Discovery of a persistent Zika virus lineage in Bahia, Brazil

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Abstract

Metagenomic next-generation sequencing coupled with capture probe enrichment was used to recover 11 whole and partial Zika virus (ZIKV) genomes from patients in Bahia, Brazil from April 2015 to January 2016, where the majority of suspected Brazilian ZIKV cases have been reported. Phylogenetic reconstructions and molecular clock analyses using the newly generated data uncovered the existence of a Bahia-specific ZIKV lineage sharing a common ancestor in mid-2014, indicating sustained circulation of this strain in Bahia since that date.

Introduction

Zika virus is an arthropod-borne RNA virus primarily transmitted by mosquitoes of the Aedes species, although transmission through blood transfusion and sexual contact has also been described (1). ZIKV can be classified in two genotypes: the African genotype, found only in the African continent, and the Asian genotype, associated with outbreaks in Southeast Asia, several Pacific islands and, more recently, in the Americas (2). In May of 2015, Brazil reported the first detection of autochthonous cases of ZIKV in the northeast of the country (3, 4). Since then, transmission has been confirmed in 22 of the 27 federal states (5).

The rapid geographic expansion of ZIKV transmission, together with its proposed association with microcephaly and congenital abnormalities (6), demand a rapid increase in

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molecular surveillance in the most affected areas. This is particularly relevant for regions
where other mosquito-borne viruses, particularly dengue virus (DENV) and chikungunya
virus (CHIKV), are known to co-circulate with ZIKV (2), as surveillance based on clinical
symptoms alone can lead to misdiagnosed cases. Accurate genetic characterization of
circulating strains can also facilitate determination of the origin and potential spread of ZIKV
infection in travelers returning from endemic countries. Previous analyses have suggested
that ZIKV was introduced in the Americas at least one year before its initial detection in
Brazil (1). Bahia state comprised 93% of suspected ZIKV cases in Brazil in 2015 (2), and
has reported cases of ZIKV-associated fetal microcephaly, although no genetic information
aside from one complete genome is available from the region to date (2, 7). Here we report
epidemiological findings obtained using 11 new complete and partial ZIKV genomes from
15 clinical cases seen at Hospital Aliança in Salvador, Bahia, between April 2015 and
January 2016.

**Materials and Methods**

Symptomatic patients were diagnosed with acute ZIKV infection by positive
qualitative RT-PCR testing from serum using primers targeting the NS5 gene (8).
Metagenomic next-generation sequencing (NGS) libraries were constructed following
DNase treatment of nucleic acid extracts as previously described (9, 10), followed by
pathogen detection using the SURPI bioinformatics pipeline (11). Clinical samples with
titers exceeding $10^4$ copies per mL by ZIKV quantitative RT-PCR using the QuantiTect
SYBR Green RT-PCR kit (Qiagen) and primers targeting the fiber gene (12) produced
sufficient metagenomic data to permit complete ZIKV genome assembly. For the remaining
samples at lower titers, metagenomic NGS libraries were enriched for ZIKV sequences
using a set of 299 XGen biotinylated lockdown capture probes (IDT Technologies)
designed to tile across all ZIKV genomes (>10 kB) in the National Center for Biotechnology
Information (NCBI) GenBank database as of February 2016, and curated for redundancy at a 99% similarity cutoff. Enrichment was performed using the XGen lockdown protocol and SeqCap EZ Hybridization and Wash Kit (Roche Molecular Systems) according to the manufacturer's instructions.

Using the MAFFT program, the 11 sequences from Bahia (GenBank accession numbers KU0940224, KU0940227, KU0940228, and KX101060-KX101067) were aligned together with all published and available near-complete ZIKV genomes and longer sub-genomic regions (>1500nt) of the Asian genotype as of April of 2016. Both maximum likelihood (ML) and Bayesian phylogenetic inferences were performed using PhyML and BEASTv1.8.2 programs, respectively. The ML phylogeny was reconstructed using the general time reversible nucleotide substitution model with a proportion of invariant sites (GTR+I). Statistical support for phylogenetic nodes was assessed using a bootstrap approach (with 100 replicates). A Bayesian molecular clock phylogeny was estimated using the best fitting evolutionary model determined in Faria, et al (1); specifically, a strict molecular clock GTR+I substitution model, a Bayesian skyline coalescent prior and a non-informative continuous time Markov chain (CTMC) reference prior for the molecular clock rate.

Results

In total, 11 ZIKV partial and complete genome sequences were recovered, with average coverage of 69.4 ± 2.0% and coverage ranging from 40 - 100% (Table 1). Nearly all isolates from Bahia (11 out of 12) clustered together within a single strongly supported clade (posterior probability 1.00, bootstrap support 96%, Clade C, Figure 1). This support for the Bahia clade is notable given that the majority of ZIKV genomes are incomplete, and this uncertainty is accounted for in the phylogenetic inference. The tree topology obtained is in accordance with previous findings (1,4,6) and the time to the most recent common
ancestor (TRMCA) of the epidemic in the Americas is similar to that previously estimated
(1) (Clade A, Figure 1). Our analyses using the new genomes generated here indicate that
ZIKV was introduced in Bahia between March and September 2014. An isolate from
Maranhão, northeast Brazil, located 1000 km from Bahia, is ancestral to the Bahia clade
(bootstrap support 66%, posterior probability 1.00, Clade B, Figure 1). The TRMCA of this
northeast Brazil clade (comprising the Bahia clade and the Maranhão sequence) is
estimated to be between September 2013 and April 2014, at a very early stage of the
epidemic, strengthening the evidence for the hypothesis that ZIKV in the Americas
originated from Brazil (1). One previously reported sequence from Bahia (7) clustered
(posterior probability=0.99) with an isolate from Belém, Pará state in northern Brazil, which
is 3000 km from Bahia. This may represent a separate introduction of ZIKV to Bahia state,
as the patient had no history of traveling abroad and denied any sexual contact.

Conclusions
Our results suggest an early introduction and presence (mid-2014) of ZIKV in the
state of Bahia, Brazil. The size and statistical support for this cluster make it very likely that
this lineage represents a large and sustained chain of transmission within the state. The
majority of the cases of the Bahia-specific ZIKV lineage clustered closely to a sequence
from Maranhão, and we found evidence for an additional autochthonous transmission event
to Bahia from Pará state. Thus, ZIKV in Bahia in mid-2014 is likely to have been introduced
from other regions in Brazil rather than from outside the country. The emergence of ZIKV in
Bahia state in mid-2014 reported here is consistent with viral infection in pregnant women
during the first trimester corresponding to the initial cases of fetal microcephaly reported in
Bahia beginning in January 2015 (13), although the peak period of microcephaly did not
occur until November of that year.
We cannot yet determine whether the lineage (clade C) identified here comprises the majority of ZIKV cases in Bahia, or, alternatively, whether multiple genetically distinct lineages co-circulate in the state. Brazil currently faces a major public health challenge with co-circulation of ZIKV, DENV, and CHIKV throughout the country (2-4, 14, 15). Additional molecular surveillance in the Americas and beyond is urgently needed to trace and predict the transmission of ZIKV virus.

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Conflicts of Interest

C.Y.C. is the director of the UCSF-Abbott Viral Diagnostics and Discovery Center and receives research support from Abbott Laboratories, Inc. and Metabiota, Inc. The other authors disclose no conflicts of interest.
References

Table 1. Clinical sample information from patients with acute ZIKV infection.

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Isolate</th>
<th>Patient</th>
<th>Geographical location</th>
<th>ZIKV qRT-PCR cycle threshold</th>
<th>Viral Load (copies/mL)</th>
<th>Collection date</th>
<th>% Genome Recovery (Coverage)</th>
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<tr>
<td>KX101066</td>
<td>Bahia01</td>
<td>Female, 72 years old</td>
<td>Brazil: Bahia, Salvador</td>
<td>34.6</td>
<td>1,042</td>
<td>16-May-2015</td>
<td>84.66</td>
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<td>KX101060</td>
<td>Bahia02</td>
<td>Male, 37 years old</td>
<td>Brazil: Bahia, Salvador</td>
<td>32.5</td>
<td>4,086</td>
<td>5-May-2015</td>
<td>74.22</td>
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<tr>
<td>KX101061</td>
<td>Bahia03</td>
<td>Male, 35 years old</td>
<td>Brazil: Bahia, Salvador</td>
<td>32.8</td>
<td>3,272</td>
<td>5-May-2015</td>
<td>77.25</td>
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<tr>
<td>KX101062</td>
<td>Bahia04</td>
<td>Male, 40 years old</td>
<td>Brazil: Bahia, Salvador</td>
<td>34.1</td>
<td>1,464</td>
<td>1-Jun-2015</td>
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<tr>
<td>KX101063</td>
<td>Bahia05</td>
<td>Male, no data</td>
<td>Brazil: Bahia, Camaçari</td>
<td>33.7</td>
<td>1,901</td>
<td>10-Dec-2015</td>
<td>42.52</td>
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<td>KU940228</td>
<td>Bahia07</td>
<td>Female, 37 years old</td>
<td>Brazil: Bahia, Salvador</td>
<td>13.7</td>
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<td>29-Aug-2015</td>
<td>100</td>
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<td>KU940227</td>
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<td>33.3</td>
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<td>KU940224</td>
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<td>–</td>
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<td>Bahia12</td>
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<td>Brazil: Bahia, Salvador</td>
<td>34.2</td>
<td>1,327</td>
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<td>Bahia15</td>
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<td>–</td>
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<td>25-Jan-2016</td>
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Figure 1. Timescale of the ZIKV outbreak in the Americas. A molecular clock phylogeny is shown of the ZIKV outbreak lineage estimated from complete and partial (>1500nt) coding region sequences. For visual clarity, five basal Southeast Asia sequences, HQ23499 (Malaysia, 1966), EU545988 (Micronesia, 2007), KU681082 (Philippines, 2012), JN860885 (Cambodia, 2010) and KU681081 (Thailand, 2013) are not displayed. Blue horizontal bars represent 95% Bayesian credible intervals for divergence dates. A, B and C denote clades discussed in main text and numbers next to them denote posterior probabilities and bootstrap scores (in %). Circle sizes represent, at each node, the posterior probability support of that node. Taxa are labeled with accession number, sampling location, and sampling date. Names of sequences generated in this study are in bold. The inset graph on the left shows the posterior distributions of the estimated ages (TMRCAs) of clades A, B and C, estimated in BEAST using the best fitting evolutionary model.