SUPPLEMENTAL METHODS

Chikungunya virus-specific real-time reverse-transcription polymerase chain reaction. The presence of chikungunya virus (CHIKV) mRNA was determined using a TaqMan real-time reverse-transcription polymerase chain reaction (RT-PCR) assay as described[1] with primer/probe sets designed to detect Asian and East/Central/South African (ECSA) genotypes of CHIKV (R.S. Lanciotti, personal communication): primers, forward (CHIKV 3855) 5′-GAGCATACGGTTACGCAGATAG-3′, reverse (CHIKV 3957–Asian) 5′-TACTGGTGATACATGGT GGTTTC-3′, and (CHIKV 3957–ECSA) 5′-TGCTGGTGA CACATGGTGGTTTC-3′; probes, (CHIKV 3886 FAM-Asian) 5′-FAM-ACGAGTAATCTGCGTACTGGGACGTA-BHQ1-3′ and (CHIKV 3886 FAM- ECSA) 5′-FAM-ACGAGTCAT CTGCGTATTGGGACGCA-BHQ1-3′.

Sanger sequencing. Viral RNA was extracted from 140 μL of cell culture supernatant or 140 μL of patient serum using the QIAamp Viral RNA Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's protocol, and RNA was eluted in 60 μL of RNase-free water. Primers for amplifying and sequencing the *E1* gene were designed according to reference genome British Virgin Islands 99659 (GenBank accession no. KJ451624). The SuperScript III One-Step RT-PCR System (Invitrogen, Carlsbad, CA) was used for reverse transcription of viral RNA and subsequent amplification of viral complementary DNA (cDNA). Specifically, 5 μL of extracted viral RNA was denatured at 90°C for 1 minute and immediately placed on ice. Subsequently, 0.25 μL each of forward and reverse primers (Supplemental Table 2), 11.3 μL of RNase-free water, 17.5 μL of 2X Reaction Buffer (Invitrogen), and 0.7 μL of SuperScript III RT/Platinum Taq High Fidelity Enzyme Mix (Invitrogen) were added to the denatured RNA. Viral RNA was reverse transcribed (50°C for 60 minutes), and the resulting cDNA was amplified via one cycle of denaturation (94°C for 2 minutes), 33–35 cycles of denaturation (94°C for 15 seconds), annealing (55–57°C for 30 seconds), and extension (68°C for 3 minutes), and a final extension (68°C for 10 minutes). Amplicons from each sample were visualized using a 1% agarose gel to confirm the presence of a ~2.4-kb DNA fragment. The PCR product was sequenced via the Sanger method at the University of California, Berkeley's DNA Sequencing Facility (https://mcb.berkeley.edu/barker/dnaseq/home) using the primers listed in Supplemental Table 2.

Deep sequencing. Three autochthonous serum samples from October 2014 were deep sequenced. For each sample, cDNA was synthesized as follows: 4 μL of 5X first-strand buffer, 2 μL dithiothreitol (DTT; 0.1 M), 2 μL deoxynucleotide triphosphates (dNTPs) (10 mM), 1 μL SUPERase•In RNase Inhibitor (Life Technologies, Carlsbad, CA), 1.5 μL Superscript Reverse Transcriptase III (Invitrogen), 0.5 μL random hexamers (1.1 μg/μL; Life Technologies), and 3 μL RNase-free water were added to 6 μL denatured nucleic acids. Initial annealing of hexamers was performed at 25°C for 2 minutes, and RNA was reverse transcribed at 42°C for 60 minutes. The reaction was inactivated via a 5-minute incubation at 95°C. Next, cDNA was amplified using multiple displacement amplification (MDA). In brief, two MDA reactions were set up for each sample, with each MDA reaction containing 10 μL cDNA, 2 μL dNTP mix (25 mM), 5 μL of 10X phi29 reaction buffer (New England Biolabs, Ipswich, MA), 2.75 μL random hexamers, 1.25 μL DTT (100 mM), 30 μL RNase-free water, and 1 μL bovine serum albumin (10 mg/mL). DNA was denatured by heating the reaction at 95°C for 5 minutes, followed by immediate placement on ice. Subsequently, 1.25 μL phi29 polymerase was added to each tube, and reactions were incubated at 30°C for 4 hours. Reactions for each sample were combined, and amplified DNA was purified using the QIAquick PCR Purification kit (Qiagen) as described in the manufacturer's protocol. DNA was eluted in 30 μL Buffer EB (Qiagen). Libraries for high-throughput sequencing were prepared from 1.5 ng of purified DNA using the Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA) according to the recommended protocol. Libraries were pooled in equimolar ratios and sequenced using the HiSeq2000 platform (Illumina) at the QB3 facility located at the University of California, Berkeley (http://qb3.berkeley.edu/qb3/gsl/services-lp.cfm) to generate 100-bp reads. Reads for each sample were mapped to full-length CHIKV sequences from the National Center for Biotechnology Information (NCBI) using the "Bowtie2" software.[2] "Samtools"[3] and in-house Python scripts (Python Software Foundation) were used to generate pileups and consensus nucleotide.

SUPPLEMENTAL REFERENCES

1. Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, Campbell GL, 2007. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis 13:* 764–767.
2. Langmead B, Salzberg SL, 2012. Fast gapped-read alignment with Bowtie 2. *Nat Methods 9:* 357–359.
3. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R; Genome Project Data Processing Subgroup, 2009. The Sequence Alignment/Map format and SAMtools. *Bioinformatics 25:* 2078–2079.

SUPPLEMENTAL TABLE 1

List of Nicaraguan CHIKV samples with Sanger *E1* sequencing results

Strain name	GenBank no.	Sample type	Case type	Source (study)	Date of symptom onset (month/day/year)	Date of sample collection (month/day/year)	Hospitalized (Y/N)	*E1* sequence homology with BVI_99659†	
								nucleotide	Amino acid
7333	KT192708	Isolate	Imported	National surveillance	08/18/2014	08/19/2014	Y	100%	–
7334	KT192709	Isolate	Imported	National surveillance	08/18/2014	08/19/2014	Y	100%	–
7444	KT192710	Isolate	Imported	National surveillance	08/19/2014	08/21/2014	N	100%	–
7745	KT192711	Isolate	Imported	National surveillance	08/20/2014	08/24/2014	Y	100%	–
7975	KT192712	Isolate	Imported	National surveillance	08/24/2014	08/26/2014	N	100%	–
5872	KT192713	Isolate	Autochthonous	Cohort	10/16/2014	10/16/2014	N	100%	–
4548	KT192714	Isolate	Autochthonous	Cohort	10/20/2014	10/20/2014	N	100%	–
1686	KT192715	Isolate	Autochthonous	Cohort	10/21/2014	10/21/2014	Y	100%	–
11539	KT192716	Serum	Autochthonous	National surveillance	10/29/2014	10/31/2014	Y	100%	–
11540*	KT192717	Serum	Autochthonous	National surveillance	10/29/2014	10/31/2014	Y	100%	–
1758	KT192718	Isolate	Autochthonous	Hospital	11/3/2014	11/5/2014	Y	C10415T; G11130T	E1-A145V; E1-P383 silent
1507	KT192719	Isolate	Autochthonous	Cohort	11/9/2014	11/9/2014	N	100%	–
1771	KT192720	Isolate	Autochthonous	Hospital	11/12/2014	11/13/2014	Y	100%	–
1086	KT192721	Isolate	Autochthonous	Cohort	11/26/2014	11/26/2014	N	T10080C	E1-L33 silent
4184	KT192722	Isolate	Autochthonous	Cohort	11/26/2014	11/27/2014	N	T11076C	E1-F365 silent
1800	KT192723	Isolate	Autochthonous	Hospital	12/1/2014	12/3/2014	Y	G10665A	E1-T228 silent
1802	KT192724	Isolate	Autochthonous	Hospital	12/1/2014	12/3/2014	N	G10852A; G11130T	E1-V291I; E1-P383 silent
1803	KT192725	Isolate	Autochthonous	Hospital	12/2/2014	12/4/2014	Y	100%	–
1806	KT192726	Isolate	Autochthonous	Hospital	12/8/2014	12/9/2014	Y	100%	–
1807	KT192727	Isolate	Autochthonous	Hospital	12/8/2014	12/9/2014	Y	T11076C	E1-F365 silent
4367	KT192728	Isolate	Autochthonous	Cohort	12/23/2014	12/24/2014	N	100%	–
3370	KT192729	Isolate	Autochthonous	Cohort	12/25/2014	12/26/2014	N	100%	–
4784	KT192730	Isolate	Autochthonous	Cohort	1/3/2015	1/4/2015	N	100%	–
1817	KT192731	Isolate	Autochthonous	Hospital	1/5/2015	1/6/2015	Y	A10465G	E1-I162V
1820	KT192732	Isolate	Autochthonous	Hospital	1/7/2015	1/8/2015	Y	G11130T	E1-P383 silent
7448	KT192733	Isolate	Autochthonous	Cohort	2/4/2015	2/5/2015	N	G11130T	E1-P383 silent
1851	KT192734	Isolate	Autochthonous	Hospital	2/8/2015	2/10/2015	Y	100%	–
7763	KT192735	Isolate	Autochthonous	Cohort	2/15/2015	2/15/2015	N	T11076C	E1-F365 silent
1852	KT192736	Isolate	Autochthonous	Hospital	2/16/2015	2/18/2015	Y	G11130T	E1-P383 silent
1853	KT192737	Isolate	Autochthonous	Hospital	2/16/2015	2/18/2015	Y	100%	–
1854	KT192738	Isolate	Autochthonous	Hospital	2/18/2015	2/20/2015	Y	100%	–
7050	KT192739	Isolate	Autochthonous	Cohort	3/20/2015	3/21/2015	N	100%	–
7676	KT192740	Isolate	Autochthonous	Cohort	4/21/2015	4/22/2015	N	T11076C	E1-F365 silent

CHIKV = chikungunya virus; Y = yes; N = no.
*Full-length genome obtained from Hi-Seq; sequence available from GenBank, accession no. KT192707.
†Nucleotide numbering is according to BritishVirginIslands/99659/KJ451624/2014.

SUPPLEMENTAL TABLE 2

CHIKV primers used for RT-PCR and Sanger sequencing

Primer*	Sequence	RT-PCR	Sequencing
CHIK_CW_9544-63 f	TGGCCGCAGTTATCCACAAA	X	X
CHIK_CW_10341-60 f	AGCATACAGGGCTCATACCG		X
CHIK_CW_11143-60 f	GTCAATTACCCGGCGTCA		X
CHIK_CW_11164-83 f	ACCACCCTCGGGGTCCAAGA		X
CHIK_CW_11954-27 r	AGAGTTCGGTATGCTATGCCTACATCTC	X	X

CHIKV = chikungunya virus; RT-PCR = reverse-transcription polymerase chain reaction.
*Primers were designed based on the reference genome of CHIKV strain 99659 from the British Virgin Islands.