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WEST NILE VIRUS SURVEILLANCE PROGRAM IN SERBIA

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Abstract

Serological and virological examination of the presence of human and animal infection caused by West Nile Virus (WNV) as well as the presence of the virus in vectors, which has been conducted during the past few years, confirmed an active virus circulation in the territory of the Republic of Serbia. Based on the obtained results and anticipated intense circulation of WNV, which poses substantial risks for both public and animal health in Serbia, and having in mind its crucial role in the protection of public health, Veterinary Directorate of the Ministry of Agriculture and Environmental Protection infront the Veterinary Service launched and funded the national WNV monitoring program starting from April 2014. The Program encompassed the entire territory of the Republic of Serbia and was conducted by scientific and specialized veterinary institutes and field veterinary service in close collaboration with qualified entomologists and ornithologists. The principal objective of the monitoring – surveillance program is early detection of WNV in monitored regions, timely reporting of the virus presence and activation of human health service institutions and local authorities aimed at establishing the control measures - eradication of mosquitoes, informing the local community and taking all relevant preventive measures for human health protection. The surveillance program of the WNV occurrence and spread is based on direct and indirect surveillance of WNV in natural environment. Indirect surveillance encompasses serological testing of seronegative sentinel horses and poultry for the presence of WNV infection, and it is performing continuously and periodically during the most

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intensive mosquito activity (May – September). The number of sentinel animals that should be tested was defined at the district level, according to the rate of anticipated risk of WNV infection. Direct surveillance was performed through periodical and continuous testing of pooled mosquitoes samples collected at two-week intervals during peak mosquito season (May – September) and samples of wild birds (tissues of dead birds and throat swabs of captured live susceptible bird species). The number of samples was stipulated according to the anticipated risk rate in particular regions.

Key words: West Nile virus, surveillance program, Serbia

PROGRAM NADZORA PRISUSTVA VIRUSA ZAPADNOG NILA U SRBIJI

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Kratak sadržaj

Sprovedena serološka i virusološka ispitivanja prisustva infekcije uzrokovane virusom Zapadnog Nila (WNV) kod različitih vrsta životinja i ljudi, kao i vektora virusa u proteklih nekoliko godina su potvrdila aktivnu cirkulaciju ovog virusa na području Republike Srbije. Na osnovu ovih rezultata i realne predpostavke o nastavku intenzivne cirkulacije WNV i opasnosti za, pre svega, javno zdravlje ali i zdravlje životinja na području zemlje, veterinarska služba na čelu sa Upravom za veterinu Ministarstva poljoprivrede i zaštite životne sredine je prepoznala svoju značajnu ulogu u zaštiti javnog zdravlja i od aprila 2014. godine je pokrenula i finansirala nacionalni program monitoringa WNV. Pomenuti program nadzora na celokupnom području Republike Srbije sprovode veterinarski naučni i specijalistički instituti i nadležne veterinarske stanice u saradnji sa entomolozima i ornitolozima. Osnovni cilj programa monitoringa je rana detekcija prisustva WNV na nekom području i pravovremeno alarmiranje humane zdravstvene službe i lokalnih samouprava radi kontrole – suzbijanja koma-

raca, informisanja stanovništva i preduzimanja svih mogućih preventivnih mera zaštite ljudi. Program monitoringa – nadzora pojave i širenja WNV se zasniva na indirektnom i direktnom praćenju prisustva WNV u prirodi. Indirektno praćenje virusa se vrši serološkim testiranjem na WNV seronegativnih – sentinel konja i živine, koja se vrše kontinuirano i periodično u periodu najveće aktivnosti komaraca (maj – septembar). Broj sentinel životinja koje se prate je definisan na nivou svakoga okruga Republike Srbije i to u odnosu na visinu rizika od pojave infekcije WNV. Direktno praćenje prisustva WNV se vrši periodičnim i kontinuiranim ispitivanjima zbirnih uzoraka komaraca uzorkovanih svake dve nedelje u periodu njihove najveće aktivnosti (maj - septembar) i divljih ptica (tkiva uginulih i briseva živih prijemčivih vrsta divljih ptica) na prisustvo ovoga virusa. Broj uzoraka za ispitivanje je takođe određen po okruzima na osnovu visine rizika.

Ključne reči: virus Zapadnog Nila, program nadzora, Srbija

INTRODUCTION

West Nile virus (WNV) is a neurovirulent mosquito-borne *Flavivirus* with zoonotic potential, which is maintained in nature in an enzootic transmission cycle between avian hosts and ornithophilic mosquito vectors. The virus occasionally infects other vertebrates, including humans and horses, in which it may cause sporadic disease outbreaks that may result fatal. West Nile virus (WNV) was first isolated from a febrile woman in the West Nile district of Uganda in 1937 (Smithburn et al., 1940) and today is considered as the most widespread flavivirus in the world, endemic in Africa, Asia, Europe, Middle East, Australia and Americas (Trevejo and Eidson, 2008; Calistri et al., 2010; Weissenböck et al., 2010; Papa et al., 2011).

WNV infections have been described in a wide variety of vertebrates (Komar et al., 2003). The virus is maintained in an enzootic cycle between ornithophilic mosquitoes, mainly of the *Culex* genus (Hayes et al., 2005; Ziegler et al., 2012), but also *Aedes* and *Ochlerotatus* genus, and certain wild bird species (Savini et al., 2012; Ziegler et al., 2012). WNV was found in more than 150 species of wild and domestic birds (van der Meulen et al., 2005). Wild birds are important to public health because birds migrating across national and intercontinental borders and becoming a long-range virus vectors (Linke et al., 2007). Following infection, many bird species produce levels of viraemia that are sufficient for transmitting the virus to mosquitoes (Komar et al., 2003). Human and mammals, especially horses, are occasional, dead end hosts and play limited roles in the natural cycle because viraemia is generally too low to

infect mosquitoes (Dauphin et al., 2004; Valiakos et al., 2011), however severe neuroinvasive disease and occasionally with fatal outcomes can occur.

In Europe, until the 1990´s WNV had caused sporadic outbreaks with rare reports of encephalitis but its epidemiological behaviour changed when it re-emerged in Romania, Russia and the Mediterranean basin causing dozens of humans and horses deaths (Castillo-Olivares and Wood, 2004; Blitvich, 2008; Calistri et al., 2010). Also, only recently the strains of WNV lineage 2 were identified in Europe: in 2004 and 2005 in goshawks and birds of prey in Hungary, in 2007 in Volgograd, Russia, and in 2008 and 2009 in goshawks and a falcon in Austria (Bakonyi et al., 2006; Erdélyi et al., 2007; Platonov et al., 2008; Wodak et al., 2011). Since 2008, WNV has been heavily spreading throughout central and southeastern Europe, constituting a serious veterinary and public health problem for Europe (Barbic et al., 2012; Ziegler et al., 2012).

In Serbia, WNV situation was mostly unknown until 2009. Serological testing of horses sampled during 2009-2010 by ELISA based on WNV recombinant envelope E (rE) protein and PRNT showed for the first time in Serbia that 12% of 349 horses from northern part of country presented specific neutralizing WNV antibodies. Positive horses were found in 14 of the 28 municipalities studied, which are up to 200 km distant (Lupulović et al., 2011). In another study, presence of WNV specific antibodies was found in 28.6% (72) of 252 examined horse sera samples collected from 7 different stables and locations in Vojvodina province and Belgrade area, during 2007-2011. WNV seroprevalence ranged per stable from 13.3% up to 40% seropositive animals (Medić et al., 2014). In addition, just one year later, to asses WNV presence in the environment immediately after the human WNV outbreak in 2012, during November and December of 2012, presence of anti-WNV IgG antibodies were examined by commercial ELISA test in blood sera samples of 130 horses from 6 stables and 1 settlement in Vojvodina province, northern Serbia. Positive results were obtained in 49.23% (64/130) samples. Per stable, percent of seropositive animals was from 35% to 64% (Petrović et al., 2014). This prevalence (49.23%) obtained in horses during 2012 was much higher than that found in horses during 2009 and 2010 (12%), including the confirmed seroconversion in at least 8 horses tested negative in 2010, thus confirming an intensive WNV circulation in 2012 on the territory of Serbia (Petrovic et al., 2014). Similarly, 96 horses from 5 tested stables during 2012 were tested again during 2013 with the same methodology. High prevalence of 46.88% (ranged between stables from 23.53-75.0%) with new cases of seroconversion were detected also indicating an intensive WNV circulation in 2013 (unpublished data).

WNV circulation in Serbia was also confirmed in wild birds as virus natural host. In total, 92 blood sera and 81 pooled tissue samples were collected

from 133 dead and live captured wild resident and migratory birds (45 species within 27 families) from January until September 2012 in Vojvodina Province - northern part of Serbia. WNV antibodies were detected by ELISA and PRNT in 7.6% (7/92) blood sera and virus presence was confirmed in tissue of 8 out of 81 (9.87%) and blood of one bird. Most of the antibody or virus positive birds were strictly resident, suggesting endemic presence of WNV in Serbia (Petrovic et al., 2013). By phylogenetic analysis of genomic sequences, all WNV isolates were classified as a lineage 2 strains that clusters with the viruses responsible for the most recent human and animal outbreaks reported in neighbouring countries (Petrovic et al., 2013).

The first studies on the presence of WNV in mosquitoes as virus vectors date back to the period 2005-2010. A total of 56757 mosquitoes (841 pools of 50 individual insects) originating from 66 localities in 29 settlements in Vojvodina were examined. The presence of WNV genome was established in only three pooled-samples of mosquitoes collected during 2010 in the territory of Detelinara (part of the city of Novi Sad). The isolate was typed as lineage 2 WNV (Petric et al., 2012). This study was furher done during 2012 and 2013, when significantly increased prevalence of WNV in mosquitoes was established. Even more than 9% of the mosquito pools examined during 2012-2013, mainly of species *Culex pipiens* was tested positive for WNV presence (unpublished data).

The history of WNV infection among human population in Serbia is mostly unknown, and only scarce historical data exists. First serological investigation of WNV infection presence in human population in Serbia was conducted in 1972 and antibodies against WNV were found in 2.6% - 4.7% of human sera (Bordjoški et al., 1972). In another study, antibodies against WNV were detected, depending on location, in 1 to 8% of tested human sera in Serbia (Vesenjak-Hirjan et al., 1991). After a gap of many years, more recent serological examinations show presence of anti-WNV IgG antibodies in 18 out of 451 (3.99%) human collected from 2005 to 2010 in Vojvodina province with yearly rates varying between 1.97% and 6.04% (Petrić et al., 2012). Except this data, as to our knowledge, no clinical manifestation of disease was ever reported in Serbia until 2012. In August 2012, an outbreak of WNV infection in humans was reported for the first time ever in Serbia (EpiSouth Weekly Epi Bulletin - N°232 and N°240, 2012; ECDC, 2012), being the first time that WNV infections in the country have been associated with clinical symptoms. As of November 30, 2012, a total of 71 West Nile fever cases were reported, among which 42 were clinically and laboratory confirmed, and in 9 cases resulted fatal (lethality of 12.7%). All the cases were detected in central

and northern part of the country, 72% of them in the Beograd district (ECDC, 2012; Obrenovic et al., 2013; Popovic et al., 2013). This epidemic continued, and became even more severe during 2013. As of November 2nd, 2013, a total of 303 West Nile fever cases were reported, among which 202 were clinically and laboratory confirmed, and 103 were classified as probable cases. Infection in 35 cases resulted fatal (lethality of 11.6%). Almost all of the cases were also detected in central and northern part of the country (Institute of Public Health of Serbia, 2014).

The aforementioned serological and virological examinations confirmed active circulation and endemic presence of WNV in the territory of the Republic of Serbia. Based on the obtained results and anticipated intense circulation of WNV that poses substantial risks for both public and animal health in Serbia, and having in mind its crucial role in the protection of public health, Veterinary Directorate of the Ministry of Agriculture and Environmental Protection infront the Veterinary Service launched and funded the national WNV surveillance program starting from April 2014. The methodology of implementation and management of this surveillance program is presented in this article.

METHODOLOGY OF WNV SURVEILLANCE PROGRAM IN SERBIA

The surveillance program encompassed sentinel species (poultry and horses), mosquitoes (particularly species *Culex pipiens,* which were confirmed as most prevalent WNV vectors in our region) and wild bird species, which are natural virus reservoirs and populate the natural habitats in Serbia, either temporarily or permanently. The surveillance program was conducted throughout the year according to the provided guidelines. Active surveillance was performed by serological examination of sentinel poultry and horses and by testing of virus presence in samples of mosquito vectors (sampled by dry-ice baited traps in the period of most prominent vector activity using special traps), as well as in the samples of all collected dead wild birds belonging to the species susceptible to WNV (tested throughout the year). Passive surveillance encompassed serological (testing of paired serum samples) and virological examination of clinically ill horses manifesting signs of CNS dysfunction.

The active and passive surveillance encompassed all municipalities in the Republic of Serbia. The selection and distribution of sampling localities in each county-region is defined by epizootiological services of scientific and specialized veterinary institutes according to the assessment of the risk of exposure to WNV. By assessing the exposure risk, the following is taken into consideration:

1. already available results of serological examination of horses;
2. existence of areas suitable for mosquitos such as standing waters, rivers, water flows, canals etc.;
3. settlements with recorded human infections (according to the data obtained from the Institute of Public Health of Serbia – "Batut" and regional Institutes of Public Health in the relevant territory)

Based on the available data on the presence and circulation of WNV in the Republic of Serbia, the districts, i.e. Counties, were categorized according to risk of WNV infection outbreak (Table 1).

Table 1. Categorization of districts - Counties in the Republic of Serbia according to risk of WNV outbreak based on available results of laboratory examination of horses and vector mosquitoes, as well as of human cases

Regions – Counties with particularly high risk	**Regions – Counties with moderate risk**
North Bačka County	North Banat County
South Bačka County	West Bačka County
Middle Banat County	Šumadija County
South Banat County	Pomoravlje County
Srem County	Bor County
City of Belgrade	Zaječar County
Mačva County	Zlatibor County
Kolubara County	Moravica County
Podunavlje County	Raška County
Braničevo County	Rasina County
	Nišava County
	Toplica County
	Pirot County
	Jablanica County
	Pčinj County

ACTIVE SURVEILLANCE

1. Serological surveillance

Serological surveillance implicated sampling and examination of blood sera of sentinel horses and poultry for the presence of WNV specific antibodies (sentinel animals are individuals that have not been in contact with WNV, that is, do not possess specific antibodies against WNV in the blood). Serological examination is performed using ELISA technique. The testing is performed in authorized scientific and specialized veterinary institutes.

1.1. Serological surveillance of sentinel poultry

Serological testing of sentinel poultry encompassed blood samples of poultry kept in extensive breeding system (backyard poultry), where only poultry hatched during the year of testing shall be tested on anti-WNV antibody presence. The tested population should be mainly located in the suburban areas (predominantly rural settlements). In high-risk regions (10 Counties – Table 1), the sampling is performing in ten settlements with highest risk, i.e. five poultry blood samples were collected per one settlement, from at least one husbandry with extensive poultry keeping system (backyard poultry). The sampling and examination thereof is performing during the period May-September, i.e., in 6 sampling ocassions: one in May (by the end of the month), one in June, two in July, one in August (middle of the month) and one in September (until 15th September), meaning ones or twice monthly, depending on the risk of infection in the relevant period of the year. In regions/Counties with lower risk of WNV infection outbreak (15 Counties – Table 1), the sampling is performing in 6 high-risk settlements by collecting up to 5 blood sera per one settlement from at least one husbandry with extensive (backyard) keeping system. The sampling and examination thereof is performing during the period June-September, i.e., in 4 sampling ocassions: one in June, one in July, one in August (middle of the month) and one in September (until 15th September). Throughout entire surveillance period (from May/June until September), the obtaining of blood samples had to be done at the same locations for all sampling ocassions. The term "location" in this Program considered the area of selected settlements. Sampling plan is presented in Table 2.

1.2. Serological surveillance of sentinel horses

During the preparatory period of WNV surveillance in horses and with an aim of selecting appropriate sentinel animals, mandatory serological surveillance of horses was conducted from March to May 2014 to identify WNV-

seronegative animals, which are to be used as sentinel animals in the WNV surveillance program.

Serological testing of sentinel horses' blood sera implicated collecting of up to 50 samples from as many as possible locations (minimum 3) in each high-risk County (10 Counties – Tables 1 and 2) and up to 30 samples from as many as possible locations (minimum 3) in each lower-risk region (15 Counties – Tables 1 and 2). The collected samples are testing for the presence of anti-WNV antibodies by applying ELISA test. The sampling should be performed successively from the same sentinel animals, three times (first sampling – during June; second sampling – during July; third sampling – during August 2014).

2. *Virological surveillance*

Virological surveillance encompassed sampling and examination of organs and tissues of dead birds or throat swabs of captured live wild birds (susceptible species), as well as examination of pooled samples of vector mosquitoes (species *Culex pipiens*) for the presence of West Nile Virus – WNV. The virus presence was also tested in samples of brain and cerebrospinal fluids from dead horses with clinically manifested neurological dysfunction (passive surveillance). Virological testing is performing by molecular methods (*real-time RT-PCR* or *RT-PCR*) in the National reference laboratory for WNV in Specialized Veterinary Institute „Kraljevo" (VSI Kraljevo), as well as in virology laboratories of Scientific Veterinary Institute „Novi Sad" (NIV-NS) and Scientific Veterinary Institute of Serbia (NIVS).

2.1. Virological surveillance of wild birds

Dead wild birds found in the natural environment, particularly the resident species most susceptible to infection, e.g. *Corvidae* (magpie, crow, raven, rook. etc.), raptors (northern goshawk, falcon and eagle) and singing birds as well as birds died in rehabilitation centres, zoos or bird breeding farms (mostly raptors such as falcons, eagles, hawks…) were submitted to the aforementioned laboratories for testing for the presence of WNV. If dead birds were unavailable for laboratory examination, the sampling in high-risk regions (May – October) could be performed from captured live WNV-susceptible birds species by obtaining throat swabs or by hunting of certain bird population (*Corvidae*) for examination purposes (in cooperation with the hunting associations). Storage and transportation of samples to the authorized laboratory strongly requires maintenance of cold-chain conditions (refrigeration or freezing (swabs)). Samples obtained from dead birds (brain and parenchymatous

organs) and throat swabs are testing for the WNV presence using molecular methods (*real-time RT-PCR* or *RT-PCR*). Collection of dead-bird samples and testing od virus presence is performing throughout the year in high-risk regions (10 Counties – Tables 1 and 2) and in the period May-October in lower-risk regions (15 Counties – Tables 1 and 2)

2.2 Virological surveillance of mosquitoes – WNV vectors

Vector mosquitoes (*Culex pipiens*) are testing for the WNV presence by molecular methods (*real-time RT-PCR* or *RT-PCR*). The mosquitoes were examined as pooled sample (50-100 individual insects per pool) per one sampling point. Mosquitoes are collecting by dry-ice baited traps in the period of their most intensive activity (May-September) at the semi-urban and urban localities suitable for their survival and reproduction (standing waters, rivers, water flows, canals etc.) in the vicinity of susceptible animals (i.e., close to horse stables and poultry farms). The collection of mosquitoes should be performed at two-week intervals from 10 localities throughout the high-risk Counties (7 samplings, starting by end May, and then by mid and end of following months until mid September). In lower-risk Counties, the sampling should be performed monthly at 5 localities throughout the County (5 samplings, once a month, starting by end May, and then in the second half of the following months until mid September) (Tables 1 and 2). Native mosquito samples (without liquid) collected by dry-ice baited or other traps require rapid cooling (freezing) and immediate transportation to the laboratory (VSI Kraljevo, NIV-NS, or NIVS) for examination while still frozen. To the purpose of sampling and entomological examination, establishing of close cooperation with entomologists is highly recomended.

PASSIVE SURVEILLANCE

All horses with clinically manifested neurological dysfunction had to be subjected to testing for WNV infection in the framework of passive surveillance. The testing encompassed serological examination of paired samples of blood sera collected at 3-4 week intervals. The presence of WNV-specific antibodies is done in the corespondent scientific or specialized veterinary institute. In cases of lethal outcome in horses, samples of brain tissue and cerebrospinal fluid need to be submitted for laboratory examination for the presence of WNV (laboratories of VSI Kraljevo, NIVS or NIV-NS).

Table 2. WNV surveillance plan (sampling and testing)

	High-risk regions/Counties	**Lower-risk regions/Counties**
1. Testing of sentinel animals (domestic poultry and horses) aimed at early detection of WNV circulation		
Surveillance of sentinel poultry on rural households – poultry hatched in current year (backyard poultry)	Serological testing at the authorized institute in the period May-September from 10 settlements / County; 5 samples / settlement from et least one household according to described schedule. 6 samplings (1 in May; 1 in June; 2 in July; 1 in August – by middle; 1 in September (until 15 Sept)	Serological testing at the authorized institute in the period June-September from 6 settlements / County; 5 samples / settlement from et least one household according to described schedule. 4 samplings (1 in June; 1 in July; 1 in August – by middle; 1 in September (until 15 Sept)
Surveillance of sentinel horses	Serological testing of 50 sentinel horses in the authorized institute, sampling from minimum 3 localities per County. Sampling and blood testing of same horses to be performed three times (in three occasions) (June-July-August)	Serological testing of 30 sentinel horses in the authorized institute, sampling from min 3 localities per County. Sampling and blood testing of same horses to be performed three times (in three occasions) (June-July-August)

	High-risk regions/Counties	Lower-risk regions/Counties
2. Testing aimed at early detection of WNV in natural reservoirs and vectors		
Virus surveillance in wild birds	Application of *RT-PCR* or *real time RT-PCR* methodology for testing samples of dead susceptible bird species throughout the year, or up to 100 samples of purposely hunted birds or live captured susceptible bird species per County during the period May - October	*RT-PCR* or *real time RT-PCR* methodology for samples of up to 50 dead birds (WNV-susceptible species) per County during the period May - October
Virus surveillance in vectors - mosquitoes (*Culex pipiens*)	Collecting mosquitoes at 2-week intervals in the period May-September at 10 localities within the County and testing the virus presence by *RT-PCR* or *real time RT-PCR* methodology (7 samplings in the period from end May to the first half of September)	Collecting mosquitoes at monthly intervals in the period May-September at 5 localities per County and testing the virus presence by *RT-PCR* or *real time RT-PCR* methodology (5 samplings once a month in the period from second half May to the first half of September)

SAMPLING PROCEDURE, SAMPLE DISTRIBUTION AND REPORTING

According to the provisions of the Surveillance program, sampling of calculated amount of blood samples from sentinel horses and poultry from settlements, households and stables, as well as obtaining of basic epizootiological and anamnestic data is done by authorized veterinary service and epizootiolo-

gists in scientific or specialized veterinary institutes responsible for serological testing of blood samples of sentinel horses and poultry for the presence of WNV-specific antibodies.

Responsible epizootiologists of scientific and specialized veterinary institutes collected basic epizootiological and anamnestic data, as well as the samples of wild birds and mosquitoes in their regions. The samples were submitted to laboratories for testing for the presence of WNV, that is, national reference laboratory fro WNV in the VSI Kraljevo or Virology laboratories of NIV-NS and NIVS. The carcasses of susceptible species of wild birds and throat swabs of captured susceptible live wild birds, as well as mosquito samples (pooled samples consisting of 50-100 mosquitoes per one sampling ocassion and sampling locality) collected in the territories of North Bačka, West Bačka, South Bačka, Srem, North Banat, Middle Banat and South Banat Counties were submitted to the virology laboratory of the NIV-NS. The carcasses of susceptible species of wild birds and throat swabs of live susceptible wild birds, as well as mosquito samples (pooled samples consisting of 50-100 mosquitoes per one sampling ocassion and sampling locality) collected in the epizootic regions controlled by Specialized Veterinary Institutes VSI Niš, VSI Kraljevo, VSI Zaječar and VSI Jagodina were submitted to the national reference laboratory for aviary influenza, Newcastle disease and WNV in VSI Kraljevo. The samples collected in the epizootical region controlled by NIVS, that is, epizootical regions of NIVS, VSI Požarevac and VSI Šabac were submitted to the virology laboratory of the NIVS.

The Institutes communicated the regular monthly testing reports to the Veterinary Directorate by the 10th of the following month. In cases of positive seroconversion finding (positive serological finding in previously seronegative sentinel animals) during testing or establishment of virus presence in wild birds, vectors (mosquitoes) or horses (during active or passive surveillance), SUSPECTED CASE on presence of infectious disease is set up and reporting immediately, without delay, to the responsible veterinary inspector, Veterinary Directorate and to the National reference laboratory of the VSI Kraljevo. Positive and suspect samples were immediately submitted to the National reference laboratory of the VSI Kraljevo for final confirmation and diagnosis. In case of positive finding, the National reference laboratory sends the confirmatory official report to the sender and to the Veterinary Directorate. Based on positive seroconversion finding, or positive finding for virus presence, the Veterinary Directorate declares the presence of WNV, i.e., POSITIVE CASE of WNV infection in the relevant region. Veterinary Directorate communicates the information on Suspected and Positive Cases of WNV infection (confirmation of suspect infection – positive case) to the Ministry of Health.

Blood serum samples obtained from horses and poultry confirmed positive for the presence of WNV-specific antibodies, as well as the virus-positive samples of wild birds and mosquitoes must be stored in frozen state to the purpose of further examination (including potentially virus-positive samples of diseased/dead horses tested during passive surveillance process).

To provide the unremitting and timely WNV surveillance, particularly its stages that require technically complex procedures (surveillance of wild birds and vector mosquitoes), timely planning and implementation of required resources (personnel, equipment, reagents) by all participating parties is of vital importance. Surveillance of wild birds and mosquitoes, as a technically complex segment of the procedure, requires the following preceding actions and prerequisites:

- official approval for capturing and sampling of wild birds;
- participation of qualified ornithologists or at least certified ringers (binders) for performing field activities and accurate identification of wild bird species used for obtaining samples
- cooperation and working together with hunting societies and organizations;
- legally registered, i.e., reported nets for capturing wild birds;
- participation of qualified entomologists to perform accurate identification of trapped mosquitoes (at species level) to be examined for the presence of WNV and for field activities aimed at trapping and collecting of mosquitoes;
- sufficient number of adequate traps for collecting the mosquitoes (*Culex pipiens*);
- dry ice for storing and transportation of samples to the laboratory

The WNV surveillance program has been conducted during 2014 in the territory of Serbia. The program is still ongoing, and its evaluation will be performed by the beginning of 2015. Based on the data obtained in this evaluation, the effects of the Program will be assessed. We believe that the obtained data will enable potential corrections and amendments to the Program, thus making it highly effective instrument for further surveillance of this vector-borne zoonotic viral infection in the territory of Serbia.

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