Full-Length Genome Sequence of a Chikungunya Virus Isolate from the 2017 Autochthonous Outbreak, Lazio Region, Italy

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ABSTRACT  We report here the genome sequence of a human chikungunya virus isolate from the ongoing autochthonous outbreak in central Italy. The sequence (East-Central-South African lineage, Indian Ocean sublineage), which is similar to recent sequences from Pakistan and India, shows E1 and E2 signatures of strains whose main mosquito vector is Aedes aegypti, although Aedes albopictus is the vector in Italy.

Chikungunya virus (CHIKV) is an enveloped virus of the genus Alphavirus, family Togaviridae, with a single-stranded positive-sense RNA genome about 12,000 nucleotides (nt) long (1–3). CHIKV is widely distributed in tropical regions of Africa, Southeast Asia, the Indian subcontinent, the Pacific region, and, since 2013, the Americas. In European countries, autochthonous outbreaks were reported in 2007 (Emilia Romagna region, Italy) and in 2010, 2014, and 2017 (France) (4, 5). For the second time, Italy is currently experiencing autochthonous cases in the Lazio region (cities of Anzio, Latina, and Rome) and in the Calabria region (city of Guardavalle Marina) (5, 6). Autochthonous transmission is estimated to have started in Anzio in early to mid-June 2017 or earlier. As of 6 October 2017, 242 cases in Lazio (148 confirmed) and 33 in Calabria (5 confirmed) are acknowledged by the national surveillance system; 7 cases (3 confirmed) epidemiologically linked to Lazio have been reported in other Italian (n = 5) or European (n = 2) regions. As with the 2007 outbreak, it is likely that in the present outbreak, the index case is a viremic traveler yet to be identified. The massive presence of a competent vector (Aedes albopictus) and the exceptionally hot summer season of 2017 likely favored the establishment of a natural host-vector cycle.

Partial characterization of the surface glycoprotein E1 from the first human cases and from infected mosquitoes in the Lazio region indicates that the virus belongs to the East-Central-South African (ECSA) lineage and lacks the A226V adaptive mutation reported to increase replication in A. albopictus (7–11).

We report here the near-complete genome sequence of the first isolate, CHIKV/ITA/Lazio-INMI1-2017, obtained on BHK-21 cells from an acute-phase serum sample collected on 11 September 2017 from a patient living in Anzio. An 11,604-nt-long sequence was amplified in 23 overlapping reverse transcriptase-PCR amplicons and Sanger sequenced.

In the maximum-likelihood phylogenetic tree, CHIKV/ITA/Lazio-INMI1-2017 belongs to the Indian Ocean sublineage of ECSA. Unlike the strain causing the 2007 outbreak in Italy, the current strain clusters with recent Indian and Pakistan isolates, as well as with
isolates obtained in China from travelers returning from India (12). Nucleotide similarity with the sequence of isolate Pakistan 07/2016 is >99% (21 mismatches over 11,604 nt).

Focusing on the main genetic signatures described for the E1 and E2 envelope glycoproteins, CHIKV/ITA/Lazio-INMI-2017 shows the following polymorphisms compared to the reference S27 strain: K201E, M269V, D284E, I317V, and V322A in E1 and A164T, T312M, V264A, S375T, and V386A in E2. Considering the reported role of E1 and E2 polymorphisms in determining the fitness of CHIKV for the mosquito vector, it seems that, given the genetic signature of CHIKV/ITA/Lazio-INMI-2017, Aedes aegypti is the preferred vector (7–9, 12–15). This may have implications for transmission efficiency in the present outbreak, given that A. albopictus is the unique competent vector, with A. aegypti not circulating in Italy.

Further characterization of the strain involved in the present outbreak, as well as assessment of possible microevolutionary trends, will be crucial for clarifying the infection dynamics and investigating the relevance of genetic signatures for virus fitness in local mosquito vectors and the extent of transmission in humans.

**Accession number(s).** The assembled complete genome sequence of chikungunya virus isolate CHIKV/ITA/Lazio-INMI1-2017 has been deposited in GenBank under the accession number MG049915.

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**REFERENCES**

1. Burt FJ, Rolph MS, Rulli NE, Mahalingam S, Heise MT. 2012. Chikungunya: a re-emerging virus. Lancet 379:662–671. [https://doi.org/10.1016/S0140-6736(11)60281-X](https://doi.org/10.1016/S0140-6736(11)60281-X).
2. Caglione C, Lalle E, Castilletti C, Carletti F, Capobianchi MR, Bordi L. 2013. Chikungunya virus infection: an overview. New Microbiol 36:211–227.
3. Zeller H, Van Bertel W, Sudre B. 2016. Chikungunya: its history in Africa and Asia and its spread to new regions in 2013–2014. J Infect Dis 214(suppl 5):S436–S440. [https://doi.org/10.1093/infdis/jiw391](https://doi.org/10.1093/infdis/jiw391).
4. Rezza G, Nicolotti L, Angelini R, Romi R, Finarelli AC, Panning M, Cordioli P, Fortuna C, Boros S, Magarano F, Silvi G, Angelini P, Dottori M, Ciuffolini MG, Majori GC, Cassone A, CHIKV study group. 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet 370:1840–1846. [https://doi.org/10.1016/S0140-6736(07)61779-6](https://doi.org/10.1016/S0140-6736(07)61779-6).
5. European Centre for Disease Prevention and Control. 2017. Clusters of autochthonous chikungunya virus cases in Italy, first update–9 October 2017. Stockholm, Sweden: European Centre for Disease Prevention and Control. [https://ecdc.europa.eu/en/publications/documents/RRAs/chikungunya-Italy-update-9-Oct-2017.pdf](https://ecdc.europa.eu/en/publications/documents/RRAs/chikungunya-Italy-update-9-Oct-2017.pdf).
6. Ministero della Salute, Istituto Superiore di Sanita. 27 October 2017. National plan of surveillance and response to arbovirus transmitted by mosquitoes (Aedes sp.), with particular reference to chikungunya, dengue and zika viruses. [http://www.salute.gov.it/portale/temi/documenti/chi/chi/chikungunya/bollettino_chikungunya_ULTIMO.pdf](http://www.salute.gov.it/portale/temi/documenti/chi/chi/chikungunya/bollettino_chikungunya_ULTIMO.pdf).
7. Schuffenecker I, Itoen I, Michaut A, Muri S, Frangeul L, Vaney MC, Lavenir R, Paradig N, Reyes JM, Pettinelli F, Bismont L, Diancourt L, Michal S, Duquerroy S, Guigon F, Bréhin AC, Cubito N, Després P, Kunst F, Rey FA, Zeller H, Brisse S. 2006. Genome microevolution of chikungunya viruses causing the Indian Ocean outbreak. PLoS Med 3:e263. [https://doi.org/10.1371/journal.pmed.0030263](https://doi.org/10.1371/journal.pmed.0030263).
8. Bordi L, Meschi S, Selleri M, Lalle E, Castilletti C, Carletti F, Chiappini R, Di Caro A, Capobianchi MR. 2011. Chikungunya virus isolates with/without A226V mutation show different sensitivity to IFN-a, but similar replication kinetics in non human primate cells. New Microbiol 34:87–91.
9. Bordi L, Carletti F, Castilletti C, Chiappini R, Sambri V, Cavini F, Ippolito G, Di Caro A, Capobianchi MR. 2008. Presence of the A226V mutation in autochthonous and imported chikungunya virus strains. Clin Infect Dis 47:428–429. [https://doi.org/10.1086/589925](https://doi.org/10.1086/589925).
10. Venturi G, Di Luca M, Fortuna C, Remoli ME, Riccardo F, Severini F, Toma L, Del Manso M, Benedetti E, Caporali MG, Amendola A, Fiorentini C, De Liberato C, Giammattei R, Romi R, Pezzotti P, Rezza G, Rizzo C. 2017. Detection of a chikungunya outbreak in central Italy, August to September 2017. Euro Surveill 22:39. [https://doi.org/10.2807/1560-7917.ES.2017.22.39.17-00646](https://doi.org/10.2807/1560-7917.ES.2017.22.39.17-00646).
11. Bordi L, Carletti F, Lalle E, Colavita F, Meschi S, Di Caro F, Nicastri E, Scognamiglio P, Vairo F, Di Lallo D, Panella V, Capobianchi MR, Ippolito G, Castilletti C. 2018 January 15. Molecular characterization of the first autochthonous chikungunya cluster in Latium region, Italy. Emerg Infect Dis. [https://doi.org/10.3201/eid2401.171605](https://doi.org/10.3201/eid2401.171605).
12. Ho DTW, Chan DPC, Lam CY, Liang DC, Lee SS, Kam JKM. 2017 August 24. At the advancing front of chikungunya fever in Asia: two imported cases in Hong Kong with novel amino acid changes. J Microbiol Infect. [https://doi.org/10.1016/j.jmi.2017.08.006](https://doi.org/10.1016/j.jmi.2017.08.006).
13. Arankalle VA, Shrivastava S, Cherian S, Gunjikar RS, Walimbe AM, Jadhav SM, Sudeep AB, Mishra AC. 2007. Genetic divergence of chikungunya viruses in India (1963–2006) with special reference to the 2005–2006 explosive epidemic. J Gen Virol 88:1967–1976. [https://doi.org/10.1099/0.82714-0](https://doi.org/10.1099/0.82714-0).
14. Agarwal A, Sharma AK, Sukumaran D, Parida M, Dash PK. 2016. Two novel episodic mutations (E1:K211E and E2:V264A) in structural proteins of chikungunya virus enhance fitness in Aedes aegypti. Virology 497:59–68. [https://doi.org/10.1016/j.virol.2016.06.025](https://doi.org/10.1016/j.virol.2016.06.025).
15. Singh RK, Tiwari S, Mishra VK, Tiwari R, Dhole TN. 2012. Molecular epidemiology of chikungunya virus: mutation in E1 gene region. J Virol Methods 185:213–220. [https://doi.org/10.1016/j.viromet.2012.07.001](https://doi.org/10.1016/j.viromet.2012.07.001).