

# Complete Coding Sequences of Six Toscana Virus Strains Isolated from Human Patients in France

Amal Baklouti,<sup>a,b</sup> Isabelle Leparc-Goffart,<sup>a,b,c</sup> Geraldine Piorkowski,<sup>a,b</sup> Bruno Coutard,<sup>d</sup> Nicolas Papageorgiou,<sup>d</sup> Xavier De Lamballerie,<sup>a,b</sup> Rémi N. Charrel<sup>a,b</sup>

UMR "Emergence des Pathologies Virales" (EPV: Aix-Marseille University - IRD 190 - Inserm 1207 - EHESP), Marseille, France<sup>a</sup>; Fondation IHU Méditerranée Infection, APHM Public Hospitals of Marseille, Marseille, France<sup>b</sup>; Institut de Recherche Biomédicale des Armées, National Reference Laboratory for Arboviruses, Marseille, France<sup>c</sup>; CNRS, AFMB UMR 7257, Marseille, France<sup>d</sup>

**Toscana virus (TOSV) is an arthropod-borne phlebovirus belonging to the *Sandfly fever Naples virus* species (genus *Phlebovirus*, family *Bunyaviridae*). Here, we report the complete coding sequences of six TOSV strains isolated from human patients having acquired the infection in southeastern France during a 12-year period.**

Received 15 April 2016 Accepted 19 April 2016 Published 26 May 2016

**Citation** Baklouti A, Leparc-Goffart I, Piorkowski G, Coutard B, Papageorgiou N, De Lamballerie X, Charrel RN. 2016. Complete coding sequences of six Toscana virus strains isolated from human patients in France. *Genome Announc* 4(3):e00454-16. doi:10.1128/genomeA.00454-16.

**Copyright** © 2016 Baklouti et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Amal Baklouti, amal.baklouti@yahoo.fr, or Rémi Charrel, remi.charrel@univ-amu.fr.

Toscana virus (TOSV) belongs to the *Sandfly fever Naples virus* species within the genus *Phlebovirus* in the family *Bunyaviridae*. It is transmitted by phlebotomine sandflies belonging to the genus *Phlebotomus* in the Old World. TOSV is an enveloped virus with a single-strand negative-sense RNA genome consisting of three segments (large [L], medium [M], and small [S]). L encodes the virus polymerase, M encodes Gn and Gc, the two glycoproteins, and the nonstructural proteins NSm, and S encodes the nonstructural proteins NSs and the nucleoprotein (N) (1).

The phylogenetic relationships indicate that TOSV strains are subdivided into 3 lineages (A, B, and C) that are more or less congruent with the geography. Lineage A strains have been identified in Italy, France, Algeria, Tunisia, and Turkey (2–5); lineage B strains were detected in Spain, Portugal, France, Morocco, and Turkey (6, 7); lineage C was detected on a molecular basis only (no virus isolation) in Croatia and Greece. Interestingly, lineages A and B cocirculate in France and Turkey (8, 9).

Despite the large geographic area where human populations are exposed to TOSV, only 10 full-length sequences were available at the outset of this study, three for lineage A and seven for lineage B; of these 10 sequences, seven originated from sandflies and three from humans. We determined the complete coding sequence of five lineage B TOSV (TOSV-4906, TOSV-11368, TOSV-9028, TOSV-54441132704, and TOSV-5963930705) and one lineage A (TOSV-5904242804) strain. This study brings the total to 6 complete sequences of TOSV that are publicly available.

All strains were isolated in Vero cells from clinical samples collected in human patients. Viral RNA extracted from an infected Vero cell culture supernatant at passage 3 was used for next-generation sequencing (NGS) using PGM Ion Torrent (Life Technologies) after nonspecific amplification. Reads were mapped on TOSV reference sequences to produce long consensus contigs. The criteria for read validation were >50% mapping in length and >80% identity to the selected reference sequence ISS.Phl.3 (GenBank accession numbers NC\_006318 to NC\_006320) for lin-

eage A TOSV-5904242804; for the other 5 lineage B strains, sequences were mapped onto TOSV-AR 2007 (GenBank accession numbers EF656361 to EF656363).

The open reading frames (ORFs) of the 6 strains encoded N (254 amino acids [aa]), NSs (316 aa), Gn (527 aa), Gc (502 aa) and L (2,101 aa). The full-length sequences of each gene were aligned with homologous TOSV sequences using the CLUSTAL algorithm. Neighbor-joining analysis (Kimura 2-parameter and *p*-distance models) was performed by MEGA6, with 1,000 bootstrap pseudoreplications. These six TOSV strains are available for academic research purposes in the collection of the European Virus Archive (EVAg).

**Nucleotide sequence accession numbers.** The sequences of TOSV-9028, TOSV-4906, TOSV-11368, TOSV-5963930705, TOSV-5904242804, and TOSV-54441132704 have been deposited in GenBank under the accession numbers [KU904263](https://www.ncbi.nlm.nih.gov/nuclink/KU904263) to [KU922125](https://www.ncbi.nlm.nih.gov/nuclink/KU922125), [KU922127](https://www.ncbi.nlm.nih.gov/nuclink/KU922127), [KU925897](https://www.ncbi.nlm.nih.gov/nuclink/KU925897) to [KU925899](https://www.ncbi.nlm.nih.gov/nuclink/KU925899), [KU935733](https://www.ncbi.nlm.nih.gov/nuclink/KU935733) to [KU935735](https://www.ncbi.nlm.nih.gov/nuclink/KU935735), [KU935736](https://www.ncbi.nlm.nih.gov/nuclink/KU935736) to [KU933538](https://www.ncbi.nlm.nih.gov/nuclink/KU933538), and [KX010932](https://www.ncbi.nlm.nih.gov/nuclink/KX010932) to [KX010934](https://www.ncbi.nlm.nih.gov/nuclink/KX010934).

## ACKNOWLEDGMENTS

This work was supported through funds received from (i) the EU grant FP7-261504 EDENext, and this paper is catalogued by the EDENext Steering Committee as EDENext455 (<http://www.edenext.eu>), and (ii) the European Virus Archive goes Global (EVAg) project in the European Union's Horizon 2020 research and innovation programme under grant agreement 653316 (<http://global.european-virus-archive.com/>). The work of R.N.C. was done under the frame of EurNegVec COST Action TD1303.

## FUNDING INFORMATION

The PhD salary of Amal Baklouti was granted by the Fondation Méditerranée Infection.

## REFERENCES

- Charrel RN, Bichaud L, De Lamballerie X. 2012. Emergence of Toscana virus in the Mediterranean area. *World J Virol* 5:135–141.

2. Cusi MG, Savellini GG, Zanelli G. 2010. Toscana virus epidemiology: from Italy to beyond. *Open Virol J* 4:22–28.
3. Sakhria S, Bichaud L, Mensi M, Salez N, Dachraoui K, Thirion L, Cherni S, Chelbi I, De Lamballerie X, Zhioua E, Charrel RN. 2013. Co-circulation of Toscana virus and Punique virus in northern Tunisia: a microneutralisation-based seroprevalence study. *PLoS Negl Trop Dis* 7:e2429. <http://dx.doi.org/10.1371/journal.pntd.0002429>.
4. Alkan C, Allal-Ikhlef AB, Alwassouf S, Baklouti A, Piorkowski G, De Lamballerie X, Izri A, Charrel RN. 2015. Virus isolation, genetic characterization and seroprevalence of Toscana virus in Algeria. *Clin Microbiol Infect* 21:1040.e1–1040.e9. <http://dx.doi.org/10.1016/j.cmi.2015.07.012>.
5. Dachraoui K, Fares W, Bichaud L, Barhoumi W, Beier JC, Derbali M, Cherni S, De Lamballerie X, Chelbi I, Charrel RN, Zhioua E. 2016. Phleboviruses associated with sand flies in arid bio-geographical areas of Central Tunisia. *Acta Trop* 158:13–19. <http://dx.doi.org/10.1016/j.actatropica.2016.02.008>.
6. Nougairede A, Bichaud L, Thiberville SD, Ninove L, Zandotti C, De Lamballerie X, Brouqui P, Charrel RN. 2013. Isolation of Toscana virus from the cerebrospinal fluid of a man with meningitis in Marseille, France, 2010. *Vector Borne Zoonotic Dis* 13:685–688. <http://dx.doi.org/10.1089/vbz.2013.1316>.
7. Sanbonmatsu-Gámez S, Pérez-Ruiz M, Collao X, Sánchez-Seco MP, Morillas-Márquez F, De la Rosa-Fraile M, Navarro-Mari JM, Tenorio A. 2005. Toscana virus in Spain. *Emerg Infect Dis* 11:1701–1707.
8. Charrel RN, Izri A, Temmam S, Delaunay P, Toga I, Dumon H, Marty P, De Lamballerie X, Parola P. 2007. Cocirculation of 2 genotypes of Toscana virus, southeastern France. *Emerg Infect Dis* 13:465–468.
9. Elliott RM, Bouloy M, Calisher CH, Goldbach R, Moyer JT, Nichol ST, Pettersson R, Plyusnin A, Schmaljohn CS. 2000. Family *Bunyaviridae* in virus taxonomy, p 599–621. Seventh report of the International Committee on Taxonomy of Viruses, vol 22. Academic Press, San Diego, CA.