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Equine Seroprevalence Rates as an Additional Indicator for a More Accurate Risk Assessment of the West Nile Virus Transmission

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A B S T R A C T

The West Nile Virus (WNV) is a zoonotic arbovirus that has recently been causing outbreaks in many countries in southern and Central Europe. In 2012, for the first time, it caused an outbreak in eastern Croatia with total of 7 human clinical cases. With an aim of assisting public health personnel in order to improve survey protocols and vector control, the high risk areas of the WNV transmission were estimated and mapped. The study area included cities of Osijek and Slavonski Brod and 8 municipalities in Vukovarsko-Srijemska County. Risk estimation was based on seroprevalence of WNV infections in horses as an indicator of the virus presence, as well as the presence of possible WNV mosquito vectors with corresponding vector competences. Four mosquito species considered as possible WNV vectors are included in this study: Aedes vexans, Culex modestus, Culex pipiens and Ochlerotatus caspius. Mosquitoes were sampled using dry-ice baited CDC trap, twice a month, between May and October. This study suggests that the two mosquito species present the main risk of WNV transmission in eastern Croatia: the Culex pipiens – because of good vector competence and the Aedes vexans – because of the very high abundances. As a result, these two species should be focus of future mosquito surveillance and a vector control management.

***Key words**: West Nile Virus, outbreak, mosquitoes, Aedes vexans, Culex pipiens, Culex modestus, Ochlerotatus caspius, risk assessment, risk mapping, equine seroprevalence, vector competence*

Introduction

West Nile virus (WNV) is the most widely distributed arthropod-borne virus in the world . It belongs to the family *Flaviviridae*[1] and is actually mosquito-borne virus ('mobovirus')[2] which is a neuropathogen for humans, horses and birds[3]. The virus is maintained in a bird-mosquito transmission cycle primarily involving mosquitoes from *Culex* genus where birds are the amplifying reservoir hosts, while the mosquitoes are transmitting vectors[4]. Although, the majority of human WNV infections are asymptomatic, both the humans and the horses can have clinical manifestations of the infection. They are also a dead-end hosts because of their low grade of viremia[5]. First reports of the WNV human infections date back as far as the 1930s, but the dramatic expansion of the virus was registered in the last 30 years[6]. In Croatia, the first confirmed multiple clinical human cases were reported in 2012 with a total of 7 infected persons, all from the eastern part of the country. Immediately after the outbreak entomological research was conducted in order to catch the infected mosquitoes, but none of the mosquito pools tested for WNV were positive[7]. This was the first outbreak of the WNV which came rather late since almost all neighbouring countries to Croatia had confirmed the presence of WNV. In Italy, the first WNV cases were recorded in 1998[8], culminating with a big outbreak in 2008[9]. In Slovenia there has been a confirmed presence of WNV in song birds since 2003[10]. In Hungary the WN encephalitis occurred in a goose flock in 2003[11]. Finally in Serbia in 2010, 12% of horses tested for WNV were found positive[12]. Additionally, Croatia is on the route of a bird migration flyways[13]. More specifically, in the eastern part there is a big wetland

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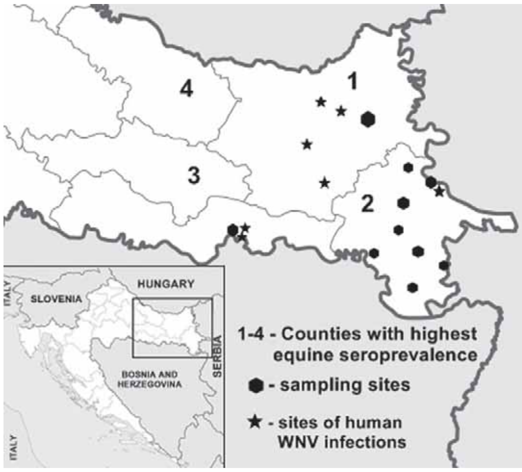

Fig. 1. Map of eastern Croatia marked with the sampling sites, the human WNV infections sites and the counties with highest seroprevalence of WNV in horses in Croatia.

known as a bird resort – the Nature Park Kopački Rit and some additional floodplains along the rivers Drava, Sava and Danube. The Croatian fauna comprises of 50 mosquito species and 15 of them have medical importance[14]. Multiple European mosquito species are considered as competent vectors of the WNV: *Culex pipiens* (Linnaeus 1758), *Culex theileri* (Theobald 1903), *Culex modestus* (Ficalbi 1890), *Ochlerotatus caspius* (Pallas 1771) and *Anopheles maculipennis* (Meigen 1818). However, only the first three species appear to play an important role as vectors in European countries[3,15,16]. Several other authors include some other European species as potential vectors of the WNV, namely *Aedes vexans* (Meigen 1830)[17], *Ochlerotatus cantans* (Meigen 1818), *Coquillettidia richiardii* (Ficalbi 1889)[18], *Anopheles plumbeus* (Stephens, 1828) and *Aedes cinereus* (Meigen 1818)[19]. With the exception of *Culex theileri* all of the listed species are found in Croatian mosquito fauna[20].

With the exception of the surveillance of dead birds, the use of sentinel animals is the most common system of monitoring the WNV circulation. Because of their susceptibility to the WNV infection, monitoring of the equine populations within the close proximity to urban human populations, might be useful for predicting the disease risk and for providing an early warning of a corresponding WNV transmission in humans[21,22]. In Croatia, the highest equine seroprevalence of the WNV infections were found in the eastern part, in the counties next to the Hungarian, Serbian, and the Bosnia and Herzegovinian state borders[5].

The aim of this study was to determine high risk areas of possible WNV transmissions based on the quantity of the virus which was derived from the seroprevalence rates of the WNV infection in horses. Furthermore, the aim was to determine the presence of a number of possible vectors: the *Cx. pipiens, Cx. modestus, Ae. vexans* and *Oc. caspius*.

Cx. pipiens and *Cx. modestus* – because they are ornitophilic species regarded as the main bridge vectors, the *Ae. vexans* – although it's not considered as good WNV vector it can become very abundant, often at the same time when the West Nile Virus activity is at its peak. It feeds readily on humans as well as on the domestic animals, and it has been found naturally infected with various arboviruses[23] and finally, the *Oc. caspius* – which even though has a low abundance in Croatia, this species was included because the specimens infected with the WNV had been confirmed in Italy[24] and France[15]. This species feeds aggressively on humans and horses and is able to engorge on birds[25]. As a result of the study, maps of areas with high risk of WNV transmission are provided with aim of assisting public health personnel in order to improve survey protocols and vector control.

Materials and Methods

Study area and mosquitoes sampling

Study area includes the following three counties in eastern Croatia: Osječko-Baranjska, Vukovarsko-Srijemska and Brodsko-Posavska county. In the Osječko-Baranjska county study was performed in and around the city of Osijek which is the county capital with population of ca. 115,000 inhabitants. In the Brodsko-Posavska county, also the capital Slavonski Brod (ca. 63,000 inhabitants) was studied while in the Vukovarsko-Srijemska county 8 municipalities were covered (area of 2,500 km^2 and 180,000 habitants) including the town of Vukovar (Figure 1). Eastern part of Croatia is a part of the Pannonian plain bounded with three rivers: Sava, Drava and Danube. Their floodplains provide many suitable habitats for migratory birds as well as the larval breeding sites for many mosquito species. Within all of the mentioned counties there were confirmed clinical cases of the WNV human infections in 2012 (Figure 1). The mosquito population surveillance was conducted separately for each county but for the purpose of this study we provided mosquito samples for each county as it follows: Osječko-Baranjska county from 16 sampling localities in 2012 (224 trap-nights), Brodsko-posavska county from 4 sampling localities during 2001[26] (32 trap-nights) and Vukovarsko-srijemska county from 8 sampling localities in 2012 (80 trap-nights).

In each sampling locality, adult mosquitoes were collected by using dry ice baited CDC traps from the evening until the morning, twice a month from May to September. This sampling period includes the two peak activity periods for the mosquitoes species considered here. After collection, all adult mosquitoes were counted and identified using the identification keys[23,27].

Estimation of the WNV transmission risk

The relative risk that a species of mosquito might infect humans with the WNV was estimated using the Kilpatrick's formula[28], which we modified by not including the minimum infection rate values for each mosquito species. This was due to the lack of information on the WNV infection rates for most of the species and localities analysed in this study since none of the pools of collected mosquitoes were positive for WNV. In the modified formula (see below) we used the equine seroprevalence rates as an indicator of the quantity of the virus circulating in a specific area and the amount of exposure of each mosquito species to the WNV respectively. The calculated risk transmission estimation represents a relative number of the WNV-infectious bites by each mosquito species in relation to mosquito abundance, to its vector competence and to the equine seroprevalence of the WNV infections. The vector competence is based on transmission rates of the WNV (the percentage of all re-feeding mosquitoes that transmitted virus by bite). The following formula was applied for each mosquito species and locality:

$$Risk = A \times S_e \times C_v$$

The A is the abundance, S_e is the equine seroprevalence and C_v is an index of vector competence. Previously reported data on vector competence from the USA[17,29,30] and France[15] were used. For the species where there was a multiple data for vector competence, a mean was calculated. The equine seroprevalence rates for the WNV infections in the studied counties were: Osječko-Baranjska – 7.00, Vukovarsko-Srijemska – 6.72 and Brodsko-Posavska – 1.41[5].

Mapping of the estimated transmission risk

An estimated transmission risk maps were generated using the geostatistical methods (ArcGIS, ESRI, 2012). The Ordinary Kriging (OK) was used as an interpolation method as a spatial best linear unbiased prediction. The structure of the spatial variance between the measurements was analyzed by calculating a sample semivariogram, approximated by the stable variogram model in case of the city of Osijek and Vukovar-srijemska county area. Spatial variability of the Slavonski Brod area was modelled by the Inverse Distance Weighting (IDW) method according to the small number of sampling localities (n = 4) and relatively small value range. Interpolated surface should be that of a location dependent variable. The root-mean-square error (RMSE) of the Kriged, or weighted estimate was used as a measure for the best model evaluation (RMSE: 2194.4 m, 34.7 m and 255.8 m for Osijek, Slavonski Brod and Vukovar-Srijemska county, respectively).

Results

A total of 51,895 mosquitoes belonging to the four investigated species were trapped with a total trapping effort of 336 trap-nights (Table 1). Among those four species the *Ae. vexans* was the eudominant species with a

TABLE 1
NUMBER OF MOSQUITOES BY SPECIES COLLECTED DURING 2012 IN CITY OF OSIJEK AND VUKOVARSKO-SRIJEMSKA COUNTY AND IN 2001 IN TOWN OF SLAVONSKI BROD

Species	Osijek	Vukovarsko-srijemska	Slavonski Brod	Total
Aedes vexans	40432	1190	2706	44328
Culex modestus	34	24	0	58
Culex pipiens	2196	324	1055	3575
Ochlerotatus caspius	3915	2	17	3934
Total	46557	1540	3778	51895

TABLE 2
ESTIMATED RELATIVE RISK OF EACH MOSQUITO SPECIES FOR TRANSMITTING WEST NILE VIRUS IN SPECIFIED STUDY AREA

Study area / WNV vector	Equine seroprevalence	Realtive abundance	Cv (reference median) %	Risk	Cummulative risk
Osijek	7.00				114.1
Ae. vexans		86.84	15.97	97.083	
Cx. modestus		0.07	54.50	0.279	
Cx. pipiens		4.72	49.38	16.304	
Oc. caspius		8.41	0.08*	0.471	
Vukovarsko-srijemska	6.72				158.5
Ae. vexans		77.27	15.97	82.928	
Cx. modestus		1.56	54.50	5.708	
Cx. pipiens		21.04	49.38	69.814	
Oc. caspius		0.13	0.08*	0.007	
Slavonski Brod	1.41				35.6
Ae. vexans		71.63	15.97	16.128	
Cx. modestus		0.00	54.50	0.000	
Cx. pipiens		27.92	49.38	19.443	
Oc. caspius		0.45	0.08*	0.005	

*disseminated infection rate used

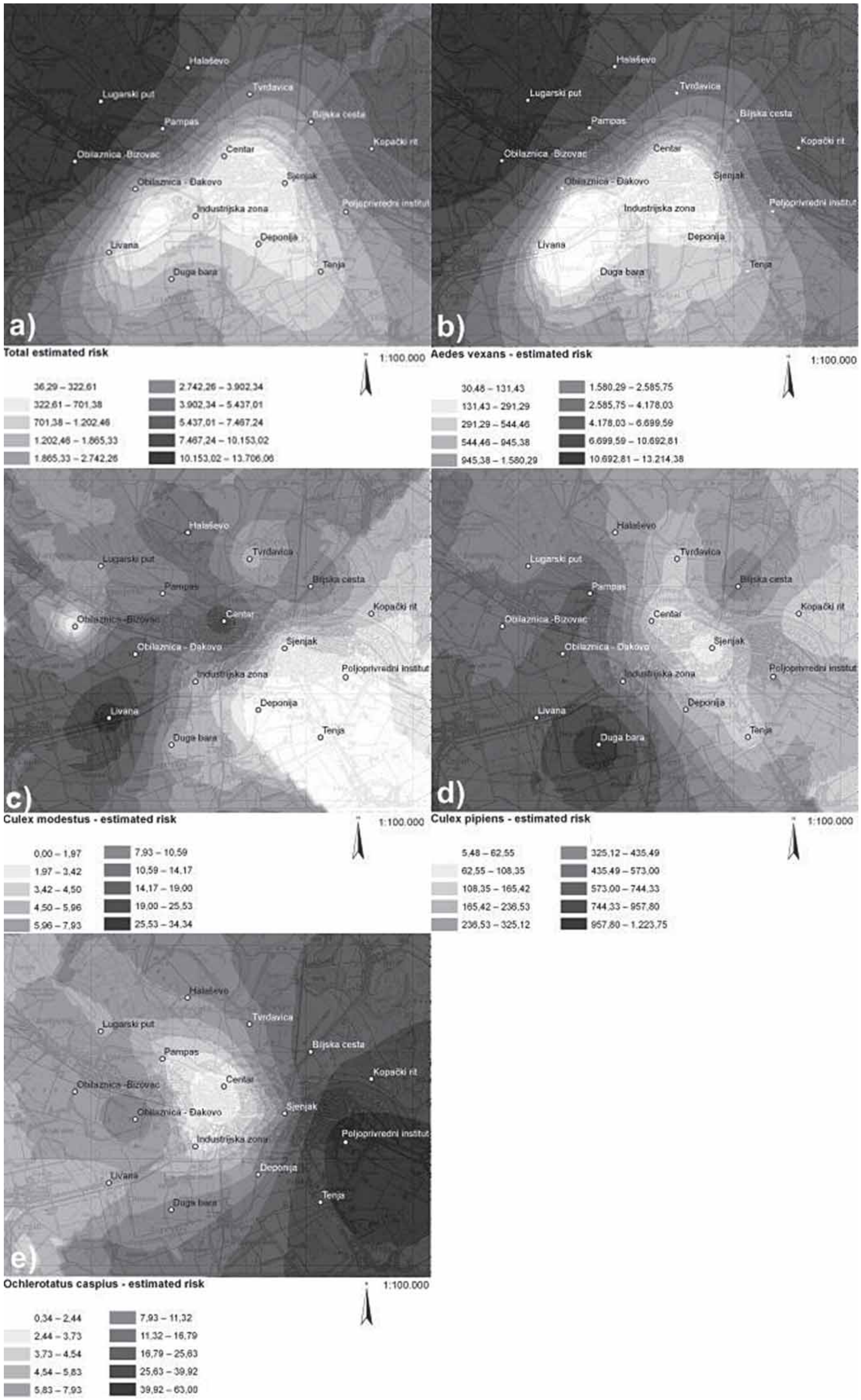

Fig. 2. Estimated risk maps of the West Nile virus transmission for the city of Osijek with 16 marked sampling sites, representing: Total estimated risk a) and an estimated risk for each species b) Aedes vexans, c) Culex modestus, d) Culex pipiens and e) Ochlerotatus caspius.

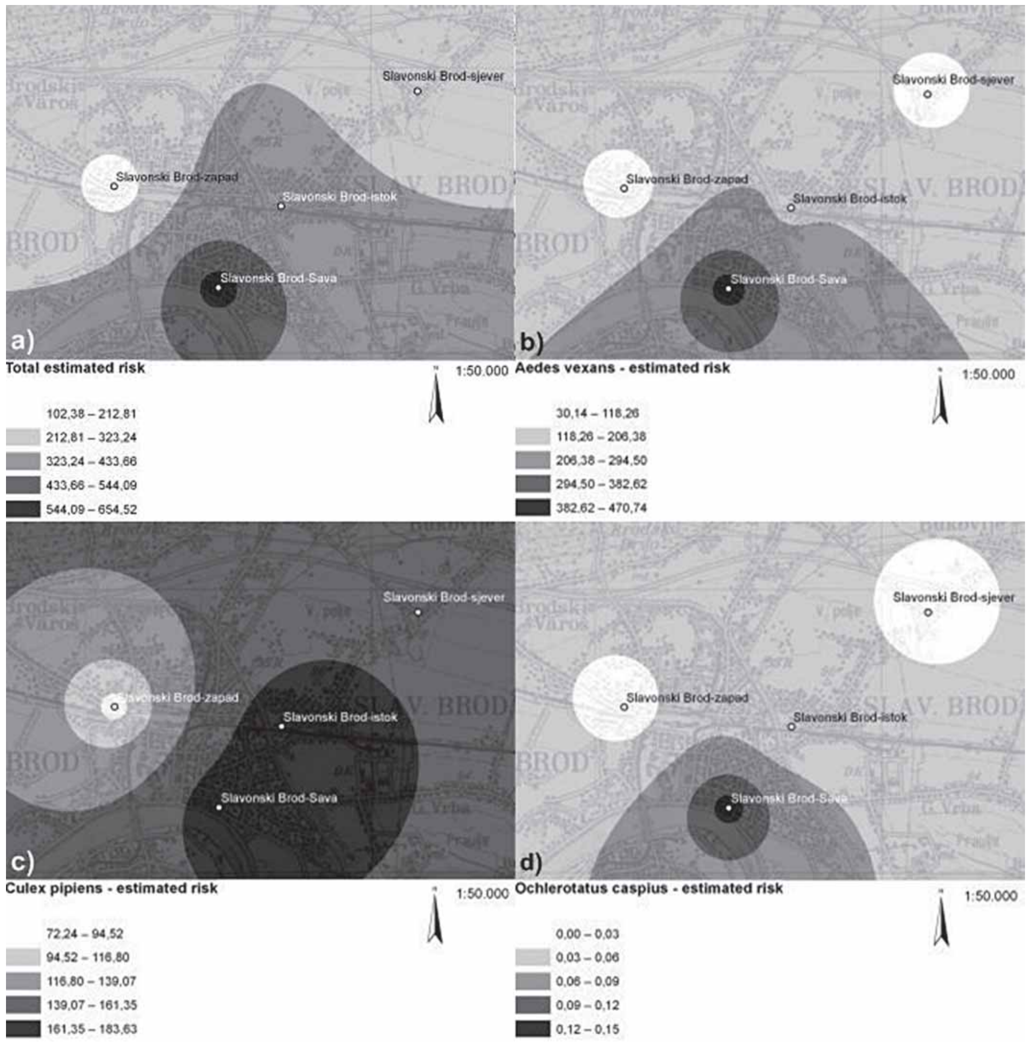

Fig. 3. Estimated risk maps of the West Nile virus transmission for the town of Slavonski Brod with 4 marked sampling sites, representing: Total estimated risk a) and an estimated risk for each species b) Aedes vexans, c) Culex pipiens and d) Ochlerotatus caspius. Because of absence of Cx. modestus map for that species is not presented.

share of 85.4% of mosquito fauna, the *Oc. caspius* and *Cx. pipiens* have similar share which is 7.6% and 6.9%, respectively, while the *Cx. modestus* is not that common with a share of just 0.01%.

When observing a relative abundance, two species stand out as possible main contributors to the risk of the WNV transmission: the *Cx. pipiens* – due to high vector competence and the *Ae. vexans* due to a very high abundance. The *Cx. modestus* has a low impact because of a small abundance while the *Oc. caspius* seems to have a negligible vector competence for the WNV (Table 2). High seroprevalence rates and a presence of the potential vectors for city of Osijek and Vukovarsko-srijemska county resulted in a high cumulative risk for these two areas. Risk in city of Osijek originates from a high abundance of the *Ae. vexans* while in the Vukovarsko-Srijemska county it has a foothold in the presence of good WNV vector – *Cx. pipiens* (Table 2).

The total estimated risk values were obtained by applying the formula for each mosquito species and summing results for the each sampling site. The values have the greatest range for the city of Osijek (36.29 – 13,706.06) where the highest number of mosquitoes per sampling site was collected (Table 2, Figure 2). For Vukovarsko-Srijemska county the values ranged from 63.67 to 1,087.76 (Figure 4), while in the town of Slavonski Brod the smallest values were obtained with the range between 102.38 and 654.52 (Figure 3).

Estimated risk maps of the WNV transmission were obtained considering the abundance of each mosquito species on an each sampling site. Therefore, the area of Osijek is represented with a map of the total estimated risk including all investigated vector species in the Figure 2a; and the maps of estimated risk for each mosquito species separately in the Figure 2b-e. The risk maps for the town of Slavonski Brod and the Vukovarsko-Sri-

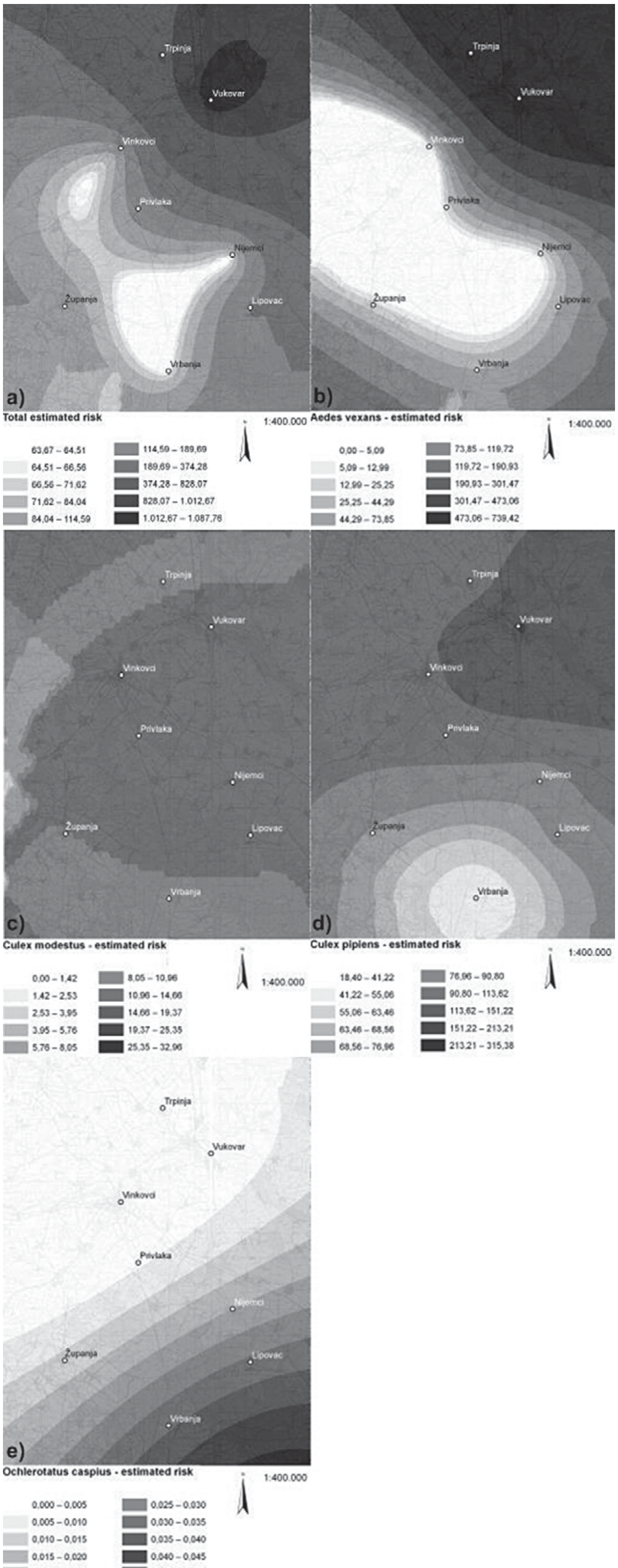

Fig. 4. Estimated risk maps of the West Nile virus transmission for the Vukovarsko-srijemska county with 8 marked sampling sites, representing: Total estimated risk a) and an estimated risk for each species b) Aedes vexans, c) Culex modestus, d) Culex pipiens and e) Ochlerotatus caspius.

jemska county are also presented accordingly. There is only one exception in the Figure 3 where due to the absence of *Cx. modestus* in the fauna of caught mosquitoes in the town of Slavonski Brod, a one risk map is omitted.

Discussion and Conclusion

Out of the four species included in this study the *Cx. pipiens* should be regarded as the main threat for public health especially in Vukovarsko-Srijemska county where its abundance is high and where equine seroprevalence of the WNV implies high concentration of the virus. To date, in the city of Osijek, the *Ae. vexans* has just been causing a lot of nuisance because of its high abundance. However, from now on it should also be considered as the species which could be a possible vector of the WNV, although its vector competence is low. Confirmation of presence of the cell fusing agent virus (CFAV) originating from the *Flavivirus* in *Ae. vexans* mosquitoes in Serbia during 2005[31] provides additional concerns regarding this species. Even though this agent is not pathogenic to humans, it is evidence of presence of *Flavivirus* in this species. The other two species, *Cx. modestus* and *Oc. caspius*, do not represent high risk due to low abundance and low vector competence, respectively.

Usage of equine populations as an early warning of virus outbreaks was applied in the USA[22,32] because equines are often spatially dispersed within human populations and are highly susceptible to the WNV infections. Furthermore, more mosquito species prefer feeding on horses rather than humans[25,33]. In urban areas of Texas during 2002, equine cases of the WNV disease occurred significantly earlier (on average 12 days earlier) than the closest human cases[22]. This is a good example of why the incorporation of monitoring equine populations for the WNV in surveillance programs should be considered.

It is unclear for how long the WNV has been present in Croatia and why there was an outbreak in 2012. The situation is similar to the rest of the Europe where the WNV outbreaks occur nearly every year but in different and often widely separated regions[33]. A possible reason could be migration of the infected birds from Africa which could be seeding the virus in different areas[34]. It is also possible that the virus remains endemic all year long in some areas, but it causes the outbreaks only under certain conditions[35]. In favour of the second possibility is the fact that the virus can overwinter in *Culex* mosquitoes[36].

The estimated risk maps of the WNV transmission which were obtained in this study could provide some valuable information to the public health institutions about where the risk of the WNV transmission is coming from and which species should be in focus. However, a further surveillance and study of the WNV transmission is necessary. Even though seroprevalence in horses is not commonly used tool for predicting WNV transmission some confirmations for the usage of our risk estimation are given; the sites of human clinical cases of the WNV infection from the towns of Slavonski Brod and Vukovar belong to the area of high estimated risk of the WNV transmission, presented in the Figures 3a and 4a. For the city of Osijek there are no confirmed cases of the WNV infection.

A risk of the WNV transmission estimated in this study could provide data to forecast the relative number of the future short-term human WNV infections, by incorporating fractions of mosquitoes blood meals taken from humans and human population density in the area[28 37]. A prediction of this kind should use a properly analysed data[38] because there are still a lot uncertainties about spreading of the WNV and calculating potential risk of an epidemic. Some mosquito species could be underrepresented by dry ice baited CDC traps which capture preferentially host-seeking females or females that have not been able to successfully complete a blood meal[28,33]. Understanding the relationship between vertebrate abundance and mosquito feeding is also crucial[39]; some data shows that the *Cx. pipiens* feeds more often on humans than previously thought[40]. The geographical profile of an area plays an important role in spreading of the WNV as well[41]. The WNV dynamics and ecology including the adaption of the WNV to infect a local mosquito vectors[42] are all still objects of many studies. All of these parameters in a heterogeneous environment[38] can cause extreme variation with 0–52% of mosquitoes transmitting the West Nile Virus at a single site between different sampling periods, and a similar variation across the populations[43]. Therefore it is important to continue the search for more indicators which can help in predicting the inevitable future of WNV outbreaks and seroprevalence rates of WNV in horses should be one of them.

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SEROPREVALENCIJA INFEKCIJE KOD KONJA KAO DODATNI POKAZATELJ ZA PRECIZNIJU PROCJENU RIZIKA ŠIRENJA VIRUSA ZAPADNOG NILA

SAŽETAK

Virus Zapadnog Nila (VZN) je životinjski arbovirus koji je odnedavno uzrok brojnih epidemija u mnogim zemljama južne i središnje Europe. U 2012., po prvi put je zabilježena epidemija u istočnoj Hrvatskoj, gdje je od groznice Zapadnog Nila oboljelo ukupno sedam ljudi. S ciljem poboljšanja javnozdravstvene zaštite pomoću nadzora i kontrole prijenosnika procijenjena su i mapirana područja visokog rizika širenja VZN. Istraživano područje obuhvatilo je gradove Osijek i Slavonski Brod, te osam općina u Vukovarsko-srijemskoj županiji. Procjena rizika temelji se na seroprevalenciji infekcije s VZN kod konja kao pokazateljem količine virusa, te prisutnosti određenih vrsta komaraca – mogućih prijenosnika VZN. S ciljem bolje procjene rizika u istraživanje su uključene četiri vrste komaraca za koje se smatra da su potencijalni prijenosnici VZN, a to su: *Aedes vexans*, *Culex modestus*, *Culex pipiens* i *Ochlerotatus caspius*. Komarci su lovljeni pomoću CDC-klopke sa suhim ledom kao atraktantom, dva puta mjesečno u razdoblju od svibnja do listopada 2012. na području Osijeka i Vukovarsko-srijemske županije, odnosno od svibnja do rujna 2001. na području Slavonskog Broda. Ovo istraživanje dokazalo je da dvije vrste komaraca predstavljaju glavni rizik širenja VZN u istočnoj Hrvatskoj, *Culex pipiens* – zbog dobre vektorske sposobnosti i *Aedes vexans* – zbog iznimno velike brojnosti. Na ove dvije vrste se treba usredotočiti pri budućim nadzorima populacije komaraca i kontrole brojnosti prijenosnika bolesti.