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|  | **Mediterranean and Black sea Program For Intervention Epidemiology Training** |  |

Case Study

A One Health approach for West Nile virus (WNV) surveillance in Serbia

*Facilitator’s guide*

**One Health Approaches to Field Epidemiology Practice in MediPIET countries**

**Belgrade, Serbia, 3-7 June 2024**



This case study was initially adapted by Dr Mitra Drakulovic, National Public Health Institute “Dr Milan Jovanovic-Batut” Belgrade, Serbia for MediPIET training purposes (MediPIET Module of Vectorborne Diseases and Zoonosis, April 2016, March 2017) from a previous existing case study for the WNV outbreak in Greece October 2011.

**Source:**

This case study is built around the first large West Nile virus (WNV) outbreak that occurred in Serbia between June and November 2013. The case study deals with the descriptive part of the outbreak investigation of WNV (Part 1) and mosquito surveillance in Serbia (Part 2).

**Authors:**

Mitra Drakulovic, based on the CS of “WNV outbreak in Greece, 2010” developed by Takis Panagiotopoulos & Kostas Danis in 2013. This CS has an additional learning objective related to mosquito surveillance principles and concepts (see Part 2), as compared with the Greek CS.

**Reviewers:**

Adela Páez, MediPIET Scientific Coordinator supported the author to adapt the original CS from Greece. The Serbian CS was further reviewed by the entire MediPIET Scientific Coordinators team at that time in April 2016 (Ahmed Zaghloul, Marie Belizaire & Nikoletta Mavroeidi). In October 2020, it has been reviewed again by the author and MediPIET Scientific Coordinators (Adela Páez, Iro Evlampidou & Angeliki Lambrou).

**Revisions:**

The case study was revised and modified for the One Health module in 2023 (Emily White Johansson and Pawel Stefanoff). This revision updated the learning objectives, removed sections on the WNV outbreak investigation, and added sections on animal and avian surveillance to complement descriptions of human and vector surveillance from the original case study. The case study was reviewed in 2024 by Mitra Drakulovic (Serbia NPHI) , Netta Beer, Timothee Dub and Pawel Stefanoff (ECDC).

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**Purpose:**

The aim of this case study is to demonstrate the application of One Health principles to the development of an integrated West Nile virus (WNV) surveillance programme in Serbia for the early detection of WNV circulation in the environment in order to better protect human and animal health.

**Intended learning outcomes:**

By the end of this case study, participants should be able to:

* Explain One Health principles and their application to surveillance of zoonotic diseases.
* Describe the WNV transmission cycle and how it informs the design of an integrated surveillance system using One Health principles.
* Explain the role of human, animal, and environmental surveillance in WNV early detection and preparedness planning to reduce transmission and protect health.
* Explain the objectives and rationale of human surveillance.
* Explain the objectives of vector surveillance and define mosquito-based indicators.
* Explain the objectives of avian surveillance including its strengths and limitations.
* Explain the objectives of host mammal surveillance including its strengths and limitations.

**Duration of the case-study**: [2-2.5 hours]

**Part 1: Establishing a national WNV surveillance programme in Serbia**

*[expected duration: 30 minutes]*

On a Wednesday afternoon, 31 July 2012, a physician at the Clinic of Infectious Disease in Belgrade, called the Serbian Centre for Disease Control (SCDC) and reported an unusual cluster of cases (n=14) with neuroinvasive disease, manifested as meningitis in 11 cases and encephalitis in 3 cases during the previous 15 days. This is compared to the mean of seven hospitalised cases per month for the previous three years.

On the same day, 14 serum and 10 cerebrospinal fluid (CSF) specimens from the 14 hospitalised patients were sent for further testing to the Reference Laboratory for Arboviruses “Torlak” in Belgrade. The following day, the results showed that IgM antibodies against West Nile virus (WNV) had been detected in all 14 serum specimens and 10 CSF specimens.

This was the first reported outbreak of WNV infection in humans in Serbia. Prior to 2012, the WNV situation in Serbia was mostly unknown due to a lack of routine diagnostic testing of suspected human cases. There was no routine WNV surveillance system in Serbia.

Some research on WNV had been conducted in Serbia before 2012 to understand virus circulation in humans, animals, mosquitoes, and birds. This included four serological surveys of WNV antibody prevalence in humans and animals. In 2010, the first serological survey in humans identified WNV antibodies in 4% of the population in South Backa. At the same time, studies with mosquito testing identified WNV RNA in 3 of 50 analysed *Culex pipiens* mosquito pools in this same district. Also in 2010, a second serological survey of horses found neutralizing WNV antibodies in 12% of racing horses in nine Serbian districts in the northern part of the country. One year later, a third survey found WNV prevalence in horses to be 29% in seven Serbian districts in Vojvodina Province. In 2012, the fourth survey confirmed WNV presence in wild resident and migratory birds in Serbia. Seven (8%) of the 92 serum samples in birds had anti-WNV IgG antibodies.

You are working at the National Institute of Public Health and your manager asks you to participate in the working group to assess the WNV risk for the Serbian population. The first task of your team is to assist the Ministry of Health in setting up surveillance system(s) to inform risk assessments and disease control programmes. You realised, however, that surveillance of human cases alone is not sufficient based on this prior research and your knowledge of the WNV transmission cycle.

Question 1: Explain One Health principles and how they apply to designing a WNV surveillance programme in Serbia.

*[Expected time of discussion: 5 minutes]*

In 2021, the One Health High Level Expert Panel defined One Health as: *An integrated, unifying approach that aims to sustainably balance and optimise the health of people, animals, and ecosystems. It recognises the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent.’* Given theWNV transmission cycle, an integrated surveillance approach is needed to connect partners and integrated surveillance across human, animal, and environmental sectors.

Since humans are accidental hosts, human surveillance cannot provide sufficient information on the presence of the virus that can cause disease in both humans and animals (problem relevant for both sectors)

The One Health approach recognises that the health of people is closely connected to the health of animals and the environment. Most emerging infectious disease risks originate at the human-animal-environment interface. In coordination with the Ministry of Health, your team decides to contact other sectors. When talking with entomologists and veterinarians, you review together how the virus circulates in nature.

WNV is a flavivirus maintained in a transmission cycle between mosquitoes and birds. The virus is transmitted by infected mosquitoes (mainly of the genus *Culex*) to birds who are its natural hosts. Birds play a role in the geographic dispersion of WNV. Humans, horses, and other mammals are incidental or dead-end hosts (e.g., they do not transmit the disease to mosquitoes or other species due to low viral loads) and are at risk of developing sometimes fatal neurological disease. While human infections are mainly caused by the bite of an infected mosquito, some infections may also occur through transplantation and blood transfusion.

Considering the complex WNV transmission cycle, your team is now extended to involve representatives from the human, animal, and environmental sectors. Together, you decide to collect data from all sectors simultaneously and set up an integrated surveillance programme.

Question 2: Based on the WNV transmission cycle, who should be the key stakeholders or agencies involved in surveillance and what are their main responsibilities for the surveillance programme?

*[Expected time of discussion: 5 minutes]*

Remind participants the information given previously on West Nile virus transmission, the role of mosquitoes and birds in the transmission cycle, the fact that horses (as well as humans) are dead-end hosts, and that horses can manifest neurological symptoms.

**Humans:** The National Institute of Public Health and its regional and local network are responsible for surveillance of human cases.

**Animals**: Veterinary Directorate and the Ministry of Agriculture and Environmental Protection (Now called the Veterinary Directorate of Ministry of Agriculture, Forestry and Water Management), is responsible for implementing active surveillance using sentinel animals (e.g., horses, backyard chickens) as a complementary early warning system for new human cases; passive surveillance of clinical cases of neurological disease in horses is a mandatory notifiable condition.

**Wild or domestic birds**: Veterinary Directorate and the Ministry of Agriculture and Environmental Protection (Now called the Veterinary Directorate of Ministry of Agriculture, Forestry and Water Management), monitors dead wild and residential birds as an early indicator of WNV circulation to help inform public health preparedness plans to protect human and animal health.

**Mosquitoes**: The Veterinary Directorate of Ministry of Agriculture, Forestry and Water Management is responsible for mosquito-based surveillance to determine the presence and abundance of competent vectors, and the proportion infected with arboviruses as an early indicator of WNV circulation in a local area.

Between 2012 and 2014, the National Institute of Public Health, Serbian Centre for Disease Control, and the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection (Now called The Veterinary Directorate of Ministry of Agriculture, Forestry and Water Management), with the scientific support of your working group, launched the national integrated WNV surveillance programme in Serbia. There are four main surveillance components: human surveillance, mosquito vector surveillance, avian surveillance, and animal surveillance.

Different agencies and stakeholders in Serbia at national, regional, and local levels are involved and responsible for these different surveillance components including: the Serbian Centre for Disease Control (SCDC) and the National Institute of Public Health with its regional and local networks, and in close collaboration with regional blood centres, reference laboratories, and healthcare institutions; the Veterinary Directorate of the Ministry of Agriculture and Environmental Protection (Now called The Veterinary Directorate of Ministry of Agriculture, Forestry and Water Management), in close collaboration with medical entomologists, ornithologists and veterinary institutes.

Question 3: What do you think is the overarching objective of an integrated WNV surveillance programme from a One Health perspective?

*[Expected time of discussion: 5 minutes]*

Remind participants the information given previously on West Nile virus transmission, the role of mosquitoes and birds in the transmission cycle, the fact that horses (as well as humans) are dead-end hosts, and that horses can manifest neurological symptoms.

The overall objective of the national integrated WNV surveillance system is the early detection of WNV presence in a local area through vector, animal, avian surveillance for timely implementation of WNV control measures (e.g., inform the public to protect themselves from mosquito bites, reduce standing pools of water) to better protect human and animal health. For example, early detection of WNV presence can alert clinicians so they are more attentive for relevant symptoms and refer for testing.

In Serbia, the main objective of the integrated surveillance programme is the early detection of WNV presence in wild birds (natural hosts) and mosquitoes (virus vectors) and serological testing in sentinel animal populations such as horses (incidental hosts) for the timely reporting to the public health service and local authorities to inform clinical and mosquito control preparedness.

While this is a national surveillance programme, your interdisciplinary team proposes different surveillance strategies for high- and low-risk areas within Serbia. The selection and distribution of sampling localities for active surveillance in each district would be defined according to the WNV risk assessment for all districts in the country.

**Figure 1: Categorisation of districts in Serbia according to the risk of WNV outbreak**

Map

Description automatically generated

**Part 2: Surveillance of human cases**

*[expected duration: 40 minutes]*

Your team at the National Institute of Public Health reviewed key facts about WNV infection, diagnosis, clinical manifestations, and risk factors to inform the WNV surveillance system for human cases.

Approximately 80% of people with WNV infection are asymptomatic. About 20% of infected people experience West Nile fever, a moderately severe disease characterised by high fever, red eyes, headache, and muscle ache. It may also present with mild fever and malaise. Less than 1% of infected people develop West Nile virus neuro-invasive disease (WNND), which is characterised by meningitis, encephalitis, or acute flaccid paralysis and has a case fatality rate of 4-12%. WNND mostly occurs in elderly or immunocompromised individuals.

Laboratory diagnosis is performed using one of the following: (1) isolation of WNV from blood or CSF; (2) detection of WNV nucleic acid in blood or CSF; (3) WNV-specific antibody response (IgM) in CSF or – for probable cases – in serum; (4) WNV IgM high titre and detection of WNV IgG and confirmation by neutralisation.

In response to the first outbreak in 2012, your team at the National Institute of Public Health decided to establish a surveillance system for human cases. The system was set up in close collaboration with the network of local and regional Institutes of Public Health and health care institutions. While human case surveillance was established in 2012, the integrated WNV surveillance programme was not fully launched in Serbia until 2014.

Question 4: What do you think should be the objectives of surveillance of WNV infections in humans, given that the initial picture of the outbreak was that of a localised one in a district of Serbia?

*[Expected time of discussion: 5 minutes]*

* Record new cases of WNV disease as completely as possible,
* Define disease incidence in Serbia as a whole, and, most importantly, to assess an incidence rate in different districts within Serbia and to identify areas in higher risk to raise awareness and to conduct control measures (“place”),
* Identify newly affected areas promptly (“place”).
* Follow the evolution of disease incidence over time, to inform about the intensity and/or continuation of control measures (“time”),
* Identify personal risk factors (e.g., age, gender, health/disease status etc.) and to focus control measures more appropriately (“person”),
* Identify potential sources of infection,
* Identify main clinical manifestations (emerging disease).

Even though it was a localised outbreak, your team decided to establish a national surveillance system for human cases to continuously monitor the WNV epidemiological and clinical situation in Serbia. This is important, since the disease risk can change over time due to various factors, including meteorological and environmental changes.

Based on the WNV epidemiological and clinical situation, the overall surveillance objectives are to: (1) record new cases of WNV infections and deaths as completely as possible at national and district levels to help identify high-risk districts for targeted interventions; (2) identify newly affected areas promptly; (3) monitor the evolution of WNV incidence over time to understand WNV intensity and the need for continuation of control measures; (4) identify personal risk factors and focus control measures more appropriately; (5) identify potential sources of infection (e.g., mosquito bite, blood transfusion); (6) identify main clinical manifestations.

Question 5: Based on the clinical picture and epidemiological situation, develop a case definition for human WNV surveillance.

*[Expected time of discussion: 5 minutes]*

Discuss issue of sensitivity vs. specificity of clinical criteria. Sensitive clinical criteria are justified given that a positive test result is a prerequisite for WNV diagnosis.

High sensitivity includes more cases (“casting a wider net”). The risk of having a too sensitive definition is that there might be false-positives included (i.e. non cases that are identified as cases).

High specificity includes a narrower definition to ensure exclusion of non-cases. The risk is that there might be false-negatives (i.e. true cases might be excluded).

Raise issue of epidemiological criteria. Since it is the first time that the disease is diagnosed in the country, limited data on WNV epidemiology in horses and birds will be available (see below).

Refer to the below case definition provided to participants on the next page.

For comparability with other European countries, your working group proposed adoption of the 2008 European Union case definition of WNV infection.

A **confirmed case** was defined as a person meeting any of the following **clinical criteria**:

* encephalitis,
* meningitis,
* acute flaccid paralysis,
* fever without specific diagnosis

and at least one of the four **laboratory criteria**:

* isolation of WNV from blood or CSF,
* detection of WNV nucleic acid in blood or CSF,
* WNV-specific antibody response (IgM) in CSF,
* WNV IgM high titre and detection of WNV IgG and confirmation by neutralisation.

A case was considered **probable** if the patient met any of the above clinical criteria and showed WNV-specific IgM antibody response in serum. Contrary to the ECDC case definition (<https://www.ecdc.europa.eu/en/west-nile-fever/facts>), which, in addition to the above, applies epidemiological criteria for classifying a clinical case as probable (“residing, having visited or having been exposed to mosquito bites in an area where WNV is endemic in horses or birds”), epidemiological criteria were not used in the case definition, due to the absence of recent surveillance data in horses and birds.

Cases reported as encephalitis (including meningoencephalitis), meningitis, or acute flaccid paralyses were classified as WNND.

Question 6: What kind of surveillance system would you recommend for WNV infections in humans in this setting? Should the system be active or passive?

*[Expected time of discussion: 5 minutes]*

The investigators recommended enhanced WNV surveillance activities. Ideally, Serbia could implement

* an active surveillance (i.e., daily inquiries to hospitals) during the WNV activity season, e.g., from June to mid-November, and
* **passive surveillance in the remaining period (from November 15th to May 30th)**.

Surveillance should encompass case-based reports includoing demographic, underlying conditions, clinical, laboratory and epidemiological information. Upon identification of a probable or confirmed case of WNV infection, a focus investigation should be carried out with an in-depth interview of the patients or their proxies, e.g., family members or friends, medical providers or by reviewing their medical records, to assess clinical progression of disease and potential risk factors and sources of infection. Surveillance should be aimed at cases of all degrees of severity, but focused particularly on hospitalised West Nile neuro-invasive disease (WNND) cases.

In July 2012, your team recommended that WNV become a nationally notifiable human disease. A probable or confirmed case from the laboratory or health care institution must be notified to the local Institute of Public Health, which would then inform regional and national authorities of the notified case.

The team also recommended enhanced surveillance activities for human cases. Ideally, during the WNV activity season (e.g., June to mid-November), there would be active surveillance with daily inquiries to hospitals about cases while passive surveillance of notified cases would be conducted year-round.

Active surveillance is a more sensitive system and provides more comprehensive data. However, it is also labour-intensive and more expensive to implement. Your team was worried that an active surveillance system would not be sustainable, and this could lead to problems with comparability of data over time if the surveillance approach was changed in the future.

In addition, while the surveillance system includes all notifications of confirmed or probable WNV cases across the spectrum of severity, it was decided that a main surveillance focus would be on hospitalised cases with West Nile neuro-invasive disease (WNND).

Question 7: Why do you think WNND was the focus of surveillance? What are some advantages and limitations of this approach?

*[Expected time of discussion: 5 minutes]*

Mild WNV infections are less likely to seek medical care and be identified. Thus, diagnosed and reported mild WNV infections will probably represent a small fraction of all non-WNND cases. In contrast, WNND cases, because of the disease severity, are more likely to be hospitalized and diagnosed, and therefore reported more consistently.

Advantages: “tip of the iceberg” approach to surveillance is used for many conditions with mild / asymptomatic manifestations for consistent reporting; existence of clear criteria for WNND case definition; less dependence on local availability of health services and health care seeking behaviour and thus better comparability of data over time and among different geographic locations; appropriate estimates of disease burden.

Limitations: only a very small fraction of WNV infections lead to WNND, so most WNV cases will not be captured using a ‘tip of the iceberg’ approach. The time lag from WNV infection to WNND onset leads to low timeliness in detecting affected areas or persons. Also, in less affected areas, physicians may be less familiar with WNV symptoms and diagnostic procedures. Therefore, the disease may be overreported in known endemic regions, since the physicians will insist on diagnosing patients with less pronounced symptoms, and underdiagnosed in less affected areas, because only most severe cases would be reported.

A frequently cited saying “Absence of evidence is not evidence of absence” is true for many rare focally distributed diseases.

While all confirmed or probable WNV cases across the spectrum of severity are recorded in the surveillance system, your team decided to focus surveillance reporting on WNND cases for several reasons. Mild WNV cases are less likely to seek medical care and be identified. Therefore, mild WNV cases reported into the surveillance system would likely represent only a small fraction of all non-WNND cases. In contrast, WNND cases, because of the disease severity, are more likely to be hospitalised and diagnosed, which leads to more complete and consistent reporting in the surveillance system.

A major advantage of this ‘tip of the iceberg’ approach is that there are clear criteria for the WNND case definition, and more complete and consistent reporting for WNND cases. Surveillance is less dependent on local availability of health services or health care seeking behaviour for mild illnesses with very low hospitalisation rates. This leads to better comparability of surveillance data over time and for different districts within Serbia. It also provides the most appropriate and comparable WNND disease burden estimates for different districts and over time.

There are also disadvantages since this approach only monitors the most severe form of WNV disease, which is a tiny fraction of all WNV cases. The full burden of WNV in Serbia will remain unknown using this approach. There is also a time lag from WNV infection to WNND onset leading to a lack of timely data for detecting newly affected areas. To get more timely data, your team decided to separately analyse non-WNND cases since these cases would be notified more quickly after the infection date than WNND cases.

Question 8: To meet the stated surveillance objectives (see previous question 4), what kind of epidemiological data would you collect on human cases within the surveillance framework? What could be the possible data sources?

*[Expected time of discussion: 5 minutes]*

Necessary information would include clinical manifestations with date of onset of symptoms, laboratory results of cases, demographic characteristics (age, gender), place of residence, place of potential exposure (including travel history during the incubation period, blood transfusions), underlying chronic medical conditions and potential risk factors (e.g., occupation, spending time outdoors or in the countryside) and non-use of protective measures against mosquitoes, etc. This could be done using a standardized reporting form.

Possible sources of information include:

Surveillance system and review of hospital records,

Diagnostic laboratories,

Interviews of the patients or their proxies, i.e. family members or friends

Interviews with medical providers or by reviewing patients’ medical records.

Your team discussed the types of epidemiological data to collect within the surveillance framework. You came to the following decisions.

Cases notified to the surveillance system include demographic characteristics (e.g., age, gender) and place of residence from healthcare institution records or laboratory reports.

Clinical data is obtained from a review of hospital records including clinical manifestations, date of symptom onset, date of hospital admission and discharge, diagnoses and procedures conducted during the hospitalization, and any pre-existing medical conditions.

In-depth patient interviews conducted upon case notification use a standard reporting form to obtain additional patient information to understand potential sources of infection and risk factors, including occupation, place of potential exposure, spending time outdoors or in the countryside, and non-use of protective measures against mosquitoes.

**Part 3: Vector surveillance**

*[expected duration: 20 minutes]*

Your interdisciplinary team also discussed how to conduct vector surveillance in a systematic way. Again, the team reviewed the basic facts with its entomologist members.

The WNV transmission cycle is maintained in nature between birds and mosquitoes. Infected mosquitoes (mainly *Culex*) transmit the virus to birds who are its natural hosts, while humans, horses, and other mammals are incidental or dead-end hosts (e.g., they have low viral loads and do not re-transmit the virus to mosquitoes or other species).

Based on this WNV transmission cycle, the Veterinary Directorate of Ministry of Agriculture, Forestry and Water Management established a mosquito vector surveillance programme in 2014 in parallel to the human surveillance system.

Question 9: Based on the WNV transmission cycle, what do you think should be the objective of mosquito vector surveillance within the integrated WNV surveillance programme?

*[Expected time of discussion: 5 minutes]*

Remind participants the information given previously on West Nile virus transmission, and the role of mosquitoes and birds in the transmission cycle.

Mosquito-based surveillance includes the systematic collection of mosquito samples and screening for arboviruses presence to provide timely information about the circulation of WNV in vectors in a local area so that preparedness measures may be undertaken to protect human and animal health.

Mosquito surveillance allows investigators to track WNV as it spreads across a country and becomes established within different areas. By monitoring local mosquito populations and virus activity in vectors, investigators can use mosquito surveillance to quantify the intensity of virus transmission in different areas over time. A predictive index of WNV infection risk in humans may also be developed based on vector surveillance that is comparable over time and for different districts.

See detailed description on the next page.

The team at the Serbian National Centre for Disease Control decided that the main objective of mosquito surveillance would be to help detect risk of WNV in a local area so that preparedness measures may be implemented in a timely manner to protect human health.

In the absence of effective WNV treatments or a vaccine, protecting human health primarily depends on preventing WNV transmission. Public health authorities must implement measures early and timed before peak transmission season to help reduce the risk of infected mosquitoes from biting people.

Human case reports alone are not sufficient to predict infection risk or prevent outbreaks. Outbreaks can develop quickly, and most human cases occur and are notified up to several weeks after the peak transmission season. The incubation period from infection to onset of symptoms is 2-14 days in humans, and there is a further time lag for seeking medical care, diagnosis and reporting the case into the surveillance system.

Mosquito surveillance can help identify conditions associated with WNV risk about 2 to 4 weeks before reported human cases occur. This provides a critical early warning system for preparedness measures to be implemented including vector control interventions and public education programmes.

Mosquito surveillance also allows investigators to track WNV as it spreads across a country and becomes established within different areas. By monitoring local mosquito populations and virus activity in vectors, investigators can use mosquito surveillance to quantify the intensity of virus transmission in different areas and over time. A predictive index for WNV infection risk in humans may be developed for different areas too.

Question 10: Based on these objectives, what kind of mosquito surveillance system would you recommend for Serbia? What are the relevant indicators?

*[Expected time of discussion: 5 minutes]*

* Active vector surveillance
* Monitor the presence and abundance of competent mosquito vectors by area,
* Monitor the WNV infection prevalence in the vector population.
* Indicators: vector density, number of positive pools, and prevalence of WNV in vectors. See next page for details on indicators.

Frequency and distribution of sampling sites should depend on the WNV risk assessment for the district. Mosquitoes are usually collected using light traps between May and September for the early detection of WNV in the virus vector that will provide timely information to inform control programs.

In 2012, your team working with the Serbian National Centre for Disease Control recommended an active mosquito vector surveillance to monitor local mosquito populations and virus prevalence in vectors, including genetic characterisation of the WNV strain in mosquitos.

Mosquito surveillance provides key indicators for WNV surveillance programmes including the presence and abundance (referred to as density) of competent mosquito vectors (e.g., those mosquitoes capable of transmitting WNV); and the proportion of competent vectors infected with WNV, or the prevalence of virus in the mosquito population.

Between May and September 2012, you were invited as an observer to the launch of the new system together with the team of entomologists. The team placed light traps at all 13 sampling sites across the country. Light traps are commonly used to attract a wide range of mosquitoes. Each week, the team placed 4-6 traps at each sampling site that were left overnight. The team completed 25 overnight sampling events during the peak transmission season. To obtain the most reliable virological results, traps were emptied immediately after each 12-hour period, and collected specimens were placed on dry ice. In the laboratory, mosquitoes were tested in pools of 50 mosquitoes.

Out of a total 6,369 female mosquitoes in 180 pools, only 10 pools tested positive for WNV-RNA: 9 pools were *Culex pipiens* and one pool was *Anopheles maculipennis*. Phylogenetically, WNV lineage 2 sequences were obtained from the mosquito pools during the 2012 outbreak.

WNV strainsare classified into two main lineages (lineage 1 and lineage 2). As of 2012, at the time of this outbreak, lineage 1 had been identified in most outbreaks in humans and horses in Europe as well as in North America. However, prior to this outbreak, lineage 2 was mostly identified circulating in Sub-Saharan Africa, Madagascar, and South Africa. Across Europe, lineage 2 had only been identified in 2004 and 2005 in goshawks in Hungary, in 2007 in humans in Volgograd, Russia and in 2008 in wild hawks and a captive kea in Austria.

**Part 4: Avian surveillance**

*[expected duration: 30 minutes]*

Your interdisciplinary working group discussed the possible use of avian surveillance data. You review together the published evidence on the role of birds as the main natural WNV reservoir.

Hundreds of wild and domestic bird species are susceptible to WNV infection, particularly *Corvidae* (e.g., magpie, crow, raven, rook), raptors (e.g., northern goshawk, falcon, eagle), and common pheasants and songbirds. Migratory birds have been found to spread WNV infection over long distances during their migrations. While WNV is more often found in migratory birds, wild resident birds may act as amplifiers of local WNV strains and are important for maintaining WNV circulation in the local area.

WNV is transmitted to birds through the bite of an infected mosquito, and mosquitoes in turn may also become infected by biting infected birds. Some predator or scavenger birds may become infected by eating other infected dead birds. Most birds survive WNV infection, although some species (e.g., jays, crows) may die from the WNV infection.

Prior to 2012, the extent of WNV circulation in migratory and wild resident birds in Serbia was unknown. In collaboration with your interdisciplinary working group, investigators from the Veterinary Directorate decide to assess the presence of WNV in wild birds in Serbia. This study was conducted in January to September 2012 (prior to and during the outbreak period) and its results led to the establishment of routine avian surveillance as part of the integrated WNV surveillance programme in 2014.

Question 11: Based on role of birds in the WNV transmission cycle, what do you think should be the objective of routine avian surveillance within the integrated WNV surveillance programme?

*[Expected time of discussion: 5 minutes]*

Remind participants the information given previously on West Nile virus transmission, the role of mosquitoes and birds in the transmission cycle.

Avian surveillance provides an early warning of viral activity in the natural reservoir host so that public health authorities have time to implement control measures to reduce WNV transmission and better protect human and animal health. Serological and molecular investigations may detect the presence of WNV in migratory and wild residential birds, while also characterizing the genomic strains of the virus found in birds.

Importantly, if serology is used on wild birds this is not always easy to interpret since investigators may not know where the bird got infected (locally or elsewhere) and the exact time point for infection. For example, WNV was detected in migratory birds through serological analysis in Sweden, but this was not a sign that WNV was circulating locally within Sweden.

See next page for detailed explanation.

Investigators at the Veterinary Directorate decided to set up serological and molecular surveillance in birds to confirm the presence and circulation of WNV in the bird population in Serbia, and to characterize the genomic strains of the virus in circulation.

A total of 133 wild resident and migratory birds were investigated between January and September 2012 in Vojvodina province. The birds belonged to 45 species within 27 families. Blood sera (n=92) and pooled tissues from respective birds (n=81) were tested. WNV RNA was detected in a total of 9 wild birds (8%) including three Northern Goshawks, two White-tailed Eagles, one Legged-gull, one Hooded crow, one Bearded Parrot-bill, and one Common Pheasant. All WNV isolates were typed as lineage 2 strains same as vector surveillance results.

Based on these findings, a routine avian surveillance system was established in Serbia to provide an early warning of viral activity in the natural reservoir host so that your team at the National Institute of Public Health could implement control measures *in a timely manner* to reduce WNV risk to humans and animals during the peak season.

Question 12: Based on these objectives, what kind of avian surveillance system would you recommend for Serbia? When and where should avian surveillance be conducted? Should the system be active or passive?

*[Expected time of discussion: 5 minutes]*

In Serbia, avian surveillance focuses on dead wild birds in the natural environment, and particularly resident species that are highly susceptible to WNV infection – see description on next page.

Dead birds reported to the Veterinary Directorate would be tested for the presence of the virus by molecular methods as part of an active surveillance system. The selection and distribution of sampling localities in each district depends on whether the district is defined as a high- or low-risk area for WNV transmission.

See next page for detailed explanation.

Investigators at the Veterinary Directorate decided that avian surveillance would focus on dead wild birds found in the natural environment, and particularly resident species most susceptible to WNV infection, or mainly *Corvidae* (e.g., magpie, crow, raven, rook), raptors (e.g., northern goshawks, falcon, eagle) and common pheasants and songbirds. If these bird species were found dead in rehabilitation centres, zoos, or breeding farms, they would also be submitted for WNV testing as part of the routine surveillance system.

The surveillance encompassed the entire territory of Serbia and was conducted by veterinary institutes and field veterinary services in close collaboration with ornithologists. For the integrated WNV surveillance programme, birds would be tested for the presence of the virus by molecular methods.

The selection and distribution of sampling localities for active surveillance in each district was defined according to a risk assessment for WNV exposure. In high-risk areas, avian passive surveillance was conducted throughout the year. In addition, active surveillance was carried out by shooting or live trapping up to 100 susceptible bird species and tested for WNV testing from May to October. In low-risk areas, only passive surveillance was conducted by collecting up to 50 dead wild birds and tested for WNV from May to October each year.

Question 13: What are some strengths and limitations to routine avian surveillance within WNV programmes?

*[Expected time of discussion: 5 minutes]*

Strengths: Serological confirmation of WNV in the natural reservoir hosts for the virus. Genomic characterization of WNV strains circulating in the local environment. Timely detection of WNV in a local area to serve as an early warning system for public health authorities to enact control measures.

Limitations: Requires capacity at the local level to let the public know to observe and report dead birds, and to then invest in systems to respond to reports, record data, and collect birds for testing. The quality of the surveillance depends on public awareness and efforts to observe and report dead birds, and for authorities to rapidly investigate each report. In addition, if serology is used on wild birds this is not always easy to interpret since investigators may not know where the bird got infected (locally or elsewhere) and the exact time point for infection. For example, WNV was detected in migratory birds through serological analysis in Sweden, but this was not a sign that WNV was circulating locally within Sweden.

See next page for detailed explanation.

Your interdisciplinary working group discussed how this new avian surveillance system could complement other surveillance systems and any challenges that would need to be addressed.

First, avian surveillance could provide serological confirmation of WNV presence in the natural reservoir of the virus. Second, it will allow for the genomic characterization of WNV strains circulating in the local environment. Third, it can provide an early warning system to public health authorities to implement control measures and better protect human health.

However, avian surveillance also requires capacity at the local level to let people know where to report dead birds, and systems for responding to reports, recording data, and collecting birds for testing. Laboratory capacity for bird necropsies and testing is also required. The quality of the system relies on public awareness and active efforts by local residents to observe and report dead birds to authorities, and for authorities to investigate each report. Rural areas are also a challenge for avian surveillance with fewer people around to observe and report dead birds to authorities.

Since avian surveillance requires human and financial resources, authorities may also need to decide if all dead birds reported should be submitted for WNV testing and create a more sensitive surveillance system. Otherwise, surveillance could focus on specific bird species more susceptible to infection and thus develop a more specific surveillance system. Finally, to be useful, such a system requires ongoing and unrestricted exchange of data between institutions in the veterinary and public health sector.

**Part 5: Animal surveillance**

*[expected duration: 30 minutes]*

Your working group further discussed with the Veterinary Directorate how to best use the animal surveillance for ongoing and systematic WNV surveillance.

Horses are frequent incidental hosts for WNV. Like human cases, most horses infected with WNV do not show signs of illness or only exhibit mild symptoms. The incubation period in horses is 1-2 weeks and approximately 10% of WNV-infected horses develop neurological symptoms such as ataxia, weakness, and paralysis, which may lead to fatal neurological disease in a small proportion of horses.

Several vaccines are available for horses that reduce the risk of viremia, clinical symptoms, and mortality from WNV. In Serbia, however, at the time of the outbreak in 2012, horses were not vaccinated against WNV. Because of conversion and anti-WNV antibody presence, horses are often used in WNV surveillance programmes. Other animals such as dogs or backyard chickens may also be used for sentinel animal surveillance.

Prior to 2010, the WNV situation in animals was mostly unknown in Serbia. The first serological testing of horses sampled in 2009-2010 showed that 12% of 349 horses sampled from the northern part of the country had neutralising WNV antibodies. WNV antibody positive horses were found in 14 of 28 municipalities spread over 200 km. In another study, the presence of specific antibodies against WNV was found in 29% (n=72) of 252 horse sera samples taken from 7 large horse stables located in Belgrade and Vojvodina Province. Seroprevalence ranged from 13% to 40% across stables sampled in these areas.

Question 14: Based on role of animals in the WNV transmission cycle, what do you think should be the objective of equine surveillance within the integrated WNV surveillance programme?

*[Expected time of discussion: 5 minutes]*

WNV disease in animals is notifiable to the Veterinary Directorate. WNV animal surveillance assists with export certification, meeting international reporting obligations, and informs public health on possible risk areas for WNV transmission.

Sentinel animal surveillance of horses and other mammals provides an early indication of WNV activity in a local area. It also helps to address gaps in other surveillance systems (human, vector, bird) such as in rural communities with few people to observe and report dead birds, or in other geographic areas where bird or vector surveillance is not set up. It may also provide WNV case confirmation in areas where a human case has not yet been detected and reported.

See next page for more detailed explanation.

Since 2012, WNV is a notifiable disease in animals in Serbia. All veterinary laboratories are required to report suspected or confirmed WNV cases in all animal species. WNV animal surveillance also assists with export certification requirements, meeting international reporting obligations to the World Animal Health Organisation, and informing public health on possible risk areas for WNV transmission.

Equine surveillance data is among the most consistently reported data. Equine data helps to address gaps in other surveillance systems (human, vector, bird) for geographical regions where mosquito or avian surveillance are not in place, or in rural communities where low population density means there are fewer people to observe and report dead birds. Equine surveillance may also provide WNV case confirmation in areas where no human cases may have yet been diagnosed and reported.

Question 15: Based on these objectives, what kind of animal surveillance system would you recommend for Serbia? Should the system be active or passive?

*[Expected time of discussion: 5 minutes]*

Both passive and active animal surveillance is used in Serbia.

Passive surveillance: WNV is a notifiable condition for clinical symptoms in horses in Serbia. This assists with export certification, meeting international reporting requirements, and informs public health authorities about WNV risk in local areas.

Active sentinel animal surveillance: In Serbia, horses and backyard chickens are used for sentinel animal surveillance. The aim is to serologically detect the presence of WNV in sentinel animals in order to provide early detection of WNV in a local area, and to help fill gaps in environmental surveillance in rural areas or other geographic regions where it is not set up. The frequency and distribution of sampling in localities within districts depends on the risk assessment for the area, and whether the district is categorized as high- or low-risk district.

See next page for detailed explanation.

Animal surveillance consists of both passive surveillance, consisting of serological and virological testing of horses with clinical manifestations of disease, and active sentinel surveillance of horses and backyard chickens (serological surveys).

For passive surveillance, all veterinary laboratories are required to report suspected or confirmed WNV cases in all animal species, including horses, to the Veterinary Directorate in Serbia. Passive surveillance includes serological testing of paired serum samples (i.e., collected from the same animal at different times), and virological examination of clinically ill horses with symptoms of neurological dysfunction.

For active surveillance, horses are used as sentinel animals for serology testing as indirect indicators of viral presence in Serbia. Active surveillance of sentinel horses is conducted differently in high- and low-risk districts in Serbia. In high-risk areas up to 50 horses are sampled in a minimum of 3 localities per district (compared to 30 horses for low-risk areas). The sampled horses are repeatedly tested for the serological presence of WNV during the peak seasonal period in June, July, and August. Only horses that tested negative for WNV IgM antibodies in April-May (prior to WNV transmission season) were included as sentinel animals for WNV surveillance.

Question 16: What are some strengths and limitations of active sentinel horse surveillance within an integrated WNV surveillance programme?

*[Expected time of discussion: 5 minutes]*

Strengths: Passive surveillance is mandated reporting to the Veterinary Directorate and provides information to inform public health programmes. Active sentinel animal surveillance provides early indications of WNV activity in a local area, and also helps to fill gaps in other surveillance systems as previously discussed.

Limitations: Need to ensure sentinel animals are not already immune to WNV through prior infection or vaccination. As WNV circulation increases over time, this may be a challenge for the horse population in particular. Not possible to use horse surveillance if vaccination programs are in place.

Horses are highly susceptible to WNV infection and provide evidence of WNV circulation in rural communities where vector surveillance may not be set up, or where avian surveillance may be challenging due to low population density. Active sentinel horse surveillance also provides complementary animal case data from areas where human cases may not have been reported. This way, the authorities can increase the awareness among the community and the healthcare workers.

Although highly susceptible, horses are less suitable for use as sentinel animals in areas where vaccination of the horse population against WNV is widely used. In Serbia, horses are not vaccinated against WNV and are therefore suitable for use as sentinel animals for WNV surveillance in Serbia.

However, due to WNV circulation in previous years, there was a high percentage of already seropositive horses, so the adequacy of the number of seronegative (sentinel) horses that could be used in the WNV surveillance program was already a challenge in 2012.

This problem in sentinel horse surveillance can be addressed by the inclusion of other animal species as sentinel animals such as backyard chickens, cattle, and pigs. Your team at the Veterinary Directorate are now working to investigate these other options for future animal surveillance in Serbia.

**Part 6: Epilogue**

*[expected duration: 5 minutes]*

In 2018, the national integrated WNV surveillance programme revealed the highly intensive circulation of WNV in Serbia that year. In 2018, Serbia recorded the highest number of human WNND cases (n=415), leading to 36 deaths. In total, human WNV cases were reported in 17 out of 25 districts in Serbia reflecting a further spread of WNV circulation into central, eastern and western parts of the country.

The first human case was reported on July 2nd and the last one on November 17th. As early as July, there were 70 human cases registered in 8 districts. In 2018, one-third of cases were recorded by early-July. This was an unusually early start to the WNV season.

Most human cases were preceded by the detection of WNV in horses, wild birds, and mosquitoes. The first evidence of WNV circulation was obtained by detection of the virus in mosquitoes on June 19th in four districts, and 2-3 days later in an additional 3 districts. This was the first round of mosquito collection and testing within the integrated WNV surveillance programme. Around the same time, the first WNV positive tracheal swab of a wild bird was detected on June 20th. In the following days, the first WNV IgM antibody-positive horse was identified on June 30th. In total, WNV circulation in mosquitoes, horses, or wild birds was detected in 16 out of 25 districts in Serbia in 2018.

The detection of WNV in mosquitoes preceded the first human case by 2 to 8 weeks in 9 districts; detection of WNV positive wild birds preceded human cases from one day to 2 weeks in 3 districts; and the identification of WNV IgM antibodies in horses occurred 2 days to 8 weeks before the first human case in 7 districts in 2018.

However, in 5 (29%) districts the surveillance programme did not detect early WNV circulation before the occurrence of human cases. This may have been due to the unusually early start and intensive WNV season in 2018. Investigators are considering adding meteorological data and predictions to help establish the most appropriate annual timing for WNV surveillance in wild birds, horses, and mosquitoes.

During the 2018 season, the Veterinary Directorate and Ministry of Agriculture, Forestry and Water Management continuously communicated surveillance results to the National Institute of Public Health who then conveyed recommendations for risk communications, mosquito control and public protective measures to regional institutes of public health and district public health authorities responsible for local implementation.

Overall, the results demonstrate the success of the integrated WNV surveillance programme in most districts as an early warning system for public health authorities to implement control measures to reduce the risk of WNV exposure and protect human health.

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