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Pregled | Review

# The Role of Mosquito Surveillance in Monitoring Arboviral Infections in Croatia

## Uloga praćenja komaraca u kontroli arbovirusnih infekcija u Hrvatskoj

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### Abstract

In accordance with the noticed growing trend in the number of vector-borne disease cases, the extent of public health significance of this problem is also increasing. However, the strengthening of capacities for suppression of these outbreaks is present. With the aim of protecting population from arbovirus infections, there is a need for continuous monitoring and evaluation of risk factors related to vectors and vector borne diseases and human health, for which data is acquired through monitoring programs. In order to address these issues, several activities must be integrated and taken into consideration: vector surveillance, entomological activity, early detection system, preventive measures, counter-epidemic measures and virus detection in vectors, reservoirs or infected animals. There is a legal framework regarding mosquito monitoring on a local, as well as national level. Additionally, at the initiative of the Croatian Institute of Public Health, invasive mosquito species monitoring is being implemented and continuously conducted since 2016.

### Sažetak

U skladu s uočenim trendom porasta broja slučajeva zaraznih bolesti koje se prenose vektorima, dolazi do povećanja razmjera javnozdravstvenog problema, no s druge strane i do jačanja kapaciteta u svrhu suzbijanja navedenih bolesti. S ciljem zaštite populacije od arbovirusnih infekcija nameće se potreba za kontinuiranom provedbom praćenja i procjenom rizika utjecaja vektora i vektorskih bolesti na zdravlje, za što je neophodno prikupljanje podataka kroz programe praćenja. U problematici zaraznih bolesti koje se prenose vektorima potrebno je integrirano obuhvatiti aktivnosti koje se odnose na nadzor nad vektorima, entomološke djelatnosti, sustav ranog otkrivanja, preventivno suzbijanje, protuepidemijske mjere te praćenja prisutnosti virusa u vektorima, rezervoarima ili zaraženim životinjama. U Hrvatskoj je zakonom propisano praćenje komaraca na lokalnoj i na državnoj razini te je na inicijativu Hrvatskog zavoda za javno zdravstvo uspostavljen monitoring invazivnih vrsta komaraca koji se kontinuirano provodi od 2016. godine.

## Introduction

Mosquitoes are currently in the focus of global entomological research, because, in addition to being nuisance, they are of great public health significance due to their vector potential for transmitting arboviral infections. Microorganism transfer between hosts is increased due to mosquito feeding biology, with reference to their feeding on multiple successive hosts. Malaria, dengue, West Nile virus (WNV) infection, Zika virus (ZIKV) infection, yellow fever and filariasis are the most common mosquito-borne diseases. According to the World Health Organization data, vector illnesses account for more than 17% of infectious diseases and cause more than 700,000 deaths annual-

ly. The risk of vector-borne diseases infection in the European region is relatively low; however, there is a growing trend in their incidence and geographical distribution on a global scale<sup>[1]</sup>.

To this day, 52 mosquito species have been identified in Croatia, of which only a few species carry a significant vector potential for disease transmission<sup>[2]</sup>.

Although mosquito presence in a given area is not considered as an issue, it represents a basic precondition for transmission of vector pathogens. Distribution and mosquito population density, as well as infectious diseases transmission potential, are strongly influenced by climatic factors, especially air temperature, humidity, as well as precipitation amount and

frequency. The climate change and trend in the rise of global temperature reflect on vector-borne diseases distribution and lead to new patterns of these diseases. The existing preconditions for mosquito maturation and reproduction and the present vector potential of mosquitoes combined with possible occurrence of pathogens could significantly increase the infectious disease spreading, particularly of arbovirus infections, such as WNV, dengue, ZIKV infection, chikungunya and others<sup>[3, 4]</sup>.

The first autochthonous dengue fever case in Croatia was recorded in the Pelješac peninsula in 2010; and indigenous WNV infection cases recorded in 2012, 2013, 2014, 2015 and 2016 have shown vulnerability to transmission of these diseases in areas where vector species were present and active<sup>[5, 6]</sup>.

Additionally, recent chikungunya outbreaks in Italy and France and the increasing prevalence of WNV cases in Europe, as well as ZIKV infection occurrence, jointly show susceptibility to transmission of these diseases on a global level<sup>[7]</sup>.

In order to reduce the vulnerability of the population and impact on human health, the introduction of mosquito-borne diseases risk assessment is necessary, with knowledge and data obtained from the monitoring programs.

### Mosquito Surveillance in Croatia

The mosquito monitoring system represents an important part of the global response to vector-borne diseases. Activities such as human health surveillance, as well as reservoir and mosquito control, are the key elements in risk assessment and management of health threats both to humans and animals<sup>[1]</sup>.

The legislative framework for vector surveillance in Croatia is provided by the Programme of Measures to Control Pathogens, Harmful Arthropods (*Arthropoda*) and Harmful Rodents of which Planned, Organized and Systematic Control using Disinfection, Desinsection and Deratization is of Public Health Importance for the Republic of Croatia (Programme of Measures)<sup>[3]</sup>.

The changes and amendments to the Programme of Measures to Control Pathogens, Harmful Arthropods (*Arthropoda*) and Harmful Rodents of which Planned, Organized and Systematic Control using Disinfection, Desinsection and Deratization is of Public Health Importance for the Republic of Croatia (Amendments to the Programme of Measures) came into force in July 2018 and provided legislation that brought mosquito monitoring on a national level, as opposed to earlier practice<sup>[8, 9]</sup>.

Among the many species inhabiting the territory of Croatia, special attention in recent years is being

focused on invasive mosquito species, especially the Asian tiger mosquito *Aedes (Stegomyia) albopictus* (Skuse, 1894), first time recorded in Zagreb in 2004. It is a species that meets the criteria for high potential for the transmission of dengue and chikungunya virus and is a potential vector for Zika virus transmission. The invasiveness of this species is proved by a number of factors such as expressed competitive ability, ecological plasticity, high survival capabilities, and on the other hand, lack of effective mosquito control measures.

Following the Croatian Institute of Public Health (CIPH) initiative, national monitoring of invasive mosquito species in Croatia was established in 2016 and continuously carried out in 2017 and 2018 in the entire territory. The aim was to monitor invasive mosquito species and to collect data on distribution of the species in the entire territory of Croatia, in order to create a distribution map of breeding sites and to create a unique national database with the purpose of conducting risk assessment for vector-borne diseases. In 2017 and 2018, the inspection of ovitraps was carried out every 14 days from May to November, according to the Protocol provided by the CIPH. One to two grams of biological insecticide per trap was used in the water<sup>[10, 11]</sup>. Biological insecticide/larvicide based on *Bacillus thuringiensis* subsp. *israelensis* strain H-14 (3000 ITU/mg, i.e. 37.4% w/w) under the trade name VectoBac WG was distributed to county public health institutions by the CIPH solely for research purposes. The collected samples were delivered twice a month to determination centres of county public health institutions where biologists specialized in identifying mosquitoes (medical entomologists) were available.

Oviposition traps were placed on typical sites representing points of entry for mosquitoes, such as: tyre-repairing workshops, cemeteries, border crossings, gas pumps, railway and bus stations, parks, private houses near the main roads, residential buildings and public services in the county of the relevant public health institutions. GPS coordinates or addresses with the description of the locations where trap containers were placed were recorded and delivered to the CIPH. National monitoring carried out in 2018 confirmed the presence of Asian tiger mosquito in all counties, even though it was previously believed that the Asian tiger mosquito inhabited only the coastal areas and the islands of Croatia (Figure 1).

The monitoring revealed another species, *Aedes (Finlaya) japonicus* (Theobald, 1901), and its expansion in Croatia was confirmed in 14 counties. This species was recorded for the first time in September 2013 in Đurmanec and in the border crossing zone of Macelj in the Krapina-Zagorje County<sup>[12]</sup> (Figure 2). The collected data through national monitoring of in-

FIGURE 1 DISTRIBUTION OF *Aedes albopictus* IN THE TERRITORY OF CROATIA IN THE PERIOD BETWEEN THE FIRST FINDING IN 2004 AND 2018  
SLIKA 1 RASPROSTRANJENOST KOMARCA *Aedes albopictus* NA PODRUČJU HRVATSKE U RAZDOBLJU OD PRVOG NALAZA 2004. DO 2018. GODINE

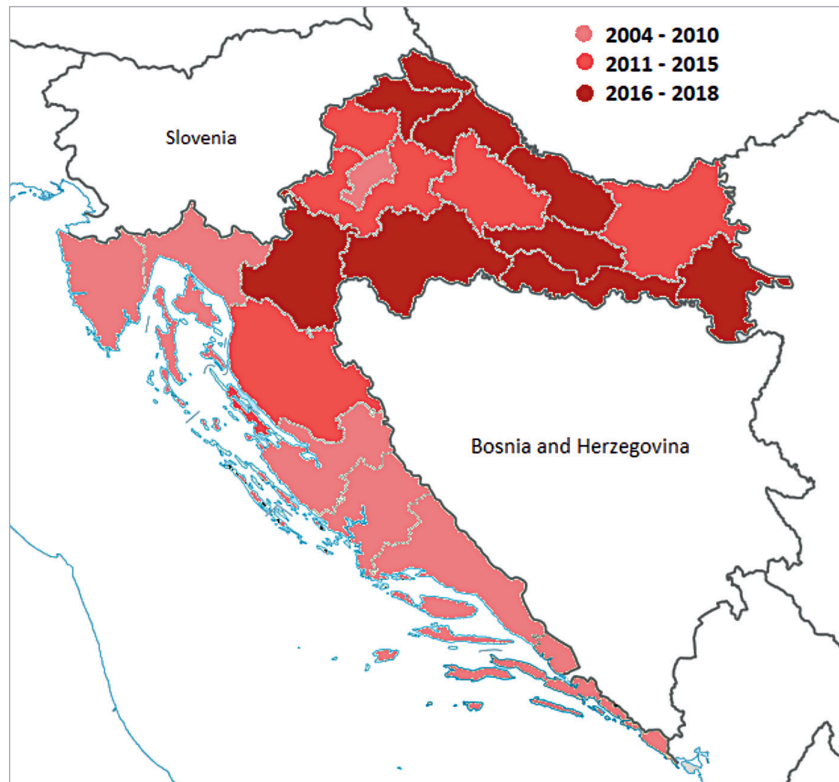
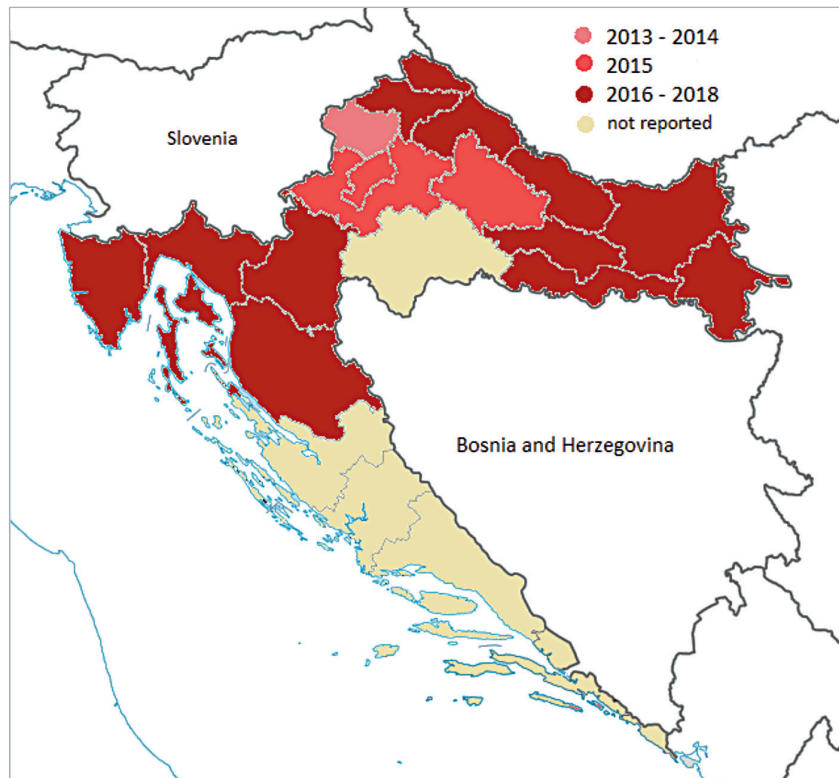


FIGURE 2 DISTRIBUTION OF *Aedes japonicus* IN THE TERRITORY OF CROATIA IN THE PERIOD BETWEEN THE FIRST FINDING IN 2013 AND 2018  
SLIKA 2 RASPROSTRANJENOST KOMARCA *Aedes japonicus* NA PODRUČJU HRVATSKE U RAZDOBLJU OD PRVOG NALAZA 2013. DO 2018. GODINE



vative mosquito species in Croatia are annually sent to the VectorNet database which publishes the latest information (up-to-date) on the distribution of vectors in Europe and shows the distribution of vector species on a regional level.

In the areas where invasive mosquito species are established, priority is set on integrated mosquito and disease surveillance, as well as further vector spreading prevention. This especially applies to the Asian tiger mosquito, who has become an indigenous species in the coastal areas of Croatia and the islands.

### Significance of Mosquito Monitoring for Emerging and Re-Emerging Arboviral Infections in Croatia

The number of arboviral infections, as well as other vector-borne diseases reveals a growing trend in the last few decades. The issue of these disease outbreaks requires multisectoral cooperation between human and veterinary medicine, public health, entomology, forestry and meteorology sector<sup>[13]</sup>.

The collaboration between physicians and veterinarians with the purpose of prevention of zoonoses in Croatia has been existent for decades. However, co-operation on arboviral zoonoses surveillance, with the exception of several separate scientific studies, has not been systematically established until recently. In 2011, encouraged by scientific research that has provided evidence of WNV circulation among horses, the Croatian Ministry of Agriculture introduced a monitoring program of this important zoonosis. The monitoring is continuously carried out, resulting in the detection of Usutu virus (USUV) circulation among horses, before any recorded human cases<sup>[13]</sup>.

The WNV occurs in nature by circulating between birds and mosquitoes, with occasional cases of infecting humans and horses. Different species of wild birds are natural reservoirs of the virus, and the vectors are *Culex* genus mosquitoes. The *Culex Pipiens* Complex has been recorded as the most common vector of WNV and USUV so far. Most cases of WNV infection in humans are asymptomatic; however, a severe neu-

roinvasive disease may develop with a fatality rate of up to 10%.

The first detection of WNV antibodies in humans in Croatia dates to 1980, and systematic virus monitoring was started in a pilot study of seroprevalence in horses in 2010<sup>[14]</sup>.

The first case of WNV neuroinvasive disease was reported in September 2012 in the eastern part of Croatia, and by the end of 2012 there were 7 cases of WNV infection recorded, indicating that permanent use of preventive epidemiological measures, including vector control, are necessary<sup>[1]</sup>.

According to the CIPH data, 20 new cases of neuroinvasive disease occurred in the Zagreb City and Zagreb and Međimurje counties in 2013, and in 2014 one WNV case occurred in the Zagreb City area. One case was recorded in 2015, while in the east of the country two indigenous cases were recorded in 2016<sup>[15]</sup>. Despite the small number of cases in 2016, the first fatal case of WNV infection was recorded. In 2017 and 2018, there were 8 and 61 cases of WNV infection in Croatia, respectively.

The number of human cases in Croatia and EU for the period between 2012 and 2018 is presented in the Table 1.

EU neighbouring countries reported 580 human cases: Serbia (415), Israel (128), Turkey (23) and Kosovo (14). To date, 181 deaths due to WNV infection have been reported by Greece (47), Italy (46), Romania (43), Serbia (35), Kosovo (3), Turkey (3), Bulgaria (2), the Czech Republic (1) and Hungary (1).

According to the CIPH data in 2018, the majority of cases were recorded in the Osječko-Baranjska County and in the area of Zagreb and Zagreb County. Other counties with confirmed human cases of WNV infection are Vukovarsko-Srijemska, Varaždinska, Brodsko-Posavska, Koprivničko-Križevačka, Karlovačka, Međimurska, and Virovitičko-Podravska County. Taking into account the place of residence in the incubation period, three cases referred to infection outside of Croatia. Most recorded cases were the neuroinvasive form of the disease, with five recorded deaths.

TABLE 1 NUMBER OF WEST NILE VIRUS INFECTIONS IN THE EU AND CROATIA IN THE PERIOD FROM 2012 TO 2018

TABLICA 1 BROJ SLUČAJEVA INFEKCIJE WEST NILE VIRUSOM U EU I HRVATSKOJ U RAZDOBLJU OD 2012. DO 2018. GODINE

Year	2012	2013	2014	2015	2016	2017	2018
Number of infections in the EU	937	785	210	315	492	288	1684
Number of infections in Croatia	7	20	1	1	2	8	61

Izvor podataka/Source of data: ECDC, CIPH, CRONEUROARBO

Compared to 2017, on the EU level, the recorded equid cases increased by 30%. During the 2018 transmission season, 285 outbreaks among equids were reported by Italy (149), Hungary (91), Greece (15), France (13), Spain (9), Austria (2), Romania (2), Germany (2), Slovenia (1) and Portugal (1)<sup>[16]</sup>.

Surveillance of medically significant mosquito-borne viruses is an important part of public health practice. Field specimens, especially mosquitoes collected as part of surveillance programs should be tested for the presence of viral nucleic acid by standard or real-time reverse-transcriptase polymerase chain reaction (RT-PCR)<sup>[17]</sup>.

A study conducted in the north-western part of Croatia in the 2015-2018 period sampled and analyzed adult mosquitoes of the following species: *Ae. albopictus*, *Culex Pipiens* Complex, *Aedes (Aedimorphus) vexans* (Meigen, 1830) and *Ochlerotatus (Ochlerotatus) sticticus* (Meigen, 1838), which were tested for the presence of arboviruses. USUV RNA was detected in one out of 80 *Ae. albopictus* pooled specimens from the Zagreb area. Pooled samples of mosquitoes *Culex Pipiens* Complex from the City of Zagreb and Međimurje County were tested for the presence of WNV and USUV RNA. USUV RNA was detected in a single sample from the Međimurje County and in a single sample from Zagreb<sup>[2]</sup>.

Given that no such research has been continuously carried out in Croatia, in order to improve the monitoring system, continuation of the research on the presence of arboviruses in mosquitoes throughout its territory is necessary, with continuous monitoring of the presence of mosquito eggs as well as adult mosquitoes.

## Discussion

Before the eradication of malaria in the 1950s, mosquitoes had the major role in the transmission of this disease in Croatia. However, their role as vectors of arboviral infections has recently become increasingly prominent.

Proper monitoring of changes in the presence and abundance of mosquito species is important for providing valuable information to health and public health institutions in order to take proper measures to control the mosquito population. Mosquito borne diseases are (again) a threat to European countries and pose an obvious challenge when it comes to vector-borne diseases. The reappearance of pathogens through the population of autochthonous mosquitoes, such as the WNV, is a major issue in several European regions<sup>[18]</sup>.

Collaboration of experts from different sectors at the local, national and global level to achieve the best

outcomes for the preservation of human health is represented through the „One Health” approach. Through close collaboration of experts from various fields, joint projects have also been initiated to further strengthen the surveillance of arboviral zoonoses. Given the emergence of arboviral infectious diseases, their emergence and spread in Croatia are expected in the future as well. The currently established monitoring system encourages intensive professional and scientific cooperation of experts from various disciplines across Croatia and represents an adequate response to current challenges. The continuation of its improvement and alignment with the new challenges, in accordance to the generally accepted „One Health” initiative, is the only guarantee for the preservation of human and animal health<sup>[13]</sup>.

In Croatia, the Croatian Ministry of Health Reference Center for Diagnosis and Surveillance of Viral Zoonoses was established in 2016 at CIPH. Active surveillance of arboviruses in mosquitoes is conducted as a part of multidisciplinary „One Health” initiative (integrated monitoring of humans, mosquitoes, wild birds, horses and poultry). In addition to detection of acute infections, seroprevalence studies as well as detection of viruses in vectors are continuously conducted to determine the circulation of arboviruses.

According to the principles of health care and the powers granted by the state, CIPH monitors and analyses the epidemiological situation in Croatia. CIPH is responsible for continuous collection of complete data on the presence of mosquito species in Croatia, and for the development of vector and vector breeding sites maps as well as development of a unique national database necessary for risk assessment for vector borne diseases. For assessing the risks of emerging arbovirus infections, in 2016 CIPH established a national system for monitoring invasive mosquito species where collected data could contribute to earlier detection of invasive mosquito species, enabling timely and adequate planning for controlling the invasive mosquitoes.

The important outcomes of the national monitoring indicate an improvement to the implementation of mosquito control measures in Croatia as well as to preparedness and response for vector-borne diseases in the country and the EU. By submitting collected data to the VectorNet, CIPH contributes to collecting and compiling data on the distribution and surveillance of mosquito species. This information can be used to produce comprehensive online maps, providing the stakeholders and general public with most up-to-date information on mosquito distribution, and can also support relevant ECDC risk assessment on the diseases associated with mosquitoes.

VectorNet database is a common database on the presence and distribution of vectors and pathogens in vectors in Europe and the Mediterranean basin, through developing a network of experts and organisations from the medical, public health and veterinary domains. It is maintained as joint initiative of the European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA), which support the collection of data on vectors and pathogens in vectors, related to both animal and human health<sup>[19]</sup>.

During the implementation of the national monitoring, eggs of another invasive mosquito species, *Aedes (Finlaya) japonicus* (Theobald, 1901) originating from East Asia, were sampled from the traps. Since the first recorded finding in France in 2000, it was also recorded in Belgium, Switzerland, Germany and Slovenia. Although it is not of public health concern, determining the presence and distribution of this species has shown the efficiency of monitoring in the scope of early detection of new species through the network of county public health institutions surveillance. Although still not present in Croatia, the greatest public health concern represents the *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) due to its high vector potential for carrying ZIKV<sup>[21, 22]</sup>.

According to the Act on the Protection of Population from Infectious Diseases, timely planning and implementation of preventive disinfection, disinsection and deratization (DDD) measures at the local community level is important. In order to hinder and limit mosquito spreading, as well as reduce the risk of disease outbreaks, continued supervision is necessary, with ensuring the implementation of disinsection measures and, most importantly, educating citizens on responsible behaviour that prevents creating conditions for mosquito growth and reproduction. Guidelines for taking additional mosquito disinsection measures can be developed from the collected data on the invasive mosquito distribution<sup>[23, 24]</sup>.

Although monitoring and mosquito control in Croatia are legally regulated, some shortcomings in the practice are evident, such as insufficient number of counties implementing disinsection measures according to legislative and professional practice. Therefore, in the case of the emergence of neuroinvasive diseases caused by the WNV, a detailed analysis of the mandatory reports on the implementation of DDD measures in Croatia in 2015, revealed that only several counties conducted mosquito monitoring and obligatory preventive measures in accordance with professional practice, i.e. only with larvicidal treatment combined with, as appropriate, adulticidal treatment of mosquitoes.

The information flow on occurrence and zoonoses cases between the veterinary and human medicine sectors in Croatia is also regulated by law. Even though the obligations of veterinary inspectors in informing human health authorities of zoonosis cases among animals are prescribed by the Act on the Protection of Population from Infectious Diseases and the Ordinance on the Manner of Tracking, Reporting and Reporting on Animal Diseases, the cases are often not reported to the public health institutes<sup>[25, 26]</sup>. Hence, it is necessary to initiate the procedure with the intent to implement the prescribed provisions of the Law without additional legal changes. In addition to the diseases listed in the Act and according to the CIPH proposal, depending on the epidemiological situation, it is necessary to proceed with notifications in cases of other illnesses.

Apart from vector control measures, health and safety measures for the protection and control of population, goods and traffic travelling to countries with endemic diseases, or coming to Croatia from areas where such diseases are significant, are conducted according to International Health Regulations (IHR, 2005) and the Croatian Act on the Protection of Population from Infectious Diseases<sup>[25, 27]</sup>.

## Conclusions

The need to continue national monitoring and control of mosquitoes as carriers of arboviral infections is obvious. Because of the growing trend in the occurrence of vector infectious diseases arises the need for a systematic epidemiological-virological-entomological analysis of arbovirus infection cases in Croatia with the aim to protect the health of the population.

Monitoring mosquitoes on a national level provides necessary information for the development of appropriate and timely responses, and strengthening of entomological and laboratory capacities for the analysis of the presence of mosquito viruses, which will strengthen the system in order to prevent new disease cases.

The implementation of mosquito monitoring at the national level provides a successful framework that, with networking of experts from different fields and a multidisciplinary approach, can undoubtedly help to further improve the system in Croatia with the aim of an effective monitoring program.

Establishing national mosquito surveillance for monitoring the presence and abundance of mosquitoes will strengthen the ability for early detection of the new species presence as well as cooperation and integration at the European level, thus contributing to the European surveillance network.

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