Early Genomic Detection of an Outbreak of Dengue Virus 2 Cosmopolitan Genotype in Angola, 2018

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Abstract

We used portable genome sequencing to investigate reported dengue virus transmission in Angola. Our results reveal autochthonous transmission of dengue serotype 2 (Cosmopolitan genotype) in Jan 2018.

Keywords

Dengue virus, Angola, nanopore sequencing, epidemiological surveillance

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Introduction

In Africa, the burden of disease caused by *Aedes*-borne virus infections may be similar to that of the Americas (1, 2). However, the transmission and genetic diversity of arthropod-borne viruses in Africa remains poorly understood due to a paucity of systematic surveillance. Moreover, syndromic surveillance may confound symptomatically similar illnesses (3, 4) and current serological diagnostic tests can be masked by cross-reactivity to other circulating flaviviruses (5). Improved genomic surveillance of disease causing viruses is necessary to better understand the dynamics of transmission in Africa.

During 2013, Angola experienced a large dengue outbreak that was concentrated in Luanda Province (6). Cases detected in returning travellers showed that the virus rapidly disseminated from Angola to Europe, Asia and the Americas (7). Whilst infections were dominated by serotype 1 viruses (8), all dengue viral serotypes were reported in returning travellers from Angola (9).

Although dengue is likely endemic in Angola, patterns of DENV transmission in the country outside the 2013 epidemic are poorly characterized. The lack of genomic characterization restricts our understanding of DENV diversity within Angola, and the frequency and directionality with which dengue viruses are exchanged with other countries. Here, we report a new approach for portable sequencing of dengue virus serotype 2 (DENV2), and use this method to characterize the first viral genome generated in Angola.

Epidemiological data

To investigate the timing and frequency of dengue occurrence in Angola, rapid diagnostic SD-Bio Line Dengue Duo (NS1 Ag+) tests were conducted to detect dengue specific IgM

and IgG presence. Samples were collected between 1st Jan 2016 and 15th May 2018 from patients presenting symptoms associated with DENV in central Luanda (n=6690, 3203 male). Samples were originally obtained for routine diagnostic purposes from persons visiting local clinics. In these cases, we used residual samples without informed consent with the ethical approval of the National Ethical Committee, Ministry of Health, Angola.

A total of 210 patients tested positive for recent dengue viral infection by IgM. Including 91 patients who also tested positive by IgM presence, a total of 555 patients tested positive for historical dengue viral infection by IgG presence. These data suggest that DENV transmission is not uncommon in Luanda province. During Jan 2016 and mid May 2018, we found 119 primary acute infections, classified here as IgM+ and IgG. Acute infections were detected in all months of the year during 2016. Most acute infections were detected between January and August 2016 and during April and May 2018 (**Fig. 1A**).

Molecular diagnostics

153 sera samples collected from 21st July 2017 to 31st Jan 2018 were tested for DENV RNA by real-time quantitative PCR (RT-qPCR) at the Instituto Nacional Saúde Pública (INSP), Luanda, Angola using the CDC DENV1-4 Real-Time RT-PCR assay kit on an Applied Biosystems 7500 Fast machine, according to the manufacturer's instructions. Of these, one sample yielded a RT-qPCR cycle threshold of 22.5 for DENV-2. This sample was retrieved from a 48-yo male Luandan resident who visited a clinic on 18th Jan 2018. The patient reported travelling to Mussulo Island between 24 Dec 2017 – 2 Jan 2018, a resort 30 km from Luanda.

Genome sequencing

The RT-qPCR positive sample was subjected to viral genomic amplification and sequencing using a new multiplex PCR primer scheme designed to amplify the entire coding region of DENV2. Published genomes of non-sylvatic DENV2 were aligned and used to generate a 90% consensus sequence that formed the target for primer design. Primers that generate overlapping amplicons of length 980bp, with overlap 20bp were designed following (10) (see **Technical Appendix** for primer scheme). cDNA synthesis, multiplex PCRs using 35 cycles, library preparation and sequencing were conducted following (10).

Generation of consensus sequences was performed following (10). The 90% consensus sequence defined above was originally used as a reference genome for mapping sequencing reads, but this reference was later refined to a more appropriate reference genome (Genbank Accession LC121816) using BLAST searching of the provisionally mapped data. The median sequencing depth was 11,448 reads, and 75% of the genome had a depth of at least 2419 reads. In total, 96% of the coding region of DENV2 (named isolate AO-1) was sequenced.

Phylogenetic analyses

Phylogenetic trees were estimated to explore the relationship of the sequenced AO-1 genome (GenBank accession number XXX) to other isolates. 1395 DENV2 genome sequences with associated date and country of collection were retrieved from GenBank. From this data set, a subset was generated that included all identified African sequences (n=35), 200 globally sampled sequences (randomly sampled from the remaining 1360 sequences), and the novel AO-1 sequence. These sequences were aligned using MUSCLE, as implemented in Geneious 9.0.5 (11). A maximum likelihood (ML) phylogenetic tree was estimated using a GTR+4Γ

substitution model in RAxML 8.2.10 (12). 500 non-parametric bootstrap replicates were performed to evaluate statistical support for phylogenetic nodes.

Most DENV sequences available in GenBank are partial gene sequences. We therefore supplemented the above whole genome alignment with all African DENV2 sequences >1000bp that belonged to the same genotype as the novel Angolan sequence. Sequences were aligned to the envelope region of DENV2, and a separate phylogeny was estimated from this alignment using the models specified above.

Phylogenetic estimation strongly supports placement of the Angolan genome in the Cosmopolitan genotype of DENV2 (**Fig. 1B**). The Angolan strain forms part of a well-supported monophyletic clade that comprises genomes sampled in East Africa, and is most closely related to DENV isolated from a returning traveler from this region. Inclusion of partial sequences in the phylogeny indicates that viruses from this clade have been present in Africa since at least 2013 (**Fig. 1B**).

Finally, we generated maps of the distribution of currently available DENV sequence data, in order to explore the global distribution of the DENV2 Cosmopolitan genotype and to identify geographic gaps in DENV genomic surveillance that might bias phylogenetic interpretation. All DENV sequences >100bp of any serotype, with known location of sampling (including returning travelers), were downloaded from GenBank. DENV2 sequences were genotyped using the Genome Detective online classification tool (http://www.genomedetective.com). The majority of sequenced dengue viruses in Africa belong to DENV2 (49%), of which 70% belong to the Cosmopolitan genotype (**Fig. 1C**). We find that whilst 16% of all global clinically apparent dengue infections have been estimated to occur in Africa (1), DENV1-4

sequences from Africa currently represent <1% of the available global DENV sequence data. No data exists from the Democratic Republic of Congo, which has been epidemiologically linked with Angola during past arboviral outbreaks (13). Additional data will help to address transmission dynamics of DENV2 in the country and to identify common routes of virus importation into Angola.

Conclusions

The DENV2 sequencing approach reported here may represent a useful tool for genomic characterization and molecular epidemiology of DENV2 outbreaks in Africa and elsewhere. Based on phylogenetic evidence and the geographic distribution of detected genotypes, the DENV2 Cosmopolitan genotype detected in Angola is likely endemic in Africa. The AO-1 genome analyzed here probably represents an early transmission event from an ongoing DENV2 epidemic in Luanda. Further sequencing of DENV in the region is required to determine whether the Cosmopolitan genotype is endemic to Angola, or if it represents a more recent introduction from elsewhere (for example, from East Africa or from other unsampled locations).

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Figure 1

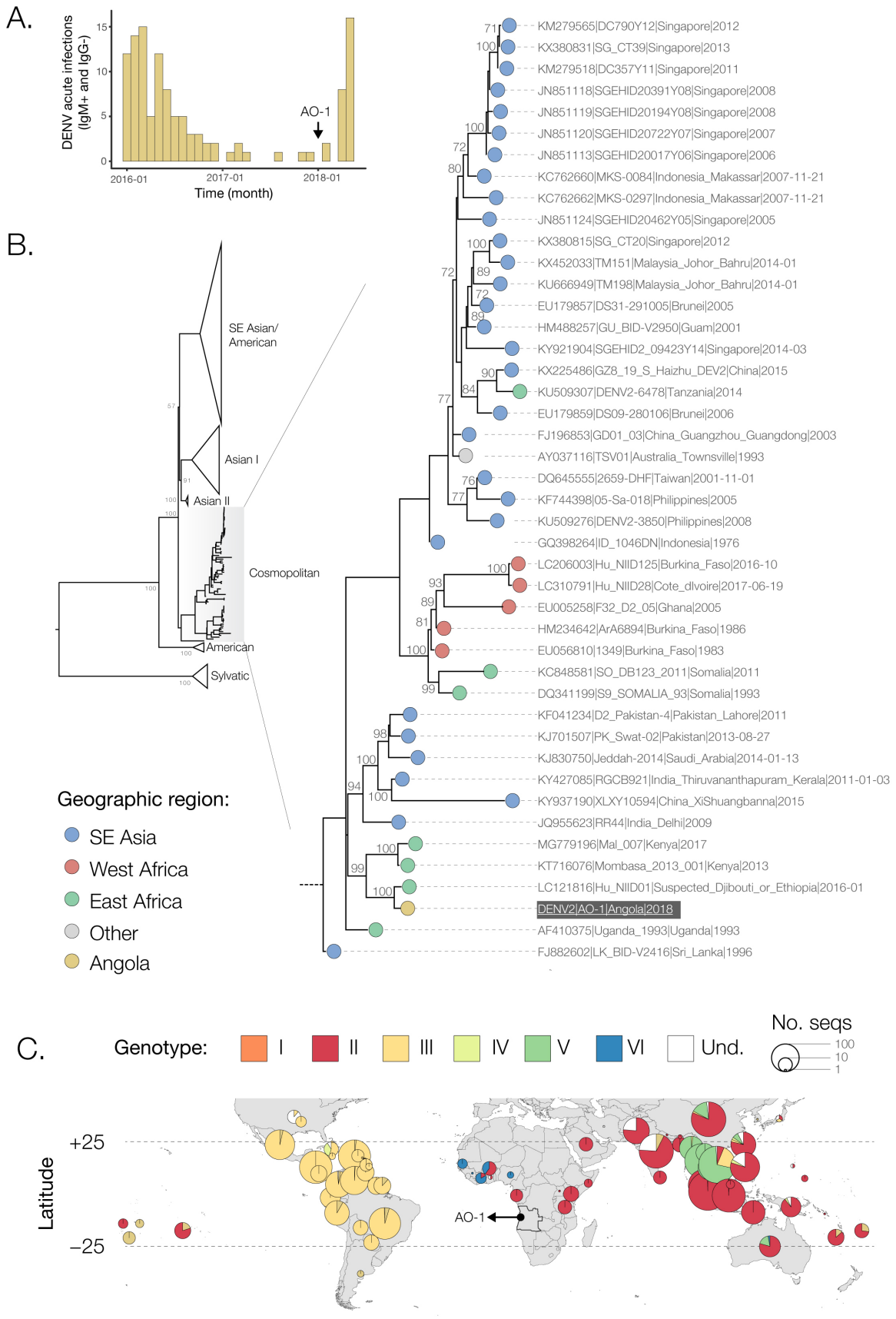
A.
DENV acute infections (IgM+ and IgG-)
AO-1
Time (month)
2016-01
2017-01
2018-01
B.
SE Asian/ American
Asian I
Asian II
Cosmopolitan
American
Sylvatic
KM279565|DC790Y12|Singapore|2012
KX380831|SG_CT39|Singapore|2013
KM279518|DC357Y11|Singapore|2011
JN851118|SGEHID20391Y08|Singapore|2008
JN851119|SGEHID20194Y08|Singapore|2008
JN851120|SGEHID20722Y07|Singapore|2007
JN851113|SGEHID20017Y06|Singapore|2006
KC762660|MKS-0084|Indonesia_Makassar|2007-11-21
KC762662|MKS-0297|Indonesia_Makassar|2007-11-21
JN851124|SGEHID20462Y05|Singapore|2005
KX380815|SG_CT20|Singapore|2012
KX452033|TM151|Malaysia_Johor_Bahru|2014-01
KU666949|TM198|Malaysia_Johor_Bahru|2014-01
EU179857|DS31-291005|Brunei|2005
HM488257|GU_BID-V2950|Guam|2001
KY921904|SGEHID2_09423Y14|Singapore|2014-03
KX225486|GZ8_19_S_Haizhu_DEV2|China|2015
KU509307|DENV2-6478|Tanzania|2014
EU179859|DS09-280106|Brunei|2006
FJ196853|GD01_03|China_Guangzhou_Guangdong|2003
AY037116|TSV01|Australia_Townsville|1993
DQ645555|2659-DHF|Taiwan|2001-11-01
KF744398|05-Sa-018|Philippines|2005
KU509276|DENV2-3850|Philippines|2008
GQ398264|ID_1046DN|Indonesia|1976
LC206003|Hu_NIID125|Burkina_Faso|2016-10
LC310791|Hu_NIID28|Cote_dIvoire|2017-06-19
EU005258|F32_D2_05|Ghana|2005
HM234642|ArA6894|Burkina_Faso|1986
EU056810|1349|Burkina_Faso|1983
KC848581|SO_DB123_2011|Somalia|2011
DQ341199|S9_SOMALIA_93|Somalia|1993
KF041234|D2_Pakistan-4|Pakistan_Lahore|2011
KJ701507|PK_Swat-02|Pakistan|2013-08-27
KJ830750|Jeddah-2014|Saudi_Arabia|2014-01-13
KY427085|RGCB921|India_Thiruvananthapuram_Kerala|2011-01-03
KY937190|XLXY10594|China_XiShuangbanna|2015
JQ955623|RR44|India_Delhi|2009
MG779196|Mal_007|Kenya|2017
KT716076|Mombasa_2013_001|Kenya|2013
LC121816|Hu_NIID01|Suspected_Djibouti_or_Ethiopia|2016-01
DENV2|AO-1|Angola|2018
AF410375|Uganda_1993|Uganda|1993
FJ882602|LK_BID-V2416|Sri_Lanka|1996
Geographic region:
SE Asia
West Africa
East Africa
Other
Angola
C.
Genotype:
I
II
III
IV
V
VI
Und.
No. seqs
100
10
1
Latitude
+25
−25
AO-1

Figure Legend

Figure 1. A) Number of acute infections of dengue virus in Luanda from Jan 2016 to mid May 2018. **B)** Midpoint rooted maximum likelihood phylogeny of DENV2 whole genomes. Support for branching structure is shown by bootstrap values at nodes. The Cosmopolitan genotype clade containing the Angolan DENV2 sequence is expanded. **On the right side,** an ML phylogeny of DENV2 Cosmopolitan genotype envelope gene sequences (1479bp), rooted on a sylvatic genotype outgroup that was included during phylogenetic tree estimation (not shown). Colors indicate geographic location of sampling. The Angolan DENV2 is highlighted. Support for branching structure is shown by bootstrap values at nodes (bootstrap scores >70 shown). **C)** Geographic distribution of availability of dengue serotype 2 sequence data (>100bp). Pie chart radii are log-proportional to the number of sequences available in each country, and are colored by genotype (und. = undefined).

Technical Appendix

Primer Name	Primer sequence (5'-3')
DENV2_1_LEFT	AGCAGATCTCTGATGAATAACCAACG
DENV2_1_RIGHT	TTTTTGCCATCGTCGTCACACA
DENV2_2_LEFT	TCGCTCCTTCAATGACAATGCG
DENV2_2_RIGHT	CCATTCTCAGCCTGCACTTGAG
DENV2_3_LEFT	ACATTGGTCACTTTCAAAAATCCCC
DENV2_3_RIGHT	TGAAGGGGATTCTGGTTGGAACT
DENV2_4_LEFT	ATAGTGGTTGCGTTGTGAGCTG
DENV2_4_RIGHT	CGGCAGCACCATTCTGTTATGA
DENV2_5_LEFT	TCATGCAGGCAGGAAAACGATC
DENV2_5_RIGHT	TCTCAAGAGTAGTCCAGCTGCA
DENV2_6_LEFT	TGGAAATCAGACCATTGAAAGAGAAAGA
DENV2_6_RIGHT	TGGTCAGTGTTTGTTCTTCCTCTT
DENV2_7_LEFT	CCAATCCTGTCAATAACAATATCAGAAGAT
DENV2_7_RIGHT	TGATGGCTGGGGTTTGGTATCT
DENV2_8_LEFT	AGATCGAAGATGACATTTTCCGAAAGA
DENV2_8_RIGHT	CCCATGTATATGTACTGGTCATTTTCATT
DENV2_9_LEFT	ATGCCAGTGACCCACTCTAGTG
DENV2_9_RIGHT	CCACCACTGTGAGGATGGCTAT
DENV2_10_LEFT	ACCAGAAAAACAGAGAACACCCC
DENV2_10_RIGHT	CCACTTCCTGGATTCCACTTTTCT
DENV2_11_LEFT	GGAGCTGGACTTCTCTTTTCCAT
DENV2_11_RIGHT	GACGTCCCAAGGTTTTGTCAGC
DENV2_12_LEFT	AGAGCATGAAACATCATGGCACT
DENV2_12_RIGHT	GTGCCTCTTGGTGTTGGTCTTT
DENV2_13_LEFT	TGGGACACAAGAATCACACTAGAAG
DENV2_13_RIGHT	CCGCACCATTGGTCTTCTCTTT