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Mosquito Survey during West Nile Virus Outbreak 2012 in Northeast Croatia

Enrih Merdić[1], Goran Vignjević[1], Nataša Turić[1], Mirta Sudarić Bogojević[1], Josip Milas[2], Ivana Vrućina[1] and Željko Zahirović[1]

[1] »J. J. Strossmayer« University, Department of Biology, Osijek, Croatia
[2] Institute of Public Health Osijek-Baranya County, Osijek, Croatia

A B S T R A C T

During the August and September 2012, seven human cases of the West Nile neuro-invasive disease were reported in Croatia. Medical entomology research on a potential vectors during the outbreak was supported by the Ministry of Health. A mosquito survey has been done in 64 sites in three eastern Croatian counties (Osijek-Baranja County, Vukovar-Srijem county and in Brod-Posavina county). Dry ice baited CDC traps were used for mosquito sampling in a period from the 10th to 25th September 2012. A total of 1785 mosquitoes were collected and 5 species were determined. The most numerous species were Aedes vexans with 1634 specimens, a Culex pipiens c., the potential vector of WNV, was present with 6.39%, in 114 specimens. That species was present in 43 out of 64 investigated sites. Vector control included both the control of mosquito larvae and the adults. Treatments have been done on 184 small breeding sites and on 2900 ha of an urban area.

***Key words**: mosquitoes, vectors, WNV outbreak, Croatia, mosquito control*

Introduction

During the August 2012 information about human infections with West Nile Virus (WNV) in Serbia upset the people in the neighboring countries, including Croatia. Following an outbreak of WNV infection in Greece[1] and Romania in 2010[2], and especially due to recent outbreak in Serbia (2012), the surveillance for West Nile neuroinvasive disease (WNND) was significantly enhanced in Croatia. All health authorities were asked to increase their vigilance. In addition, the case definition for suspected cases used for routine surveillance was modified: a history of travel to Serbia was added.

In Croatia, the first presence of WNV was in 1980, proven through serological testing of healthy people. In the north-eastern part of Croatia 1.2% of the 816 tested were seropositive, while in Dalmatia this was 3.4% out of 2377 samples[3]. Since 1980 and until the end of the first decade of 21st century there is no information about this virus in Croatia. After a big research of seroprevalence on horses in 2010, presence of this virus was determined in almost every lowland county (seroprevalence 3.5%)[4]. Even bigger seroprevalence (3.7%) was determined during the considerably smaller researches completed in 2011 (unpublished data).

The two main parameters which enable the disease transmission are the fauna and biology of mosquitoes and the biology of pathogen (WN virus)[5]. Only the optimal conditions of the both parameters enable the transmission and spreading of the disease. The WNV circulates in two ecosystems in Europe, both sylvatic and the urban. For an efficient local transmission, mosquito species are needed which are capable of sustaining and transmitting the virus within the indigenous bird population. Ornithophilous mosquito species will be the most relevant species for the WNV establishment in an enzootic cycle in temperate Europe[6]. For transmission to humans and horses, vectors are needed that have a more opportunistic feeding behavior; feeding both on birds and mammals and serving as bridge vectors. The vector capacity is different in different mosquito species. Up to now, in 43 mosquito species the WNV has been detected[7–9]. Among them, 10 can be potential vectors in Croatia. Very successful vector of WNV is *Culex pipiens*[10]. According to prevalence and abundance in Croatia, all territory of Croatia can be considered as a risk area for the WNV transmission. In the east Croatia, *Cx. pipiens complex.* is present in mosquito fauna at a level of between 5

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to 10%[11]. The both *Cx. pipiens c.* and *Ae. albopictus* in the Adriatic part of Croatia are dominant species[12].

After a first human case of the WNND reported by the General Hospital Slavonski Brod, and especially after a second human case reported by the Osijek University hospital, the Ministry of Health organized several meetings with the aim of a fast reaction. At these meetings a decision to start epidemic surveillance was made, which included both mosquito survey and mosquito control in the area where the human cases were noted.

In this paper a result of mosquito research and review of incurred treatments during the outbreak in northeast Croatia is given.

Materials and Methods

Dry ice baited CDC traps were used for mosquito sampling in 64 sites in the northeast Croatia from the 10th to the 25th of September 2012. Sampling in Osijek-Baranja County was done in 43 localities (16 in Osijek), in Vukovar-Srijem county in 17 localities, and in Brod-Posavina county in 4 localities, once per locality. The traps were set up in an afternoon in the villages and towns close to the human settlements and several traps were set up in the backyards of the houses were the patients came from. The traps operated for at least 12 hours including dusk and down. All the traps were set up in an appropriate microhabitat including shaded areas, bushes, backyards etc. Sampled mosquitoes were determined according to the keys[5]. One part of the sampled mosquitoes was stored in an entomological collection in »J. J. Strossmayer« University, Department of Biology, and the other part was frozen (–70 °C) and prepared for the further analysis.

In addition to those samplings, mosquitoes sampled in a regular mosquito monitoring in Osijek throughout the September were added. Monitoring of the mosquitoes was based on a biweekly monitoring with a same method on 16 sites in and around Osijek area. The traps were put on grid with a distance of 2.5 km from each trap.

Mosquito control methods include both larviciding and adulticiding. For larvicide treatments Vectobac WDG was used and applied manually. For areal adulticiding deltametrin 0.8g/ha or 0.5 l/ha water mixture using electric powered microner AU6600 with RPM 15000 was used. The all treatments were implemented in the period between the 18th and the 29th of September 2012. The ULV spraying against the adult mosquitoes had been done in 5 infected areas, two times in a row and within three days. Treatments in total of 2900 ha started on the 20th and finished the 28th of September 2012. Control sampling had been done two days after the whole action

Results

Mosquitoes were sampled in three northeast Croatian counties (Figure 1). Traps were distributed with an increased attention of sampling in the places of outbreak,

Fig. 1. Study area in east Croatia. OBC – Osijek-Baranya County, VSC Vukovar-Srijem County, BPC – Brod-posavina County, small squares – position of CDC traps, X – place of human case of WNVD.

but also in the wider area too. In Brod-Posavina county the sampling was made in Slavonski Brod only because the patients from that area considered it imported or accidently infected (unpublished results). A total of 1785 mosquitoes had been caught and 5 species identified. The most numerous species was *Ae. vexans* accounting to 1634 specimens caught (Figure 2), and the biggest number of these mosquitoes, (393 specimens) sampled in the village of Nard. Potential vector, *Cx. pipiens c.* was a second most numerous with the share of 6.39%. The rest of species *Oc. caspius, An. maculipennis* complex and *An. hyrcanus* shared only 1.8% of the total. In 43 out of 64 sampling sites presence of *Cx. pipiens c.* was noted, although abundance was very small and ranged from 1 to 10 specimens. The localities with the biggest number of these mosquitoes were Duga bara – Osijek with 10, Livana with 9, and Čepin and Lipovac with 8 specimens. Research results in the counties are shown in Tables 1–4.

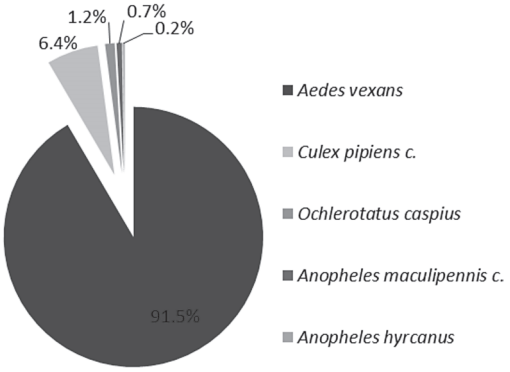

Fig. 2. Mosquito faunistic compound during WNV outbreak in east Croatia 2012.

TABLE 1
SAMPLING SITES AND FAUNISTIC COMPOSITION OF MOSQUITOES IN OSIJEK-BARANYA COUNTY DURING WNV OUTBREAK IN CROATIA 2012 (WITHOUT OSIJEK)

Locality	Habitat	*Ae. vexans*	*An. hyrcanus*	*An. maculipennis c.*	*Cx. pipiens c.*	*Oc. caspius*	Total
Sarvaš	Cemetery, shaded	4					4
Erdut	Rural house backyard, shaded				2		2
Draž	Bushes by the road				6		6
Beli Manastir	Bushes by the road				1		1
Kopačevo	Near cemetery, shaded	31	1		1	10	43
Kneževi vinogradi	Agricultural, shaded		1		4		5
Budimci 1	Rural house backyard, shaded			1	1		2
Budimci 2	Rural house backyard, shaded	2					2
Čepin	Rural house backyard, shaded				8		8
Kuševac	Rural house backyard				3		3
Vuka	Rural house backyard, shaded			1	4		5
Đakovo	Bushes by the road	2			2	1	5
Našice	City park	3					3
Bistrinci	Forest edge	88		1	2		91
Bizovac	Bushes by the road					5	5
Koška	Bushes by the road						0
Donji Miholjac	House backyard near fishponds	40		1	1		42
Petrijevci	Bushes by the houses	43		1			44
Belišće	Near cemetery, shaded	210			1		211
parNard	Forest edge	393					393
Belišće 1	Urban house backyard, shaded				1		1
Belišće 2	Urban house backyard, shaded				1		1
Belišće 3	Cemetery, shaded				1		1
Belišće 4	City gas station, shaded				5		5
Budimci 1	Rural house backyard, shaded				1		1
Budimci 2	Rural house backyard, shaded				1		1
Budimci 3	Rural house backyard, shaded				2		2
Budimci 4	Rural house backyard, shaded				0		0
Total		816	2	5	48	16	887

The Public Health Institute of Osijek and the Baranya county made both the adulticide and larvicidae treatments. The larval mosquito control (small water bodies) had been done in places where human cases were noted and out of 2108 checked, and in only 8.7% (184 breeding sites) the larvae had been found and treated with a *Bti* based preparation. In the village of Budimci up to 19.8% of the breeding sites were active, mostly on an open cesspool, a trench drain, and similar water bodies (Table 5). Number of adult mosquitoes before treatment ranged from 0 to 8 and after treatment from 0 to 1 (Table 6).

Discussion

During the WNV outbreak mosquito fauna was indicating the most common fauna composition for the late summer in this study area. The domination of the floodwater mosquitoes was expected since this generation of the floodwater mosquitoes hatched at the beginning of August, which itself is a result of a high water level of the river Drava at the end of July 2012. Except for the high water level of the river Drava in July, the 2012 was a dry year, with very few active small and medium breeding sites which produced *Cx. pipiens c.* mosquitoes. The mosquito species and mosquito abundance in this study coincides with the previous research in the same area. The absolute dominance of *Ae. vexans* is present in this as well as the previous studies[13]. The share of *Cx. pipiens c.* as a potential vector of the WNV accounted to 6.39% of the total population in this study and it coincides with the previous long term studies in Osijek (5.85%)[14].

Up to now 43 mosquito species have been recorded as the WNV vectors[7,8]. In Croatia, there are 10 species of mosquitoes that are considered potential bridge vectors

TABLE 2
SAMPLING SITES AND FAUNISTIC COMPOSITION OF MOSQUITOES IN THE CITY OF OSIJEK DURING WNV OUTBREAK IN CROATIA 2012

Locality	Habitat	*Ae. vexans*	*An. hyrcanus*	*An. maculipennis c.*	*Cx. pipiens c.*	*Oc. caspius*	*Oc. sticticus*	Total
Lugarski put I	River embankment, shaded	191	1		2			194
Obilaznica – Bizovac	Agricultural	50			1	4		55
Halaševo	Agricultural, shaded	337		1	1			339
Pampas (uz nasip)	River embankment, forest	57			1		1	59
Obilaznica – Đakovo	Small orchard, shaded	24			1			25
Livana	Rural house backyard, shaded				9			9
Tvrđavica	Rural house backyard, shaded	61		3		1		65
Centar	Urban house backyard, shaded	1						1
Industrijska zona	Urban, small shade	10			6			16
Duga bara	Bushes by the canal	2			10			12
Biljska cesta	Bushes by the canal	14		1				15
Sjenjak	Urban house backyard, shaded							0
Smetlište (Brijest)	Agricultural, shaded				6			6
Kopački rit	Flooding area, shaded	32			1			33
Poljoprivredni institut	Cemetery, shaded	10						10
Tenja	Rural house backyard, shaded				1			1
Total		789	1	5	39	5	1	840

TABLE 3
SAMPLING SITES AND FAUNISTIC COMPOSITION OF MOSQUITOES IN VUKOVAR-SRIJEM COUNTY DURING WNV OUTBREAK IN CROATIA 2012

Locality	Habitat	*Ae. vexans*	*An. maculipennis c.*	*Cq. richiardii*	*Cx. pipiens c.*	Total
Ilok	Bushes by the road, near river		1	1		2
Vrbanja	Rural house backyard, shaded	3	1		2	6
Nijemci	Rural house backyard, shaded					0
Drenovci	Rural house backyard, shaded	4			2	6
Vukovar	Urban house backyard, shaded	2			2	4
Bošnjaci	Rural house backyard, shaded					0
Babina greda	Agricultural, shaded					0
Županja	Rural house backyard, shaded				2	2
Vinkovci	Urban house backyard, shaded					0
Trpinja	Agricultural, shaded	2				2
Lipovac	River embankment, shaded				8	8
Privlaka	Rural house backyard, shaded				1	1
Šarengrad	River embankment, shaded	2				2
Gunja	Forest edge	7			1	8
Sotin	Rural house backyard, shaded	6			1	7
Ivankovo	Rural house backyard, shaded				3	3
Stari Mikanovci	Cemetery, shaded				4	4
Total		26	2	1	26	55

for the WNV[15]. Potential vectors of the WNV in Croatia can be divided into three groups based on mosquito abundance: first – very numerous (*Ae. vexans*, and *Cx. pipiens c.*), second – moderately numerous (*Oc. caspius*, *Oc. cantans*, *Ae. cinereus* and *An. maculipennis* complex), and third – few in number (*Cx. modestus*, *Coquil-*

TABLE 4
SAMPLING SITES AND FAUNISTIC COMPOSITION OF MOSQUITOES IN BROD-POSAVINA COUNTY DURING WNV OUTBREAK IN CROATIA 2012

Locality	Habitat	*Ae. vexans*	*Cx. pipiens c.*	Total
Slavonski brod-zapad	House backyard			0
Slavonski brod-istok	House backyard, shaded		3	3
Slavonski brod-Sava	River embankment, shaded	1		1
Total		1	3	4

TABLE 5
RESULTS OF SURVEY OF SMALL WATER BODIES DURING WNV OUTBREAKE IN EAST CROATIA 2012

Site	Larviciding (*Bti*)		
	No. of objects checked	No. of positive breeding places	%
Budimci	243	48	19.8
Nijemci	448	48	10.7
Belišće	1197	68	5.7
Kuševac	220	20	9.1
Total	2108	184	8.7

lettidia richiardii and *An. plumbeus*)[15]. According to this *Cx. pipiens c.* and *Ae. vexans* could be considered as the only potentially efficient vectors. Since *Ae. albopictus* has not been recorded in the northeast Croatia and *Ae. vexans* has a small vector capacity, the most likely vector is *Cx. pipiens c.* which has stable dominant population in the eastern Croatia. None of the 114 *Cx. pipiens c.* mosquito specimens sampled in this study, was WNV positive (unpublished data). The mosquitoes were analyzed through a presence of viral RNA which indicated the possible presence of other viruses, but an additional analysis is required. A possible reason for such result may be a small number of analyzed mosquitoes, also confirmed in the literature[16]. The probability to isolate viral RNA from a small number of mosquitoes is very low.

Appearance time of WNND in Croatia coincides with same outbreaks in the neighboring countries such as Hungary and Romania[17,2]. The late summer is a period of the year when ecological conditions of vectors and pathogens overlap. For any risk assessment, besides this overlap, the abundance of the main vector, nearness of birds and virulence of virus, also must be taken into consideration.

Out of 5 recorded species *Cx. pipiens c.* is reported field or laboratory vector for infectious pathogens, including the West Nile virus (WNV), Tahyna virus (TAHV), Sindbis virus (SINV), Usutu virus (USUV), Batai virus (BATV) and *Dirofilaria immitis* in Europe. Like the WNV, SINV and USUV are maintained in an avian-mosquito cycle. The vertebrate reservoirs are largely passeriformes (WNV, USUV) birds, with migratory members being responsible for wide geographic distribution[18,19]. *Ae. vexans* has many attributes of an ideal vector species. It is widely distributed, can become very abundant, often at the same time when virus activity is at its peak, it feeds readily on humans and domestic animals, and it has been found naturally infected with various arboviruses[20]. Natural infections in the North America with several viruses (including WNV) have been reported but in Europe *Ae. vexans* is involved in the transmission of the Tahyna virus only[21,22].

According to normal seasonal dynamic of mosquitoes in the area mosquito abundance drastically decline toward end of season (September-October)[13]. Specimens of *Cx. pipiens c.* during September decrease oviposition and prepare for hibernation (in adult stage) seeking for suitable places. On the other side, abundance of the most numerous species in this research, *Ae. vexans*, also decreased but preferably due to cold weather, which is noted in the end of September. These reasons support results of vector control that only 8.7% of potential breeding sites were found positive. Below to this, small num-

TABLE 6
RESULTS OF ADULT MOSQUITO SAMPLING BEFORE AND AFTER ADULTICIDE TREATMANTS DURING WNV OUTBREAK IN EAST CROATIA 2012

Site	Sampling date before treatment	No. of mosquitoes before treatment	Treated area (ha)	Sampling date after treatment	No. of mosquitoes after treatment
Budimci	18. Sept.	4	220+220	30. Sept.	1
Nijemci	10. Sept.	0	310+310	30. Sept.	0
Belišće	18. Sept.	8	490+490	30. Sept.	0
Kuševac	11. Sept.	3	220	30. Sept.	0
Vukovar	10. Sept.	4	320+320	30. Sept.	0
Total		19	2900		1

ber of adult mosquitoes decreased due to both end of season and control measurements.

All authority activities connected with the WNV outbreak, and consequently the mosquitoes, are based on two laws: Civil Protection of Infectious Diseases Law (Official Gazette 79/07, 113/08, 43/09) and a Health Care Law (OG 150/08, 71/10, 169/10, 22/11 i 84/11) as well as the Rule Book of Ways of Conducting DDD Measurements (OG 128/11). Both activities, the medical entomological survey and the epidemic mosquito control had been done according to the obligations of law. All treatments were very effective because of the two reasons, well-designed treatments and an end of season and a cooler weather that followed. However, one question still remains and that is žWhat about obligated preventive disinsection (also prescribed by the law)?' The lack of funds at the local level disables any treatments. Certainly this WNV outbreak should stimulate activities of prevention.

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E. Merdić

»J. J. Strossmayer« University, Department of Biology, J. Huttlera 4, 31000 Osijek, Croatia
e-mail: enrih@biologija.unios.hr

ISTRAŽIVANJE KOMARACA ZA VRIJEME POJAVE BOLESTI IZAZVANE VIRUSOM ZAPADNOG NILA U HRVATSKOJ 2012. GODINE

SAŽETAK

Tijekom kolovoza i rujna u Hrvatskoj je prijavljeno 7 oboljelih od neuroinvazivnih bolesti izazvanim virusom Zapadnog Nila. Entomološka istraživanja komaraca potencijalnih vektora potaknuta su od strane Ministarstva zdravstva. Istraživanje komaraca obavljeno je na 64 postaje u tri istočnohrvatske županije. Uzorkovanje je obavljeno u Osječko-baranjskoj županiji na 43 postaje (od toga 16 postaje je bilo u Osijeku), u Vukovarsko-srijemskoj na 17 postaja i u Brodsko-posavskoj županiji na 4 postaje. Komarci su uzorkovani CDC kopkama uz suhi led kao atraktant od 10. do 25. rujna 2012. godine. Sveukupno je uzorkovano 1785 jedinki komaraca i determinirano je 5 vrsta. Najbrojnija vrsta je bila *Aedes vexans s* 1634 jedinke, a vrsta *Culex pipiens,* potencijalni vektor, je bila prisutna u ukupnom udjelu komaraca s 6,39%. Ova vrsta bila je uzorkovana na 43 (67%) od ukupno 64 postaje. Protuepidemijska dezinsekcija uključivala je larvicidno tretiranje i adulticidno tretiranje. Sveukupno je pregladano 2108 objekata a u 184 utvrđena su legla vrste *Cx. pipiens c.* i na njima obavljen tretman. Adulticidni tretman je obavljen u mjestima odakle su zabilježeni pacijenti zaraženi virusom zapadnog Nila na ukupnoj površini od 2900 ha.