First Complete Genome Sequences of Zika Virus Isolated from Febrile Patient Sera in Ecuador

Márquez, S; Carrera, J; Pullan, S T; Lewandowski, K; Paz, V; Loman, N; Quick, J; Bonsall, D; Powell, R; Thézé, J; Pybus, O G; Klenerman, P; Eisenberg, J; Coloma, J; Carroll, M W; Trueba, G; Logue, C H

DOI:
10.1128/genomeA.01673-16

License:
Creative Commons: Attribution (CC BY)

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Link to publication on Research at Birmingham portal

Publisher Rights Statement:

General rights
Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

• Users may freely distribute the URL that is used to identify this publication.
• Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
• Users may use extracts from the document in line with the concept of ‘fair dealing’ under the Copyright, Designs and Patents Act 1988 (?)
• Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy
While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBITRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Download date: 01. Aug. 2019
First Complete Genome Sequences of Zika Virus Isolated from Febrile Patient Sera in Ecuador

Colegio de Ciencias Biológicas y Ambientales, Universidad San Francisco de Quito, Quito, Ecuador; Public Health England, Porton Down, United Kingdom; Clinical Laboratory, Hospital Delfina Torres de Concha, Esmeraldas, Ecuador; Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom; Peter Medawar Building for Pathogen Research, University of Oxford, Oxford, United Kingdom; Primer Design Ltd., Southampton, United Kingdom; Department of Zoology, University of Oxford, Oxford, United Kingdom; School of Public Health, University of Michigan, Ann Arbor, Michigan, USA; University of California, Berkeley, California, USA

ABSTRACT
Here, we present the complete genome sequences of two Zika virus (ZIKV) strains, EcEs062_16 and EcEs089_16, isolated from the sera of febrile patients in Esmeraldas City, in the northern coastal province of Esmeraldas, Ecuador, in April 2016. These are the first complete ZIKV genomes to be reported from Ecuador.

The emergence and diagnostic detection of arthropod-borne viruses have been increasingly reported in Ecuador, and include dengue virus (DENV), chikungunya virus (CHIKV), and, most recently, Zika virus (ZIKV) (1). In 2016 alone, 2,693 suspected, 839 laboratory-confirmed, and 15 imported cases of ZIKV were reported (2). The early clinical manifestations resulting from infection with ZIKV closely resemble those caused by DENV and CHIKV (3), and without differential diagnosis by serological, molecular, or sequencing means, misdiagnosis based on clinical presentation alone may occur (4, 5).

Here, we report the complete genome sequences of two ZIKV strains, EcEs062_16 and EcEs089_16, isolated from the sera of two febrile patients in the coastal province of Esmeraldas, Ecuador, in April 2016. ZIKV was detected in both patient sera samples using the Genesig Dengue, Zika and Chikungunya Virus Multiplex real-time assay kit on a BioRad CFX96 system, following the manufacturer’s guidelines (Genesig, United Kingdom).

ZIKV was propagated from sera by inoculation of C6/36 cell monolayers (Aedes albopictus; ECACC, United Kingdom). Supernatant was removed after 7 days, purified, and nucleic acid extracted using the QIAamp viral RNA minikit (Qiagen Gmbl, Germany); 5 μL of purified RNA was used as template in ten 20-μL reactions, each with a set of 10 primer pairs designed to amplify ~1.5-kb overlapping amplicons covering the whole genome. Sample EcEs089_16 produced insufficient product from the 1.5-kb amplicon scheme, and therefore the 400-bp tiling amplicon protocol devised by Quick and Loman (http://www.zibraproject.org/data) was utilized to produce sufficient product for sequencing. Sequencing libraries were prepared from 1 μg of total material, comprising equimolar amounts of each of the 10 amplicons for EcEs062_16 or 500 ng of product from each of the two multiplex reactions for EcEs089_16.

The Nanopore sequencing kit SQK-NSK007 (Oxford Nanopore Technologies, United Kingdom) was used to produce both sequencing libraries, according to the manufacturer’s R9 amplicon sequencing protocol. EcEs062_16 and EcEs089_16 libraries were sequenced on FLO_MIN104 and FLO_MIN105 flow cells, respectively. Sequencing was performed on an Mk1b MiniION device. Bases were called in Metrichor using 2D
Basecalling RNN for SQK-NSK007. Consensus sequences of 10,646 bp and 10,616 bp for EcEs062_16 and EcEs089_16, respectively, were generated using the ZiBRA analysis pipeline (http://www.zibraproject.org/data). Direct RNA sequencing of culture supernatant using Illumina technology provided fuller length sequences of 10,812 bp and 10,810 bp, respectively, for EcEs062_16 and EcEs089_16.

The EcEs062_16 and EcEs089_16 genomes clustered together with a sequence isolated from Paraiba state, Brazil (KX280026, posterior probability 0.96, bootstrap support 75%), rather than with sequences from neighboring Colombia and Peru. Esmeraldas city is one of the furthest continental points west of the Paraiba region (4,874 km by air). Neither patient reported travel history to Brazil, and Ecuador does not share a land border with Brazil; therefore, the epidemiology of ZIKV’s introduction into and movement within Ecuador merits further investigation. We aim to further validate the potential of portable sequencing as a diagnostic tool to assess the temporal movements of ZIKV throughout South America, as we previously described for Ebola in West Africa (6).

Accession number(s). The complete genomic sequences have been annotated and deposited in GenBank, under accession numbers KX879603 to KX879604, and on http://nextstrain.org/zika.

ACKNOWLEDGMENTS

We acknowledge Mauricio Ayoví for transferring samples from Esmeraldas to USFQ, Quito; Pablo Endara (USFQ) for his comments and observations; Rory Bowden of the Wellcome Trust Centre for Human Genetics in Oxford for sequencing time in their busy lab; and Peter Thaves of ECACC for the provision of C6/36 cells.

This project was partially funded by Public Health England (PHE), USFQ, and the University of California, Berkeley. All real-time PCR reagents were provided freely by Primer Design, Ltd. PHE European Collection of Authenticated Cell Cultures (ECACC) generously provided C6/36 cell lines. No external funding was provided for this work.

REFERENCES


