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Short communication

Genomic surveillance of dengue virus in Benin

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ABSTRACT

Objective: Dengue is a widespread viral infection transmitted from mosquitoes to humans, mainly in tropical and subtropical climates. In Benin, only dengue virus (DENV) serotype 2 infection has been previously described in humans. This study aimed to investigate DENV infection and serotypes in suspected patients.
Methods: Plasma samples from 464 patients attending health centers in February 2023 with clinical symptoms and suspected for dengue infection were included, and analyzed for DENV by real time quantitative Polymerase Chain Reaction (Dengue Altona 3.0 kit). PCR positives samples were further characterized by whole genome sequencing and phylogenetic analysis to identify the circulating DENV serotype.
Results: The RT-qPCR results showed that four patients (D6, D23, D28, D44) were positive with the cycle threshold values less than 40 (31.3, 34.7, 14.7 and 14.3) respectively. Full-length DENV sequences were obtained for D6, D28 and D44. One patient (D6) was infected with DENV-1 serotype, and the two others (D28 and D44) were positive for DENV-3. Phylogenetic analysis shows that the new DENV-1 sequence is close to those obtained in Burkina Faso in 2022 and Nigeria in 2023, and the two DENV-3 sequences form a separate cluster with sequences obtained in Burkina Faso in 2022.
Conclusion: We showed for the first time, the presence of dengue serotype 1 and serotype 3 infection in Benin. These results send a strong signal to health authorities and show that arbovirus surveillance efforts must be integrated into pathogen monitoring programs.

1. Introduction

Dengue is an infectious disease caused by dengue virus (DENV), a single-stranded RNA virus (Medina et al., 2012). This arbovirus, belonging to the *Flavivirus* genus, is transmitted by mosquitoes of the *Aedes* genus. It is estimated that around 390 million people are infected each year, with around 96 million symptomatic cases (Bhatt et al., 2013), and around 25,000 deaths per year. This infection occurs mainly in tropical and intertropical areas, where vectors are present. DENV infection increased in recent years worldwide. Four major serotypes (DENV-1 to DENV-4) are known, and all are present in Africa (Eltom et al., 2021). An accurate and rapid diagnosis is necessary to confirm the infection for patient care as well as for public health surveillance systems. Indeed, dengue infection is confused with other infections such as malaria, typhoid fever, chikungunya, Zika or yellow fever, because of similar clinical signs.

Probable cases of dengue fever were described in 1987 among German humanitarian workers returning from Benin (Eisenhut et al.,

Abbreviation: EDTA, Ethylene diamine tetraacetic acid; RNA, Ribonucleic acid; DNA, Deoxyribonucleic acid; MAFFT, Multiple Alignment using Fast Fourier Transform.

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1999). In 2019, the first cases of dengue fever infection with serotype 2 in humans have been reported in Benin (Allanonto et al., 2021). Although the other serotypes have not yet reported in humans in Benin, studies showed infection with serotypes 1 and 3 in travelers returning from Benin to France in 2019 (Fourié et al., 2020), and to Japan in 2010 (Ujiie et al., 2012), indicating a probable presence of these serotypes in Benin. Finally, the presence of DENV-3 has been reported in 2022 in mosquitoes in Benin (Tchibozo et al., 2022). Here, we report the full genome characterization of DENV-1 and 3 strains that circulate in Benin.

2. Materials and methods

2.1. Ethics statements

Ethics approval was obtained from the Comité d'Ethique pour la Recherche en Santé (N°82/MS/DC/SGM/CNERS/SA). All participants were informed about the study objectives as well as the procedures and provided written consent for participation.

2.2. Sample collection

Blood samples were collected from mid to end of February 2023, a dry season period, on 464 febrile patients attending health centers tested negative for malaria and typhoid, which constitute the main inclusion criteria. Peripheral venous blood was collected on EDTA tubes in two towns in northern Benin (Tchaourou and Papane) and South Benin (Cotonou) and stored in a refrigerated cooler until transportation to the *National* Laboratory for Viral Haemorrhagic Fever of Benin where the samples were tested for presence of DENV. Information including age, sex, location and clinical signs were collected.

2.3. RNA extraction and laboratory diagnosis

Viral RNA was extracted from all plasma using the Qiamp Viral RNA kit following the manufacturer's instructions, after dengue TDR test as shown in the Supplementary fig. 1. The extracted RNAs were amplified using RealStar® Dengue RT-PCR Kit 3.0 (Altona) for DENV diagnosis. The cycle threshold (CT) values representing the number of reaction cycles needed to replicate enough viral RNA was detected. All sample with CT values lower than 40 were considered as positive and used for sequencing.

2.4. Library preparation and DNA sequencing

RNAs from positive samples were firstly quantified using Qubit Fluorometer (Thermo Fisher Scientific, USA) and converted to complementary DNA with the LunaScript RT SuperMix Kit (NEB, New England Biolabs, USA) including random hexamer and oligo-dT primers. Double-stranded DNA was generated by using NEBNext® Ultra™ II Non-Directional RNA Second Strand Synthesis Module (NEB). The purified resulting ds-cDNA was enzymatically fragmented and converted to Illumina-compatible dual indexed libraries with the Twist Library Preparation EF kit v2.0 (Twist Biosciences, USA). Quality control of libraries was performed using Qubit and D1000 ScreenTape with the 4150 TapeStation (Agilent Technologies, USA). The purified libraries were pooled and enriched by using the Twist target enrichment standard hybridization protocol with the Twist Comprehensive Viral Research panel (Twist BioScience, USA). The panel is made of one million unique probes targeting more than three thousand viruses. The post-capture amplified and purified library was diluted and sequenced on Illumina iSeq100 platform (2 × 150 cycles). DENV genome classification into serotype and Fasta sequences were obtained after viral genome assembly and variant calling using online CZID (https://czid.org/). New DENV sequences Benin_D6_2023, Benin_D28_2023 and Benin_D44_2023 were submitted to GenBank under accession numbers PQ014897, PQ012565 and PQ012567 respectively.

2.5. Phylogenetic analysis

The new DENV genome sequences were aligned with known representatives of the different genotypes within a serotype, uploaded from GenBank. Alignments were performed by using MAFFT version 7 (https://mafft.cbrc.jp/alignment/server/), then manually checked and curated under AliView. The phylogenetic trees were constructed with the maximum likelihood method with 1000 bootstrap iterations as implemented in IQ-Tree (http://iqtree.cibiv.univie.ac.at/). ModelFinder was used to set up the best fitting model. The consensus trees were displayed with FigTree v1.4.4. The envelope E protein sequences were obtained after alignment to the DENV-1 reference (NC_001477.1) and DENV-3 reference (NC_001475.2), visualization under AliView and curation.

3. Results

3.1. Dengue virus serotypes and phylogenetic analyses

In total, four samples (D6, D23, D28 and D44) from 464 suspected patients were detected positive for DENV infection with CT value less than 40 and further analyzed (Supplementary fig. 1). The characteristics of the study population and main patients symptoms are presented in the Table 1. Sequence analysis was unsuccessful for sample D23. Phylogenetic analysis showed that patient D6 was infected with DENV-1 and patients D28 and D44 were infected with DENV-3, all genotype III. The DENV-1 sequence of D6 (Fig. 1) is most closely related to that from Burkina Faso in 2022 and Nigeria in 2023, and located in a cluster composed of sequences isolated in 2023 in Chad. Interestingly, the sequence is distant from DENV-1 sequence obtained from a French traveler in 2019 (Benin_EPI_ISL_773718_2019). Phylogenetic analysis based on the nucleotide sequence of the envelope E protein showed that this portion of DENV-1 of D6 is genetically close to DENV-1 sequences from Chad in 2023 (Supplementary fig. 2). The two DENV-3 sequences from Benin are genetically similar, sharing the same nucleotide and amino acid percentage identity (data not shown). As depicted in Fig. 2, they cluster in the same subclade as sequences reported from Burkina Faso in 2017 and Senegal in 2018, but form a separate mini cluster with previous sequences from Burkina Faso in 2022. Phylogenetic analysis when targeting the E protein showed that this portion of DENV-3 sequence of patients D28 and D44 remain genetically close to DENV-3 sequences from Burkina Faso in 2022 (Supplementary fig. 3).

3.2. Specifics mutations in DENV nucleotides and amino acids on new sequences from Benin

Herein, we assessed whether sequences from Benin present mutation not observed in other sequences included in the phylogenic analysis. No specific change in DENV-1 nucleotides and amino acids was observed. Mutations were observed on C protein (T150C), E protein (T2074C and T2413G), NS2A protein (G3628A), and NS3 protein (T5551C) of two

Table 1
Characteristics of the study populations.

	Participant D6	Participant D23	Participant D28	Participant D44
Age in years	34	29	47	18
Sex	Female	Male	Male	Female
Location	Cotonou	Dassa	Cotonou	Cotonou
Temperature	41,2	40,8	38,6	40,2
Anorexia	Yes	Yes	Yes	Yes
Muscle pain	Yes	Yes	Yes	Yes
Migraine	Yes	Yes	Yes	Yes
Vomiting	Yes	No	Yes	No
Headache	Yes	Yes	Yes	Yes
Skin rash	No	No	No	No

Fig. 1. Maximum likelihood (ML) phylogenetic tree based on complete genome of dengue virus type 1, available on GISAID (https://gisaid.org/) and on viprbrc (https://www.viprbrc.org). The tree was drawn using IQ-TREE web server for 1000 ultrafast bootstrap replicates under the GTR + F + I + G4 substitution model. The topology was visualized by FigTree, version 1.4.4. Only bootstrap values ≥80 were shown on the tree. The genome highlighted in red corresponds to the DENV sequences from Benin in 2023, and that highlighted in blue corresponds to the DENV sequence from the traveler returning from Benin to France. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

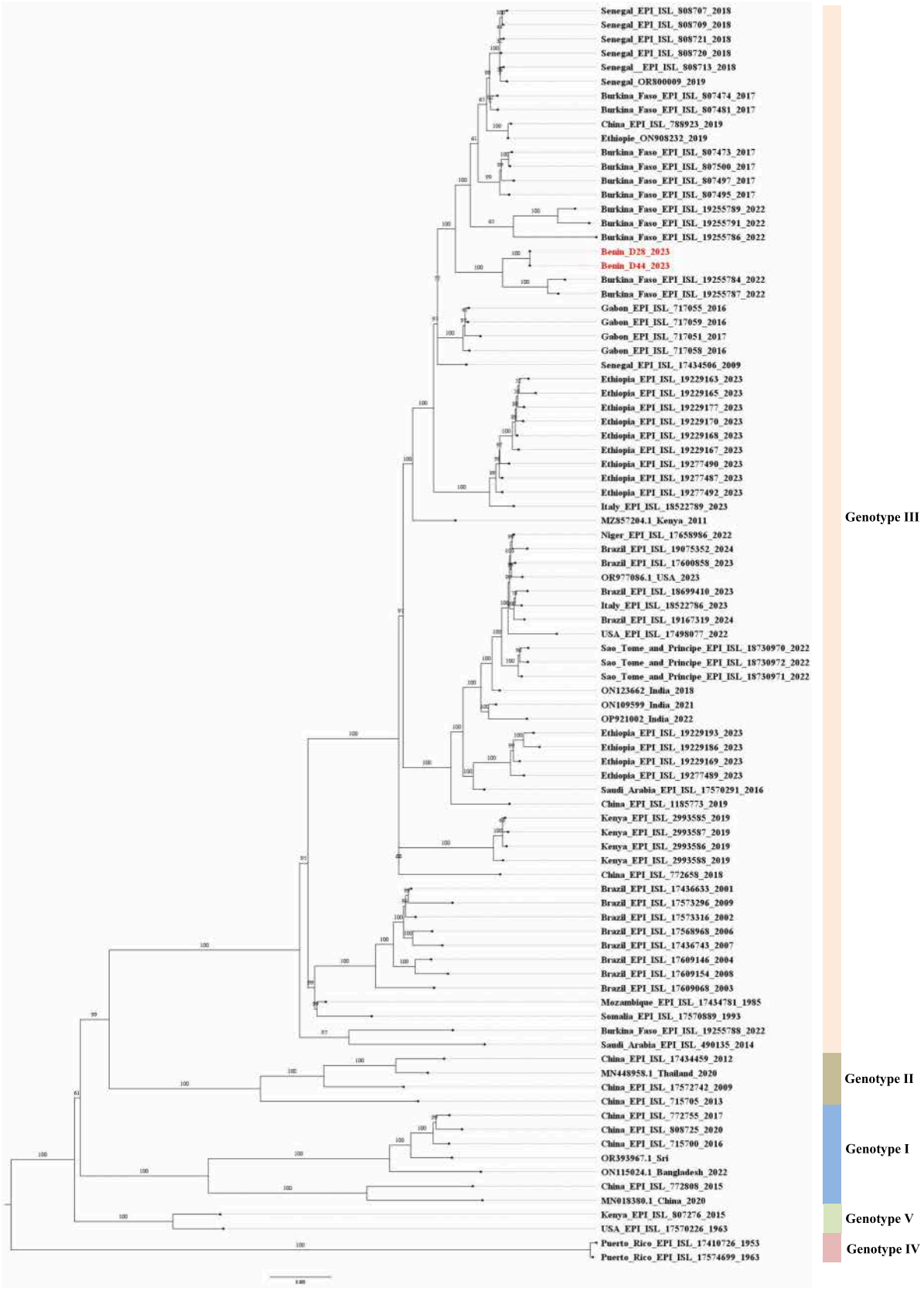

Fig. 2. Maximum likelihood (ML) phylogenetic tree based on complete genome of dengue virus type 3, available on GISAID (https://gisaid.org/) and on viprbrc (https://www.viprbrc.org). The tree was drawn using IQ-TREE web server for 1000 ultrafast bootstrap replicates under the GTR + F + I + G4 substitution model. The topology was visualized by FigTree, version 1.4.4. Only bootstrap values ≥80 were shown on the tree. The genome highlighted in red corresponds to the DENV sequences from Benin in 2023. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

DENV-3 sequences (supplementary Table 1A). Additionally, other mutations on C protein (A384G), E protein (T1249C), and on NS5 protein (A8599T) were observed on the two DENV-3 sequences of Benin and only on the two sequences of Burkina Faso found in the same mini clade. As shown in the supplementary table 1B, changes in amino acids were observed in the envelope E protein (T169K) and in NS2A protein (M53I and V189M).

4. Discussion

We describe for the first time, the circulation of DENV-1 and DENV-3 in humans in Benin. Indeed, only samples from 4 patients out of the 464 included or from positive samples for the dengue RDT (4/98) were sequenced. Given this high proportion of PCR negative test in RDT positive samples, we speculate that our samples were collected in late stage of dengue fever when virus had been cleared in most patients. The two serotypes found were previously reported in foreigners who had stayed in Benin, questioning about their probable presence in the country, although cases were never officially reported in Benin. Remarkably, the serotype 1 sequence from 2023 is not linked to the isolated one from the French foreigner in 2019. The information on the traveler's stay before his visit to Benin was not described, it cannot be excluded that the dengue infection was contracted outside Benin, since its sequence is close to strains from other West African countries, mainly the sequence from Burkina Faso in 2017. The serotype 1 sequence from patient D6 is very close to strains isolated in Burkina Faso in 2022 and Nigeria in 2023, were dengue infection is endemic (Tizhe et al., 2022; Manigart et al., 2024). The high risk of transmission may be due to the geographical proximity of Benin and Nigeria and Burkina Faso, with a significant migratory flow. Moreover, the increasing urbanization was found to favor conditions for dengue transmission (Were, 2012). The two serotype 3 sequences are genetically different from those isolated in previous epidemics in Burkina Faso or Senegal in 2017 and 2018 although clustering within the same subclade, but shows some similarity to recent sequences isolated in Burkina in 2022, forming a miniclade with them. Actually, sequences are forming clades and subclades according to the geographic origin of the samples. Given the very close linkage of the two genome sequences from Benin and Burkina Faso, the presence of only some mutations in the sequences from Benin, and others mutations in the sequences from Benin and Burkina Faso, it might represent a specific viral occurrence of DENV-3. Monitoring of these new mutations is then necessary to assess their clinical impact.

The limitations of the study include the samples collection only in three localities of the country and the duration of the collection. It would be interesting to extend it to several other localities of the country, over a longer period.

5. Conclusion

We highlight for the first-time, full-length genome sequences from Benin and show the presence of DENV-1 and DENV-3 in Benin in 2023. These results send a strong signal to health authorities and show that arbovirus surveillance efforts must be integrated into pathogen monitoring programs.

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CRediT authorship contribution statement

Anges Yadouleton: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Odilon Nouatin:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Islamiath Kissira:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Parfait Houngbegnon:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Gilles Cottrell:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Nadine Fievet:** Writing – review & editing, Supervision, Methodology. **Stephane Sohou:** Writing – review & editing, Methodology. **Christelle Butel:** Writing – review & editing, Resources. **Laetitia Serrano:** Writing – review & editing, Resources. **Emilande Guichet:** Writing – review & editing, Resources. **Nicole Vidal:** Writing – review & editing, Resources, Formal analysis, Data curation. **Eric Delaporte:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Ahidjo Ayouba:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Martine Peeters:** Writing – review & editing, Resources, Project administration, Funding acquisition. **Achille Massougbodji:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

All data supporting the reported results are available on request.

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Appendix A. Supplementary data

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