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Detection of human case of sylvatic dengue virus 2 during routine surveillance of fever in Senegal, Kolda 2021

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Abstract
A human case of dengue virus 2 was detected from a febrile patient living in the Sare yoba, Kolda region (Southern Senegal). Phylogenetic analysis based on the partial sequence of the NS5 gene reveals that the virus belongs to the DENV2 Sylvatic genotype and is closely related to a strain (JF260983/ 98.98% identity) detected in Spain from a tourist who travelled to Guinee Bissau (bordering Kolda region) in 2009. This highlights a potential recent underreported circulation of sylvatic Dengue in the southern part of Senegal and calls for re-enforced integrated surveillance among humans, non-human primates and arboreal mosquitoes throughout a one-health approach.

Keywords: Febrile patient, Kolda, Circulation, Sylvatic Dengue virus 2, 2021, One health

Background

Dengue is the most prevalent arboviral disease in tropical and subtropical areas. It is caused caused by dengue virus (DENV) the etiological agent exist in four antigenically and phylogenetically distinct serotypes (DENV1-4) (1). Infection with any DENV serotypes cause infections ranging from flu like illness (Dengue fever) to a life threatening infection known as Severe Dengue (2). The WHO estimates 1/3 of the worlds population is at risk of dengue infection (2). Each of the existing dengue serotypes are maintained in two different ecologically and evolutionary distinct transmission cycles, namely the human cycle and the sylvatic cycle. The human cycle, for which only humans play a role of reservoir, is maintained between domestic and peridomestic mosquitoes in contrast to the sylvatic cycle, which

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involves non-human primates and arboreal mosquitoes (3). Despite the central and basal role that sylvatic strains of DENV play in evolution and emergence, there is no report of continuous and sustained transmission (4).
In Senegal the landscape of DENV circulation was long dominated by the occurrence and maintenance of sylvatic cycles mainly in Southern part of the country (Kedougou area) (5). In 2009 a shift occurred with the first reported urban DENV epidemic in Dakar. This this was followed by recurrent and yearly multifocal and multiserotype circulation of human cycle strains (6). Here, we report a case of DENV2 infection from which phylogenetic analysis based on a partial sequence of the NS5 gene reveal that it is closely related to a strain circulating in Guinee Bissau in 2009.

The Study

In collaboration with the Senegalese ministry of health), the Unit of epidemiology clinical research and data sciences at Institut Pasteur de Dakar, our laboratory (the Virology Unit at the Institut Pasteur de Dakar is conducting syndromic surveillance of fever around the country through a program namely 4S Network (Sentinel Syndromic Surveillance Network in Senegal) (7). As part of this nation wide surveillance project, samples from febrile patients are collected an shipped on a weekly basis to the WHO collaborating center for arbovirus suspicion diagnosis. On November 2021, a patient suspected of arboviruse infection presented in Sare Yoba health district located in Kolda region southern Senegal (***Figure S1***). It was a male patient aged 28 years old presenting with symptoms including headaches, myalgia, asthenia, arthralgia and chills. The performed malaria rapid diagnostic test yielded a negative result. A veinous blood sample was collected following 2 days of fever patient and shipped to the Virology Unit at the Institut Pasteur de Dakar for diagnosis. At IPD, the blood sample was centrifuged at 2000rpm for 5 minutes and serum was harvested and aliquoted on 2ml tubes. RNA extraction was performed using the Qiagen viral RNA kit using 140 µl of input serum following the manufactures recommendations. Extracted nucleic acid was subjected to screening for seven arboviruses as previously mentioned by Dieng and colleagues (8) from which only Dengue virus gave a positive result. In order to define the incriminating serotype, DENV+ RNA was subjected to a multiplex qRT-PCR assay using TibMolBiol Modular Dx Dengue typing kit (Cat-No. 40-0700-24) (5). Surprisingly, this assay failed to define the dengue serotype. To define the virus serotype/genotype, we successfully amplified a partial NS5 gene sequence using FU1/FD3 (9) ; the obtained amplicon was approximately ~ 1Kb. It was purified using Ampure beads at a ratio 1:0.8, a sequencing library was prepared for the Oxford Nanopore MinION using the rapid barcoding kit (SQK RBQ110.96), loaded onto a R9 flow cell and sequenced using a MinION MK1C device.
Raw data were collected and basecalled using guppy. Adapters were trimmed and reads were mapped to a DENV reference genome (NC_001474.2). A NCBI Blast search of the obtained sequence matched with sylvatic DENV2 (JF260983;). To

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determine the evolutionary history we download representative sequences of described DENV2 genotypes. Theobtained dataset was aligned using MAFFT (10) and maximum likelihood (ML) tree constructed using IQTREE (11).

The obtained ML (***Figure 1***) tree clearly shows that based on partial NS5 gene our strain fell within the West African DENV 2 sylvatic genotype and is closely related to a strain linked to hemorrhagic DENV detected in Spain in 2009 from a tourist who travelled to Guinea Bissau through Senegal (12) instead of strains of DENV2 cosmopolitan responsible of latest DENV2 epidemic in Senegal (6,13). This is the first identification of circulating sylvatic DENV-2 in Senegal after the year 2000 (14). Interestingly, the Kolda region share borders with Niokolokoba National parc where monkey species are known to be reservoir of sylvatic DENV-2 (3) (Papio papio, Erythrocebus patas) were reported (15). Additionaly, experimental findings from the surrogate human models of infection and from cultured cells suggest that there is little or no adaptive barrier for the emergence of sylvatic DENV in human populations, possibly reflecting the evolution of DENV as an opportunistic virus that is capable of infecting a wide range of primate species (3).

This finding, in addition to the present detection of this sylvatic genotype in Kolda area (sharing border with Guinea Bissau), highlight a potential unnoticed circulation of Sylvatic DENV2 in Southern Senegal. This is corroborated by the fact that the patient was not traveling, confirming that the case was autochtonous.

In 2021, a national seroprevalence study conducted in 14 administrative regions in Senegal lead the detection of 5 DENV IgM positive sample in Kolda region (U*npublished data*) No samples have been reported to be qRT-PCR positivy prior to this case.

Interestingly, clinical infection with sylvatic strains are indistinguishable from human transmission cycle strains. This can lead to underreporting of sylvatic dengue virus (12) since genomic surveillance of circulating DENV strain in Senegal, as well as in Africa, is limited (16). All parameters mentioned above support the high likelihood that potential for spillover of sylvatic DENV from Africa or Asia in the human transmission cycle. This calls for determining the genetic diversity of circulating DENV strains, which is crucial before any vaccination policy can be implemented. Indeed, determining which contemporary genotypes are in circulation in a given area is crucial to ensuring effective diagnostics and developing preventive and therapeutic countermeasures.

Conclusion

DENV is now hyperendemic in Senegal with the co-circulation of DENV1-3 belonging to human transmission cycles? and is marked by yearly epidemics that may constrain the emergence of sylvatic DENV. Nevertheless, as human DENV has the potential to enter human cells and cause hemorrhagic disease, an integrated one health approach between human, mosquitoes and non-human primate is urgently needed in regions other than Kedougou area located in southern part of Senegal.

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This could improve Dengue fever surveillance through active existing human malaria-like illness surveillance within the 4S network.

Finally, to be able the discriminate between sylvatic and epidemic DENV strains, real time genomic surveillance of DENV could play a key role on virus surveillance around the country. This will help us to better understand evolutionary history, transmission and spread with complex transmission dynamics involving both urban and sylvatic DENVcycles.

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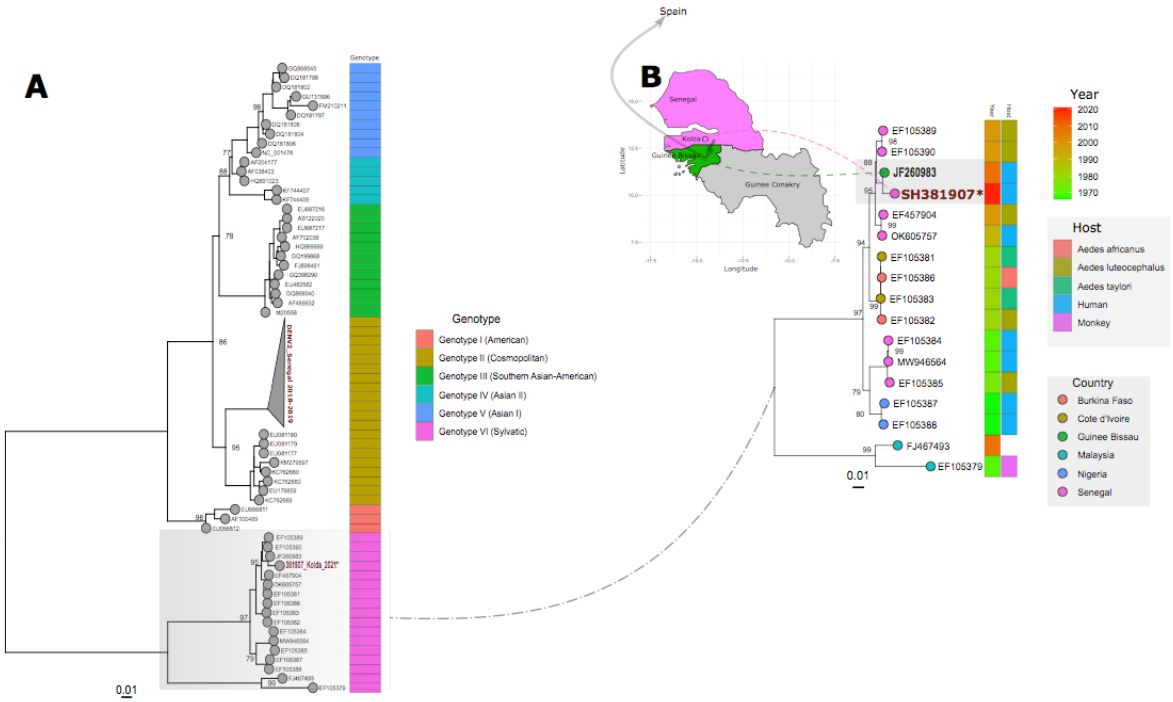

Figure 1 : Maximum Likelihood (ML) phylogenetic tree based on 1073 bp of the NS5 gene. Panel A shows the obtained sequence during this work belongs to the Sylvatic genotype of DENV-2. Panel B is an expansion of the Sylvatic genotype highlighted in grey on panel A ; the heatmap shows respectively the year of isolation and the host of used sylvatic DENV2 strains during phylogenetic analysis. Tips are colored according to the country or provenance. The arrow shows the itenerary of patient linked to sequence JF260983 who travelled to Guinee Bissau through Senegal before returning to Spain where he was diagnosis.

Ethical considerations

In this study, we used samples collected as part of approved ongoing surveillance conducted by the Institut Pasteur de Dakar (a World Health Organization Collaborating Centre for Arboviruses and Haemorrhagic Fever Reference and Reasearch). The Senegalese national ethical committee approved the protocol as a less than minimal risk research, and written consent forms were not required. All samples from humans were deidentified before we performed virus detection, characterization and analysis.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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