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# SEARCHING FOR SOLUTIONS IN AQUACULTURE: AQUAPONICS

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## Abstract

Aquaponic production combines intensive production with waste recycling and water conservation. Aquaponic join recirculating aquaculture with hydroponics to use nutrient waste from aquaculture as an input to plant growth. Traditional aquaculture systems treat or dispose nutrient-rich wastewater. In aquaponics, the waste products from the fish are converted by a bio-filter into soluble nutrients which are absorbed by the plants, and allow "clean" water to be returned back to the fish. Thus, it produces valuable fish protein with a minimal pollution of fresh water resources, while at the same time producing horticultural crops. Fish in aquaponic production systems can be raised in ponds, tanks, or other containers. Plants are grown separately in hydroponic tanks, submerged in water but suspended in gravel, sand, perlite, or porous plastic films, as well as on floating rafts. Systems vary greatly in design and construction, but most perform the following key functions: finfish and plant production, removal of suspended solids, and bacterial nitrification. This review discusses applications, effects and perspective of aquaponics.

**Key Words:** fish, aquaponics, aquaculture

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# U POTRAZI ZA NAJBOLJIM REŠENJEM: AKVAPONIKA

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## Kratak sadržaj

Akvaponika kombinuje intenzivnu proizvodnju sa reciklažom otpadnih materija i očuvanjem vode, i povezuje akvakulturu sa hidroponikom u cilju korišćenja hranljivih materija iz akvakulture za rast biljaka. U tradicionalnoj akvakulturi, otpadna voda bogata hranljivim materijama se ne koristi. U akvaponici, produkti nastali prilikom gajenja riba se pomoću biofiltera konvertuju u rastvorljive materije koje biljke apsorbuju, a "čista" voda se vraća ponovo u proizvodnju. Na ovaj način se omogućava proizvodnja visoko vrednih animalnih proteina, uz minimalno zagađenje vode, dok se istovremeno dobijaju proizvodi hortikulture. Postoji veliki broj sistema za gajenje, različitog dizajna i konstrukcije, ali se svi baziraju na zadovoljavanju ključnih funkcija: proizvodnji riba i biljaka, uklanjanju rastvorenih materija i bakterijskoj nitrifikaciji. U radu su prikazani primena, efekti i perspektive akvaponike.

**Ključne reči**: Akvaponika, akvakultura, ribe

## INTRODUCTION

Aquaponics has been considered as a sustainable agriculture system that amalgamates aquaculture and hydroponics in an enclosed symbiotic environment (Nelson, 2008). The word 'aquaponics' is derived from a combination of 'aquaculture' and 'hydroponics', and refers to the integration of hydroponic plant/vegetable production with aquaculture. It is a bio-integrated system linking recirculating aquaculture with hydroponic production of plants such as vegetables, culinary or medicinal herbs. Aquaponics may provide an effective and efficient means to provide both animal protein (fish) and mineral and vitamin sources (fresh vegetables) to populations where water/and or fertilizer resources are limited with a minimum of environmental pollution.

The basic principals of aquaponics is that fish are fed "waste plant and animal material", which they convert into protein. The waste material from the fish is then used by plants as the nutrient source, and the water is then recirculated back to the fish tank. An essential component of this is a biofilter (between the fish and the plants) which essentially comprises bacteria which converts the waste products from the fish into soluble nutrients for the plants. An absolutely critical component of this is the conversion of urea (excreted by the fish) into nitrite, and then nitrate because high levels of urea in the water are toxic to fish. The solid waste (fish faeces and unconsumed food) is usually filtered off and converted into soluble nutrients in a separate bypass.

**Design**

Aquaponic systems are usually designed as an enclosed recirculating system, but a few systems can be open, depending upon environmental factors. Fish or other aquatic organisms are reared in tanks and excrete nutrient-rich waste or effluents into the water. Metabolic byproducts excreted by fish, unionized ammonia NH3-N, ionized ammonia NH4+-N, or combined equal Total Ammonia Nitrogen (TAN) are oxidized and broken down into nitrite (NO2 --N) by nitrifying bacteria of the genera Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosovibrio. Genera that oxidize nitrite to nitrate (NO3--N) include Nitrobacter, Nitrococcus, Nitrospira, and Nitrospina (Timmons and Ebeling, 2007). These nitrifying bacteria are also known to be light sensitive (Yoshioka and Saijo, 1984). Mineralization also occurs, releasing essential inorganic nutrients into the water for plant uptake (Timmons and Ebeling 2007). These dissolved nutrients accumulate and reach concentrations equal to hydroponic nutrient solutions (Timmons and Ebeling 2007). The water is continuously circulated to hydroponically grown crops that absorb non-toxic nutrients from the water to fulfill growth requirements. The water is then circulated back to the rearing tanks where the process starts again.

There are multiple aquaponic system designs that have been analyzed and utilized for crop production. Depending upon the system scale there are five main components to an aquaponic system: rearing tank, solids removal, biofilter, hydroponic subsystem, and sump (Rakocy and Hargreaves, 1993). Some systems are able to eliminate one or two of the components, and scale and primary production focus are the key factors determining the system design. Some aquaponic systems are able to efficiently operate with the use of hydroponic subsystems acting as a biofilter. This is possible with the aid of media such as hydroton, pea gravel, and expanded shale (Rakocy 1984; McMurtry et al. 1990). Floating raft hydroponics also known as DWC, which utilize polystyrene sheets and net pots for plant support, may also provide adequate biofiltration provided the hydroponic subsystem is large enough (Rakocy 1995). When utilizing media within hydroponic subsystems, care must be taken to prevent an overload of suspended solids; therefore, media filled subsystems are not ideal for commercial scale production (Timmons and Ebeling 2007).

One of the most important components of an aquaponic system is the hydroponic subsystem: media filled, NFT, and DWC (Lennard and Leonard 2006). A media filled hydroponic subsystem contains a grow bed filled with a soilless medium for plant support.

Popular soilless media include hydroton (expanded clay pebbles), gravel, sand, and perlite. The NFT system consists of troughs that expose suspended plant roots (net cup) to a thin film of water. DWC is similar to the media filled subsystem but instead of using media in the hydroponic bed, a floating raft (polystyrene sheets and net cup) supports the plants.

Currently there are two main irrigation methods for hydroponic subsystems, flood and drain or continuous flow. Flood and drain system uses a siphon to periodically drain water when it reaches a specified level. A continuous flow system allows water to constantly run throughout the system (Rakocy et al. 2006). Lennard and Leonard (2006) found that hydroponic subsystem design and water flow have a significant effect on Green oak lettuce (Lactuca sativa) yield where media>DWC>NFT; NFT systems were 20% less efficient in nitrate removal. Lastly, producers should realize that differing aquaponic or hydroponic methods (system designs) do not alter the genotypic characteristics of plants. Production will not surpass genetic limitations regardless of growing techniques, and plants will reach peak production when optimum requirements are met (nutrient assimilation, light, temperature, etc.).

**Fish**

There is no real limitation on the types of fish which can be used. Today it is common to grow Nile tilapia (*Oreochromis niloticus*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), and various carp species (*Cyprinus sp*.), in aquaponic systems. Tilapia appear to be one of the most popular species of fish reared in aquaponic systems, because the warm temperatures for optimal growth of tilapia are also needed for the growth of plants (Rakocy and McGinty, 1989).

Other species of fish that are reared in aquaponic systems in include largemouth bass (*Micropterus salmoides*), sturgeon (*Acipenser spp*.), baramundi (*Lates calcarifer*), sunfish (Family *Centrarchidae*), bream (*Abramis brama*), pacu (Family Characidae), red claw lobster or crayfish, and ornamental fish such as angelfish (*Pterophyllum scalare*), guppies (*Poecilia reticulata*), tetras (Family Chiracidae), gouramis (Family Belontiidae), swordfish (Family Xiphiidae), mollies (Family Poeciliidae).

**Plants**

Common plants that do well in aquaponic systems include various lettuce

(Lactuca spp.), tomato (Solanum spp.), spinach, and herb species including sweet basil (*Ocimum basilicum*), mint, watercress, chives, and most common house plants. Species of plants that have higher nutritional demands and will do well only in heavily stocked, well established aquaponic systems include tomatoes, peppers, cucumbers, beans, peas, and squash, among others (Rakocy, 1999).

Many of the fruit vegetables (tomato, pepper, cucumber, melon, etc) appear to require higher levels of nutrients in hydroponics, than the leafy vegetables. Nutrient wastes from tanks are used to fertilize production beds via the water. The roots of plants and associated rhizosphere bacteria remove nutrients from the water. These nutrients, generated from the feces of fish, algae and decomposing feed, are contaminants that could otherwise increase to toxic levels in the tanks. Instead they act as liquid fertilizer for hydroponically grown plants. In turn, the hydroponic beds function as biofilters, and the water can be recirculated to the tanks. Bacteria in the gravel and associated with the roots of the plants have a critical role to play in the cycling of nutrients; without these organisms, the system would stop functioning (Diver, 2006).

Aquaponic plants are subject to many of the same pests and diseases that affect field crops, although they seem to be less susceptible to attack from soilborne pests and diseases. Because plants may absorb and concentrate therapeutic agents used to treat parasites and infectious diseases of fish, these products cannot be used in aquaponic systems.Even the common practice of adding salt to treat parasitic diseases of fish or to reduce nitrate toxicity would be deadly to plants. Instead, non-chemical methods are used, ie., biological control (resistant cultivars, predators, antagonistic organisms), barriers, traps, manipulation of he environment, etc.). It also seems that plants in aquaponic systems may be more resistant to diseases that affect those in hydroponic systems. This resistance may be due to the presence of some organic matter in the water, creating a stable, ecologically balanced growing environment with a wide diversity of microorganisms, some of which are antagonistic to pathogens that affect the roots of plants (Rakocy, 1999).

## CONCLUSIONS

Aquaponic system is advantageous compared to other agriculture production systems, and has become very popular today (Rakocy et al. 2006). Since aquaponic systems are designed as enclosed recirculating systems, their agricultural waste and environmental footprints decrease, compared to conventional agriculture practices. Furthermore, utilization of plants as a secondary crop reduces the pollution load (waste concentration) through nutrient uptake and assimilation (Timmons and Ebeling 2007). Nitrate accumulation has been shown to be reduced by 97% within aquaponic systems compared to regular recirculating aquaculture systems (RAS) (Lennard 2006). Since water within systems is recirculated, the quantity of water needed to run the system is minute compared to most fish and crop production systems. On average, 98% of the water in aquaponic systems is recycled for the duration of operation (Al-Hafedh et al. 2008). The periodical input of water is only necessary when too much water has evaporated from the system. Aquaponic systems decrease the amount of space needed to produce two crops at once. This allows plants and fish to be raised together within a relatively small environment.

Aquaponics can range from an in-home counter top system to large scale commercial systems. Additionally aquaponics on average utilizes less than 1% of land compared to conventional agriculture systems. Along with space, aquaponic systems use fewer resources than average crop and fish production systems due to symbiotic relationships (Treadwell et al. 2010). For example, aquaponics utilizes 90-99% less water than conventional agriculture systems. Also, carbon dioxide ($CO_2$) from fish rearing tanks can also be used to increase crop production within an indoor facility (Timmons and Ebeling, 2007). Furthermore, aquaponic systems can be deployed in various environments allowing for year round crop production, and potentially a closer farmer-to-consumer interaction. Lastly, successful aquaponic systems utilize secondary crops that are of economic importance or beneficial to the aquatic organisms being produced (Timmons and Ebeling, 2007).

As with all food production systems, there are a few disadvantages with aquaponic systems. First, the ratio of hydroponic growing area compared to fish rearing surface area is relatively large. Ratios have been used ranging from 1:1 to 10:1, which are dependent upon the scale of the system, primary species of focus, and space. Another disadvantage includes the labor involved with plant management. The majority of aquaculturists do not have horticulture experience or knowledge, so additional personnel is often needed. Furthermore, due to the close relationship between fish and plants within an aquaponic system, poor management practices can easily affect the sensitive system. Pesticides cannot be utilized within systems and thus, biological control or natural methods must be used to eliminate plant pests (Timmons and Ebeling 2007). When entering into a competitive market, aquaponic producers should evaluate competitors and their species of production. It has been stated that hydroponics can produce heads of lettuce cheaper than what aquaponic systems can produce (Ako and Baker 2009). Lastly, materials utilized for aquaponic production (hydroton, fish feed, etc) are not considered sustainable. For example, hydroton (clay) is mined from the earth, and fish feed may come from wild caught fisheries or commodity crops. These materials utilize nonrenewable resources for production and may also contribute to environmental pollutants.

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# DEVELOPMENT OF A MODEL FOR SURVEILLANCE AND CONTROL OF FLAVIVIRUSES IN HUMAN POPULATION

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## Abstract

The aim of this study is development of a model for monitoring and control of vector-transmitted diseases, which manifest increasing tendency during the last few decades. The main infection reservoirs are domestic and wild animals as well as various birds, and the disease is transmitted to humans indirectly - by vectors. Recently detected epidemics of Dengue virus, Chikungunya and West Nile virus in countries where these diseases are not common strongly indicate the expansion of infections transmitted by mosquitoes and other vectors, and are the consequence of climatic changes, international trade and travelling. Currently, there are neither vaccines nor specific antiviral therapy for these infections, while the efforts put on vector control did not halt the rapid increase and global spread of the disease. Serological studies and molecular investigation on humans, mosquitoes, horses and birds have suggested the activity of flaviviruses in Serbia. The obtained information on flavivirus infections in our region are of use in modelling the control and vector monitoring and the prevention of these infections in humans.

**Keywords:** flavivirus infections, diagnosis, prevention

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**IZRADA PREDLOGA MODELA ZA PRAĆENJE I KONTROLU FLAVIVIRUSA U HUMANOJ POPULACIJI**

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## Kratak sadržaj

Cilj rada je izrada predloga modela za praćenje i kontrolu transmisivnih vektorskih bolesti koje su poslednjih nekoliko decenija u porastu. Rezervoari su divlje i domaće životinje i raznovrsne ptice, a na čoveka se prenose posredno-vektorima. Nedavno uočene epidemije Denga virusom, Čikungunja i West Nile virusom u zemljama u kojima ove bolesti nisu uobičajene, potvrda su sve većeg širenja infekcija komarcima i drugim vektorima, a posledica su klimatskih promena u okruženju, rasprostranjenom trgovinom i putovanjima. Trenutno ne postoje vakcine niti specifična antivirusna terapija za ove infekcije, a napori da se konrolišu vektori nisu sprečili brzi porast i globalno širenje zaraze. Serološke studije i molekularna ispitivanja sprovedena kod ljudi, komaraca, konja i ptica ukazale su na aktivnost flavivirusa u Srbiji. Dobijene informacije o kretanju flavivirusnih infekcija u našoj sredini od koristi su u modeliranju kontrole i praćenju vektora i preveniranju infekcija kod čoveka.

**Ključne reči:** flavivirusne infekcije, dijagnoza, prevencija

## INTRODUCTION

The aim of this paper is development of a model proposed for monitoring and control of vector-transmitted diseases, which demonstrated increased incidence in the last decades. The main infection reservoirs are domestic and wild animals as well as various birds, and the disease is transmitted to humans indirectly - by vectors. Recently detected epidemics of Dengue virus, Chikungunya and West Nile virus in countries where these diseases were not present before, are the signal of a potential threat of the spread of infections transmitted by mosquitoes and other vectors, and are in close connection with climatic changes in the area, international trade and travelling.

In the last decades, mosquito-transmitted flavivirus infections have gained substantial public health importance because of wide geographical distribution, epidemic emergence outside of the endemic areas and difficult forms of diseases that may cause. *Flaviviridae* family encompasses 53 virus species, among which Dengue and West Nile virus, transmitted by mosquitoes, and Tick-borne encephalitis virus (TBEV), transmitted by ticks, are of particular importance.

Dengue (DEN) is a viral infection caused by an ARBO virus from *Flaviviridae* family. Transmission to humans occurs by a mosquito from the genus *Aedes,* mostly *Aedes aegypti*, and less frequently by *Aedes albopictus.* Dengue has 4 serotypes: 1 - 4, which produce cross-immunity. It causes an illness that ranges from mild to very severe that is manifested by a shock syndrome and hemorrhagic fever with lethal outcome. Currently, there are neither vaccines nor specific antiviral therapy. Dengue is endemic in at least 100 countries all over the world (Asia, Africa, American continent, the Caribbean, Pacific). According to the World Health Organization (WHO), 50-100 millions people in the world are infected by Dengue virus annually. (http://www.who.int/csr/resources/publications/dengue/Denguepublication/en/) Efforts aimed at vector control did not prevent rapid increase of this dangerous disease and its spread throughout the world. Risk factors for the increase of Dengue infection incidence are migration of population from undeveloped endemic countries into developed countries, increase in travelling and tourists' number, urbanization that favours the spread of urban mosquitoes such as *Aedes albopictus*. A danger of Dengue fever is now evident even in Europe, since first autochthonous cases were registered in France and Croatia in 2010 (Gjenero et al., 2011; La Ruche et al., 2010). Serological and entomological investigation on Pelješac peninsula in Croatia, where first autochthonous cases of Dengue were registered, proved the presence of specific Dengue antibodies in healthy residents and the domination of *Aeds albopictus* species in analyzed mosquitoes (Gjenero et al., 2011).

West Nile virus fever is a zoonosis caused by an ARBO virus from *Flaviviridae* family and Japanese encephalitis serogroup. Virus reservoirs in natural environment include hundreds of bird species belonging to 20 families in the order of *Passeriformes,* while vectors involve about 60 mosquito species, mostly from the genus *Culex*. Other vertebrates may be infected with this virus; however, their role in the transmission has not yet been elucidated (Hrnjakovic-Cvetkovic et al., 2014). Under favourable environmental conditions, viral turnaround cycle is intensified and may lead to infection in horses and humans, which are accidental hosts unsuitable for infecting mosquitoes. In most cases, the infection is asymptomatic or proceeds as a mild condition, but some 1% of infected individuals develop a neurological disease - meningitis, encephalitis and myelitis that rapidly progress to severe conditions with fatal outcomes, especially in the elderly (Mostashari et al., 2001). Sporadic cases and epidemics were recorded in Africa, Middle East, Europe and Asia. Since 1999, when first human cases were recorded in New York, infection has rapidly spread across USA and has become endemic in North America. This spread has instigated intensive research in the area of diagnostics and production of human vaccine. Vaccines for horses have already been developed, whereas those for human use have not yet been registered. Serological studies and molecular investigations in humans, mosquitoes, horses and birds suggested the activity of the virus in Serbia (Petric et al., 2012; Lupulovic et al., 2011; Petrovic et al., 2012). Since 2012, when first epidemics was registered in Serbia (Popović et al., 2012), human cases have been reported every year in the period from August to October. Until October 20, 2014, the Institute of Public Health of Serbia "Slobodan Jovanović Batut" recorded 56 laboratory-confirmed cases of human neuroinvasive diseases caused by West Nile Virus, among which 9 cases of elderly patients with chronic conditions resulted in a lethal outcome (Institute of Public Health of Serbia, 2014).

In the group of mammalian tick viruses, tick-borne meningo-encephalitis virus (TBEV) has been ranked into the family Flaviviridae, genus Flavivirus, with three subtypes: Far East, Siberian and European subtype. Endemic focal points of this viral infection can be found from Western Europe to China and Japan. Those in Europe are primarily located in Austria, Slovenia, Switzerland, *Czech Republic* and some countries of ex-Soviet Union. In the last 30 years in Europe, new focal points and a 400% increase in new cases in endemic areas were registered (ECDC, 2014). Increase in incidence in *Czech Republic* was explained exclusively by a change in climatic conditions (Daniel et al., 2010). Principal vector species in Europe is tick *Ixodes ricinus.* In ticks, virus is transmitted by transovarial route, and all stages from larva, via pupa to adult, may be infected and transmit the infection to vertebrates and humans by bite while feeding. The reservoirs include small rodents, mostly members of the species *Apodemus,* but also some wild and domestic animals, on which adult ticks feed. The disease progress may manifest biphasic course. The European subtype causes milder forms of disease and biphasic course can be noticed in 20-30% of infected population (ECDC, 2014). After the first phase of illness, characterized by unspecific symptoms - fever, fatigue, malaise, muscle pain, headache – the first symptoms of meningitis, meningoencephalitis, poliomyelitis, and polyradiculoneuropathy with Guillain- Barré like paralysis may arise. More difficult forms of the disease frequently affect elderly patients. First serological studies in Serbia pointed out that the activity of the virus in the territory of Vojvodina, where the seroprevalence of IgG antibody is 7.9% in the residents of South Backa region (Vojvodina, Serbia), while no TBEV-positive serum samples were detected in the region of Nis (Hrnjakovic-Cvjetkovic et al., 2014).

Usutu virus is an ARBO virus from the family Flaviviridae, genus *Flavivirus,* Japanese encephalitis serogroup. It is maintained (like the West Nile virus) throughout the transmission cycle between wild birds and ornithophilic mosquitoes, mostly from the genus *Culex.* While in the African birds infection is asymptomatic, the virus is highly virulent for European birds, and causes lethal encephalitis, necrotic hepatitis and degenerative changes in heart and neural tissue (Bakonyi et al., 2007). USUTU virus infection has been established in many bird species in Italy, Austria, Germany, Switzerland, Spain, Hungary, *Czech Republic* and Poland (Arbeitskreis, 2014). In humans, the virus can cause fever and rash. In immunocompromised patients, pronounced neurological manifestations and a fulminant hepatitis may arise (Cavrini et al., 2009). Medical importance of this ARBO virus in immunocompetent individuals remains to be evaluated. Serological investigation done by ELISA IgG test in humans showed the presence of anti-USUTU virus antibodies in 4.5% samples out of 88 healthy subjects of South Backa region, Vojvodina, Serbia (Hrnjakovic-Cvjetkovic et al., 2014).

## DIAGNOSTIC METHODS

A clinician's doubt whether there is a vector-transmitted infection, requires laboratory confirmation. The laboratory methods encompass the direct ones determining the virus isolation in cell culture or amplification of the virus genome, and indirect ones, that is, serological methods that detect the presence of specific antibodies. Flavivirus isolation may be performed from blood or cerebrospinal fluid on different cell lines, but only at the early stage of infection, when a patient is in viraemic phase characterized by high virus titre in the blood. In West Nile virus infection, viraemia is typically present during the first four days of illness (Dauphin and Zientara, 2007). Real-time reverse transcription-polymerase chain reaction (RT-qPCR) is a method of choice for the determination of flaviviruses in human plasma, serum and cerebrospinal fluid.

Serological diagnosis for all flavivirus infections that produce encephalitis in humans has a similar approach. In the last few years, specific ELISA tests have been developed for the detection of IgM and IgG antibodies against certain flaviviruses. IgM antibodies against West Nile Virus emerge 2-8 days after the onset of illness, similarly to USUTU virus infection. However, a weak point of the ELISA tests is that they cannot completely distinguish between the WNV antibodies and antibodies against other flaviviruses, especially those of the same antigenic complex. Antibodies can persist in the serum for several months after infection. IgM antibodies in WNV infection may be present in serum for as long as a year after the infection (Roehrig et al., 2003). Establishment of an accurate serological diagnosis is difficult, especially in the areas where more than one flaviviruses are circulating, so cross-reactions are possible, as is the case with WNV and TBEV in several European countries. The problem of cross-reaction exists between anti-WNV and anti-USUTU virus antibodies, where expected cross-reaction is higher in IgG than in IgM antibodies. For that reason, it is essential to implement diagnosis of USUTU viral infection and tests that are not yet commercially available for the determination of specific IgM antibodies. By the tests available so far (for IgG antibodies only, for now), both acute and convalescent sera should be analyzed for the presence of USUTU virus, thus monitoring seroconversion of IgG antibodies. Cross-reactions could be resolved by a parallel testing on various flaviviruses using plaque reduction neutralization tests according to the specially defined protocols (Beaty et al., 1995). These tests are more specific as compared to ELISA tests, but they can be performed only in Biosafety level 3 facilities (BSL III). The fact that USUTU virus infection in humans can cause severe neurological syndromes imposes the strong and urgent need for new, accessible and rapid molecular detection methods.

Serological tests are essential for the determination of infection in cases when the viraemic phase is completed. Highly specific tests are required, particularly in countries where the circulation of both USUTU virus and WNV is present (Austria, Belarus, Bulgaria, Czech Republic, Croatia, France, Hungary, Italy, Moldova, Portugal, Romania, Russia, Serbia, Slovakia, Spain and Ukraine). New methods are designed for the identification and differentiation of USUTU virus from other ARBO viruses, particularly members of the Japanese encephalitis serogroup circulating in Europe. Virus monitoring by molecular methods is better with regard to the specificity since there are no cross reactions, but is limited by short viraemic phase. Serology enables the detection of antibodies, which last longer, nonetheless with lower specificity for now, using the inhibition of hem-agglutination or ELISA tests. Each positive serum should be confirmed by plaque reduction neutralization test, a methods that is complex, expansive, time-consuming and accessible only to Biosafety level 3 facilities.

## CONCLUSION

Ever more frequent reports on the circulation of mosquitoes and ticks transmitting the diseases characteristic for tropical regions (WNV, USUTU, Dengue, Chikungunya etc.) in the European Union advocate the necessity of a more intense and careful preparedness and control of these diseases by new diagnostic procedures and more specific serological tests even in our country. The surveillance program of flaviviruses in our country exists for WNV and encompasses human, veterinarian and entomological monitoring with the aim of early detection of infections in human population, applying serological tests and detection of a viral genome in blood, cerebrospinal fluid in any suspected case of acute meningoencephalitis. The surveillance in animals has been done by passive and active surveillance of horses, chickens and non-migrating wild birds, and entomological surveillance by weekly and monthly trapping of mosquitoes depending on the determined activity in birds, humans and horses, and testing on specific antibody or virus presence. The obtained information about the fluctuations of flavivirus infections in our environment are valuable for modelling the control and surveillance of vectors. It is expected that informative campaigns will lead to the increase in personal protection, and adequate screening tests would prevent infections in the blood, tissue and organ donors.

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