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Another Dimension

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Severe Encephalitis Caused by Toscana Virus, Greece

To the Editor: In late June 2012, a previously healthy, 49-year-old woman was admitted to the emergency department of Trikala General Hospital in Trikala, Greece, with confusion and delirium. A few hours before admission, she had had a grand mal seizure; she had experienced gastroenteritis with fever (38°C) 5 days earlier. On admission, she was intubated and transferred to the intensive care unit, where she underwent mechanical ventilation and sedation.

The patient was a resident of Genesi village (350 m altitude), 22 km west of Trikala in the Thessaly region. She had not traveled abroad or to other area of Greece. Results of blood and cerebrospinal fluid (CSF) laboratory testing were unremarkable except slight leukocytosis (leukocytes 11,330 cells/mm^3, 92% neutrophils) and slightly elevated serum lactate dehydrogenase level (240 U/L). Brain imaging showed edema (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/8/14-0248-Techapp1.pdf), which resolved 48 hours after admission. The patient was awakened on day 3 of hospitalization and extubated on day 4. Treatment included anticonvulsants, mannitol, antimicrobial drugs (vancomycin and ceftriaxone), acyclovir, and corticosteroids. The patient fully recovered and was discharged from the hospital on day 12 with short-term antiepileptic medication.

Because West Nile virus (WNV) infections emerged in 2010 in Greece and outbreaks have recurred (*1*), serum and CSF samples from the patient were sent for testing to the National Reference Centre for Arboviruses. Antibodies against WNV were not detected. Reverse transcription nested PCR was conducted by using generic primers for flaviviruses,

enteroviruses, and phleboviruses. PCR for phleboviruses (*2*) resulted in a PCR product of the expected size, and the sequence was most closely related to those of isolates belonging to the *Sandfly fever Naples virus* (SFNV) species (Figure). The sequence also had the highest homology (85%) with a Toscana virus (TOSV) strain belonging to lineage C that had been obtained from a patient with central nervous system infection in Croatia in 2008 (*3*). The TOSV sequence derived from the patient in this report was submitted to GenBank (accession no. KJ418710). On the basis of a partial sequence comparison (202 nt in the polymerase gene), we found that TOSV lineage C differs from lineages A and B by 29% and 30%, respectively.

Forty-one days after symptom onset, a second serum sample was taken from the patient and tested in parallel with the first serum sample by indirect immunofluorescence to detect IgM and IgG antibodies against 4 phleboviruses: TOSV, SFNV, sandfly fever Sicilian virus (SFSV), and Cyprus virus (Sandfly Fever Virus Mosaic 1; Euroimmun, Lübeck, Germany). The first sample yielded negative results, but the follow-up sample showed IgM and IgG against TOSV and SFNV (both belonging to SFNV serocomplex). Neutralization testing to differentiate TOSV and SFNV was not performed because PCR and sequencing confirmed the TOSV infection.

Sandfly-borne phleboviruses (family *Bunyaviridae*) are endemic in Mediterranean countries, and at least 3 serotypes are associated with disease in humans: TOSV, SFNV, and SFSV. Among these, TOSV is associated with neurotropism, a major cause of meningitis and encephalitis in the Mediterranean region (*4*). Recent studies in Greece showed that the seroprevalence of TOSV (and antigenically related viruses) ranges from 0% to 60%; the higher levels are found in the islands and the coastal regions (*5*–*7*).

A study conducted in 2 Greek islands (Lefkas and Corfu, where Corfu virus was isolated) showed that the sandfly species with the widest distribution was *Phlebotomus neglectus* (31.2%), followed by *P. similis* (25.1%) and *P. tobbi* (15.3%) (*8*). In Thessaly region, where the case we report occurred, a faunistic study of sandflies showed that *P. perfiliewi* and *P. papatasi* (known vectors of TOSV) accounted for 83.4% and 3.93%, respectively, of the sandflies collected (*9*). Another phlebovirus, Adria virus (belonging to the Salehabad serocomplex), which was initially detected in sandflies collected in Albania, was detected in a febrile child with seizure in Thessaloniki in northern Greece (*10*). Concerning TOSV, however, although seroconversion has been previously observed in patients in Greece, RNA has not been detected.

For this patient, TOSV was detected by using phlebovirus generic primers. The TOSV sequence found in Greece differs greatly from other TOSV sequences, even from the genetically closer Croatian TOSV sequence (15%). To avoid false-negative results, the high genetic diversity among TOSV strains must be taken into consideration when using TOSV-specific primers.

In conclusion, a novel variant of TOSV has been detected in Greece. Further studies are needed to obtain a whole-genome sequence of the Greek TOSV strain and to identify the vector(s) of the virus. TOSV is a highly variable neurotropic phlebovirus, a characteristic that must be taken into account by laboratory scientists. Clinicians should be aware of the possibility of phlebovirus infections in Mediterranean countries and should include these viruses in the differential diagnosis of febrile illnesses observed during the warm seasons, especially in patients who exhibit neurologic symptoms.

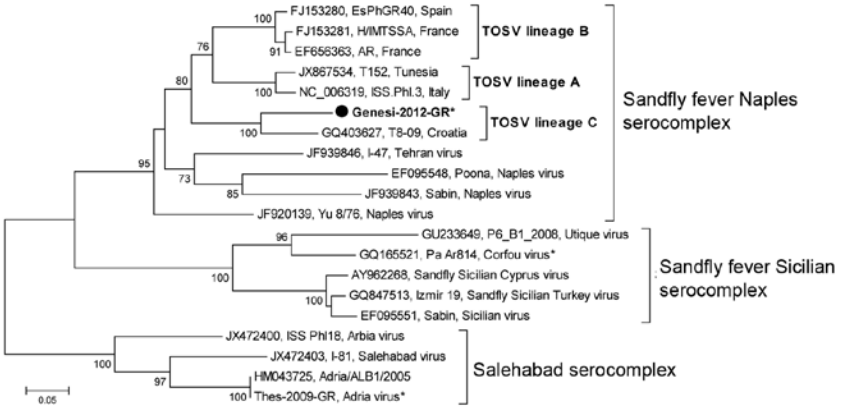

Figure. Neighboring-joining tree constructed on the basis of a 202-bp fragment of the large RNA segment of sandfly-borne phleboviruses. Black circle indicates Toscana virus strain detected in this study in a patient in Greece; asterisks (*) indicate phleboviruses detected in Greece. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. Evolutionary analyses were conducted in MEGA5 (http://www.megasoftware.net). Scale bar indicates substitutions per nucleotide position.

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Phylogenetic Analysis of West Nile Virus Genome, Iran

To the Editor: West Nile virus (WNV) is a single-stranded, positive-sense RNA virus (≈11 kb) that is taxonomically classified within the family *Flaviviridae*, genus *Flavivirus*. WNV is found in Africa, Eurasia, Australia, and North America (*1*).

Comprehensive studies on phylogenetic relatedness of WNV strains have showed that WNV can be grouped into 5 lineages. Lineage 1 contains WNV strains from different regions, including northern, western, and central Africa; southern and eastern Europe; India; and the Middle East. Lineage 1 is subdivided into 3 clades. Clade 1A contains strains from Europe, northern Africa, the United States, and Israel, clade 1B contains Kunjin virus from Australia. Lineage 2 contains isolates from west, central, and eastern Africa and Madagascar. There is evidence that lineage 2 circulates in some regions of Europe (e.g., Italy, Austria, and Greece) (*2*,*3*). Lineage 3 contains Rabensburg virus 97–103, which was isolated in 1997 from *Culex pipiens* mosquitoes in South Moravia in the Czech Republic. Lineage 4 contains a new variant of WNV (strain LEIVKrnd88–190), which was isolated in 1998 from *Dermacentor marginatus* ticks in a valley in the northwestern Caucasus Mountains of Russia. Lineage 5 contains an WNV isolate from India (strain 804994) (*4*,*5*). In this study, we compared the phylogenetic relationships of WNV circulating in Iran to other WNV strains by using a partial WNV sequence isolated from an Iranian patient.

WNV was obtained from a blood sample from an Iranian patient who had encephalitis and was hospitalized in 2009 in Isfahan in the central highlands of Iran. The patient reported no history of animal contact, insect bites, blood transfusions, transplantations, and travel. He exhibited fever, headache, hypertension, and vomiting. On initial examination, he had a body temperature of 40°C. Laboratory investigations on the day of admission showed a leukocyte count of 240 cells/mL, a protein level of 52 mg/dL, and a glucose level of 50 mg/dL in a cerebrospinal fluid sample.

Further examinations were undertaken, and samples were sent to the Arboviruses and Viral Hemorrhagic Fevers Laboratory at Pasteur Institute of Iran in Teheran. For an IgG ELISA, wells in test plates were coated overnight with mouse hyperimmune ascitic fluid. Native antigen was added, and wells were incubated and washed. Test samples and peroxidase-labeled anti-human or anti-animal immunoglobulin were added. After incubation for 10 min, optical densities were read (*6*).

Viral RNA was extracted by using the QIAmp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) from serum of the patient. A reverse transcription PCR was conducted by using a One-Step RT-PCR Kit (QIAGEN). Samples were subjected to 1 cycle at 50°C for 30 min to synthesize cDNA; 95°C for 15 min; and 95°C for 30 s, 54°C for 30 s, and 72°C for 60 s; and a final extension at 72°C for 5 min (*6*). The serum sample was positive for IgG against WNV. Molecular tests showed positive results for WNV.

The PCR product was sequenced by using the Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied