

IDENTIFICATION OF FLAVIVIRUSES AND PHLEBOVIRUSES FROM INSECTS IN SOUTHWEST OF SPAIN.

Del Ventura – Villarroel F. (1), Ruiz S. (2), Herrero L. (1), Figuerola J. (3), Hernández L. (1), Moreno J. (2), Soriguer R. (3), Sánchez-Seco M.P.* (1), Vázquez A.* (1)

(1) Institute of Health Carlos III, Majadahonda, Spain.

(2) Servicio Control Mosquitos, Huelva, Spain.

(3) EBD-CSIC, Seville, Spain

Background

In Spain, the flavivirus West Nile (WNV) and the phlebovirus Toscana (TOSV) are autochthonous. They cause neurological disease in humans and are transmitted by mosquitoes (*Culex sp.*) or sandflies (*Phlebotomus sp.*), respectively. It seems that these arboviruses are circulating in several areas from Andalucía. In the province of Cádiz in 2010, human infections due to both viruses have been diagnosed during the first WNV outbreak occurred in humans in our country. Moreover, WNV lineage 1 has been detected in *Cx. perexiguus* from Huelva in 2008 and a new lineage was described in 2006 from *Cx. pipiens* in the same area. TOSV was detected in *Phlebotomus sp.* in Granada in 2004. In addition to these viruses, whose pathogenicity has already been demonstrated, other related viruses have been detected in our territory, such as Granada (GRV) and Usutu (USUV), for which its public health importance has not been determined yet. The proposed objective in this work was to study the presence of arboviruses in vectors from several provinces of Southwestern Spain.

Methods

Insects belonging to the genera *Phlebotomus* or *Culex* were captured in several Andalusian provinces (Huelva, Sevilla and Cádiz) in 2013. Insects were pooled by species, sex, collection site, and date. The arboviral screening was performed with two generics nested RT-PCR to detect flavivirus or phlebovirus genome. A total of 2.628 insects (190 pools), belonging to the *Psychodidae* and *Culicidae* families, were analyzed. Viral RNA was extracted using the kit QIAamp Viral RNA extraction (Qiagen). Amplification products were sequenced, and sequences were compared with all published sequences recovered from GenBank. They were aligned using CLUSTAL X, and phylogenetic analyses were performed using the MEGA package (version 5.0).

Results

Both, flaviviruses and phleboviruses were detected in this work. TOSV was detected in *Phlebotomus sp.* from Cadiz, and its sequence showed a level of homology of 98% with other sequences of TOSV detected previously in Spain. WNV was detected in three pools of *Cx. perexiguus* from Sevilla, and these sequences were similar to the WNV sequence detected in 2008 in *Cx. perexiguus* from Huelva. Moreover, sequences related to the phlebovirus GRV and to the insect flavivirus SCxFV, were detected.

Conclusion

In this work, we show the presence of WNV and TOSV in their vectors from some areas in Spain for the first time. These results confirm that these viruses are circulating in a wider area in the Southwest of Spain.