with advanced disease. Such testing could improve diagnostics, reduce false-negative results, and strengthen the reliability of epidemiologic data during ongoing and future outbreaks.

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Molecular Characterization of Autochthonous Chikungunya Cluster in Latium Region, Italy

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We report partial molecular characterization of isolates from an autochthonous chikungunya virus cluster in the Latium Region of Italy. E1 sequences from 3 patients differ substantially from sequences from the 2007 outbreak in Italy and lack the A226V substitution associated with increased viral fitness in the *Aedes albopictus* mosquito vector.

Local transmission of chikungunya virus (CHIKV) has been confirmed in the Lazio region of Italy, with 2 related autochthonous clusters in the cities of Anzio and Rome (1). This event is the second known autochthonous outbreak of CHIKV in Italy; the previous one occurred in 2007 in the Emilia Romagna region and included >205 cases of CHIKV infection during July 4–Sept 27, 2007 (2). During the time between the 2 epidemics in Italy, autochthonous transmissions were described in France in 2010, 2014, and 2017; these events have focused attention on this infection because of the *Aedes albopictus* mosquito vector establishing itself in parts of the Mediterranean basin and beyond. *Ae. albopictus* mosquitoes are assumed to be the vector in the ongoing outbreak in Italy because *Ae. aegypti* mosquitoes are not circulating in this country. The Laboratory of Virology of Lazzaro Spallanzani National Institute for Infectious Disease (INMI) in Rome, as the regional reference laboratory for arboviral infections, is in charge of CHIKV diagnosis and surveillance for the Latium region.

CHIKV diagnosis is based on the detection of the viral genome by real-time reverse transcription PCR (RT-PCR) and of virus-specific antibodies by serologic tests. We conducted sequence analysis of endpoint PCR amplicons from selected case-patients to confirm the identity of the virus detected by real-time RT-PCR and performed virus isolation whenever possible. The first case-patient detected at INMI, a resident of Anzio (60 km from Rome) with no recent travel history abroad, was admitted to the Tropical Infectious Disease Unit on August 30, 2017, with suspected measles. Hospital staff suspected an arboviral disease on September 3, and on September 5, a serologic test was positive for chikungunya virus (E. Nicastri, unpub. data). The official notice of autochthonous cases in this region was disclosed on September 8, 2017. As of September 26, a total of 183 CHIKV infections have been reported (109 confirmed and 74 probable) (3), consistent with an extensive autochthonous outbreak. An intensive diagnostic campaign and backtracing of cases is under way to establish the extent of the outbreak.

Phylogenetic analyses of CHIKV strains obtained during outbreaks in different geographic regions (Réunion, Seychelles, Mauritius, Madagascar, and Mayotte) have identified the independent acquisition of a common mutation, A226V, in the E1 glycoprotein (4). This mutation, together with mutations M269V and D284E in E1 glycoproteins, has been described as a molecular signature of the Indian Ocean CHIKV outbreak (5,6). The A226V mutation in particular appeared in >90% of isolates after December 2005 and has been demonstrated to increase viral fitness in *Ae. albopictus* mosquitoes, expanding the potential for CHIKV to diffuse (5,6). In a previous study, we described the presence of this mutation in CHIKV in specimens from patients referred to our diagnostic facility, 5 imported to Italy and 2 linked to the 2007 outbreak in Italy (7). In this study, we extend the genetic analysis to samples from the ongoing outbreak.

We obtained a partial sequence of the E1 coding region directly from clinical samples of 3 patients being managed at INMI. These include the patient from Anzio and 2 additional cases belonging to a family cluster of 3 cases. On September 1, a 3-year-old child, born in Rome with neither travel history nor connection with Anzio, was brought to INMI with complaints of high-grade fever, arthralgia, rash, fatigue, headache, and retro-orbital pain. His parents reported a similar febrile syndrome 24 and 48 hours later. Samples collected on September 6 for all 3 patients tested positive for CHIKV by serology and real-time RT-PCR. We obtained E1 sequences from both parents and submitted sequences for all 3 patients to GenBank (accession nos. MF988056–8).

Further, we conducted a phylogenetic analysis that included 42 available CHIKV sequences from different parts of the world, including sequences previously analyzed in our laboratory (Figure). The analysis, based on a partial E1 sequence, showed that the virus involved in the online Latium region outbreak belongs to the broad group comprising isolates from the East/Central/South African (ECSA) clade and clusters with the Indian Ocean lineage (Figure). This finding is similar to what was observed for the 2007 Italy outbreak, but the current sequences are placed in a separate branch of the phylogenetic tree (bootstrap value 0.83). This branch also includes recent (2016) isolates from Pakistan and India, suggesting a more recent origin of the new epidemic strain, compared with the previous one affecting Italy. It is noteworthy that, unlike the isolates obtained from the 2007 outbreak in the Emilia Romagna region, the E1 sequences from the ongoing outbreak lack the A226V mutation, as do all the recent isolates placed on the same branch of the phylogenetic tree. Further study will establish the relevance of this and other genetic signatures to the fitness of the virus for the local mosquito vectors and will determine the extent of transmission cycles in humans.

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Dr. Bordi is a research scientist at the Lazzaro Spallanzani National Institute for Infectious Diseases in Rome. Her research activity is related to emerging and reemerging infections, focusing on virological aspects of host–pathogen interactions, and development of tools and protocols for the diagnosis of emerging viral diseases in the context of national research programs.
Inonotus in Patient with Hematologic Malignancy

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We report a lung-invasive fungal disease with possible cutaneous needle tract seeding in a patient with a febrile neutropenia caused by the Basidiomycetes mold Inonotus spp. Although rare, Inonotus spp. should be added to the list of microorganisms causing invasive fungal disease in neutropenic patients with hematologic malignancies.

A 33-year-old man in Madrid, Spain, with chronic myeloid leukemia in lymphoid blastic phase underwent allogeneic stem cell transplantation (SCT) from a matched unrelated donor in 2011. Four years later, he had an extramedullary pulmonary relapse, after which he began intensive reinduction chemotherapy. After the second cycle, prolonged severe aplasia developed in the patient. Invasive fungal disease (IFD) was suspected because of the presence of persistent fever despite broad-spectrum antimicrobial drugs and the appearance of a new pulmonary nodule (Figure, panel A; online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/1/17-1265-Techapp1.pdf) while the patient was receiving prophylactic micafungin (50 mg/d). Serologic fungal biomarkers were negative. A percutaneous pulmonary biopsy sample was taken, and empirical liposomal amphotericin B (3 mg/kg/d) was started (December 2015). Histology showed unspecific inflammatory tissue, and microbiology cultures were negative.

Salvage human leukocyte antigen–haploidentical SCT was performed in January 2016. Fever persisted during conditioning therapy, and a solitary cutaneous millimetric erythematous lesion appeared at the biopsy puncture site (Figure, panel B). Histopathology of the skin lesion showed dermal infiltration by periodic acid Schiff–positive elements compatible with fungal hyaline hyphae with parallel walls, regular septa, and branched hyphae with occasional bulb-like expansions; angioinvasion; and necrosis. Fungal culture of the specimen was negative, but panfungal PCR and further sequencing (I) revealed the presence of Inonotus spp. Voriconazole was added, and the lesion resolved in days. Neutrophil engraftment was achieved on day 12 post-SCT, with complete donor chimerism.

On day 34, the pulmonary lesion progressed, but we could not prove IFD as the cause of concomitant pleural effusion. Despite intensified antifungal therapy, surgical debridement was required to resolve the empyema. The patient was discharged on oral posaconazole (300 mg/d) that was eventually replaced by micafungin.

A computed tomography scan performed 6 months after the SCT showed persistence of a single mass on the left

References
9. Serologic fungal biomarkers were negative. A percutaneous needle tract seeding in a patient with a febrile neutropenia caused by the Basidiomycetes mold Inonotus spp.
10. Although rare, Inonotus spp. should be added to the list of microorganisms causing invasive fungal disease in neutropenic patients with hematologic malignancies.

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Molecular Characterization of Autochthonous Chikungunya Cluster in Latium Region, Italy

Technical Appendix

Technical Appendix Figure. Phylogenetic tree of E1 sequences of chikungunya virus obtained from 3 patients belonging to an ongoing cluster of autochthonous cases occurring in the Latium region of Italy, compared with reference sequences. The phylogenetic tree was built with the partial sequences of E1 coding region (722 bp, nucleotide positions 10178–10899 with respect to the S27 reference sequence, GenBank accession no. AF369024) obtained from these 3 patients (boldface, labeled Latium 2017;
GenBank accession nos. MF988056–8), in the context of E1 sequences representing the 3 major described CHIKV lineages: ECSA (including the Indian Ocean lineage), Asia-Caribbean, and West Africa (indicated at right). An additional 7 partial E1 human sequences previously obtained in our laboratory are noted (boldface); Italy 2007 label indicates the cluster of E1 sequences correlated with the 2007 outbreak in Italy, including 1 insect isolate (EU244823ITA07-RA1). Reference sequences are shown with GenBank accession numbers, geographic origin, and year of sampling. Letters in parentheses (A or V) indicate the amino acid observed at position 226 of E1 protein. We computed evolutionary distances using the Kimura 2-parameter method and inferred evolutionary history using the neighbor-joining method. We used the closest relative alphavirus O'nyong'nyong virus as an outgroup. Bootstraps were generated using 1,000 replicates; only those $>70$ are shown. Scale bars represent the genetic distance (substitution per nucleotide position). ECSA, East/Central/South African.