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Regional Review

An overview of mosquitoes and emerging arboviral infections in the Zagreb area, Croatia

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Abstract

Mosquito control in the Zagreb area has been conducted for many years, whereas the fauna has only been investigated in the last 20 years. So far 30 mosquito species have been detected in the city area. *Culex pipiens* form *molestus* is the dominant mosquito species in indoor breeding sites. In forested areas and areas exposed to flooding, the active period is early spring and the dominant species are *Ochlerotatus sticticus*, *Ochlerotatus cantans*, *Ochlerotatus geniculatus* and *Aedes vexans*. The eudominant mosquito species found in the artificial breeding sites are *Culex pipiens* and the Asian tiger mosquito, *Aedes albopictus*. Invasive *Ae. albopictus*, present in the Zagreb area since 2004, has expanded to a larger area of the city during the last three years. The recent emergence of the human West Nile virus and Usutu virus neuroinvasive disease in Zagreb and its surroundings highlighted the role of mosquitoes as vectors of emerging arboviruses.
The paper focuses on mosquito species and arboviral infections detected in humans and animals in the Zagreb area, Croatia.

Key words: mosquito species; vectors; arboviruses; Zagreb; Croatia.

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Introduction

Mosquitoes are currently in the focus of world's entomological research because of their impact on human health as vectors of many medically important pathogens. Blood-sucking mosquitoes are able to acquire pathogens from infectious blood of the viremic vertebrate and to transmit it to another host by bite if the mosquito is ecologically and physiologically suitable for transmission. In order to enable the pathogens/parasites to proliferate and/or to develop to the infective stages in the vector, vectors have to be highly competent. For successful transmission, multiple blood-meals are needed [1]. Isolation of several emerging and re-emerging arboviruses from mosquitoes such as yellow fever virus (YFV), dengue virus (DENV), West Nile virus (WNV), chikungunya virus (CHIKV), Usutu virus (USUV) and Zika virus (ZIKV) highlights their role as arboviral vectors [2-8].

Mosquito-borne diseases have not been reported in Croatia since 1950s, although antibodies were sporadically detected in humans and animals [9-13]. In 2010, the first autochthonous cases of dengue fever were detected in the area of the Peljesac peninsula (south Adriatic coast) [14]. The same year the indigenous dengue fever was detected in France [15]. In 2007, an outbreak of chikungunya fever occurred in north-eastern Italy which proved presence of CHIKV in local *Aedes albopictus* mosquitoes [16].

The invasive Asian tiger mosquito, *Ae. albopictus*, one of the most important vectors of DENV and CHIKV is well-established in Croatia's coastal territory [17,18] and the Zagreb area.

Although cases of WNV fever were recorded since 1960s in several European countries, the incidence was largely unknown [19]. In the 1990s, WNV has spread throughout Europe and the Mediterranean basin causing

a series of outbreaks (Algeria, Morocco, Romania, Tunisia, Italy, Israel, France and Russia) associated with severe complications, including neuroinvasive disease [19-27]. Since 2008 outbreaks in humans and horses have been frequently detected in many European countries: Greece, France, Hungary, Italy, Romania, Russia and Serbia [20,27-29]. In 2012, the first outbreak of human WNV neuroinvasive disease was reported in Croatia with seven confirmed cases in three north-eastern counties [30]. In addition, acute asymptomatic infections in horses were noted in the same counties where human cases occurred [31]. Similar to WNV, there is increasing evidence of animal USUV infections in Europe since the first reports of its identification in 1996 [32]. In 2009, USUV neuroinvasive infection was documented in two immunocompromised patients in Italy [33,34]. In 2013, first cases of human USUV neuroinvasive disease were detected during the WNV outbreak in Croatia [35].

Although sporadic human cases of ZIKV infection were reported since 1950s from countries in Africa and Asia, this virus received little attention until the outbreak on Yap Island, Federated States of Micronesia in 2007 [36]. Subsequent outbreaks in French Polynesia (2013-2014), Pacific islands and Brazil (2015), have shown the propensity of ZIKV to spread outside its usual geographical range [37-41]. *Aedes aegypti* was considered to be major epidemic vector outside Africa [42]. For the first time, ZIKV was detected in *Ae. albopictus* mosquitoes during the Gabonese outbreaks (2007-2010) [8].

Geographic location and diversity of the Zagreb area

The Croatian capital Zagreb (45º15'N and 15º30'E) covers an area of 641.355 km^2, and Zagreb County covers an area of 3078 km^2. The major part of Zagreb is located at an altitude of 112 m and is rich in landscape diversity. Diversity is the result of interaction of natural (bedrock, soil, water, climate, vegetation) and anthropogenic (soil use, archeology, architecture) factors: hills alternate with lowland landscape. Lowland areas are developed into urban and suburban areas along the Sava River. The central part of the city is a densely built urban area while the northern part is located on the slopes of Medvednica mountain characterized by forest vegetation and smaller urban settlements. Eastern, southern and western parts of the city area are mostly represented by agricultural areas. The area of the city abounds in the surface waters. Numerous streams originating in Medvednica flow into the Sava River. There are seven artificial lakes and several artificial watercourses in the city area [43].

According to Köppen classification, climate in Zagreb can be described as moderate, with hot summers, without extremely dry periods. The least amount of precipitation is recorded in winter months (February 41.7 mm) with two equal maximum rainfalls during summer (June 93.9 mm and August 92.5 mm) and the mean annual amount of precipitation is 859.5 mm. The average temperature of the hottest month is below 22°C and the average temperature of more than four months of the year is above 10°C. Average temperature of the coolest month is above -3°C, and below 18°C [44].

Mosquitoes (Diptera, Culicidae) in the Zagreb area

In Croatia, a total of 51 species of mosquitoes have been described so far [45-48]. In the City of Zagreb, 30 mosquito species have been detected [46,49-57] which comprises 58.8% of the Croatian mosquito fauna (table 1). Different mosquito species such as *Anopheles maculipennis*, *Anopheles plumbeus*, *Anopheles bifurcatus* (now *Anopheles claviger*), *Culex pipiens*, *Culex bicolor* (now synonymised *Cx. pipiens*), *Culex pyrenaicus* (now *Culex territans*), *Culex hortensis*, *Theobaldia longeareolata* (now *Culiseta longiareolata*), *Theobaldia annulata* (now *Culiseta annulata*), *Ortopodomyia albionesnsis* (now *Orthopodomyia pulchripalpis*), *Finlaya geniculate* (now *Ochlerotatus geniculatus*), *Ecculex vexans* (now *Aedes vexans*) and *Ochlerotatus communis* have been reported in the Zagreb area (publications on malaria) in the first half of the 20th century [49-51]. Pavisic (1949) reported the presence of *Anopheles nigripes* (now *An. plumbeus*) in tree-holes in the forest of Maksimir and some other eastern parts of the city [52]. In a report on nuisance mosquitoes from 1951, several mosquito species were noted in the area of Zagreb: *Cx. pipiens*, *Theobaldia longiareolata, An. bifurcatus*, *Aedes nemorosus* (now *Oc. communis*), *Aedes cantans* (now *Ochlerotatus cantans*) and *Aedes punctor* (now *Ochlerotatus punctor*) [53]. After 1990 specific areas of the city have been extensively searched such as the biggest urban Maksimir Park (316 ha) where 27 species of mosquitoes have been detected. Faunistic research conducted in 1995 detected 16 species [54] and 23 species of mosquitoes were detected during 2003 and 2004, 11 of which were detected for the first time [55]. Only three species, *Ae. albopictus*, *Culiseta morsitans* and *Or. pulchripalpis* were not detected in Maksimir Park.

In the indoor breeding sites of Zagreb the species *Cx. pipiens* form *molestus* dominates [56]. This species belongs to the *Cx. pipiens* complex. This form is homodynamic (a female mosquito can lay eggs without diapause) and autogenous (a biological characteristic of a female to lay her first batch of eggs without a blood meal), which allow them to develop all year round in favorable environmental conditions. *Cx. pipiens* form *molestus* individuals are primarily antropophilic. Larvae can be found in dark, moist basements of residential and office buildings [1].

In forested and flooding areas, natural mosquito breeding sites are active only in spring (March-June) and the most numerous species are *Ochlerotatus sticticus, Oc. cantans*, *Aedes vexans* and *Ochlerotatus geniculatus.* The most common nuisance mosquito in May and June is *Oc. sticticus* [57]. Although it may produce more than one generation in a single year [1], in Zagreb it has only one [57].

The most common mosquito species in streams is *Cx. pipiens*. The eudominant mosquito species in artificial breeding sites are *Cx. pipiens* and *Ae. albopictus*.

Mosquitoes as potential arboviral vectors in the Zagreb area

Aedes albopictus

The Asian tiger mosquito, *Ae. albopictus* (*Stegomyia albopicta*) [58] is an invasive species with a rapid geographic expansion throughout Europe during the last decade, particularly in the southern parts of Europe [59,60]. Isolation of several arboviruses such as DENV, CHIKV, USUV from field-collected *Ae. albopictus* have been reported [4,6,7,61].

In recent years, *Ae. albopictus* has been the most common species in the Croatia coastal area and now is widespread from Istria in the north through Dalmatia and islands to Dubrovnik in the south [17,18]. The first record of *Ae. albopictus* in the Zagreb area was made in October 2004 during the regular inspection of the mosquito breeding sites. Mosquito larvae were found and collected from an artificial container in a forest located in the western suburb of the city [46]. This was also the first finding of *Ae. albopictus* in Croatia. It is well known that the most common route of spread of this species into new areas is through the used tire trade [62]. Therefore, it is not surprising that in the next year the species was found in Zagreb, in two companies engaged in importing, restoring and selling tires. The companies are located in the western suburb of the city at a distance of five kilometers from the first finding. In 2005, the species was not detected in the area of first detection. Results of the monitoring conducted from 2005 to 2010 have shown that *Ae. albopictus* was present in the smaller limited area near the companies dealing with the export of used tires. In 2011, the first finding in the new area of the city was recorded, particularly in its northwestern suburb. In the following year, 2012, this mosquito species expanded rapidly into several western districts, which required further activities and research on establishment and expansion of the species. According to the European guidelines for the surveillance of invasive mosquitoes (2012) [63], the spread of *Ae. albopictus* was monitored in the period from June to October 2013 and 2014 using the ovitrap method. It was demonstrated that this species had been widespread throughout the city (except northeastern parts). The highest density of the population was found in the western districts, in the settlements with family households. Seasonal dynamics surveillance recorded the highest activity in late July and in August.

Although *Ae. albopictus* has been present in the City of Zagreb for a decade, citizens' complaints to the

Table 1. Mosquito species detected in Zagreb area.

Mosquito species
Anopheles claviger Meigen 1804
Anopheles maculipennis complex (Meigen 1818)
Anopheles plumbeus (Stephens 1828)
Aedes cinereus (Meigen 1818)
Aedes vexans (Meigen 1830)
Aedes albopictus (Skuse 1894)
Ochlerotatus geniculatus (Olivier 1791)
Ochlerotatus annulipes (Meigen 1830)
Ochlerotatus behningi (Martini 1926)
Ochlerotatus cantans (Meigen 1818)
Ochlerotatus caspius (Pallas 1771)
Ochlerotatus cataphylla (Dyar 1916)
Ochlerotatus communis (De Geer 1776)
Ochlerotatus detritus (Haliday 1833)
Ochlerotatus excrucians (Walker 1856)
Ochlerotatus punctor (Kirby 1837)
Ochlerotatus riparius (Dyar and Knab 1907)
Ochlerotatus sticticus (Meigen 1838)
Ochlerotatus rusticus (Rossi 1790)
Culex pipiens complex (Linnaeus 1758)
Culex theileri (Theobald 1903)
Culex hortensis (Ficalbi 1889)
Culex martinii (Medschid 1930)
Culex territans (Walker 1856)
Culiseta longiareolata (Macquart 1838)
Culiseta morsitans (Theobald 1901)
Culiseta annulata (Schrank 1776)
Coquillettidia richiardii (Ficalbi 1889)
Orthopodomyia pulchripalpis (Rondani, 1872)
Uranotaenia unguiculata (Edwards 1913)

nuisance by this species has been recorded only in the last few years. The presence of the Asian tiger mosquito has considerably increased the involvement of the public health professionals in surveillance of this species and increased activities of the Mosquito Control program. Since the first finding of *Ae. albopictus* in tire companies, the companies have been included in the Mosquito Control program which is regularly carried out. All the vulcanizing shops and tire supply companies are currently included in the Mosquito Control program. Elimination of mosquito breeding sites, field surveys at addresses of citizens complaining about mosquito nuisance, education of the general population and children in schools are currently conducted.

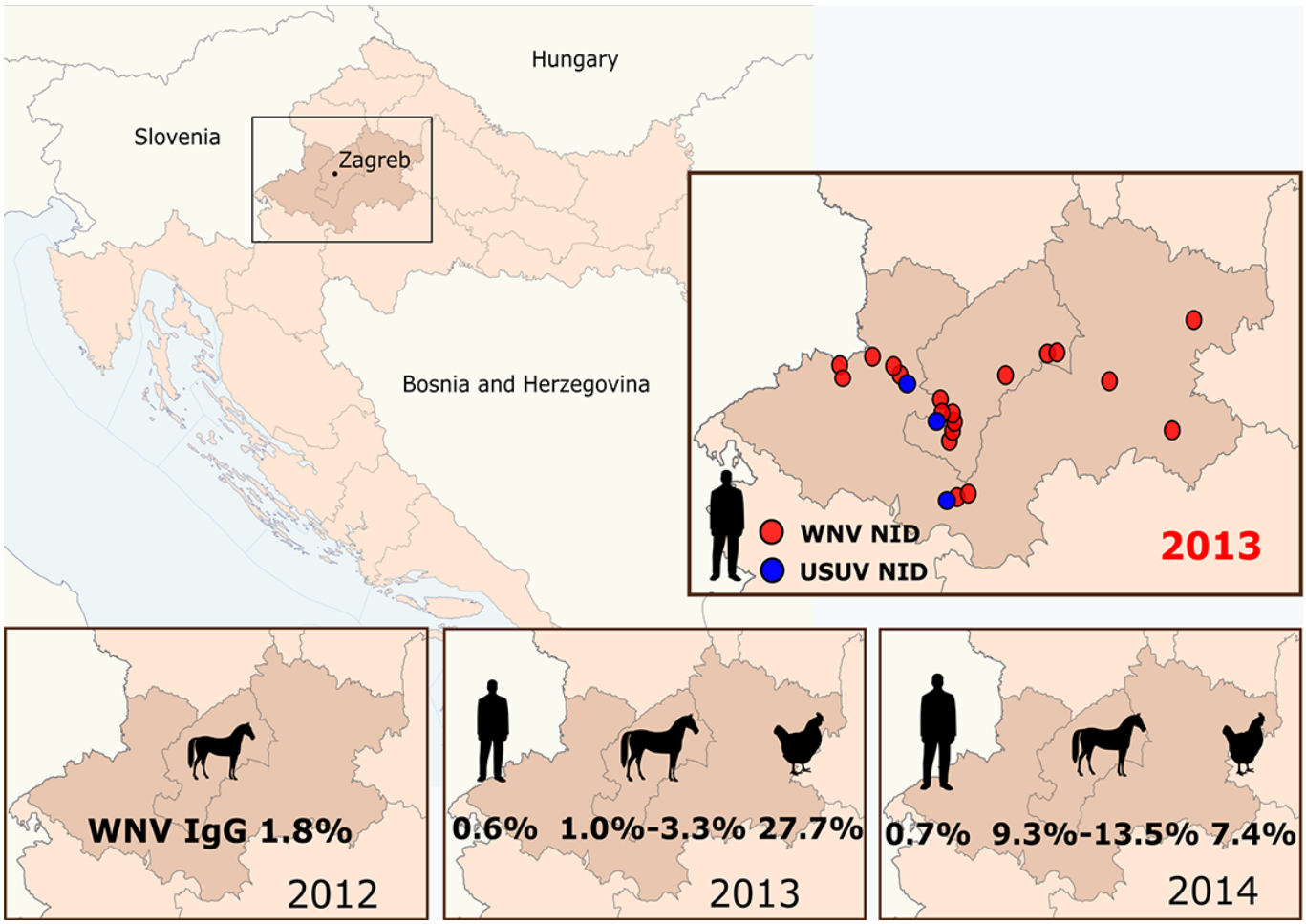
Culex pipiens

The *Culex pipiens* complex, widespread and omnipresent species of mosquitoes, has been acknowledged as a main vector in recent epidemics caused by WNV in Europe [19,22,25,64].

According to various authors the *Cx. pipiens* complex comprises several species, subspecies, forms or biotypes [1,65] of which *Cx. pipiens* form *pipiens* and *Cx. pipiens* form *molestus* are represented in the areas of Croatia and Zagreb [45,55,56]. Females as well as larvae of these forms are morphologically difficult to discern. Both forms feed on humans, birds and other mammals, but with different preference: the *molestus* form has preference to feed on humans while the *pipiens* form is primarily avian-feeding. They produce higher number of generations depending on various conditions. Both forms hibernate at the stage of female in basements, dark technical floors of buildings, shelters and drainage systems [1,65].

A recently published study suggested that hybrid offsprings between the *pipiens* and *molestus* forms are an important for epizootic transmission of WNV in North America [66]. During the 2012 outbreak in Croatia, mosquitoes were sampled within the area of appearance of WNV neuroinvasive human cases in three north-eastern counties. All tested *Cx. pipiens*

Figure 1. Geographical distribution of arboviral diseases detected in Zagreb area.



complex pools were negative for WNV RNA using pan-flavivirus RT-PCR [67].

Apart from the *Cx. pipiens* individuals, WNV has also been isolated from other species of mosquitoes in Europe: *Culex modestus*, *Coquillettidia richiardii*, *Oc. cantans*, *Ochlerotatus caspius*, *Anopheles maculipennis* complex, *Ae. albopictus*, *Ae. vexans*, *Aedes cinereus* [64,68,69]. All specified species are present in Croatia, but have significantly lower vector potential. An attested vector of WNV in experimental conditions is also *Aedes japonicus* [70], an invasive species first discovered in Croatia in 2013 in the Krapina-Zagorje County (northwestern Croatia) [48].

USUV is another flavivirus transmitted mainly by the *Cx. pipiens* complex. However, the virus has been isolated from numerous other mosquito species that include *Cx. hortensis, Cx. territans, Cs. annulata, Ae. vexans, Aedes rossicus, Ae. albopictus* [6,71].

Mosquito control in the Zagreb area

In the Zagreb area, an organized mosquito control has been implemented since 1931 when the Municipal Health Department implemented the systematic mosquito control due to increasing number of people suffering from malaria. It took place all year long, during summer in stagnant water and during the winter in the buildings [72]. Since then, a mosquito control program is continuously being conducted throughout the city apart from the area of the urban Maksimir Park which is monument of the park architecture and mainly forested area.

In recent decades, the mosquito control program in Zagreb has been designed by The Department of Epidemiology of Andrija Stampar Teaching Institute of Public Health, which also performs monitoring of the program. The mosquito control program is carried out continuously throughout the whole year. The main components of the program are detection and reduction of breeding sites as well as routine larviciding in breeding sites that cannot be eliminated. Already known indoor breeding sites are controlled once per month throughout the year and larviciding and adulticiding are performed when necessary. Larviciding of floodwater and forest mosquito species breeding sites is carried out twice per month in the period from March to June, while for urban mosquito species breeding sites in the period from May to October. Adulticiding is carried out only in small well defined areas when population of mosquitoes reaches critical levels. The Department also monitors complaint calls (residents calling and notifying about large population of mosquitoes) and provide investigation and inspection of backyard and adjacent yards [73].

Overview of arboviral diseases detected in humans, horses and poultry in the Zagreb area

Two arboviral diseases have emerged in Zagreb and its surroundings during the last few years: WNV and USUV infection (figure 1). In 2013, 19 cases of human WNV neuroinvasive disease were reported and 0.6% inhabitants were found to be WNV IgG seropositive [35]. Circulation of WNV was also confirmed in sentinel horses and poultry. Seroprevalence in horses increased from 1.8% (2012) and 1.0%-3.3% (2013) to 9.3%-13.5% (2014), while acute infections were not detected [74]. However, one horse seroconverted during the 2014 transmission season [75]. In addition, 27.7% of chickens were found to be WNV IgG seropositive in 2013 compared to 7.4% in 2014. No human clinical WNV cases were reported in Zagreb area in 2014 and IgG seroprevalence was reported to be 0.7% [76]. Serologic evidence of USUV infection was reported for the first time in one horse in 2011 [77]. During the 2013 West Nile virus outbreak, three cases of human USUV neuroinvasive disease were detected in Zagreb area [35,78]. No DENV seropositive persons were found in the Zagreb area during a seroprevalence investigation in the period 2011-2012 [79]. Autochthonous cases of CHIKV infection were not detected in Croatia, however, IgG antibodies were sporadically documented in travelers to endemic areas [80]. Although ZIKV infection was not reported in the Croatian residents so far, with an increase in global travel, the risk for ZIKV importation has heightened.

Conclusions

There is a large variety of mosquito species in the City of Zagreb. Among 30 identified species, medically most important species are the Asian tiger mosquito, *Ae. albopictus,* and the *Cx. pipiens* complex. *Ae. albopictus*, a vector of DENV, CHIKV and ZIKV has been detected eleven years ago for the first time in one city district, since then it is widespread across the city. The *Cx. pipiens* complex is the primary vector for WNV. Both of these species are artificial container breeding mosquitoes and their breeding sites are mainly influenced by urbanization and humans. Arbovirus circulation monitoring as well as permanent vector control measures should be regularly performed, particularly in areas with established *Ae. albopictus* population.

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