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Utilizing phylogenetic analysis to understand Zika virus dissemination in Ecuador

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Abstract

Phylogenetic analysis is an indispensable tool for public health, especially in developing countries. By utilizing this method, developing countries can bolster surveillance efforts, and accelerate outbreak responses. The Zika virus outbreak in the Americas, including Ecuador, underscored the threat of an often underestimated pathogen. For years, the virus's dangers were largely overlooked, but the emergence of neurological complications in newborns and immune disorders like Guillain-Barré Syndrome demonstrated its potential global impact. This study aims to explore the dissemination patterns of the 2015 Zika outbreak in the Americas, with a particular focus on its effects in Ecuador.

Methods: We utilized 24 representative sequences from Africa, Asia, the Pacific, and the Americas, including two from Ecuador, for phylogenetic and lineage analysis. Additionally, using virus transmission and vector distribution maps, we determined the origin and distribution of outbreaks as well as associated risk factors.

Results: Phylogenetic and lineage analysis revealed that Ecuadorian sequences were closely related to other American sequences, forming a homogeneous group distinct from African, Asian, and Pacific island sequences. The findings indicated that the probable origin of the Zika virus spread to Ecuador was from Asia via Brazil, which appears to be the key point for the dissemination of the Zika virus throughout the Americas, including Ecuador.

Keywords: Ecuador; Zika virus; Outbreak; Phylogenetics; Public Health

1. Introduction

The Zika virus, a member of the Flaviviridae family, is a positive-strand RNA virus characterized by an icosahedral capsid of 50 nm and a single-stranded RNA genome of 11 Kb (Weaver *et al.,* 2016; Hou *et al.,* 2017). Its genome consists of a single open reading frame (ORF) flanked by two untranslated regions (UTRs), one long at the 3' end and one short at the 5' end. The ORF encodes a single polyprotein of 3424 amino acids, which is processed into seven non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) and three structural proteins (C, PrM, and E) (Faye *et al.,* 2013). While the structural proteins form the viral particle, the non-structural proteins carry out essential functions. Phylogenetic studies have shown that Zika can be differentiated into two lineages: African and Asian. These studies also reveal a great similarity to other flaviviruses such as Dengue and West Nile virus (Lanciotti *et al.,* 2008; Wang L. *et al.,* 2016).

The virus was first detected in Rhesus monkeys in Uganda 70 years ago. For sixty years, it was confined to Africa and Asia until an outbreak occurred in the Yap Islands in 2007 (Weaver *et al.,* 2016). In 2013, new outbreaks occurred in French Polynesia and other Pacific islands. In 2015, it began to spread in the Americas, with the first cases reported in Brazil's Bahia state in May 2015. Subsequently, the virus spread rapidly throughout South and Central America (He *et al.,* 2017). By November 2016, 48 countries, including several in the Americas, reported autochthonous cases of vector-

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borne infections. In the **United** States and its federal districts, 4,444 cases of Zika were confirmed by November 2016 according to ArboNET reports (Song *et al.,* 2017). In Ecuador, by epidemiological week 23 of 2017, 1,646 cases were reported, with 965 confirmed by laboratory tests and 681 by epidemiological link (National Health Surveillance Subsecretariat, 2017).

Zika virus is primarily transmitted by the bite of vector mosquitoes, although perinatal, sexual, and transfusion transmission have also been documented. A systematic review cited three cases of transmission through breast milk; however, the specific mechanisms remain unknown (Colt *et al.,* 2017). The primary vector associated with its transmission is the Aedes mosquito, but other vectors such as Mansonia, Anopheles, and Culex have also been implicated (Shragai *et al.,* 2017). The virus has two transmission cycles: sylvatic and urban/suburban (Baden *et al.,* 2016). In the sylvatic cycle, both primates and arthropods are involved, with *A. Africanus* being the main vector in Africa. In the urban cycle, *Aedes aegypti*, which has a global distribution, is the most representative vector (Shragai *et al.,* 2017). Other primary vectors detected in Africa include *Aedes africanus, Aedes apicoargenteus, Aedes luteocephalus, Aedes furcifer, Aedes taylori, and Aedes vittatus* (Chouin-Carneiro *et al.,* 2016).

2. Clinical Picture

Approximately 70% of Zika virus cases are asymptomatic (WHO, 2016). When symptoms do occur, they resemble a flu-like illness with fever, headache, arthralgia, and conjunctivitis (Lazear and Diamond, 2016). Zika infection has also been linked to severe neurological and autoimmune disorders, including a significantly increased risk of microcephaly and other congenital abnormalities in infants born to infected mothers, as well as an increase in Guillain-Barré Syndrome (Shragai *et al.,* 2017). Other conditions associated with Zika include arthrogryposis, hip dislocation, seizures, and hypotonia, collectively known as Congenital Zika Syndrome (Alvarado *et al.,* 2016). The Brazilian strain of Zika appears more aggressive than other strains, including the original one. Strains such as H/PF/2013 and FB-GWUH-2016 have also been implicated in neurogenesis disruption (Russo, Jungmann, and Beltrão-Braga, 2017). One possible explanation is antibody-dependent enhancement, where prior infection with another flavivirus, like Dengue, increases the severity of Zika infection through immune modulation (Bardina *et al.,* 2017).

The first symptomatic cases of Zika in humans were recorded in 1954 during a jaundice outbreak in Nigeria, where infection was confirmed in three patients (Macnamara, 1954). Until the early 21st century, only a few benign cases of Zika had been reported in Africa and Asia. In 2007, the first significant outbreak outside these regions occurred in the Yap Islands, with mild symptoms in about 73% of the 6,892 residents. Sequence analysis suggested the infection originated from Southeast Asia. In 2013, a new outbreak occurred in French Polynesia, affecting around 11% of the population and coinciding with multiple Guillain-Barré Syndrome cases (Nugent *et al.,* 2017).

3. Methods

- **Sequences:** All sequences were obtained from GenBank NCBI (http://www.ncbi.nlm.nih.gov/Genbank/). Twenty-four representative sequences from the Americas, Micronesia, Asia, and Africa were analyzed: Ecuador (KX879604.1, KX879603.1), Peru (KY693678.1, KY693679.1), Colombia (KX548902.1, KY785466.1), Brazil (KU729218.1, KX280026.1), Venezuela (KX702400.1, KY693680.1), Suriname (KY348640.1, KU937936.1), Malaysia (HQ234499.1), Micronesia (EU545988.1), Nigeria (HQ234500.1), Uganda (HQ234498.1), Senegal (KF383119.1), Panama (KX198135.2, KX156774.2), Mexico (KX247632.1, KY606274.1), United States (KX922707.1, KY075936.1). The Flavivirus Spondweni virus sequence (NC_029055.1) was included as an outgroup.
- **Lineage Analysis:** Viral sequences were analyzed in FASTA format using the Genome Detective Virus Typing Tool (https://www.genomedetective.com/app/typingtool/virus/). This automated platform assigns Zika virus sequences to African or Asian lineages based on phylogenetic similarity to reference strains.
- **Phylogenetic Analysis:** The sequences were aligned using MEGA 7 (Kumar, Stecher, and Tamura, 2016) and analyzed using JModeltest (Darriba *et al.,* 2012) to determine the evolutionary model (GTR+I according to BIC). Maximum likelihood trees were obtained using RaxML (Stamatakis, 2014), and Bayesian analysis was performed using MrBayes (Ronquist and Huelsenbeck, 2003). Both programs were used within the Mobyle Snap Workbench server (Monacell and Carbone, 2014).

4. Results

- **Phylogenetic Analysis:** In figure 1, the phylogeny obtained through the maximum likelihood method is shown, and bootstrap values have been added to its branches. The analysis of sequences involved in the Americas

outbreak was closer to sequences from Micronesia and Malaysia than to those from Africa (Nigeria, Uganda, and Senegal). The lineage study with the biofábrica tool showed that all American sequences belonged to the Asian lineage. Similar results were found in the Pacific islands where the sequences also corresponded to the Asian lineage (see table 1), confirming the hypothesis that the Americas outbreak originated from previous outbreaks in those islands. On the other hand, strains isolated in Uganda and Nigeria seventy and fifty years ago and a sample isolated in Senegal in 2001 belonged to the African lineage. Additionally, table 1 presents information obtained through the biofábrica program with lineage results and the genome region that constitutes each sequence. In this table, it can be observed that all sequences used represented the same genome region; only a small area corresponding to the UTR and non-structural protein NS5 was not represented (colored in light blue).

Table 1 Accession codes of sequences, lineage, and genomic representation. The table shows the accession code, country, lineage, and year of the sequences used in the work, except for the sequence used as the outgroup

Accession Code	Country	Lineage	Year Registration
KX879604.1	Ecuador	Asian	2017
KX879603.1	Ecuador	Asian	2017
KY693678.1	Peru	Asian	2017
KY693679.1	Peru	Asian	2017
KX548902.1	Colombia	Asian	2016
KY785466.1	Colombia	Asian	2017
KU729218.1	Brazil	Asian	2016
KX280026.1	Brazil	Asian	2015
KX702400.1	Venezuela	Asian	2016
KY693680.1	Venezuela	Asian	2016
KU937936.1	Suriname	Asian	2016
KY348640.1	Suriname	Asian	2016
HQ234499.1	Malaysia	Asian	1966
EU545988.1	Micronesia	Asian	2007
HQ234500.1	Nigeria	African	1968
HQ234498.1	Uganda	African	1947
KF383119.1	Senegal	African	2001
KX198135.2	Panama	Asian	2016
KX156774.2	Panama	Asian	2015
KX247632.1	Mexico	Asian	2015
KY606274.1	Mexico	Asian	2016
KX922707.1	United States	Asian	2016
KY075936.1	United States	Asian	2016

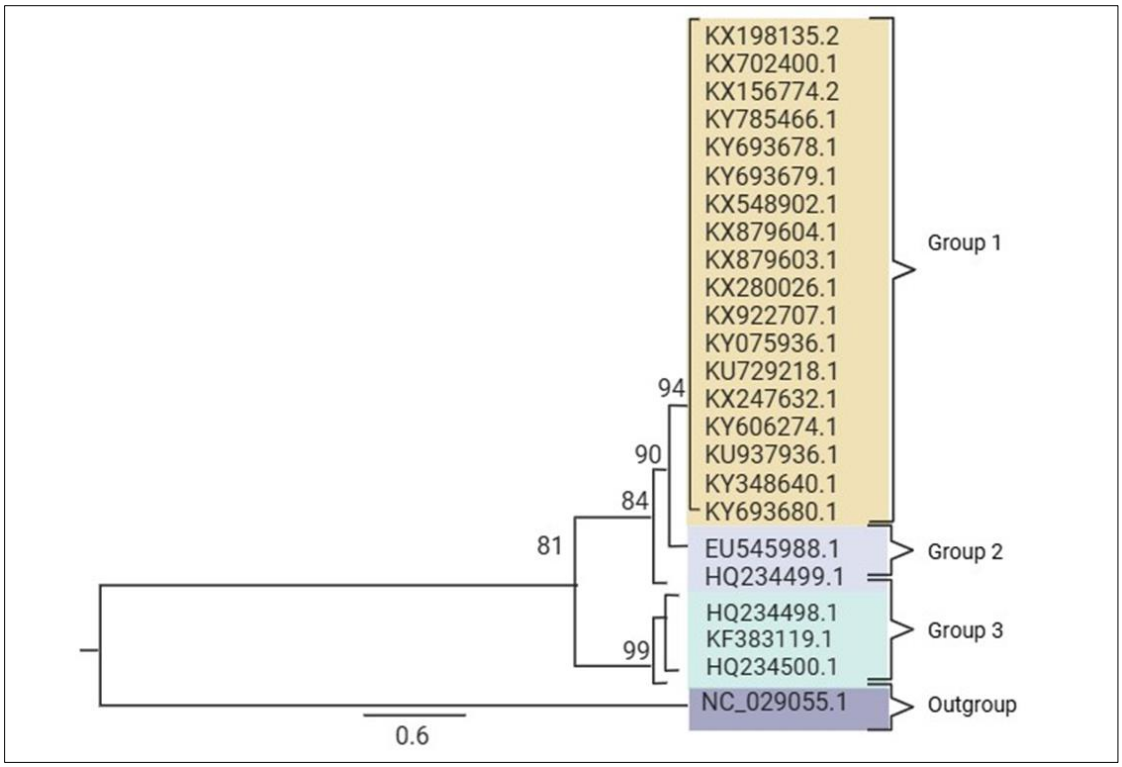

Figure 1 Maximum likelihood tree derived from Zika virus sequences of various origins. Bootstrap support values are indicated on the corresponding branches. Sequences from the Americas region are represented in Group 1, sequences from Micronesia and Malaysia are represented in Group 2, and sequences from African countries are represented in Group 3. The outgroup sequence, NC_029055.1, is represented separately. Each sequence is labeled with its GenBank accession number. The scale bar indicates the number of substitutions per site

5. Discussion

The significance of phylogenetic analysis lies in its ability to trace the dissemination patterns of the Zika virus. Through the analysis of specific sequences, it was possible to identify not only the spread within the Americas but also its origins in Micronesia and the Asian region. Transmission maps of the Zika virus indicate that the Asian lineage is the source of the outbreak in the Americas, including Ecuador. This outbreak is particularly notable due to the high number of affected individuals and its causal link to the emergence of immunological and neonatal diseases. Despite the predominance of the Asian lineage, the circulation of other lineages, such as the African lineage, has been reported in recent years. For instance, a study analyzing samples collected from febrile patients in Senegal and Nigeria between 1992 and 2016 found a seroprevalence of 6.2% for Zika, corresponding to the African lineage (Herrera *et al.,* 2017). This study demonstrated that the virus had been circulating in Africa for nearly two decades. Although the introduction of the Zika outbreak in the Americas is attributed to the Pacific islands, there remains some uncertainty regarding the exact transmission route to Brazil. Phylogenetic studies have suggested that some of the early samples originated from the Caribbean (Faria *et al*., 2016). Additionally, genomes collected from northwestern Brazil indicate that Zika had been circulating since late 2013 to early 2014, which is more than a year prior to the first official report (Faria *et al.,* 2017).

Another factor contributing to the rapid spread of the Zika virus in Latin America is the presence of the *Aedes aegypti* vector, which is highly prevalent in all regions where Zika has been reported. Additionally, other potential vectors such as *Aedes albopictus* have a broader global distribution, especially in Mediterranean and North American areas, further facilitating the virus's spread in regions where *Aedes aegypti* is absent (*Ciota et al.,* 2017). A study conducted in Singapore in 2013 highlighted *Aedes albopictus's* potential to transmit the virus and establish itself locally (Wong *et al.,* 2013). However, another study suggests that *Aedes aegypti* and Aedes albopictus have a low susceptibility to Zika infection, attributing the intensity of recent outbreaks to the high density of these vectors worldwide (Chouin-Carneiro *et al.,* 2016). Climatic conditions favorable to the Aedes vector may have also contributed to the explosive expansion of Zika in 2015. The El Niño phenomenon caused exceptional climatic changes in northwestern South America during the winter and spring (Paz and Semenza, 2016). According to the United States National Oceanic and Atmospheric Administration, temperatures in northern and eastern South America were the highest in recent years, accompanied by

a severe drought in the first half of 2015 (National Oceanic and Atmospheric Administration, 2015). Natural disasters, such as the 2016 earthquake in Ecuador, may have also triggered the virus's spread in the country; Manabí, the most affected region, reported the second-highest number of confirmed Zika cases (N=533) (National Health Surveillance Subsecretariat, 2017). A study conducted from January to July 2016 on 2,234 possible cases in both earthquake-affected areas and control areas with similar geographical characteristics and population density found an accumulated incidence of Zika infections of 11.1 per 100,000; the odds ratio of infected residents in affected areas was 8.0 (95% CI = 4.4-14.6; P < .01) (Vasquez *et al.,* 2017).

As previously mentioned, the significance of the outbreaks in the Americas and Caribbean lies in their expansion characteristics, emergence, and cellular tropism. One of the main characteristics of arboviruses is their high mutation rate, enabling them to express different phenotypic changes during their continuous evolution to adapt to different vectors and hosts. For instance, a single amino acid change in the Chikungunya virus's glycoprotein surface allows it to switch its vector to Aedes albopictus (Tsetsarkin *et al.,* 2007). In the case of flaviviruses such as Zika, an alanine to valine substitution at residue 188 of the NS1 protein determines an antigenic activity that promotes an increase in the virus's infectivity in the *Aedes aegypti* vector (Liu *et al.,* 2017). Samples from the most recent Zika outbreak in the Americas have shown to be more infectious in mosquitoes than the FSS13025 strain, previously isolated in Cambodia in 2010 (Liu *et al.,* 2017).

The latest Zika outbreak in the Americas resembles other arbovirus outbreaks such as Dengue and Chikungunya. The migration pattern associated with Zika is linked to its ability to adapt to urban vectors like *Aedes aegypti*, allowing its expansion into human environments (Fajardo, Cristina, and Moreno, 2016). Recent hypotheses propose that the genetic diversity of Zika could be responsible for its emergence, neurotropism, and expansion (Herrera *et al.,* 2017).

The alternation between human and arthropod hosts exerts selective pressure on the Zika virus population. Flaviviruses like Dengue have developed mechanisms to regulate the differentiation of non-coding RNA production in mosquitoes and humans, significantly impacting the virus's adaptability in both hosts (Khrustalev *et al.,* 2017). Nucleotide differences among the three Zika lineages suggest that genomic variants could enhance the virulence of the epidemic lineage by decreasing the number of points where RNA polymerase can be trapped during replication (Khrustalev et al., 2017). Another significant finding related to the Zika virus's virulence is the antibody-dependent enhancement (ADE) process; flaviviruses like Dengue or West Nile virus are phylogenetically related to Zika (Bardina *et al*., 2017). Studies have shown a cross-reaction of some Dengue antibodies with Zika at fixed concentrations using in vitro systems (Dejnirattisai *et al.,* 2016). A wide variability in the binding activity of the Zika E protein was detected for both Dengue and West Nile viruses in immunized plasma. For example, activated Dengue antibodies showed a binding range over 350 times greater compared to controls (Bardina *et al.,* 2017). Several outbreaks of the four Dengue strains have occurred in South America, Central America, and Southeast Asia. In several territories of the United States, both Dengue and West Nile viruses are endemic, with annual outbreaks. This highlights the importance of these findings due to the significant expansion of Zika in these territories (Bardina *et al.,* 2017).

6. Conclusion

Since the identification of the Zika remained largely silent until the outbreak in the Pacific and subsequently in the Americas. Phylogenetic analysis has proven invaluable in tracing the origin and spread of this outbreak, which is attributed to the Asian lineage. This lineage's significant expansion and high virulence in the American continent underscore the importance of understanding the phylogenetic relationships among viral strains.

In Ecuador, the spread of Zika is hypothesized to have occurred directly from Brazil, as the Asian lineage was identified in the two analyzed sequences. The high density of *Aedes aegypti*, the primary transmission vector in the Americas, and the virus's high variability contribute to the rapid dissemination and emergence of outbreaks. Phylogenetic analysis not only confirms the Asian origin of the American Zika outbreak but also reveals the close relationship of American sequences with those from outbreaks in Micronesia and the Pacific, thereby affirming both the outbreak's origin and its probable phylogenetic lineage.

The benefits of phylogenetic analysis extend beyond academic research; it is a critical tool for public health, particularly in developing countries. By providing detailed insights into the virus's transmission patterns and evolutionary dynamics, phylogenetic studies enable health authorities to implement targeted control measures promptly. Developing countries can leverage this tool to enhance surveillance, guide vaccination strategies, and improve outbreak response times. The integration of phylogenetic analysis into public health frameworks can lead to more effective disease control and prevention strategies, ultimately reducing the impact of viral outbreaks on vulnerable populations.

Compliance with ethical standards

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Disclosure of conflict of interest

There are no conflicts of interest.

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