SAND FLIES AND TOSCANA VIRUS

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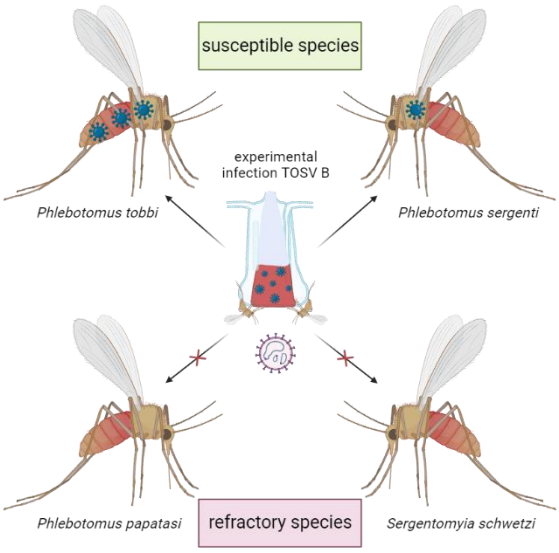

Phlebovirus toscanaense (Toscana virus, TOSV) from the *Phenuiviridae* family is an emerging but still neglected human pathogen. Infections vary from non-symptomatic forms through febrile illness to CNS disease, rarely death. The virus circulates in the Mediterranean area, where it is transmitted by sand flies (Diptera, Phlebotominae); however, there are consequently many knowledge gaps about its biology and its persistence in nature; no reservoir hosts of TOSV have been found (despite thorough examinations of various vertebrates), and vertical and sexual transmission between sand flies is not sufficiently effective to sustain the virus cycle in nature[1,2,3,4].

Based on the nucleotide sequences, TOSV is currently divided into three lineages A, B and C; for the last one no isolate and only a partial sequence has been obtained[1,5]. No differences have been observed in the nature of the host, the clinical picture, or disease severity associated with virus lineages, but they seem to a certain extent to differ in geographical distribution[2,6,7].

The only proven vectors are *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*. However, repeated findings of human cases or anti-TOSV antibodies in humans and animals in regions outside of the range of distribution of these two vectors suggests the involvement of other species of sand flies[1]. We tested the vector competence of four sand fly species: *Phlebotomus tobbi, Phlebotomus sergenti, Phlebotomus papatasi and Sergentomyia schwetzi* to two TOSV strains belonging to TOSV lineages A and B.

The strain 1500590, representing TOSV lineage A, infected vertebrate cells *in vitro*, but failed to develop in all four sand fly species tested. The strain MRS20104319501, representing lineage B, developed in *P. tobbi*, with an infection and dissemination rate of 60% and 46%, respectively. Another species that appears to be sensitive to infection, although less so than the previous species is *P. sergenti* with a total infection rate of 5.2% but a high dissemination rate of 100%. It seems that *P. sergenti* possesses an efficient midgut barrier; nevertheless, when the virus overcomes it successfully, it disseminates and establishes vector infection. Interestingly, two tested sand fly species, *P. papatasi* and *S. schwetzi*, were resistant to TOSV. For further testing, a colony of *Sergentomyia minuta*, a common species in the Mediterranean region was established[8]. We possess a reverse genetic system which will allow us to focus our research on parts of the genome that play an important role in virus-vector interactions[9].

In addition, we studied antiviral immunity in sand flies using *Phlebotomus papatasi*-derived cell lines. Following TOSV infection, distinctive 21 nucleotide virus-derived small interfering RNAs were detected. Silencing the exo-siRNA effector Ago rendered the exo-siRNA pathway inactive. Thus, our data show that this immune pathway is active in sand flies as an antiviral response against TOSV[10].

Acknowledgement
This work was supported by the project National Institute of Virology and Bacteriology (Programme EXCELES, ID Project No. LX22NPO5103) – Funded by the European Union –Next Generation EU.

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