigations and determination of potential associated pathogen.

At the IPD, blood samples were centrifuged at 2000 rpm for 5 minutes for sera/plasma collection and viral RNA extracted using the Qiagen RNA kit according to the manufacturer's instructions.

The resulting RNA was screened by targeting major arboviruses commonly circulating in Africa, including dengue virus (DENV), chikungunya virus (CHIKV), yellow fever virus (YFV), zika virus (ZIKV) and Rift Valley fever virus (RVFV). Molecular tests performed led to the detection of 3 DENV cases. This led to the notification of DENV circulation during a huge religious mass gathering, the Grand Magal de Touba. The Senegalese Ministry of Health (MoHS) raised concerns about the urgency of screening suspected Dengue cases in order to contain the spread and allowing early case detection and management. MBS-Lab which previously showed utility in epidemic response (13) was deployed in Touba at October 17th 2018 upon the request of the MoHS.

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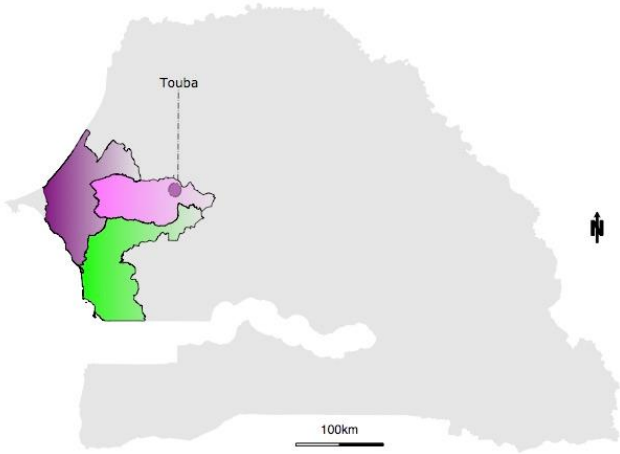

Figure1: Map showing Touba (pink) area where occurred the DENV-3 outbreak, at the same period some sporadic DENV cases were reported in Thiès (Purple) and Fatick (Green).

Patient enrolment and Sample collection

In keeping with standard WHO case definitions, DF can be stratified into 3 different categories according to clinical severities. These are: (*i*) Dengue without warning signs (DWiWS) defined as suspicious dengue case with acute fever (>38.5°C) and at least two of the following symptoms: headache, retro-orbital pain, nausea/vomiting, muscle and joint pains, rash, petechiae or leukopenia; (*ii*) Dengue with warning signs (DWWS) defined as dengue case with 1 or more of the signs: intense abdominal pain, persistent vomiting, fluid accumulation (ascite, plural, and/or pericardial effusion), mucosal bleeding, lethargy, lipothymia, liver enlargement, or progressive increase in hematocrits; and (*iii*) severe dengue (SD) defined as dengue case with 1 or more of the following manifestations: shock or respiratory distress defined (DSS), severe bleeding or severe organ compromise. The two latest categories can be fatal and required strict observation and medical intervention (5). For each patient visiting healthcare centers across Touba city and matching inclusion criteria, 5 ml of venous blood was collected into a 5ml tube. Collected samples with standardized form fulfilled with epidemiological and clinical data were transported to the MBS-Lab located at Djemoul healthcare center where confirmation tests were achieved. Any suspected cases with a positive result by qRT-PCR dengue and/or IgM signal confirmed by Plaque reduction neutralization assay was considered a confirmed case.

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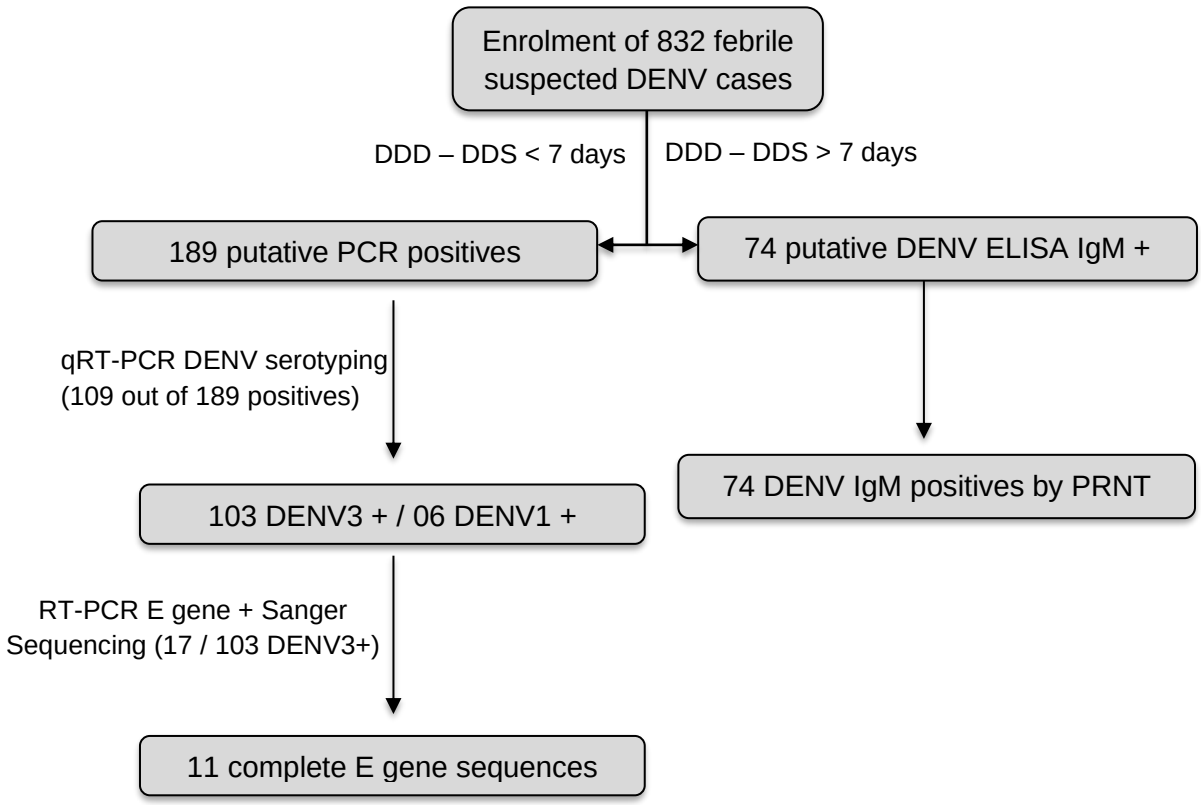

Figure2: Algorithm of patients enrolment and obtained results

RNA extraction:

A total of 832 samples were collected from suspected DENV patients between 01st October 2018 to 16th November 2018 from healthcare centres across Touba city. Viral Ribonucleic Acid (RNA) were extracted from 140µl of sera using the Qiagen (Qiagen, Hilden, Germany) viral RNA kit according to manufacturer's recommendations. RNA was then eluted in 60µl of molecular grade water and stored at -20 until use.

Dengue virus detection by qRT-PCR:

In order to confirm acute dengue infection qRT-PCR was performed on extracted RNA. The molecular detection was performed from patients with fever history of at least 07 days by using set of primers described by Wagner and colleagues (14), the reaction was performed inside the MBS-Lab with a Smartcycler thermocycler (Cepheid, Sunnyvale, California,USA) using Quantitect kit (Qiagen, Hilden,Germany). Briefly, the viral RNA detection was performed using the following temperature profile: reverse transcription at 50°C for 10min, initial denaturation of 95°C for 15min, followed by 45 cycles of denaturation at 95°C for 15sec, 60°C for 1min. Samples with Ct values < 37 were considered as positives.

Dengue virus antibody detection by Serological assays

Serological testing was carried out on samples from suspicious patients at the end of acute phase by enzyme-linked immunosorbent assay (ELISA) IgM and by 90% plaque-reduction neutralization test (PRNT) for confirmation, respectively.

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For IgM detection, plates were coated with monoclonal capture antibody (anti-human IgM), incubated overnight at +4°C and then washed at least twice with wash buffer (1X phosphate-buffered saline (PBS) supplemented with 0.05% Tween-20) and tap dried. After blocking with freshly prepared blocking buffer (1X PBS with 5% of not fat dry milk), plates were incubated at room temperature for 2 hours, washed twice at tap dried. Sera were heat-inactivated (56°C for 30min) and diluted (1:100) in a 1X PBS and added to the test wells, in addition to control dengue antigens and incubated at room temperature for 2 hours. Detection was done by added substrate solution (TMBS) and incubated in a humidified box protected from light, which was further stopped after sufficient coloration by adding sulfuric acid. Absorbance was read with a plate reader (spectrophotometer) at the wavelength of 492 nm. Results were interpreted by comparing test OD with positive and negative controls with a cut-off of 0.2. (15).
On IgM positives samples, the 90% plaque reduction neutralization test (PRNT90) was performed to determine the maximum serum dilution (1:8 to 1:1024) needed to reduce arbovirus plaque formation by 90% among Vero cells. The cut-off value for PRNT positivity was defined as 90% (PRNT90). Thus, the DENV strain New Guinean C was used. Corresponding previously heat inactivated sera were serial diluted with Gibco Dulbecco's Modified Eagle Medium (DMEM) containing 2% fetal calf serum and 1% of Penicillin/Streptomycin. Virus suspension was mixed to each serum dilution and incubated at 37°C for 60 min before their transfer to Vero cells and additional incubation at 37°C for 60 min. Next, 0.3% agarose solution was added, and plates were incubated at 37oC for 3-5 days. Reactions were then revealed using a 2% naphthol blue-black solution. Titers ≥10 were considered positive.

Data management and real time case reporting:
In order to allow early reporting of confirmed dengue cases and real time monitoring of epidemic evolution as well as identifying hotspots, a web-based platform was developed, in collaboration with the epidemiological team. This application, called ''Teranga" (https://teranga.pasteur.sn//) written in php (Backend); html, javascript (Frontend) can be accessed anywhere using laptop, tablet or smartphone connected to internet. Basically, the platform provides an online form where any type of data, including epidemiological as well as clinical data can be registered and linked to a unique identifier. Results can be exported as an Excel file with the possibility to do representation as graphs (barplot, epidemic curves, map of confirmed cases) that are updated in real time. This allows daily access of healthcare giver to DENV results of enrolled patients in their respective health districts.

Complete E gene sequencing

To get insight about the origin of the DENV-3 strain mainly responsible for the epidemics, the E gene of a random selection of 17 positive samples were sanger sequenced. cDNA synthesis was performed using AMV kit (Promega, Madison,USA). Briefly, 10µl of extracted RNA of viral RNA was mixed with 1 µl of the random hexamer primer (2 pmol) and the mixture was heated at 95°C for 2min. Reverse transcription was performed in a 20 µL reaction mix containing 2.5 U RNasin (Promega, Madison, USA), 1 µL of deoxynucleotide triphosphate (dNTP) (10 mM each DNTP), 5 U of AMV reverse transcriptase (Promega, Madison, USA) by incubating at 42°C for 60min. PCR products were generated using sets of primers described previously (16), which allow for the amplification of overlapping fragments of the full E gene of DENV-3. 5µl of cDNA were mixed with 10µL of 10× buffer, 3µl of each primer, 5µl of dNTPs 10 mM, 3 µl of MgCl2, and 0.5 µl of GoTaq polymerase (Promega, Madison, USA). The obtained amplicons were purified using a QIAquick Spin PCR Purification kit (Qiagen,Hilden, Germany) and submitted for bidirectional sequencing then sent for bi- directional sequencing using an ABI 377 automated sequencer (Applied Biosystems) using the same PCR primers set used for PCR reactions. The raw data were edited and merged using Geneious Prime to get the complete E gene sequences.

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Dataset Phylogenetic and Phylogeographic analysis:

In addition to 11 successfully complete E gene obtained during our study the complete E gene sequences of DENV-3 available in Genbank with complete metadata (country of isolation and date of isolation) were downloaded from the National Center of Biotechnology Information (NCBI) in genbank format and then converted to fasta using a custom perl script. The sequences were then filtered to exclude identical sequences (https://biopython.org/wiki/Sequence_Cleaner) resulting to a non-redundant set namely dataset-1. This primary dataset was then used to run a Maximum Likelihood tree using FASTREE. From this initial tree a cluster of viral strains closely related to our isolates were extracted to get a dataset-2 of 40 sequences used for Bayesian inference). For each described dengue genotypes among DENV-3 serotype four to ten sequences were selected and combined to Senegalese DENV-3 sequences (dataset-3) for the purpose of genotyping. Phylogenetic analysis was conducted on dataset-3 using the maximum likelihood (ML) phylogenetic approach implemented in IQ-TREE v.1.5.5 software with automatic model selection conducted by ModelFinder according to the Bayesian information criterion (BIC). The robustness of the tree topology was tested during 1000 non-parametric bootstrap analyses. The final tree was visualized and plotted using FigTree v.1.4.3 (http://tree.bio.ed.ac.uk). All sequence data used in this work are presented in the following format: accession number_region of sampling_date of isolation.
For phylogeographic inference the temporal signal (i.e., molecular clock structure) was investigated on dataset-2 using Tempest v.1.5.3 (***Rambaut A, 2016***) ; spatiotemporal spread of DENV-3 during this outbreak was reconstructed under a Bayesian framework. Time scaled Bayesian phylogenetic analysis was performed using a Markov Chain Monte Carlo (MCMC) algorithm implemented on the BEAST v1.10.4 (17). Briefly the BEAST analysis was run for 50 million MCMC steps using a GTR+G substitution model, an uncorrelated relaxed clock with log-normal distribution and a Skyline tree prior. Trees were sampled every 5000 generations ; the convergence was assessed using Tracer v1.7.1 and TreeAnnotator v1.10.4 was then used to generate maximum clade credibility trees with a burn-in value setting to 10 %. The tree was visualized and annotated using Figtree v1.4.4 (.http://tree.bio.ed.ac.uk/software/figtree/).

Results

Epidemiology, Demographic and Clinical characteristics

A total of 832 sera samples were collected from 22 healthcare districts around the Touba city for Dengue diagnosis. Most of the suspected cases were collected in Guede district with 122 suspected cases followed by Thiawene with 108 cases in contrast Oumoul Khoura and Moussobe recorded the lowest number of suspected cases with respectively 16 and 13 cases.
The overall infection rate was 31,61 % ; among confirmed cases the highest number were recorded in Guede (n = 31), followed by Darou Khoudoss (n = 28), Keur Niang (n = 27) and Thiawene (n = 22) (***Figure 4***). The sex ratio M/F for the confirmed cases was 1.02 ; There was no statistically significant difference between positivity and gender was observed ($p = 0.111$; Pearson's χ2-test). According to age, most of the confirmed cases belong 15 – 30 years age group (45.8 %) followed by the < 15 years (36.3 %), with the lowest positivity group to the virus were recorded in age group 45 – 80 years (5.2 %) ; the rate of positivity varied significantly according to the age group ($p < 0.001$; Pearson's χ2-test) (table 1).
Among positives cases 25 patients presented severe life-threatening symptoms, including haemorrhagic signs (n = 15 ; 5,7%), meningoencephalitis (n = 8 ; 3 %) and shock syndrome (n = 2 ; 0.8%)

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Table1. Epidemiologic and clinical characteristics of suspected and confirmed dengue cases, Touba-Senegal, 2018.

	Confirmed cases (N=263)	Negative (N=569)	Total (N=832)	p value
Age				
Median	18	21	20	< 0.001[1]
Q1, Q3	9.5, 26.0	12.0, 32.0	12.0, 30.0	
Group age, in year				
Unknown	12	27	39	
<15	91 (36.3%)	165 (30.4%)	256 (32.3%)	< 0.001[2]
[15,30[	115 (45.8%)	204 (37.6%)	319 (40.2%)	
[30,45[	32 (12.7%)	114 (21.0%)	146 (18.4%)	
[45,80[	13 (5.2%)	59 (10.9%)	72 (9.1%)	
Sex				
F	130 (49.4%)	315 (55.4%)	445 (53.5%)	0.111[2]
M	133 (50.6%)	254 (44.6%)	387 (46.5%)	
Clinical signs and Symptoms				
Fever	213 (81.0%)	369 (64.9%)	582 (70.0%)	< 0.001[2]
Join pain	154 (58.6%)	326 (57.3%)	480 (57.7%)	0.732[2]
Asthenia	149 (56.7%)	347 (61.0%)	496 (59.6%)	0.237[2]
Headache	243 (92.4%)	495 (87.0%)	738 (88.7%)	0.022[2]
Retrorbital pain	60 (22.8%)	123 (21.6%)	183 (22.0%)	0.698[2]
Bleeding	15 (5.7%)	19 (3.3%)	34 (4.1%)	0.109[2]
Muscle pain	133 (50.6%)	311 (54.7%)	444 (53.4%)	0.272[2]
Abdominal pain	73 (27.8%)	168 (29.5%)	241 (29.0%)	0.601[2]
Meningo-encephalitis	8 (3.0%)	11 (1.9%)	19 (2.3%)	0.320[2]
Shock syndrome	2 (0.8%)	0 (0.0%)	2 (0.2%)	0.037[2]
Rash	10 (3.8%)	16 (2.8%)	26 (3.1%)	0.445[2]
Profession				
Bureaucrat	21 (8.0%)	52 (9.1%)	73 (8.8%)	
Trade-market	17 (6.5%)	54 (9.5%)	71 (8.5%)	
Farmer	6 (2.3%)	21 (3.7%)	27 (3.2%)	0.256[2]
Student	68 (25.9%)	131 (23.0%)	199 (23.9%)	
Homemaker	55 (20.9%)	142 (25.0%)	197 (23.7%)	
Unspecific activities	73 (27.8%)	128 (22.5%)	201 (24.2%)	
Workers	23 (8.7%)	41 (7.2%)	64 (7.7%)	

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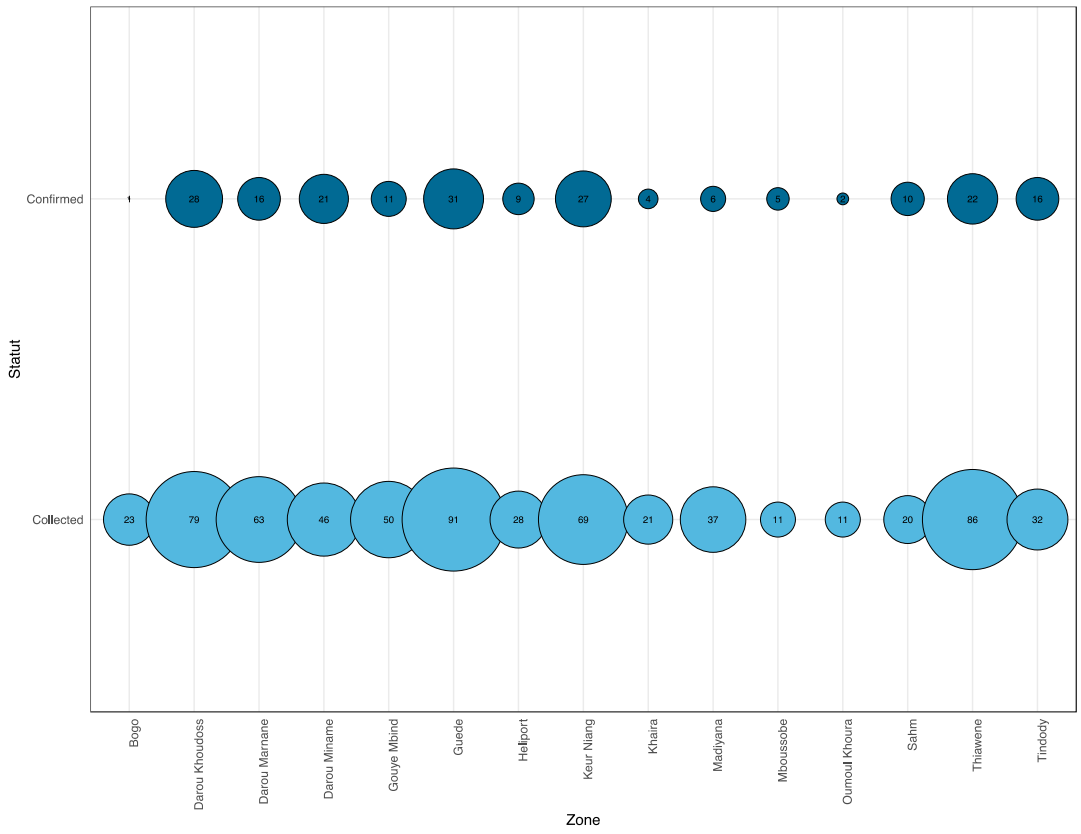

Figure3: Repartition of suspected and confirmed cases according to the healthcare districts. We represent only districts with more than 10 suspected DENV cases. Dark blue circles represents the confirmed cases (qRT-PCR and/or IgM detection followed by PRNT) and light blue circles represent suspected cases. The size of the circles is proportional to the number of cases.

The outbreak began at epidemiological week 33 and end at epidemiological week 46; the highest number of cases was recorded on week 43 (58 confirmed cases) (**Figure 4**).

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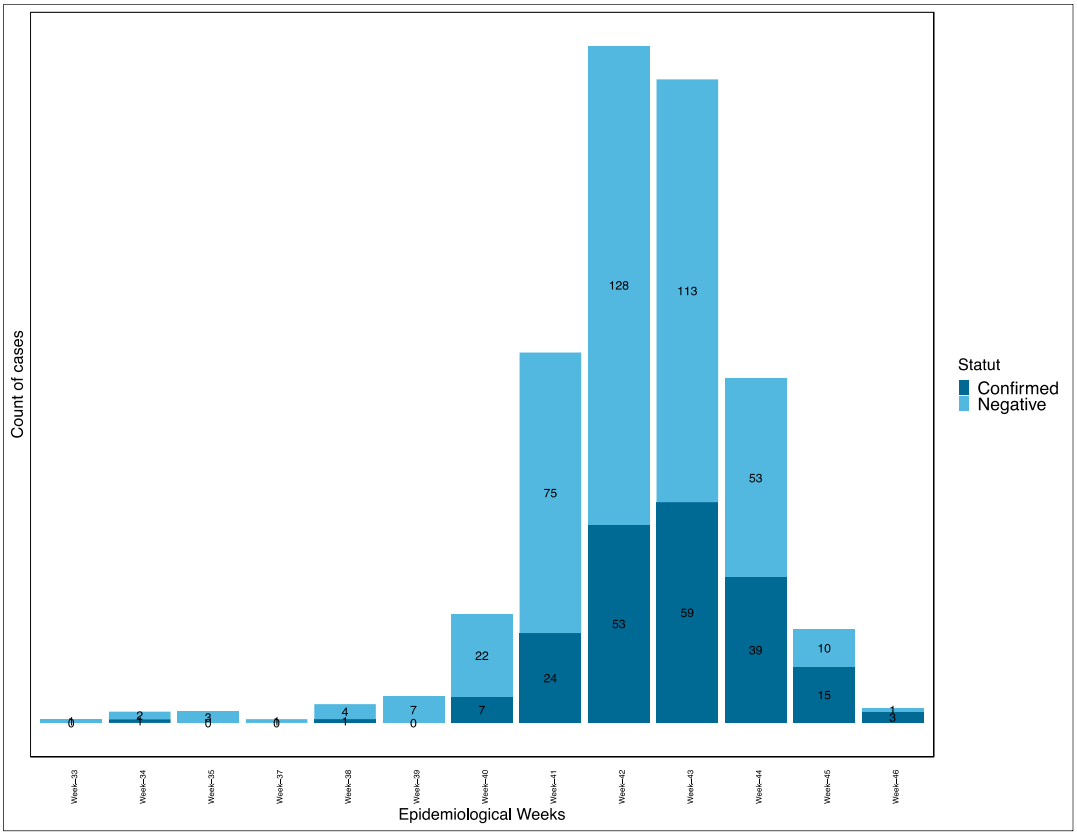

Figure 4: Epidemic curve describing the distribution of suspected and confirmed dengue cases according to the epidemiological weeks.

Detection of DENV RNA and serotyping

Among 832 enrolled suspected cases during this study, 189 (22.71%) yielded positive dengue results (qRT-PCR) (**Figure 4**). The serotyping of 109 out of all DENV RNA positive samples using real time PCR confirmed that the mainly circulating serotype during this epidemic was DENV-3 (103 / 109 ; 94.49 %).

Detection of DENV IgM

A total of 74 samples (8,89 %, n = 832 enrolled patients) were positive for DENV IgM antibodies by ELISA. Due to existing cross reactivity between flaviviruses all of them were confirmed to harbour DENV specific IgM antibodies by PRNT.

Phylogenetic and Bayesian analysis of detected DENV-3 strain

Successfully recovered full length envelope DENV-3 sequences during this work (n = 11) were combined to representative dataset of global DENV-3 sequences and subjected to phylogenetic analysis which revealed that the strain detected during this investigation belonged to DENV-3 genotype III ; these isolates were closely related to strains collected in Thiès in 2018, Fatick in 2018 and Burkina Faso in 2017 (ML tree) *(Figure 6*).

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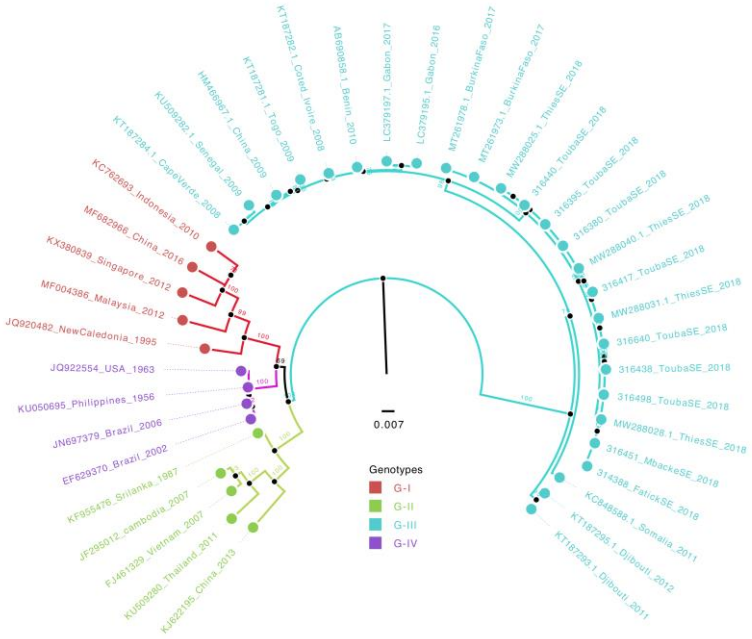

Figure 5 : Maximum likelihood phylogenetic tree inferred by using sequences from the Touba City outbreak and those from other regions of Senegal (Thiès, Fatick sampled in 2018) in combination with strains representative of the described DENV-3 genotypes. Bootstrap support (greater than 75) are represented on each nodes ; scale bar represent the number of observed substitution per site. Genotypes are represented by different colours according to the legend.

The performed phylogeographic analysis of sequenced viruses revealed that isolates form a monophyletic cluster which originated from Burkina Faso and were introduced in Touba, Senegal (location probability = 0.68; posterior = 1). It also suggests a single introduction possibly took place around 2017.07 (95 % HPD = 2016.61 – 2017.57) and then spread to this region Thiès region (Figure 6).

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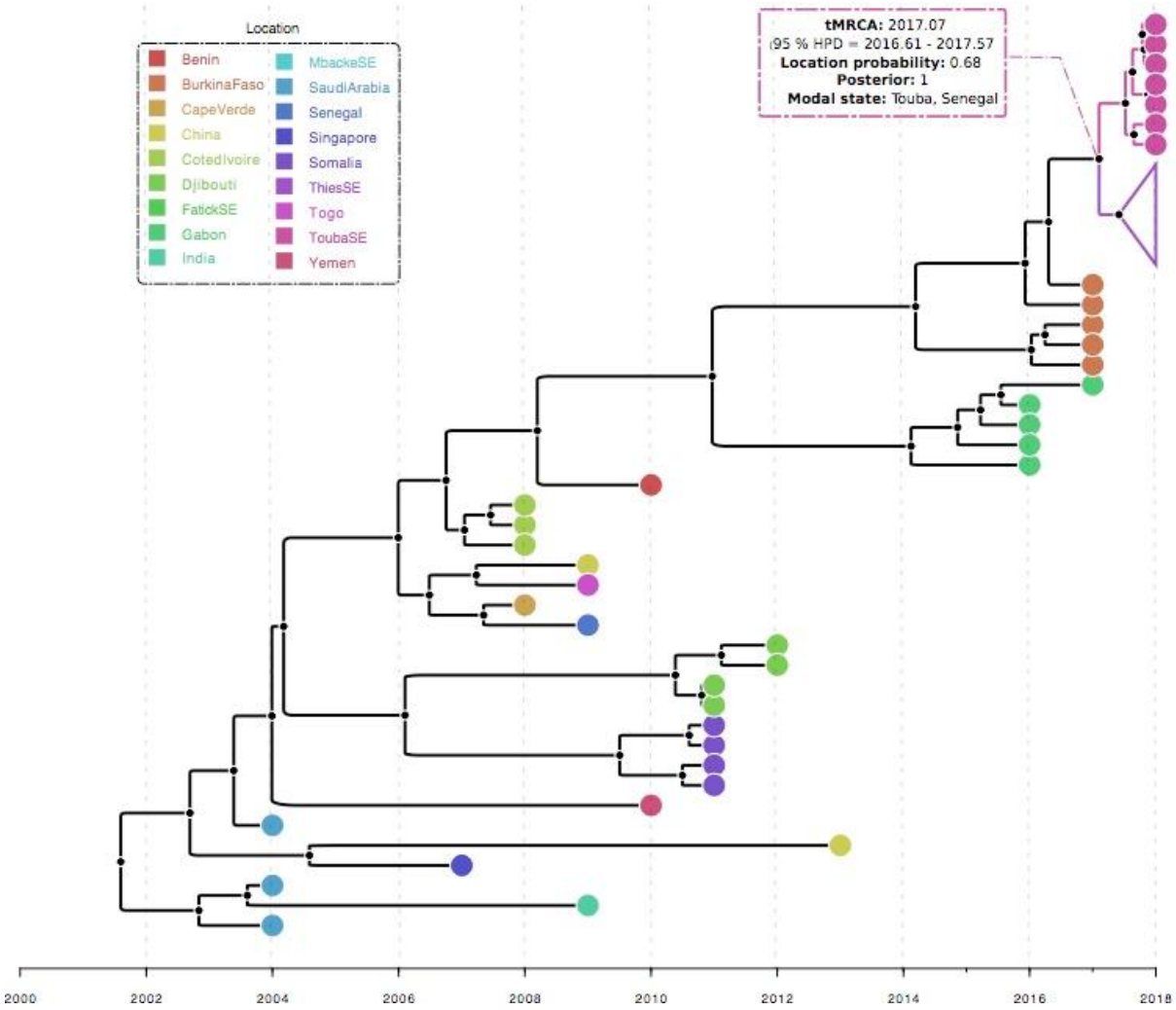

Figure 6: Bayesian discrete phylogeography of dengue 3 strains isolated in Touba 2018. Touba's strains grouped in a monophyletic cluster with sequences from Thiès (Collapsed in purple)

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Discussion :

Dengue occurrence in Senegal has long been linked to the circulation of sylvatic strains mainly in southeastern part of the country (18). In 2009, a major shift occurred with the notification of the first urban dengue outbreak associated with 196 confirmed cases and affecting Dakar, Thiès and Louga ; this outbreak was mainly caused by a DENV-3 serotype never detected before in West Africa (9) . Unprecedented recurrent outbreaks and sporadic cases marked by the co-circulation of three serotypes DENV1-3 was reported in Senegal since 2017 (19) ; the virus has recently emerged as a significant public health problem in Senegal. In October 2018, a Dengue outbreak was occurred in Senegal, more preciously at the religious city of Touba during the 124th edition of the ''Grand Magal of Touba''. This event is one of the largest religious mass gatherings in Senegal with approximately 4-5 million pilgrims from Senegal and worldwide (20). To allow rapid containment of this outbreak in a context of people mass gathering, rapid detection of suspected cases which impact patient management a Mobile Biosafety laboratory was deployed on the Holy city of Touba using the same workflow described by Dieng and Colleagues during dengue outbreak investigation in Louga city, 2017 (13). Results from epidemiological investigation revealed that the epidemic span a period of 13 weeks (from week 33 to week 46) with the highest number of confirmed cases recorded on week 43 ; the dengue positivity rate was 31.61 % with 263 confirmed dengue cases (qRT-PCR and/or IgM detection) among 832 enrolled patients. The observed positivity rate is higher than those reported from a previous DENV1 outbreak from Louga in 2017. In addition to the highest density of people in Touba during the Magal event which is known to be a risk factor to increased number of cases during infectious disease outbreak (21), the observed difference can be linked to the fact that in contrast to this study during the Louga outbreak only molecular testing of suspected DENV cases was used leading to a probable underestimation of real number of cases by missing IgM positive cases. A previous study revealed that PCR and capture IgM/IgG ELISA in combination had sensitivity to detect cases above 90% through the course of illness (22). According to the age group, the incidence is significantly higher among individual of age 15 to 30 years ($p < 0.001$; ; Pearson's χ2-test). It corresponded to the cohort of patients attending school or early working ; the higher dengue positivity on this age group may be due to their active involvement on socio economic activities (23) which may increase the chance of being exposed to mosquitoes vectors.
Performed molecular serotyping using qRT-PCR showed that during this outbreak the main circulating serotype was DENV-3 ($n = 103$) ; only six case of DENV-1 were detected among successfully serotyped DENV positives sera. Phylogenetic analysis based on the full length E gene shows that the strains belong to the African cluster of DENV-3 genotype III (figure 5) which they share with strains isolated in many African countries such as Gabon, Burkina Faso, Togo, Benin, Cote d'Ivoire, while DENV-1 isolates detected in Touba was previously studied and they belong to the genotype V (24). Interestingly during this study, we noticed 25 severe life-threatening cases associated with two death supporting previous findings reporting increased number of severe infections associated to DENV3 genotype III (DENV3/III) emergence in the Americas and Asia (25). The extent of the outbreak was probably underestimated, as Dengue infection may be asymptomatic or misdiagnosed as malaria. Furthermore, all districts did not notify correctly suspected cases and only patients with serious illness went for medical consultation. The proportion of severe dengue (SD), was slightly higher (5.7%) when compared to the reported rate (3%) during the 2009 Dengue outbreak in Senegal (9). Overall, the high rate of SD in Senegal compared to the current situation in the Americas (from 0.26% to 1.81%) is worrying.
According to previous reports DENV-3 was very common in West and Central Africa during this last decade (9,26,27). In Africa, the first detection of this serotype took place in Mozambique in 1985 (28) ;

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DENV3 is known to be endemic (29,30) and actively circulating in West Africa since 2006 (26). Studies suggest a significant association of DENV-3 with severe cases (31) raising alert to better monitoring of the serotype dissemination through pan African genomic surveillance of circulating DENV serotypes. Interestingly results from the phylogeographic analysis revealed that the DENV3 was introduced to Touba, Senegal (location probability = 0.68 ; posterior = 1) from Burkina Faso (West Africa) (figure 6) where dengue outbreak was marked by the co-circulation of DENV2 and DENV3 reported in 2016 (27). The introduction of the virus to Touba occurred around 2017.07 (95 % HPD = 2016.61 – 2017.57) which corresponds to January 2017 ; thus estimates in combination to the time of notification of the first DENV cases in Touba (October 8th 2018) support a probable cryptic DENV3 transmission in this region prior to cases notification. After introduction to Touba, the same strain probably spread to Thiès region located in the Western part of the country (***Figure 1***) where it caused the outbreak in co-circulation with DENV1-2 in late 2018 (32). Indeed, in their study Gaye and colleagues revealed that 45 % of DENV3 positives patients reported to travelled to Touba during the Grand Magal religious pilgrimage this in addition, provided genomic data during our study to support that the virus likely spread to Thiès, through viremic traveller or infected mosquitoes, where it caused the outbreak in late 2018 after its introduction to Touba in 2017. Interestingly, based on complete genome by Gaye and Colleagues (32), provided an estimated TMRCA of Thiès sequences sampled in 2018 and the most closely related African sequences from Gabonese outbreak sampled in 2016 - 2017 was around 13 years ago highlighting the absence of most of recent evolutionary history probably due to the fact that they use only seven African complete genome sequences at the time of their analysis, no DENV3 sequences from Burkina Faso 2017 and Senegal 2018 (Touba) were available. With the addition of DENV3 sequences from Burkina Faso 2017 and newly obtained sequences from Touba (n = 07), our study based on complete E gene sequences fine tune the resolution by providing a TMRCA, of recent Senegalese DENV3 sequences (Touba and Thiès 2018) with the most closely related available sequences from Burkina Faso sampled in 2017, of around 3.83 years ago (95% HPD 2.07 – 5.72 years).

Galloping urbanization and population growth at Touba, together with sanitation and water challenges and movement of populations represents many risk factors that may justify the outbreak. Therefore, dengue surveillance and preparedness should be reinforced in Senegal, particularly in Touba city during Grand Magal preparations, which coincide with the period of DENV circulation in Senegal.

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Conflicts of interest

The authors declare no confluicts of interest

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