(FL): Wingers Publishing; 1994. p. 949–83 [cited 2017 Aug 16]. http://avianmedicine.net/content/uploads/2013/03/33.pdf
8. Kaijalainen T, Ruokokoski E, Ukkonen P, Herva E. Survival of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* frozen in skim milk-tryptone-glucose-glycerol medium. J Clin Microbiol. 2004;42:412–4. http://dx.doi.org/10.1128/JCM.42.1.412-414.2004
9. Obi CL, Enweani IB, Giwa JO. Bacterial agents causing chronic suppurative otitis media. East Afr Med J. 1995;72:370–2.
10. Mittal R, Lisi CV, Gerring R, Mittal J, Mathee K, Narasimhan G, et al. Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. J Med Microbiol. 2015;64:1103–16. http://dx.doi.org/10.1099/jmm.0.000155

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Spread of Chikungunya Virus East/Central/South African Genotype in Northeast Brazil

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We investigated an outbreak of exanthematous illness in Maceió by using molecular surveillance; 76% of samples tested positive for chikungunya virus. Genetic analysis of 23 newly generated genomes identified the East/Central/South African genotype, suggesting that this lineage has persisted since mid-2014 in Brazil and may spread in the Americas and beyond.

Dengue virus (DENV), Zika virus (ZIKV), and chikungunya virus (CHIKV) co-circulate in Brazil, are predominantly transmitted by *Aedes aegypti* mosquitoes, and cause similar clinical symptoms upon infection, complicating epidemiologic surveillance. Brazil harbors the highest diversity of CHIKV in the Americas; both the Asian and the East/Central/South African (ECSA) lineages circulate in the country (*1*). Despite high prevalence of CHIKV in Brazil (352,773 notified cases during January 2016–May 2017) and its widespread distribution (*2*), little is known about its transmission. We report a molecular and genomic investigation of an outbreak of CHIKV infection in Maceió, Alagoas state, Northeast Brazil.

During March 30–May 3, 2016, ≈12,000 patients visited 2 private hospitals in Maceió; roughly 70% of them had exanthematous illness symptoms compatible with DENV, CHIKV, or Zika virus infection. We analyzed 273 randomly chosen samples by using molecular diagnostics and virus discovery methods. The study was approved by the Faculty of Medicine from the University of São Paulo Review Board, and we obtained informed consent from all participants.

Analyzed samples were from patients who were on average 37 years of age (range 1–86 years); 175 (64%) were female, and 198 (73%) resided in Maceió municipality. Diagnostic tests for DENV, ZIKV, and CHIKV confirmed that 208 (76%) were positive for CHIKV RNA (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/10/17-0307-Techapp1.pdf). In addition, 66 (24%) were positive for Zika virus RNA and 36 (13.2%) were co-infected with CHIKV and Zika virus, consistent with Zika virus circulation in Northeast Brazil in mid-2016 (*3*). We detected no DENV infections. Cycle threshold (C_t) values for CHIKV RNA–positive samples were lower (average C_t = 24.6) than those for ZIKV (average C_t = 33.5).

We applied a metagenomics next-generation sequencing protocol to 38 randomly chosen CHIKV RNA–positive samples (*4*) (online Technical Appendix). We recovered 23 CHIKV genomes (>4,000 bp) by using the MiSeq Sequencer (Illumina, Inc., San Diego, CA, USA); mean genome coverage was 72× and mean depth coverage 207× (online Technical Appendix Table 1). We also

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included 2 CHIKV RNA–positive samples from João Pessoa, Paraíba state. We did not detect the E1-A226V adaptive mutation associated with large outbreaks in Asia (*5*) in the strains we analyzed. We then appended sequences to publicly available data (659 CHIKV isolates) and used maximum-likelihood and Bayesian phylogenetic analysis to identify the origins of the outbreak (online Technical Appendix).

On the basis of available sequences of isolates from the Americas, the Maceió sequences we analyzed fell within a single strongly supported monophyletic clade (bootstrap support = 99%, posterior support = 1.00) that belongs to the ECSA genotype (Figure). Genetic analysis suggests the outbreak most likely originated from transmission cycles not previously identified in Northeast Brazil and not from a separate introduction into the Americas. Before August 2015, no CHIKV infections had been reported in Alagoas (Figure). Molecular dating analysis indicates that the outbreak was caused by a single founder strain that is estimated to have arrived in Alagoas around late April 2015 (95% Bayesian credible interval July 2014–October 2015), possibly a few months before the earliest reports of CHIKV there (Figure). Our reconstruction of the history of the ECSA genotype in Brazil using a phylogeographic approach (*6*) further suggests that this lineage was introduced into Alagoas from the neighboring Bahia state, which experienced a CHIKV epidemic during January–August 2015 (*7*).

The brief to negligible period of undetected transmission of CHIKV in Alagoas is consistent with past

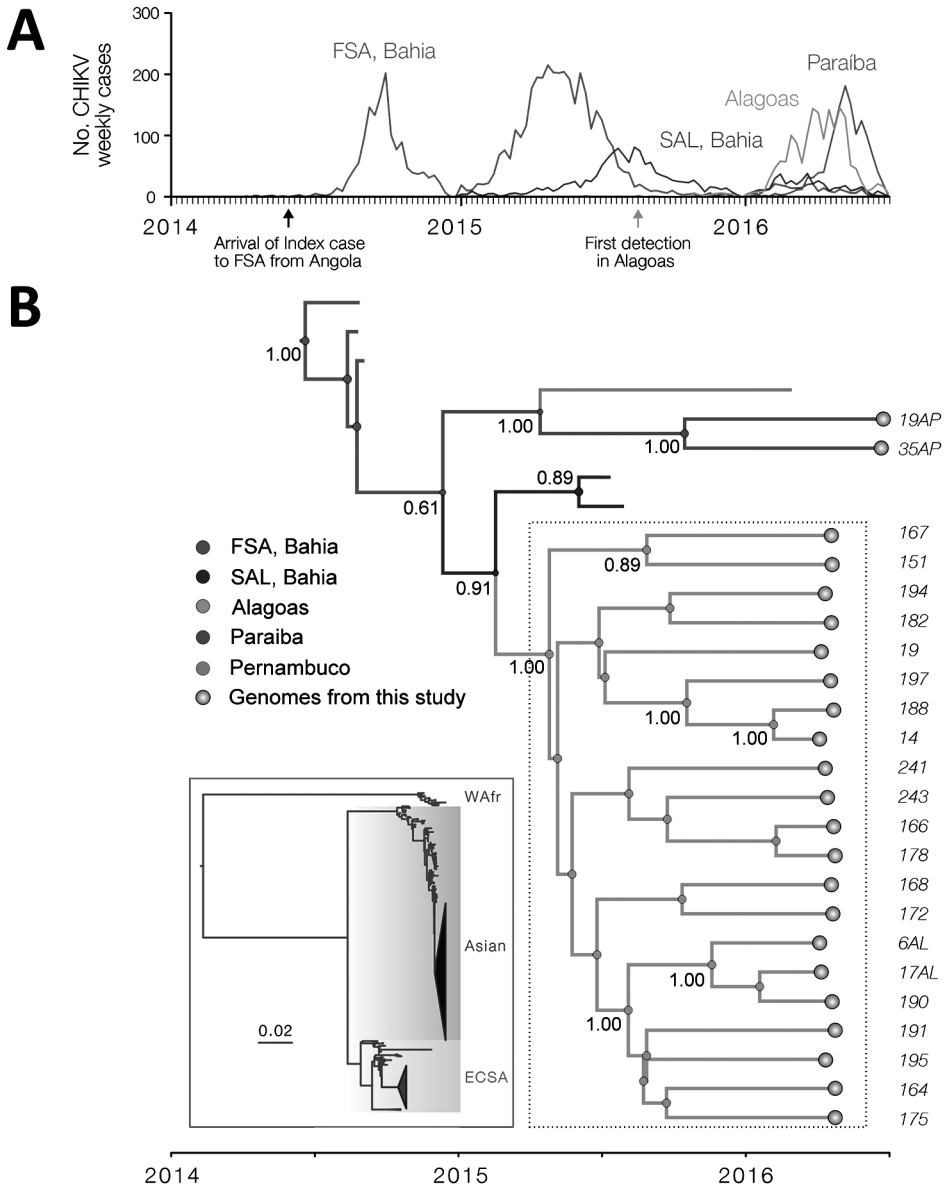

Figure. Epidemiologic and genetic surveillance of CHIKV in Northeast Brazil. A) Notified CHIKV cases for Alagoas state (Maceió municipality), Paraíba state (João Pessoa municipality), and Feira de Santana (FSA) and Salvador (SAL) municipalities (*3*), both located in the Bahia state. B) Molecular clock phylogeny obtained using 23 novel CHIKV sequences (with length >4,000 nt) collected in Northeast Brazil (dashed box). Numbers along branches represent clade posterior probability >0.75. Colors in branches represent most probable locations. At each node, size of the colored circles is proportional to location posterior probability. Inset shows a maximum-likelihood phylogeny with all publicly available CHIKV genome sequences (n = 659). The Indian Ocean Lineage (IOL) genotype has been collapsed. Triangles represent clades circulating in the Americas; the American-ECSA lineage reported in this study is shown in red and the American-Asian lineage in blue. CHIKV, chikungunya virus; ECSA, East/Central/South African genotype; WAfr, West African genotype. A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/23/10/17-0307-F1.htm).

reports (*8*) and in contrast with the unrecognized circulation in the region of Zika virus, which typically causes milder symptoms (*3*). The most common clinical signs and symptoms for the sequenced CHIKV cases were fever (87%), arthralgia (70%), headache (44%), exanthema (30%), and myalgia (26%) (Technical Appendix Table 2). CHIKV infection is often characterized by prolonged periods of disability. Further investigation is needed to study potential differences in the effects of CHIKV and Zika virus infection on public health, as well in pathology and innate and adaptive immune responses to each genotype.

The unrecognized transmission of the CHIKV ECSA genotype in Northeast Brazil is unique in the Americas. The spread of this genotype in this region will be mediated by several factors, including herd immunity, vector suitability, and human mobility. Serologic and molecular surveys in human and mosquito populations are required to characterize the factors involved in transmission and the extent of cross-protection of the Asian and the ECSA genotypes in the Americas. Although CHIKV ECSA has been found only in *Ae. aegypti* mosquitoes (*9*), a recent study has shown that *Ae. albopictus* mosquitoes in Brazil are also highly competent in CHIKV ECSA transmission (*10*). Given the widespread distribution of both vectors in the Americas (*1*), it is possible that the ECSA lineage may spread to other regions in the Americas and beyond. A better understanding of the transmission dynamics of CHIKV, DENV, and Zika virus in the Americas is essential to fully understand the risk of arbovirus-associated congenital anomalies, Guillain-Barré, and other neurological syndromes.

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References

1. Nunes MR, Faria NR, de Vasconcelos JM, Golding N, Kraemer MU, de Oliveira LF, et al. Emergence and potential for spread of Chikungunya virus in Brazil. BMC Med. 2015;13:102. http://dx.doi.org/10.1186/s12916-015-0348-x
2. Ministério da Saúde. Boletins Epidemiológicos—Secretaria de Vigilância em Saúde. Situação epidemiológica / dados dengue. 2017;48:16 [cited 2017 Jul 13] http://portalsaude.saude.gov.br/index.php/situacao-epidemiologica-dados-dengue
3. Faria NR, Quick J, Claro IM, Thézé J, de Jesus JG, Giovanetti M, et al. Establishment and cryptic transmission of Zika virus in Brazil and the Americas. Nature. 2017;546:406–10. http://dx.doi.org/10.1038/nature22401
4. Deng X, Naccache SN, Ng T, Federman S, Li L, Chiu CY, et al. An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. Nucleic Acids Res. 2015;43:e46. http://dx.doi.org/10.1093/nar/gkv002
5. Tsetsarkin KA, Vanlandingham DL, McGee CE, Higgs S. A single mutation in chikungunya virus affects vector specificity and epidemic potential. PLoS Pathog. 2007;3:e201. http://dx.doi.org/10.1371/journal.ppat.0030201
6. Lemey P, Rambaut A, Drummond AJ, Suchard MA. Bayesian phylogeography finds its roots. PLOS Comput Biol. 2009;5:e1000520. http://dx.doi.org/10.1371/journal.pcbi.1000520
7. Cardoso CW, Kikuti M, Prates AP, Paploski IAG, Tauro LB, Silva MMO, et al. Unrecognized emergence of chikungunya virus during a Zika virus outbreak in Salvador, Brazil. PLoS Negl Trop Dis. 2017;11:e0005334. http://dx.doi.org/10.1371/journal.pntd.0005334
8. Faria NR, Lourenco J, Cerqueira EM, Lima MM, Pybus OG, Alcantara LCJ. Epidemiology of chikungunya virus in Bahia, Brazil, 2014–2015. PLoS Currents Outbreaks. 2016;1 [cited 2007 Jul 13] http://currents.plos.org/outbreaks/article/epidemiology-of-chikungunya-virus-in-bahia-brazil-2014-2015
9. Costa-da-Silva AL, Ioshino RS, Petersen V, Lima AF, Cunha MDP, Wiley MR, et al. First report of naturally infected *Aedes aegypti* with chikungunya virus genotype ECSA in the Americas. PLoS Negl Trop Dis. 2017;11:e0005630. http://dx.doi.org/10.1371/journal.pntd.0005630
10. Vega-Rúa A, Zouache K, Girod R, Failloux A-B, Lourenço-de-Oliveira R. High level of vector competence of *Aedes aegypti* and *Aedes albopictus* from ten American countries as a crucial factor in the spread of Chikungunya virus. J Virol. 2014;88:6294–306. http://dx.doi.org/10.1128/JVI.00370-14

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Technical Appendix

Laboratory testing

Viral RNA was extracted from 140 µL of serum samples using QIAmp viral RNA kit (QIAGEN, Valencia, CA, USA) and RT-qPCR analyses were performed for DENV serotypes 1–4 (*1*), ZIKV (*2*) and CHIKV (*3*). Of 273 samples tested, 76% (n = 208) were CHIKV-RNA$^+$, 24% (n = 66) were ZIKV-RNA$^+$ and 13.2% (n = 36/273) were co-infected with CHIKV and ZIKV, similar to recent findings from Salvador, Bahia (*4*).

Metagenomic sequencing

To identify the cause of the outbreak, each plasma sample was subjected to centrifugation at 15,000xg for 10 minutes, filtered through a 0.45 µm filter (Merck Millipore, Billerica, MA, USA). The filtrates were treated with a mixture of nuclease enzymes to digest unprotected nucleic acids. Viral nucleic acids were extracted using Maxwell 16 Viral Total Nucleic Acid Purification Kit (Promega, Inc., Madison, WI, USA) and cDNA synthesis was performed with AMV Reverse transcription (Promega, Inc., Madison, WI, USA). A second strand of cDNA synthesis was performed using DNA Polymerase I Large (Klenow) Fragment (Promega, Inc., Madison, WI, USA). Subsequently, a Nextera XT Sample Preparation Kit (Illumina, Inc., San Diego, CA, USA) was used construct a DNA library, with each sample identifiable using dual barcodes. For size selection, we used a Pippin Prep (Sage Science, Inc.) to select a 400bp insert (range 200–600bp). The library was deep-sequenced using the MiSeq Sequencer (Illumina, Inc., San Diego, CA, USA) with 300 bped ends. BLASTx was used to identify viral sequences through their protein sequence similarity to annotated viral proteins in GenBank search, as

previously described (*5*). Of the 40 samples that were tested, we were able to obtain 23 complete or near-complete genomes (>4000bp; Genbank Accession numbers KY704933 to KY704955).

Phylogenetic analysis

Publicly available chikungunya virus genome data (>1500bp) was retrieved from Genbank on 17 Feb 2017 using an in-house script. Newly generated genomes (>4000bp) were then appended to the publicly available data and aligned using MAFFT (*6*). A maximum likelihood phylogeny was constructed for the collated dataset (n = 659) using PhyML with 500 bootstrap replicates (*7*). The correlation between genetic divergence and collection dates for the ECSA genotype subtree ($r^2 = 0.41$, rate = 8.9×10^{-4} substitutions per site per year) was inspected using TemPest (*8*). Dated phylogenies were constructed using a Bayesian framework implemented in the BEAST software package (*9*).

To reconstruct date phylogenies, we used a SDR06 nt substitution model with a non-informative Bayesian Skygrid coalescent tree prior (20 grid points) (*10*) and a strict molecular clock model. Finally, a discrete phylogeographic analysis using an asymmetric substitution model (*11*) was conducted to investigate the origins and spread across considered locations (n = 3 from Feira de Santana, Bahia, n = 1 from Pernambuco, n = 2 from João Pessoa, Paraíba, n = 2 from Salvador, Bahia, n = 21 from Maceió/Capelas, Alagoas).

References

1. Lai YL, Chung YK, Tan HC, Yap HF, Yap G, Ooi EE, et al. Cost-effective real-time reverse transcriptase PCR (RT-PCR) to screen for Dengue virus followed by rapid single-tube multiplex RT-PCR for serotyping of the virus. J Clin Microbiol. 2007;45:935–41. PubMed http://dx.doi.org/10.1128/JCM.01258-06

2. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap state, Micronesia, 2007. Emerg Infect Dis. 2008;14:1232–9. PubMed http://dx.doi.org/10.3201/eid1408.080287

3. Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, et al. Chikungunya virus in US travelers returning from India, 2006. Emerg Infect Dis. 2007;13:764–7. PubMed http://dx.doi.org/10.3201/eid1305.070015

4. Sardi SI, Somasekar S, Naccache SN, Bandeira AC, Tauro LB, Campos GS, et al. Coinfections of Zika and chikungunya viruses in Bahia, Brazil, identified by metagenomic next-generation sequencing. J Clin Microbiol. 2016;54:2348–53. PubMed http://dx.doi.org/10.1128/JCM.00877-16

5. Deng X, Naccache SN, Ng T, Federman S, Li L, Chiu CY, et al. An ensemble strategy that significantly improves de novo assembly of microbial genomes from metagenomic next-generation sequencing data. Nucleic Acids Res. 2015;43:e46. PubMed http://dx.doi.org/10.1093/nar/gkv002

6. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30:772–80. PubMed http://dx.doi.org/10.1093/molbev/mst010

7. Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 2010;59:307–21. PubMed http://dx.doi.org/10.1093/sysbio/syq010

8. Rambaut A, Lam T, de Carvalho L, Pybus OG, Exploring the temporal structure of heterochronous using TemPest, Virus Evolution 2:vew007.

9. Drummond AJ, Suchard MA, Xie D, Rambaut A. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol. 2012;29:1969–73. PubMed http://dx.doi.org/10.1093/molbev/mss075

10. Gill MS, Lemey P, Faria NR, Rambaut A, Shapiro B, Suchard MA. Improving Bayesian population dynamics inference: a coalescent-based model for multiple loci. Mol Biol Evol. 2013;30:713–24. PubMed http://dx.doi.org/10.1093/molbev/mss265

11. Lemey P, Rambaut A, Drummond AJ, Suchard MA. Bayesian phylogeography finds its roots. PLOS Comput Biol. 2009;5:e1000520. PubMed http://dx.doi.org/10.1371/journal.pcbi.1000520

Technical Appendix Table 1. Sequencing details of the chikungunya virus isolates reported in this study

Accession number	Isolate name	No. reads mapped	Mean depth coverage	Genome coverage (%)*
KY704933	6AL	19,267	175.79	79.02
KY704934	14	5,221	54.18	62.27
KY704935	17AL	2,847	44.05	73.63
KY704936	19	11,517	53.39	61.72
KY704937	151	3,064	47.40	48.03
KY704938	164	1,010	16.90	67.16
KY704939	166	36,801	467.31	94.97
KY704940	167	1,992	32.16	72.51
KY704941	168	796	16.79	52.21
KY704942	172	18,577	249.79	95.53
KY704943	175	8,561	82.14	78.84
KY704944	178	1,004	21.48	60.91

Accession number	Isolate name	No. reads mapped	Mean depth coverage	Genome coverage (%)*
KY704945	182	74,785	328.01	51.82
KY704946	188	6,927	77.66	66.64
KY704947	190	9,650	146.31	84.09
KY704948	191	13,041	62.36	56.36
KY704949	194	44,321	335.58	59.84
KY704950	195	16,140	168.99	67.76
KY704951	197	650	16.38	58.19
KY704952	241	1,192,684	2,050.39	93.76
KY704953	243	3,342	50.44	74.80
KY704954	19AP	15,832	151.30	100.00
KY704955	35AP	11,206	111.64	100.00

*Relative to reference genome sequence KU940225.

Technical Appendix Table 2. Clinical information of sequenced chikungunya virus isolates*

Isolate	CT	Sex, age (y)	Date	Location	Clinical symptoms
6AL	24.9	F, 44	30–03–16	Santa Lúcia, Maceió, AL	Fever, arthralgia
14	21.4	M, 30	30–03–16	Poço, Maceió, AL	Fever, arthralgia, muscle pain, headache
17AL	21.7	F, 50	01–04–16	Centro, Maceió, AL	Fever, muscle pain, nausea
19	19.5	F, 31	01–04–16	Santos, Maceió, AL	Fever, arthralgia, myalgia, fatigue, exanthema
151	35.8	M, 64	15–04–16	Tab. do Pinto, Maceió, AL	Fever, arthralgia, edema
164	33.9	F, 25	19–04–16	Clima Bom, Maceió, AL	Fever, arthralgia, headache
166	29.5	M, 34	17–04–16	Poço, Maceió, AL	Fever, arthralgia, headache
167	28.2	M, 30	14–04–16	Vergel, Maceió, AL	Fever, arthralgia, muscle pain
168	36.9	F, 43	14–04–16	Vergel, Maceió, AL	Fever, arthralgia, headache
172	18.2	M, 18	16–04–16	FernãoVelho, Maceió, AL	Fever, arthralgia, headache
175	19.5	F, 28	19–04–16	Poço, Maceió, AL	Arthralgia, exanthema
178	20.2	M, 41	19–04–16	Centro, Maceió, AL	Fever, pruritus
182	19.3	F, 17	14–04–16	Santa Amélia, Maceió, AL	Fever, arthralgia, maculopapular rash
188	25.8	F, 30	17–04–16	Centro, Capela, AL	Fever, arthralgia, headache, nausea
190	36.0	F, 61	15–04–16	Mangabeiras, Maceió, AL	Fever, arthralgia, myalgia, headache, retro-orbital pain
191	28.8	F, 36	19–04–16	Farol, Maceió, AL	Fever, headache, myalgia, arthralgia, leukopenia
194	23.3	F, 60	07–04–16	Pajuçara, Maceió, AL	Fever, arthralgia, myalgia, fatigue
195	17.6	F, 39	07–04–16	Poço, Maceió, AL	Fever, arthralgia, myalgia, fatigue
197	21.8	F, 21	13–04–16	Farol, Maceió, AL	Fever, myalgia, arthralgia
241	19.8	M, 11	07–04–16	Centro, Maceió, AL	Fever, headache, maculopapular rash, vomiting
243	26.2	F, 41	09–04–16	Sta. Amélia, Maceió, AL	Muscle pain, skin rash
19AP	16.5	F, 20	20–06–16	Centro, João Pessoa, PB	Vomiting, Fever, Headache, maculopapular rash
35AP	26.4	F, 32	17–06–16	Bayeux, João Pessoa, PB	Muscle pain, maculopapular rash

*Location indicates neighborhood, municipality and federal state (AL = Alagoas, PB = Paraíba). Date of sample collection is shown as dd-mm-yy. CT = qPCR-CT value.