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Was Zika introduced to Brazil by participants at the 2013 Beach Soccer World Cup held in Tahiti: a phylogeographical analysis

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Abstract

Background

Zika virus (ZIKV) was initially responsible for a limited number of punctual epidemics throughout Africa and Asia. Recently, large epidemics occurred in French Polynesia, Brazil and Pan-America. These outbreaks were associated with severe outcomes such as Guillain-Barré Syndrom and microcephaly of *in-utero* infected newborns. Previous studies demonstrated that ZIKV was introduced in Brazil from French Polynesia but failed to identify a founding event.

Method

All publicly available ZIKV full-genome sequences (n=182) were phylogenetically analyzed, using Bayesian method, to estimate the introduction date of ZIKV into Brazil.

Results

Introduction date into Brazil was estimated between 8th of July 2013 and 4th of November 2013, encompassing the Beach Soccer World Cup held in French Polynesia, in September 2013, which gathered Brazilian athletes and supporters. We also observed that ZIKV sequences from travelers infected in South-East Asia or in Pacific islands were closely related to viruses identified prior to the French Polynesian epidemic, underlining an endemic circulation of ZIKV in those countries since 2007, at least.

Conclusion

This work provides a narrower estimation of ZIKV introduction into Brazil and illustrates the need for a better exploration of ZIKV circulation and endemicity in South-East Asia, while epidemiological and prevention efforts have been mainly focused on the Pan-American epidemic.

Introduction

Zika virus (ZIKV) is an arthropod-borne virus (arbovirus) isolated for the first time in 1947 from a febrile rhesus monkey infected near the Zika forest in Uganda [1]. It is now classified into two genotypes: the old African lineage and the Asian lineage [2]. ZIKV infections are mainly asymptomatic or mild with patients displaying fever, maculopapular rash, myalgia, arthralgia and conjunctivitis [3]. ZIKV was initially responsible for a limited number of short epidemics throughout Africa and Asia, mainly documented retrospectively through serological surveys ¹. Due to the important cross-reactivity of serological assays with antibodies directed against other *Flavivirus*, these results must be interpreted with caution but suggest that ZIKV was endemic in numerous sub-Saharan countries during the second half of the 20th century and spread to several Asian countries where it was responsible of local outbreaks [3,4].

In 2007, a first large outbreak of “dengue-like illness” occurred on Yap Island, in the Federated States of Micronesia [5]. Serological and molecular analyses identified ZIKV as the cause of this outbreak with an estimation of almost 75% of infected-inhabitants. In October 2013, the first cases of ZIKV infection were reported in French Polynesia. As in Yap Island, the epidemic flared, infecting up to 49% of the general population and 66% of schoolchildren [6]. From French Polynesia, ZIKV has spread to other Pacific countries (e.g. New Caledonia, Solomon Islands, Fiji, Samoa) and also to Brazil where it was responsible for a massive and severe outbreak which fueled the current epidemic across all South and Central America [7]. During these latest outbreaks, severe complications have been described, including severe thrombocytopenia sometimes leading to deaths [8,9], Guillain-Barré Syndroms (GBS) in French Polynesia [5] and microcephaly cases in South America [10–12]. Since then, imported-cases have regularly been described in North America, Europe or Asia, underlying the threat of new epidemics in countries with competent vectors.

It is well established that ZIKV was introduced in Brazil from French Polynesia [13]. However, the circumstances of this introduction remain unknown [13,14]. Two sport events, the Confederation Cup (June 2013) and the Va'a Championship (August 2014), were evoked but ruled out by phylogenetic analyses estimating the introduction time of ZIKV into Brazil between August 2013 and April 2014 [15].

As additional sequence data is available since this study, we performed new phylogeographical analyses to investigate recent ZIKV evolution, refine the introduction date of ZIKV into Brazil and assess the potential role of all international sport events held in French Polynesia and Brazil.

Material and methods

Sequences database

All ZIKV full genome sequences available on the 1st of March 2017 were retrieved from GenBank database (<http://www.ncbi.nlm.nih.gov/genbank>). We annotated all sequences with collection date and sampling location, using data directly available in GenBank or in related publications. Poor quality, clonal or iterative sequences were discarded resulting in a dataset of 209 full genomes. As we focused on the Asian lineage's evolution, African lineage sequences (n = 27) were filtered, resulting in a final dataset of 182 geographically-annotated ZIKV sequences.

Phylogenetic analyses

Sequences were aligned using Clustal omega 1.2.4⁹ and Muscle [17], then manually curated using AliView 1.18 [18]. The presence of recombinant forms was assessed using RDP4 software [19]. The best fitting nucleotide substitution model was identified, using jModelTest v2.1.10 [20], as the general time reversible (GTR) model with a gamma distribution (n=4). A phylogenetic tree was inferred by approximate maximum of likelihood using FastTree 2.1 [21]. Bayesian phylogenies were estimated using BEAST 1.8.4 [22], with different coalescent models (Gaussian Markov Random Field (GMRF) Bayesian skyride coalescent, Skyline and Skygrid) and under strict or relaxed molecular clock models. Those Bayesian MCMC analyses were run for 100 million generations sampling every 10,000th, using the maximum likelihood phylogeny tree previously generated as starting tree. Model comparisons were performed based on the Bayes Factors and a model was retained as fitting the data best when the Bayes factor was above 5.

Effective Sampling Size (ESS) for all estimated parameters were explored in Tracer and were above 200, as recommended. After discarding 10% burn-in, log files were combined using LogCombiner. The maximum clade credibility tree (MCCT) generated with TreeAnnotator was then edited in FigTree v1.4.3 (<http://tree.bio.ed.ac.uk/software/figtree/>).

Results

To explore ZIKV epidemiology, we performed a phylogeographical analysis on 182 available ZIKV sequences belonging to the Asian lineage. No recombinant viruses were detected. The best-fit model combination was found to be the Skyline demographic model with a relaxed molecular clock. Under this model, we found an estimated nucleotide substitution rate of 1.114×10^{-3} substitutions/site/year (95% Higher Probability Densities (95HPD) = 9.93×10^{-4} - 1.254×10^{-3}). The Brazilian clade was monophyletic, as in previous studies and in favor of a single successful introduction of ZIKV in Brazil from French Polynesia (country probability = 1.0) [15,23] (Figure 1).

To determine the introduction date of ZIKV in Brazil, we estimated the time of the most recent common ancestor (tMRCA) of two nodes, A and B. Node A encompasses all South American sequences and the two closest French Polynesian sequences (GenBank accession numbers: KX447518 and KX447510). The tMRCA of this node represents the divergence time of French Polynesian and Brazilian sequences and, thus, the earliest possible date of ZIKV introduction to Brazil. Node B includes only South American sequences and represents the beginning of ZIKV expansion in Brazil and, thus, the latest possible date of ZIKV introduction to Brazil. Those two clades were well-supported (posterior probability = 1.0 for both) (Figure 1). Median tMRCA of nodes A and B are the 13rd of September 2013 (95HPD = 8th of July 2013-4th of November 2013) and the 1st of January 2014 (95HPD = 12th of October 2013-21st of March 2014), respectively (Figure 2). Thus, the ZIKV transmission from French Polynesia to Brazil may have occurred at any time point between the 8th of July 2013 and the 21st of March 2014.

Posterior probability densities of the tMRCA of those two nodes are shown in Figure 2, along with dates of sport events suspected to have led to the transmission of ZIKV to Brazil: Confederation Cup (15th-30th June 2013; I), Football World Cup (12th June-13th July 2014; II), Va'a

Championship Sprint Canoe Race (14th-18th August 2014; III) and Beach Soccer World Cup (18th-28th September 2013; IV). The Football World Cup and the Va'a Championship Sprint Canoe Race were held months after the estimated introduction time interval, while the Confederation Cup was hosted a few days before the estimated 95HPD. Only the Beach Soccer World Cup, held in Tahiti between the 18th and the 28th of September 2013, fitted the time distribution of estimated 95HPD of nodes A and B.

During this phylogeographical analysis, we also observed that seven ZIKV strains isolated recently, since 2013, presented phylogenetic branches deeply connected with ZIKV strains circulating in South-East Asia prior to the French Polynesian outbreak (posterior probabilities > 0.9 for all). These strains were isolated from 2 Singaporean patients in late 2016 [24], 3 Thai inhabitants between 2013 and 2016, and from 2 travelers returning from Thailand in 2013 and 2016 [25,26]. Those recent strains were interspersed among older isolates from South East Asia and the median tMRCA estimates of the nodes encompassing those sequences ranged from 2007 to 2012.

Discussion

In this study, we provided an updated phylogenetic reconstruction of ZIKV spreading in Asia and South America, allowing a dating of the ZIKV introduction period into Brazil with narrower confidence intervals.

With an updated sequence dataset, we estimated ZIKV introduction in Brazil between July 2013 and March 2014. Our confidence interval differs slightly from previous evaluations done by Faria et al. [23], Metsky et al. [27] and Pettersson et al. [28] (October 2013 to April 2014, August 2013 to July 2014 and October 2013 to February 2014, respectively). Differences can be largely explained by the definitions retained in each study for inferring the introduction date. Indeed, they have all estimated the introduction date as the tMRCA of the Brazilian clade [23,27,28] and a similar value was obtained in our work for this particular tMRCA (October 2013 to March 2014). In phylogenetics, the tMRCA of a clade represents the origin of a clade diversification and, for infectious diseases, it is a good estimate of the onset of a local outbreak, while the tMRCA of the node immediately preceding the node of the Brazilian clade (i.e. node A in this study) can be used to define the divergence time between French Polynesian and Brazilian sequences [29]. Thus, this latter tMRCA can be indicative of the initial introduction time before a delayed large diversification due to a massive outbreak, notably driven by seasonality of mosquito-borne diseases, as demonstrated by Faria et al. [23]. In this study, Faria et al. demonstrated the existence of long periods of ZIKA pre-detection cryptic transmission in recipient regions within South America. In such scenario, the sole use of the tMRCA of the Brazilian clade may be slightly biased and indicate the time of such massive outbreak beginning instead of the proper introduction time. Thus, these two tMRCA should be combined to better define the most probable introduction date interval. In our work, using those two tMRCA, we defined a new and slightly larger interval for ZIKV introduction into Brazil, ranging from July 2013 to March 2014.

With this new interval, and as previously published [15], the Football World Cup and the Va'a Championship were both out of the estimated time span of ZIKV introduction into Brazil. The Confederation Cup was hosted at a date slightly outside our confidence interval but cannot be entirely ruled out, due to the stochastic nature of a phylogeographical analysis. The previously uninvestigated Beach Soccer World Cup was held in Tahiti between the 18th and the 28th of September 2013, in a timeframe compatible with ZIKV introduction into Brazil and at a time showing the highest probability density for the divergence time between Polynesian and Brazilian ZIKV lineages (Figure 2). This event gathered more than 100,000 spectators and 16 national teams, including Brazil and French Polynesia. The two teams were present during all the competition and faced during the third-place play-off. To our knowledge, this event was the only mass-gathering event reuniting Brazilian and French Polynesian inhabitants at a date within the time span estimated in our phylogenetic reconstruction. During this competition, ZIKV was already circulating in French Polynesia and no prevention measures had been implemented yet as ZIKV epidemic remained undetected in French Polynesia until early October 2013 [28,30].

Interestingly, we also observed that imported-cases reported in Canada [26] and China (Genbank accession number KY328290), as well as autochthonous cases in Singapore and Thailand [24,31], all post-2013, were phylogenetically deeply connected with different Asian strains and their most recent common ancestors. The time of their MRCA were estimated from 2007 up to 2012. This ruled out a hypothetic infection of these patients by strains originating from Pacific islands or South American countries. Furthermore, those sequences were interspersed among South East Asian isolates, independently of their sampling year, and did not cluster altogether, suggesting that ZIKV has been evolving separately in several locations across South East Asia. Very few studies focused on ZIKV transmission in Asia. Indeed, despite the hypothesis that ZIKV might have spread largely in Asia, its circulation has only been documented in Vietnam in 2016 [32] in Cambodia between 2007 and 2016 [33], in Singapore in 2016 [24,34], and in Thailand from 2012

to 2014 [31]. More recently, a study also suggested an undetected endemic circulation of ZIKV in Thailand since 2002 [35]. The endemic circulation of ZIKV in these Asian countries needs to be confirmed and evaluated to assess the health risk for travelers [36] and the potential for transmission to neighboring countries with competent vectors [37].

Our study presents some limitations. Firstly, if this work highlights that the Beach Soccer World Cup is concomitant with the introduction of ZIKV in Brasil, we cannot rule out an introduction through routine human travels by a single traveler, as previously hypothesized [15]. Indeed, it is often thought that large-scale patterns in human mobility can provide more testable hypotheses about viral introduction and emergence than ad hoc hypotheses focused on specific events. However, as large-scale events are prone to facilitate outbreaks of infectious diseases [38] and as our estimated time of ZIKV introduction into Brazil corresponds sharply to the time of the Beach Soccer World Cup, we believe that the possibility that this event triggered ZIKV introduction into Brazil should be taken into account. It might be important to keep this potential risk in mind when deciding whether to implement prevention measures against arboviral infections in future mass-gathering events. Another limitation of our study is that the low number of available sequences from Asian regions may limit the accuracy of tMRCA for the deeper nodes of the tree. However, it does not alter our observation of distinct ZIKV strains in several Asian countries suggesting a largely unexplored and continuous circulation of ZIKV in Asia since 2007. This finding is consistent with the recent description of ZIKV endemic circulation in Thailand [35].

Conclusion

In this work, we suggest the Beach Soccer Worldcup held in Tahiti in September 2013 as a potential trigger of ZIKV introduction into Brazil. We also observed recent ZIKV cases phylogenetically connected to ancestors older than the South America epidemic and suggesting the endemic circulation of ZIKV in Asia. Identifying ZIKV-endemic Asian countries is of peculiar importance as most South-East Asian countries are already endemic for dengue virus. Due to the similarity of clinical symptoms, technical hurdles for diagnosis and, probably, low incidence in these countries, ZIKV circulation may remain undetected. Specific epidemiological efforts should be exerted to identify ZIKV-endemic countries, to better understand ZIKV circulation and to limit diffusion to neighboring regions with competent vectors.

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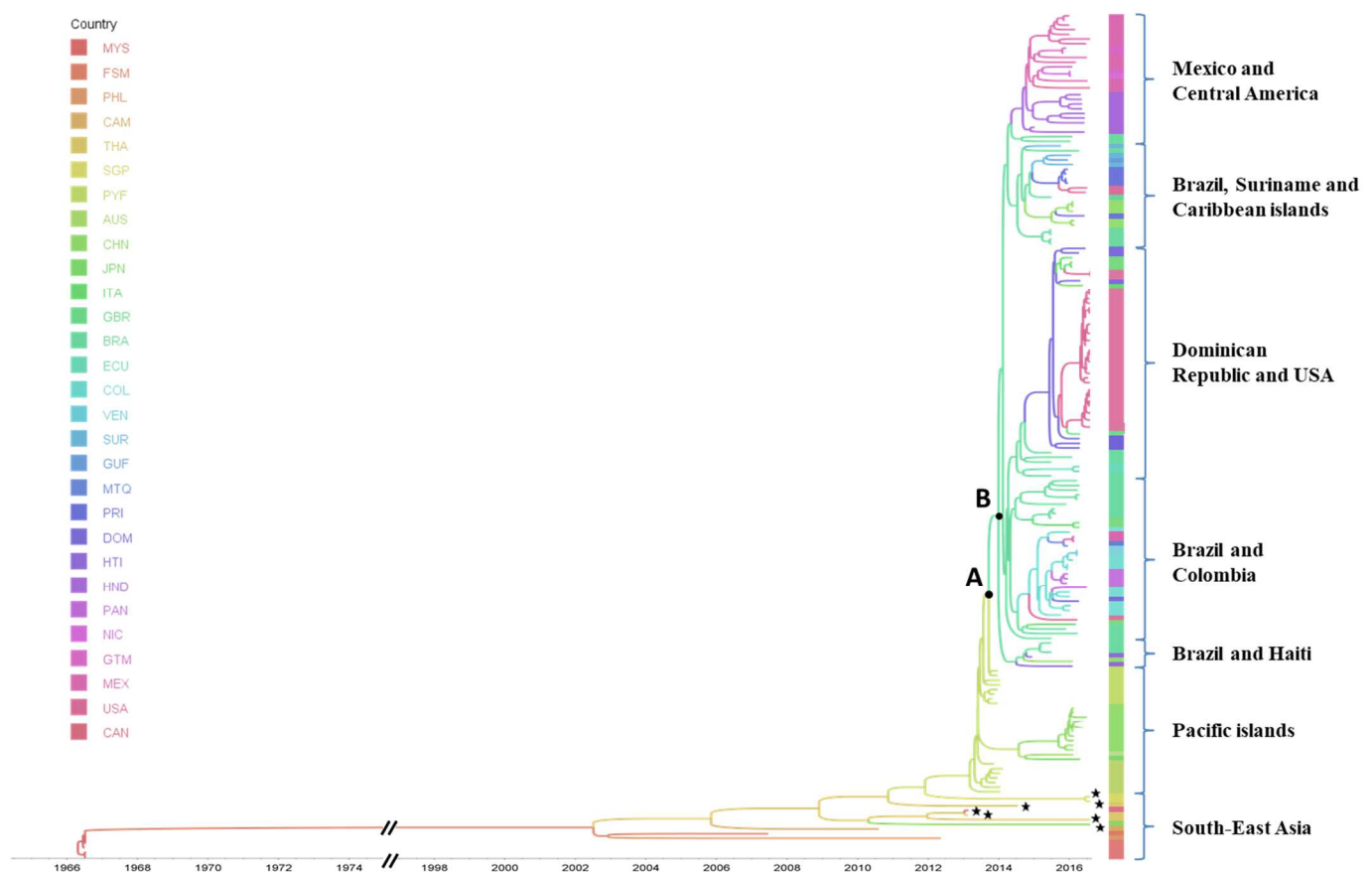


Figure 1: Phylogeography of Zika virus' Asian lineage

In this time-scaled maximum clade credibility tree, branches and nodes are colored according to the country and the most probable country of the common ancestor, respectively. The node encompassing the Brazilian clade and the two closest French Polynesian sequences (Genbank accession numbers KX447518 and KX447510) is indicated with the letter A and the one including only sequences from the Brazilian epidemic is indicated with the letter B (posterior probability = 1.0 for both). The seven ZIKV sequences obtained from South-East Asian isolates since 2013 are indicated with an asterisk.

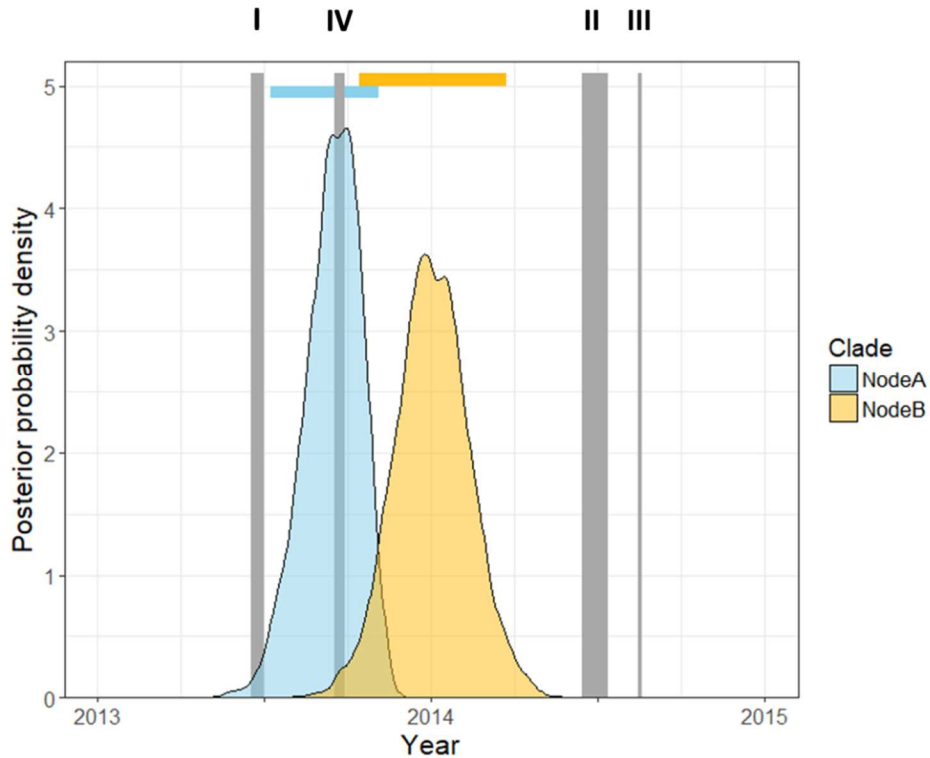


Figure 2: Posterior probability densities of the estimated ages of the most recent common ancestors (tMRCA) of nodes A and B.

Node A corresponds to the node encompassing all sequences from the Brazilian clade and the two closest French Polynesian sequences (Genbank accession numbers KX447518 and KX447510) and node B to the node of the Brazilian clade. We plotted the posterior probability densities of the tMRCA of nodes A and B (in blue and yellow, respectively), estimated with Beast v1.8.4, under a unrelaxed molecular clock and a Skyline demographic model. 95HPD of those two nodes are depicted as colored boxes above the posterior probability densities. The retained introduction interval of ZIKV in Brazil corresponds to the time interval encompassing both 95HPD (i.e. 8th of July 2013 to 21st of March 2014). Gray boxes indicate dates of mass gathering events which have rallied participants from both French Polynesia and Brazil: Confederation Cup (15th-30th June 2013; I), Football World Cup (12th June-13th July 2014; II), Va'a Championship Sprint Canoe Race (14th-18th August 2014; III) and the Beach Soccer World Cup (18th-28th September 2013; IV).