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# MOSQUITOES – VECTORS OF WEST NILE VIRUS IN CROATIA

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**Summary**

Croatian fauna consists of 50 mosquito species, and 15 of them have medical importance. Only 10 species are capable of transmitting the West Nile Virus (WNV). In Croatia the most capable vector is *Culex pipiens* c. According to species distribution and abundance all of Croatia is a risk area for WNV transmission. *Culex pipiens* c. is the dominant species (share of 5-10%) in eastern Croatia, but *Culex pipiens* c. together with *Aedes albopictus* are now eudominant species in the Adriatic area. Other species have lower vector capacity or they as a species are rare in Croatia. Ecological conditions for mosquito development during 2012 were not favourable and there were not too many mosquitoes in Slavonia, except the area along the Drava River where one flood pulse and one generation of flood mosquitoes was noted. *Culex pipiens* c. breeding sites are various small human made bodies of water and small natural bodies of water. The year 2012 was dry and few specimens of this species were noted. During WNV outbreak in 2012 in Croatia the most probable vector was *Culex pipiens* c.

***Keywords***: mosquitoes; vectors; West Nile virus; Croatia

## INTRODUCTION

The Croatian mosquito fauna consists of 50 species [1,2,3]. Out of these species, 15 have medical significance and only ten of the species are capable of transmitting the West Nile virus. The role of mosquitoes in spreading each arbovirus (viruses transmitted by arthropods), same as with the West Nile virus, is very significant. Mosquitoes are the most significant vectors, but not the only carriers of the West Nile virus. Aside from the mosquitoes this virus vectors can be Phlebotominae, Ceratopogonidae, and Ixodidae. In order for a mosquito to be a vector, some ecological, but also some ethological factors must be present. The virus enters the mosquito during feeding as part of a blood meal from an infected animal (most commonly a

bird). The virus is replicated in the stomach, ovary, nerves, fat tissue, it crosses over to the haemolymph and after that goes to the salivary glands. With new feeding the content of the salivary glands is injected into a host, where the mosquito transfers the virus as an ectoparasite [4]. The main reservoir for the West Nile virus are birds (Centers for Disease Control and Prevention - CDC, 2009.), so, related to that fact, the main vectors are mosquitoes that feed in the blood of birds - the so called ornithophilous species. Considering that mosquitoes are oligophagous (they feed on only a single group of organisms), and they feed multiple times, there is a real possibility that the next meal will be from another species from the group and that the virus will be carried over to that species. There are some extremely ornithophilous species among mosquitoes, that is, monophagous and in that case the transfer of the virus can be from one species of birds to another species of birds. Not all birds are equally exposed and susceptible to viruses. Birds in which the West Nile virus can often be found are from the order Passeriformes (perching birds) and the most common species are house sparrows (*Passer domesticus*) and various species of crows (e.g. *Corvus corone*) and the order Columbiformes (pigeons). Actually the main mechanisms for spreading the West Nile virus are bird migrations. In that context there are two cycles. The rural – sylvatic cycle which consists of the transfer of the virus among wild animals, most commonly swamp birds and ornithophilous mosquitoes. This cycle can be monitored very poorly or not at all. The urban cycle appears between synanthropic, or urban birds and urban mosquitoes. This cycle can be monitored through accidentally infected people or horses. Appearance of diseases caused by the West Nile virus has particularly been registered worldwide in the last twenty years (European Centre of Disease Prevention and Control - ECDC, 2012.).

There are two main parameters which enable the transfer of the disease: fauna and the biology of mosquitoes (vectors) in a specific area, and the biology of pathogens (protozoa and viruses) in that same area. The transfer or spreading of the disease may occur only when optimal conditions for both of these parameters exist. The vector capacity of mosquitoes is different. Some species of mosquitoes are better at transferring viruses while some transfer viruses poorly or not at all. There are about thirty species on the list of species in which the West Nile virus has been found [5]. Of these species, 10 exist in Croatia and are potential vectors, if they come into contact with infected animals.

## MOSQUITOES WEST NILE VIRUS VECTORS IN CROATIA

Species of mosquitoes noted in Croatia which can be West Nile virus vectors are: *Culex pipiens* c., *Aedes vexans*, *Aedes cinereus*, *Culex modestus, Culex theileri*, *Ochlerota-*

*tus caspius*, *Anopheles plumbeus*, *Coquillettidia richiardii*, *Ochlerotatus geniculatus*, and *Ochlerotatus cantans* [6,7].

***Culex pipiens* c.**

When describing this species it must be emphasized that it is a polytypic species, which means that the populations of this species are different according to ecological conditions and that they can be differentiated systematically on levels lower than species. (Note on systematics). Considering that, the urban type of this species (*C. pipiens pipiens* biotype *molestus)* exists in locations closer to humans, it represents more of a threat. It has adapted exceptionally well to human settlements, it has breeding sites in canals, barrels, casks, buckets, drains, severage system, etc. As an adult it spends the winter in basements of buildings and houses and in different shalters. According to research conducted over a period of ten years the share of this species in the mosquito fauna in Osijek is 5.86% [10] and in other parts of Slavonia it is 5-10%. Even though it is an ornithophilous species, because it lives so close to humans it is a common vector. The domestic mosquito *Culex pipiens pipiens* biotype *molestus* is the main vector of the West Nile virus, but it also carries the Ockelbo virus, Usutu virus, Sindbis virus, and the Japanese encephalitis virus [4]. Individual units of this species of mosquito are most probably responsible for the transfer of the virus during the appearance of neuroinvasive diseases caused by the West Nile virus in Slavonia during 2012.

**Note on sistematics**

The complex consists of several species, subspecies, forms, races, physiological variants, or biotypes according to various authors. At present it includes the names *C. pipiens pipiens* Linnaeus, *C. p. pipiens* biotype *molestus* Forskal, *C. p. quinquefasciatus* Say, *C. p. pallens* Coquillett, *C. restuans* Theobald, and *C. torrentium* Martini in the Holarctic as well as two Australian members, *C. australicus* Dobrotworsky and Drummond and *C. globocoxitus* Dobrotworsky. The females of the complex are very difficult to separate in field material. In several reared populations it took eight variables and a discriminant analysis to discern between *pipiens*, *molestus*, and *quinquefasciatus* females and overlapping was considerable. Thus, there is no reliable characteristic yet for discrimination between *pipiens* and *molestus* [4]. For that reasons in this text *Culex pipiens* c. was used to avoid errors.

***Aedes vexans***

Populations of this species may cause great difficulties for people after spring and summer floods because at those times they reproduce in such large numbers

that, according to estimates, there can be over 100 million larvae per hectare. As its name suggests, it reproduces in flood areas along rivers, swamps, lakes, where there are fluctuations in water levels. It is a good flier and it can fly up to 30 km in search of a blood meal. It is a zoophilic species and it feeds on all animals. If a human crosses its path it will feed on them as well. In nature the flood mosquito can be infected with various arboviruses, some of those are: WEE (West Equine Encephalitis) virus, EEE virus, California encephalitis virus, and in Europe Tahyna virus [4]. In relation to humans it has a significantly smaller vector capacity, since specimens of this species usually able the circulation of the virus among animals. It is a very numerous species in some parts of Croatia.

***Aedes cinereus***

Specimens of this species can be found in flood areas and the described as a species that appears together with *Aedes vexans*. Its numbers are significantly lower, therefore its vector capacity is lower. It is a moderately abundant species in Croatia.

***Culex modestus***

The larvae show a preference for shallow sunlit habitats and are frequently found on meadows, in irrigation channels, inundation areas of rivers, or rice fields with rich vegetation. Sometimes can be very numerous in Kopački rit area. The species has repeatedly been reported as an arbovirus vector of two different *Bunyavirus*, Tahyna and Lednice and is also regarded as a potential vector of WNV. Moderate abundant species in Croatia.

***Culex theileri***

A polycyclic species recorded from a broad range of elevations. The females are zoophilic, but sometimes feed on humans and bite mainly in the open, occasionally in large numbers, also entering houses and other buildings. In South Africa, Sindbis virus and WNV were isolated from wild populations. This species is very rare in Croatia.

***Ochlerotatus caspius***

This is a polycyclic, halophylic species. Sometimes only one generation per year is produced due to the nature of the breeding site. This species can be very numerous in flooded areas especially in early spring. West Nile virus, Tahyna virus, and the bacterium *Francisella tularensis*, the causative agent of tularemia, could be detected in natural populations of this species. Moderate abundant species in Croatia.

### *Anopheles plumbeus*

Larvae of *A. plumbeus* develop almost exclusively in tree-holes and represents generally rare species. Last decade reports from Germany show that some populations accomodate to new habitat - sewerage system. Although laboratory studies have shown that *A. plumbeus* can successfully be infected with *P. vivax* and *P. falciparum* and that the species is an efficient carrier of malaria, it is considered to be of minor epidemiological importance at the present time because of its ecology. It is also reported as laboratory vector of West Nile Virus. Rare species in Croatia.

### *Coquillettidia richiardii*

Larvae and pupae live submerged and obtain oxygen from the aerenchyma of aquatic plants and move very little. Females infected with WNV, and Omsk haemorrhagic fever virus were detected in wild populations. Moderate abundant species in Croatia.

### *Ochlerotatus geniculatus*

The larvae live in tree-holes at various heights and in open tree stumps of different deciduous trees as *Quercus* sp., *Fagus* sp., *Alnus* sp., *Betula* sp., and *Juglans* sp. They also occur in mixed forests in old trees and can occasionally be found in ground pools. Is is also reported as laboratory vector of West Nile Virus. Rare species in Croatia.

### *Ochlerotatus cantans*

The larvae develop rather early in spring in Southern and Central Europe, Biting females are encountered most abundantly in lowland regions from late March to June. In Croatia this species offten can be find in forests. This species is reported field vector of Tahyna virus, West Nile virus and myxomatosis. Moderate abundant species in Croatia.

## MOSQUITOES DURING THE WEST NILE VIRUS OUTBREAK IN SLAVONIA

During 2012 ecological conditions for the development of mosquitoes were not favourable, so the number of mosquitoes in Slavonia was not large, except in the Drava River area where a slightly elevated water level in July resulted in a single generation of flood mosquitoes.

During 2012, which was a drought year, there were not many breeding sites (human made small water bodies - barrels, buckets, drains, septic tanks; natural breeding sites of medium size - canals, depressions), so the number of mosquitoes was small.

During the WNV outbreak (August - September 2012) dry ice baited CDC traps were used to sample mosquitoes in 64 locations in Osijek-Baranja, Vukovar-Srijem, and Brod-Posavina Counties. During the research 5 species of mosquitoes were caught (*Table 1*). Out of the total number of 1785 mosquitoes, 114 were determined as *Culex pipiens* c. which were adequately stored and sent to virus presence analysis. Molecular analysis did not prove the presence of the West Nile virus, but the results point to the possible presence of some other viruses.

***Table 1.*** Number of mosquitoes sampled in eastern Croatia during the West Nile virus outbreak in 2012.

| Mosquito species | No. |
|---|---|
| *Aedes vexans* | 1634 |
| *Culex pipiens* | 114 |
| *Ochlerotatus caspius* | 21 |
| *Anopheles maculipennis c.* | 12 |
| *Anopheles hyrcanus* | 4 |
| Total | 1785 |

## INSTEAD OF CONCLUSION

At the moment, the West Nile virus is present in many countries in this part of Europe and its activity was detected in Croatia [8]. The most probable vector which transmits the West Nile virus to the population in Croatia during 2012 is *Culex pipiens* c. The species of *Culex pipiens* c. is the best West Nile virus vector. The species of *Culex pipiens* c. is widely spread in Croatia and in eastern Croatia it is present with the share of 5-10% [9,10] of the mosquito population. Other species represent species with lower vector capacities. The other species of mosquitoes which could be vectors according to the literature have a much lower probability, because some of the species (*Culex theileri, Anopheles plumbeus* and *Ochlerotatus geniculatus*) are rare in Croatia and some are moderately abundant (*Ochlerotatus caspius*, *Aedes cinereus*, *Coquillettidia richiardii*, *Culex modestus* and *Ochlerotatus cantans*). Except for the lower numbers and smaller areas where they are present, it is noted for most of these species that they rarely feed on birds, which considerably reduces the possibility of infection by the West Nile virus.

Although the legal regulations are set up properly, mosquito control in eastern Croatia is not well organised outside large cities (Osijek, Slavonski Brod). Mosquito control should be organised and financed by the local government and smaller units of local government usually cannot afford it. Therefore there is a high probability the virus circulation cycle, birds – mosquitoes – birds, will spread to the human population in the following years.

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Sažetak

**Komarci prijenosnici virusa Zapadnog Nila u Hrvatskoj**

U Hrvatskoj je zabilježeno 50 vrsta komaraca, a 15 ih ima medicinsko značenje, tj. potencijalni su prijenosnici različitih uzročnika bolesti. Ukupno je deset vrsta potencijalnih prijenosnika virusa Zapadnog Nila. U Hrvatskoj su najbolji vektori jedinke vrste *Culex pipiens* c. Uzmemo li u obzir brojnost i rasprostranjenost te vrste, područje cijele Hrvatske rizično je područje za prijenos virusa Zapadnog Nila. Jedinke te vrste dominantne su (udio 5 – 10% u fauni komaraca) u istočnoj Hrvatskoj, a *Culex pipiens* c., zajedno s jedinkama vrste *Aedes albopictus*, eudominantne su (udio u fauni više od 10%) vrste u jadranskom priobalju. Ostale vrste potencijalni vektori u Hrvatskoj manje su bitne zbog manje brojnosti, ograničenog rasprostranjenja i etoloških odlika. Ekološki uvjeti za razvoj komaraca tijekom 2012. nisu bili pogodni za razvoj većih populacija pojedinih vrsta u Slavoniji. Jedino uz rijeku Dravu zabilježen je veći broj komaraca, što je rezultat nešto povišenog vodostaja rijeke Drave. Legla za jedinke vrste *Culex pipiens* c. različita su manja vodena tijela koja je proizveo čovjek. Godina 2012. bila je suha, tako da je broj komaraca bio relativno malen. Za vrijeme pojave bolesti izazvane virusom Zapadnog Nila zabilježeno je pet vrsta komaraca, a najvjerojatniji vektor bio je *Culex pipiens* c. Ukupno je uhvaćeno 1.785 komaraca, a molekularna analiza na prisutnost virusa Zapadnog Nila obavljena je na 114 jedinki vrste *Culex pipiens* c. Virus nije potvrđen kod komaraca.

***Ključne riječi***: komarci; vektori; virus Zapadnog Nila; Hrvatska.