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Evidences of Mosquito-transmitted Flavivirus circulation in Piedmont, North-Western Italy

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The genus *Flavivirus* comprises several mosquito-borne species, such as the zoonotic pathogens West Nile (WN) and Usutu (USU) virus, circulating in animals and humans in the recent years in Europe.

In Piedmont, North-Western Italy, since 2009, entomological and avian surveillance was carried out to monitor mosquito population and early detect viral circulation.

Mosquito pools collected by CO₂-baited traps, were tested for the presence of *Flavivirus* strains by biomolecular analysis. Each pool, upon RNA extraction, was tested by a PCR assay for a 263 bp fragment of the *Flavivirus* NS5 gene. Positive samples were characterized by genetic sequencing. In addition, in 2012 and 2013, a serosurvey on wild birds was carried out by serum-neutralization assay in microtitre plates.

In five years, a total of 36,244 specimens, belonging to 10 mosquito species, and grouped in 1460 pools were tested.

Overall, 73 pools resulted positive for *Flavivirus*. Among them, 9 pools of *Culex pipiens* resulted correlated with European Usutu strains. The other 64 pools, belonged to 5 mosquito species (*Ae. albopictus*, *Ae. vexans*, *Cx. pipiens*, *Oc. caspius*, *Oc. geniculatus*), resulted related with various strains of Mosquito *Flavivirus*, actually under investigation.

Samples of blood from 304 birds belonging to 47 different species have been collected. Four serum samples from 4 bird species (*Anas platyrhynchos*, *Turdus merula*, *Asio otus* and *Columba livia*) had neutralizing antibodies against USUV. Ten samples from 6 species (*Otus scops*, *Ixobrychus minutus*, *Falco subbuteo*, *Luscinia megarhynchos*, *Falco tinnunculus* and *Picus viridis*) tested positive for WNV. Neutralizing antibodies for WNV were significantly more prevalent ($p < 0.001$) in trans-Saharan migrants than in resident and short-distance birds, but no migratory habit-related differences were found for USUV. Entomological surveillance shows the presence of mosquitoes with moderate-high competence for WNV and USUV, but a very low infection prevalence. In the same way, antibodies found in resident bird species suggest that both viruses are circulating in North Western Italy despite the absence of clinical cases in humans, horses and birds.