

Viral infections: from the lab to the clinic

First evidence of Toscana virus (genus phlebovirus, family bunyaviridae) in Algeria based on virus isolation and high rate of neutralizing antibodies in humans

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AIM

To assess the presence of Toscana virus in northern Algeria through virus isolation in sandflies, and to perform a seroprevalence study in humans using neutralization assays.

MATERIALS AND METHODS

Trapping of sandflies was performed at Draa El Mizan in the Kabylia region of Algeria in summer 2013 (380 m - altitude). Capture sites were selected on epidemiological data reporting laboratory-documented cases of cutaneous leishmaniasis and on the presence of domestic herbivorous animals. Pools (up to 30 sandflies) were processed for virus isolation and RT-PCR detection (2 generic systems targeting all phleboviruses and sandfly fever Naples species, respectively; 1 real-time RT-PCR system targeting Toscana virus) [Sanchez-Secco et al 2003 J Med Virol, Charrel et al 2007 Emerg Infect Dis, Weidmann et al 2009 J Clin Virol]. Complete genome sequencing was done using Next-Generation Sequencing with a Ion-Torrent PGM. Sequences were aligned with all complete sequences from Genbank and phylogenetic analysis was performed gene by gene using amino acid complete ORF and Maximum Likelihood algorithm. Two-fold dilutions (1:20 to 1:160) of human and animal sera were tested for neutralizing antibodies against Toscana virus as described [Sakhria et al 2013 PLoS Negl Trop Dis].

RESULTS

A total of 22,998 (13,431 sand flies captured alive and 9567 sand flies captured dead) during 5 nights-CDC traps (696 sand flies/ night-CDC traps). They belonged to 5 species: *Phlebotomus perfiliewi*, *Phlebotomus perniciosus*, *Phlebotomus longiscupis*, *Phlebotomus papatasi* and *Sergentomya minuta*.

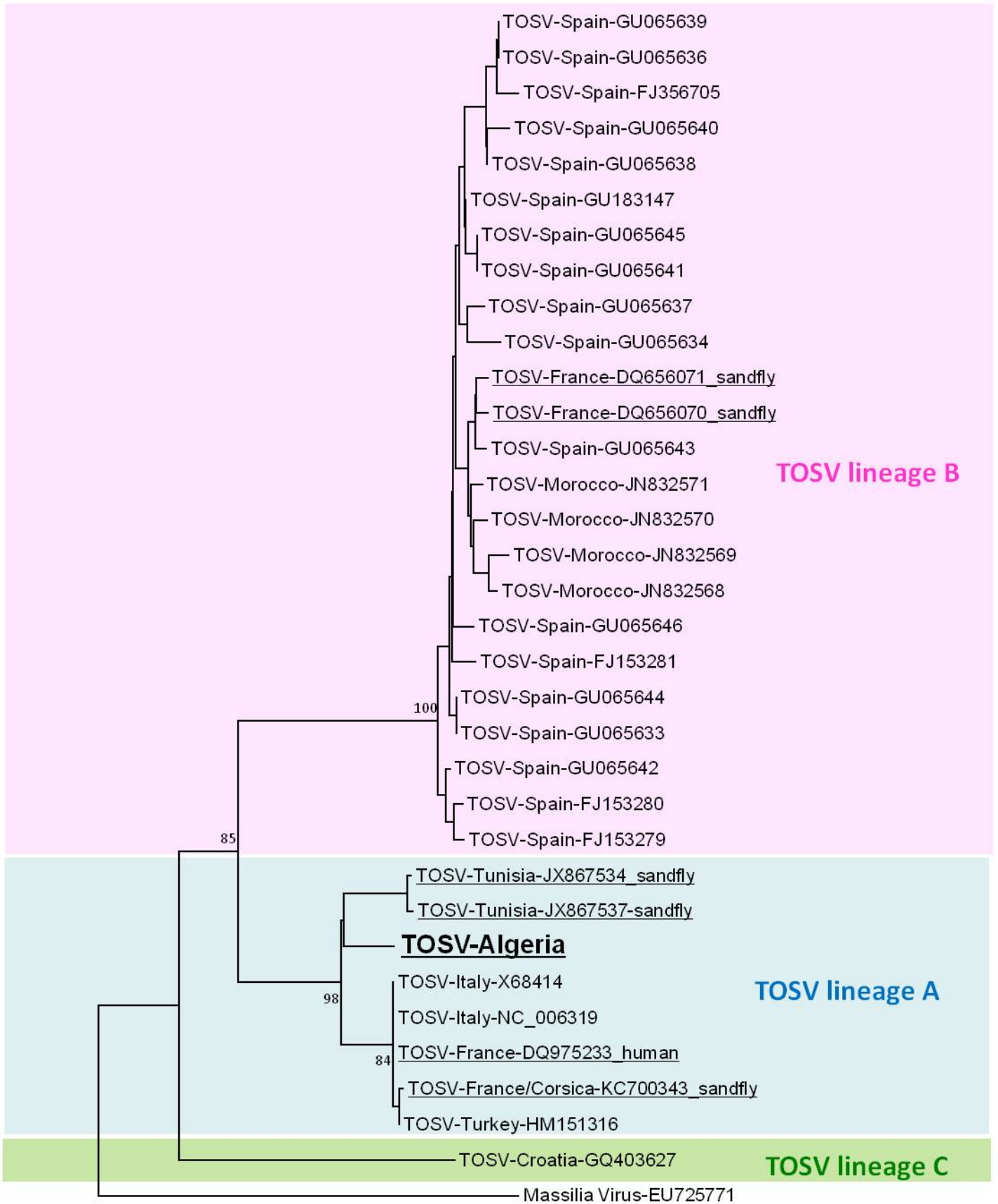
Two pools were found positive for the presence of phlebovirus RNA, which proved to be Toscana virus RNA after sequencing. One strain of Toscana virus was isolated onto Vero cells and the complete sequence was used for alignment and derived genetic and phylogenetic analyses (Figure). Genetic analysis indicates that this Algerian strain (i) grouped within the lineage A together with Tunisian, Italian, French and Turkish strains is distinct, (ii) was clearly distinct from all other sequences for which data are currently available.

A total of 370 human sera (women 247, male 123) were collected in the same region. The four classes of age (<20, 20-39, 39-59, >=60) included 23, 150, 89, and 108 persons, respectively. Microneutralisation-based (MN) seroprevalence study showed that 26 sera possessed neutralizing antibodies (7.0%).

CONCLUSIONS

This study constitutes the first undisputable evidence that Toscana virus is present in northern Algeria (Kabylia) and that it readily infects human populations living in the region. It is now important to implement diagnosis of Toscana virus in hospitals of the region to test patients presenting with either febrile syndrome or neuro-invasive infections in addition to Enteroviruses, mumps, herpesviruses and West Nile virus. Future studies are necessary to delineate the boundaries of the area where Toscana virus is circulating in Algeria.

Phylogeny of Toscana virus (partial L gene)



0.05