WNV presence and distribution

The West Nile virus (WNV) is among the most widespread mosquito-borne flavivirus in the world (Weissenböck et al., 2010). WNV was originally isolated from Uganda in 1937; afterwards epidemic outbreaks have been reported in Asia, Europe, Australia; and in August 1999 was introduced into the United States causing deaths in wild and zoo birds, horses, and humans in the New York City area. West Nile virus introduction and circulation have been demonstrated on multiple occasions in Southern Europe and Mediterranean basin since 1960s when seropositive animals or virus isolates were discovered in France, Portugal, and Cyprus (Filipe et al., 1969; Joubert et al., 1970), with WNV activity having dramatically increased over the last 10 years and spread to eastern territories without previous WNV records. If migratory birds have been associated to the introduction of viral strains from endemic areas (Calistri et al., 2010) the mechanism of virus persistence in animal hosts in Europe leading to endemization of the disease is still unknown.

The circulation of WNV in Europe may occur silently for several months, or even years, before a spill over event occurs and viral circulation becoming evident. In Europe, WNV has mainly been reported in central and south-eastern Europe, regions in which WNV infections and virulence have recently increased, and the implicated viruses have spread to new areas, including

Bulgaria and Greece in 2010, Albania and Macedonia in 2011, and Croatia, Serbia, and Kosovo in 2012, Germany in 2018.

Epidemiological situation in Italy

Following the first limited evidence of viral circulation in Tuscany in 1998, the Italian Ministry of Health issued a national veterinary surveillance plan for WNV monitoring areas at risk for WNV introduction and circulation. The surveillance system did not detect any relevant circulation of WNV until 2008, when the virus was identified in mosquitoes, birds, horses and humans in the area surrounding the Po river delta (Savini et al., 2008). Since then, WNV epidemics have been registered every year caused by genetically divergent isolates (Savini et al., 2012; Monaco et al., 2015) and, to date, 15 out of 20 Italian regions are considered endemic. (link al bollettino Italia).

Surveillance activities have been refined every year according to the epidemiology of the disease in the country thus leading to the adoption of a national plan integrating human, animal and entomological surveillance (One Health Surveillance) (Italian Ministry of Health, 2019 *1*).

In the Italian ecosystem, peak transmission of WNV to humans in Italy typically occurs between July and September, coinciding with the summer season when mosquitoes are most active and temperatures are highest. The mosquitoes cease their activity during the colder months, but it has been demonstrated that the virus is able to survive during this period in the infected mosquitoes, which overwinter indoors (Nasci et al, 2001).

To early detect WNV circulation and therefore to reduce the risk of transmission to humans by triggering both vector-control and SoHO safety measures, wild birds, corvids (Eurasian jay, Carrion crow and Magpie), poultry, horses, and mosquitoes are sampled according to a risk-based ranking of the Italian provinces.

- [1] Ministry of Health. National Integrated plan for the prevention, surveillance and control of West Nile virus and Usutu virus 2019 (NP) – DGPRE 10381. April 5th 2019.

Routes of transmission

WNV is maintained in nature by enzootic cycle between adult ornithophilic mosquitoes and several bird species. Birds are the WNV reservoir and play an essential role for the amplification and spread of virus in news areas. WNV vectors in Europe belonging to the Culex, Aedes,and Coquillettidia genera, which feeds on a wide variety of vertebrate host species. Mosquitoes became infected biting on a viraemic bird. After blood meal, WNV is able to spread through the gut wall into the haemolymph, replicates in most of the internal tissues and reaches the salivary glands. The period from the assumption of the virus until its localization in the salivary glands of the vector is named "extrinsic incubation period". Furthemore, in a few birds species susceptible to infection, a direct oral route transmission of the virus has been described (Komar et al., 2003). It is likely that practices such as assembly, feeding of nestlings, cannibalism, predation and necrophagy may allow the viral spread. In vector mosquitoes is vertical transmission is possible (Reisen et al., 2006) but the epidemiological impact considered negligible.

When ecological and climatic conditions favour substantial viral amplification within the birds-vector transmission cycle, increasing numbers of infected mosquitoes present a human/horse infection risk (epidemic cycle). trasmission.

West Nile virus is most commonly transmitted to humans by mosquitoes even though additional routes of human to human transmission have also been documented as blood transfusions (Pealer et al., 2002), organ transplants (Nett et al., 2012), exposure in a laboratory setting (Campbell et al., 2002) or the transmission from the mother to baby during pregnancy, delivery, or breastfeeding (Petersen and Hayes, 2008). It is important to note that these methods of transmission represent a very small proportion of cases thus sufficient to evoke only a sporadic occurrence of the disease. Humans are dead-end hosts since are not able to infect mosquitoes during the viremic phase of the infection. Thus, the above mentioned routes of direct transmission represent the main risk of infection dissemination among community. To minimize the risk, the screening of blood and organs for transplantation in areas with WNV circulation is a common measure to prevent any inter-human spread. Laboratory acquired infections have also been reported.

Vectors

WNV is transmitted by different genera and species of mosquitoes. The main vectors are some of the species of ornithophilic mosquitoes belonging to the genus Culex, which is always closely associated with the transmission of WNV during outbreaks. The mosquitoes cease their activity during the colder months, but it has been demonstrated that the virus is able to survive during this period in the infected mosquitoes, which overwinter indoors.

WNF vectors are mosquitos belonging to the Culex, Aedes and Coquillettidia genera (family Culicidae (link:https://efsa.maps.arcgis.com/apps/MapJournal/index.html?appid=512a03aa8df84d54a51bcb69d1b62735) (EFSA AHAW Panel, 2017b).

Host species susceptible to WNV infection

West Nile Virus has an extremely broad host range. Many species of birds act as primary hosts for WNV, though its vertebrate host range includes also species of mammals, amphibians, and reptiles (McLean et al., 2002). Not all infected hosts transmit the virus, but only those in which the virus replicates efficiently enough to reach viremias sufficiently high to infect mosquitoes through blood feeding. Birds are the main vertebrate hosts of the WNV. It is generally acknowledged that Passeriformes (especially *Corvidae*, *Fringillidae*, and *Passeridae* families), *Charadriiformes* (Laridae) and *Strigiformes* are considered highly competent hosts, although differences in viremic levels vary depending on the species and the viral strain.

Horses and humans are considered dead end hosts of the virus and do not contribute to the transmission cycle as they develop a low and transitory viremia not considered able to infect competent mosquito species.

WNV has been also associated with sporadic disease in small numbers of other species, including squirrels, chipmunks, bats, dogs, cats, white-tailed deer, reindeer, sheep, alpacas, dromedary camels, alligators and harbour seals during intense periods of local viral activity.

Some species of mammals including squirrels (*Sciurus* sp.), eastern chipmunks (*Tamias striatus*) and eastern cottontail rabbits (*Sylvilagus floridanus*) may be capable of transmitting WNV to mosquitoes, although their importance as reservoir hosts is still uncertain.

Among reptiles, clinical signs were mainly reported during outbreaks in alligators and crocodile monitor (*Varanus salvadori*) lizard. Amphibians including lake frogs (*Rana ridibunda*) and North American bullfrogs (*Rana catesbeiana*) can also be infected with WNV. As with mammals, their importance as reservoir hosts is still uncertain.