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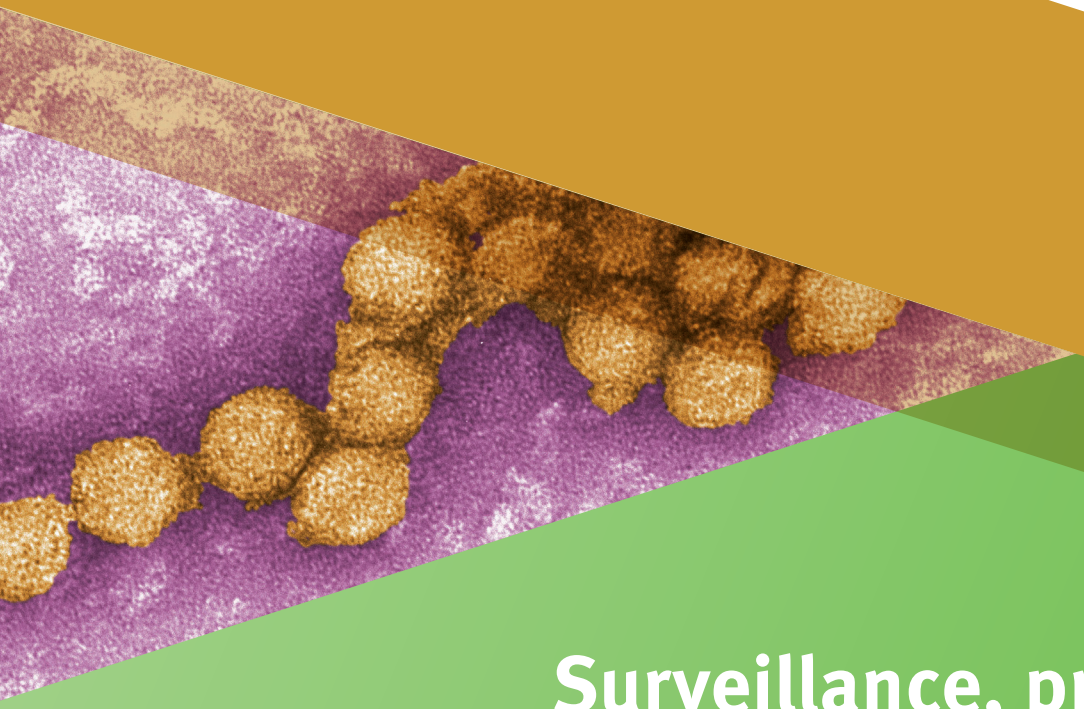

TECHNICAL REPORT

Surveillance, prevention and control of West Nile virus and Usutu virus infections in the EU/EEA

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This report of the European Centre for Disease Prevention and Control (ECDC) was coordinated by Céline M. Gossner and was written by the following ECDC and EFSA experts (in alphabetical order): Sotiria-Eleni Antoniou (EFSA), Tamás Bakonyi (ECDC), Céline M. Gossner (ECDC), Joana M. Haussig (ECDC), and Vanja Nikolac Markic (ECDC).

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Contents

Abbreviations

ADIS	Animal Disease Information System
CSF	Cerebrospinal fluid
EFSA	European Food Safety Authority
EEA	European Economic Area
ELISA	Enzyme-linked Immunosorbent Assay
EU	European Union
JEV	Japanese encephalitis virus
NAT	Nucleic Acid Test
NGS	Next-Generation Sequencing
NUTS	Nomenclature of Territorial Units for Statistics
NRL	National Reference Laboratory
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase Chain Reaction
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RT-qPRC	Quantitative Reverse Transcription Polymerase Chain Reaction
SoHO	Substances of Human Origin
TBEV	Tick-borne encephalitis virus
USUV	Usutu virus
VNT	Virus Neutralisation Tests
WNF	West Nile fever
WNV	West Nile virus
WOAH	World Organisation for Animal Health (formerly the Office International des Epizooties, OIE)

Executive summary

West Nile virus (WNV) and Usutu virus (USUV) are mosquito-borne flaviviruses, which are endemic in several EU/EEA countries. Both viruses can cause disease in human and animal hosts. Although they have similar biological features, ecology and epidemiology; there are important differences in their pathogenicity, and their impact on human and animal health.

This technical report describes the result of a project to provide an overview of the epidemiological situation of WNV and USUV, the diagnostic, surveillance, prevention and control practices applied in EU/EEA countries, and to assess their public health and animal health impact in the EU/EEA. The project was undertaken in 2021 and 2022 and used the One Health approach by involving experts from the public health, animal health and substances of human origin (SoHO) sectors. Information was collected by i.) a questionnaire survey amongst national representatives of the three sectors in the EU/EEA countries, ii.) a literature review to obtain additional/supplementary information on the epidemiological situation and applied practices in countries, and iii.) a two-day online meeting of experts in the three sectors from the most affected EU/EEA countries, as well as representatives of the European network on medical and veterinary entomology (VectorNet) and European network of expert laboratories on emerging viral diseases (EVD-LabNet), the European Food Safety Authority (EFSA), the European Commission, and experts on SoHO and vector-borne diseases at ECDC.

Experts from 29 EU/EEA countries answered the survey questions from at least one sector. The distribution of answerers from the different sectors were similar (i.e. 24 from the public health sector, 24 from the animal health sector and 26 from the SoHO safety sector). The literature review collated information from 824 relevant publications (579 papers on WNV, 23 on USUV and 222 on both viruses). The first and second day of the expert meeting were attended by 77 and 69 participants, respectively, from 13 EU/EEA countries and networks/EU institutions.

Between 2012 and 2021, 16 EU/EEA countries reported a total of 3 632 autochthonous cases of WNV infections in humans, with an exceptionally high number of cases in 2018 (n=1 551). Nine countries (Austria, Croatia, Czechia, Germany, Greece, Hungary, Italy, Romania and Spain) reported autochthonous, human WNV infections in consecutive years and/or annual incidence of ≥0.1/100 000 population in some areas, in some years. Sporadic, locally-acquired WNV infections occurred in Bulgaria, Cyprus, France, the Netherlands, Portugal, Slovakia and Slovenia. Autochthonous human cases of WNV infection have not yet been diagnosed in Belgium, Denmark, Estonia, Finland, Iceland, Ireland, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Norway, Poland and Sweden. Within the same period, WNV infections in animal hosts (predominantly detected in horses and different bird species) and vectors were reported by the same countries as the ones with human cases, except Poland, where WNV seropositivity was detected in animals (avian and equine hosts) only. It has also been detected in at least nine mosquito species in 13 countries. Most frequently, *Culex pipiens s.l.* specimens were found positive for WNV.

Eight countries (Austria, Croatia, Czechia, France, Germany, Hungary, Italy and the Netherlands) reported a total of 104 cases of USUV infections in humans between 2012 and 2021. Neurological manifestations were diagnosed in 11 cases; the remaining ones were either asymptomatic infections, febrile diseases or no information on clinical manifestation was available. USUV infections in animal hosts (predominantly in birds) were reported by 15 countries: In addition to the countries with human cases, infections in animals were detected in Belgium, Greece, Luxembourg, Poland, Slovakia, Slovenia and Spain. The virus was also detected in mosquitoes in eight countries.

All responding countries had laboratory capabilities to diagnose WNV infections both in humans and in animals, except Norway for animals. Molecular and serological diagnostic methods were used most frequently. There were different practices in the countries with confirmatory tests of positive samples. Some countries do not perform specific tests for the detection of USUV infections, but they relied on cross-reactive, pan-flavivirus (molecular or serological) assays. Confirmatory tests and differentiation from related flaviviruses were performed in several countries, but some countries used reference laboratories abroad.

Human WNV infections were under indicator-based surveillance in the EU/EEA. All countries conducted passive surveillance except Czechia, Greece and Spain, that conducted active surveillance. Most countries run human WNV surveillance all year round, except Romania, where it was only during the transmission season. Although West Nile fever (WNF) in animals is notifiable to the World Organisation for Animal health and to the European Commission, only 22 EU/EEA countries reported having surveillance system for WNF in animals. Passive surveillance in animals was applied in 21 countries, and several countries used additional surveillance methods (e.g. active surveillance, active case finding, sentinel surveillance). An integrated, One Health approach to surveillance for WNV infections was applied in nine countries. Usutu virus infections were not notifiable at the EU level in either humans or animals. A few countries had developed case definitions for human USUV infection surveillance, and Italy runs integrated, One Health surveillance system for USUV infections. Seven EU/EEA countries had established USUV surveillance systems in animals, predominantly in birds. Eight countries had surveillance systems for the detection of WNV and/or USUV in mosquitoes.

In public health, WNV infection prevention and control measures were focused on the SoHO safety aspects. Sixteen countries reported using nucleic acid test (NAT) for the screening of blood donations. Between 2016 and 2020, a total of 256 WNV contaminated blood donations were reported by 16 EU countries. Three EU countries reported a total of 29 USUV contaminations in blood donations within the same period. Stem cell, tissue and organ donations were tested for WNV infections in a subset of the countries. In most countries, preventive measures for WNV transmission via SoHO (e.g. NAT screening or donor deferral) were applied during the transmission season, and/or triggered by the first detected human case in the administrative unit. In Italy and Slovenia, detection of WNV infection in animal hosts or in mosquitoes also triggered SoHO safety measures. EU/EEA countries used ECDC weekly epidemiological updates/maps to recognise WNV affected areas in other EU/EEA countries and enlargement countries, for the implementation of SoHO safety measures on donations from travellers returning from those areas. No EU/EEA counties implemented specific SoHO safety measures for USUV infections except for deferral of proven contaminated donations.

EU/EEA countries applied diverse mosquito control strategies and practices, depending on the public health impact of WNV infections in the country/area, national legislation and resources. Some countries used preventive (usually larvicide treatments), while reactive (usually adulticide) treatments in outbreak situations were mandatory in certain countries. The geographic coverage and number of repetitive treatments also varied in different countries. In addition to mosquito control actions, some countries organised citizen education campaigns on personal protection from mosquito bites and on mosquito breeding site reduction techniques. No mosquito control measures associated with USUV infections were reported by the countries.

This study collected information on the epidemiological situation, laboratory diagnosis, surveillance, and prevention of WNV and USUV infections in four sectors (public health, animal health, SoHO safety and entomology) in the EU/EEA. The surveillance data on both WNV and USUV indicated extension of the geographical range of the two viruses in the EU/EEA countries during the study period; however, no clear annual trends of case numbers were identified. This could be explained by the diversity of drivers influencing WNV and USUV activity in a particular transmission season (e.g. temperature, precipitation, abundance of vectors, infected and susceptible avian hosts). The proportion of asymptomatic WNV and USUV infections in the human and animal host populations were mostly unknown. There were strains of different genetic lineages of both WNV and USUV circulating in Europe; however, the differences in virulence of the strains were scarcely studied.

Most EU/EEA countries had appropriate laboratory diagnostic capabilities to diagnose WNV and USUV infections, however, the differential diagnosis between the two infections was challenging, particularly when serological methods were used. Harmonisation of the diagnostic methods and access to validated commercial tests could increase the credibility and comparability of laboratory results from different sectors and countries. There were differences in the surveillance systems for humans and animals in the different countries, depending on the national policies and practices. Some countries implemented virus surveillance in mosquito vectors too; however, the benefits and costs of such surveillance should be assessed before implementing on national or regional level. As the surveillance data collection and reporting were not harmonised between countries and sectors, it hindered the comparison of surveillance data on EU/EEA level. Integrated, One Health approaches to surveillance was proven as useful systems in some countries.

West Nile virus infections were considered endemic in several EU/EEA countries. WNV had high public health impact in some countries and major implications on blood safety and security. Seasonal surveillance should be maintained for early detection of WNV activity and emergence of WNV in new areas or countries, to inform SoHO safety authorities. Prevention of WNV human cases focused on the application of blood safety measures and avoidance of mosquito bites. The animal health impact of WNF was the most significant in equines, where prevention focused on vaccinations and avoidance of mosquito bites.

Usutu virus infections were also endemic in many EU/EEA countries and there was co-circulation of both viruses in several areas. The public health and animal health impact of USUV in the EU/EEA was limited and far less than the public health impact of WNV. The information available at the time of the construction of this report does not justify implementation of USUV-specific SoHO safety measures or the implementation of USUV targeted surveillance systems at EU/EEA level. However, it is worth inviting EU/EEA countries to enhance diagnostic capacity and differential diagnosis both for humans and animals, to better understand the epidemiology of USUV infection, monitor the possible emergence of new USUV strains, which may show enhanced pathogenicity for humans, and to assess the potential implication of the co-circulation of the two viruses.

Background and objectives

The purpose of this technical report is to provide evidence to strengthen surveillance, prevention and control of West Nile Virus (WNV) and Usutu virus (USUV) infections in the EU/EEA.

The specific objectives are:

- to provide an overview of the epidemiological situation in the EU/EEA;
- to describe the laboratory diagnosis used in EU/EEA countries;
- to describe the surveillance systems in place in EU/EEA countries;
- to describe the preventive measures applied for the safety of SoHO supplies;
- to highlight gaps in laboratory diagnosis, surveillance, prevention and control practices, and suggest avenues to address these gaps.

Key facts about West Nile virus and Usutu virus and related infections

The viruses

West Nile virus (WNV) and Usutu virus (USUV) are neurotropic, enveloped, single stranded RNA viruses belonging to the Japanese encephalitis virus serocomplex within the *Flavivirus* genus, *Flaviviridae* family [1]. In the enzootic cycle, both viruses are typically transmitted among birds by mosquitoes. Humans, equids and other mammals may be incidentally infected through mosquito bites; they are considered dead-end hosts because the low-level viraemia cannot sustain onward transmission. Co-circulation of the two viruses has been reported in many European countries [2].

West Nile virus was first isolated in Uganda in 1937 [3], and it has been subsequently detected globally. Phylogenetic analyses of WNV genome sequences have identified nine different evolutionary lineages, of which only lineages 1 and 2 have been associated with disease in humans [4]. Lineage 1 WNV strains have been circulating since at least the late 1950s in Europe and in the Mediterranean Basin, where they have caused sporadic infections and outbreaks in humans and animals [5]. In 2004, a lineage 2 WNV strain emerged in central Europe [6], which caused outbreaks of neuroinvasive disease in humans and animals, and has spread to several central and southern European countries. Another lineage 2 strain emerged in eastern Europe in 2007, which has subsequently spread to southern Europe [4,7]. Lineages 1b (Kunjin virus) and 1c have so far been found only in Australia and India, respectively [8,9]. Representatives of other WNV genetic lineages have also been detected in different European countries, however they have not been associated with human or animal diseases [10-13].

Usutu virus was first isolated in 1959 from a *Culex neavei* mosquito that was captured near the Usutu River, South Africa [14,15]. The virus was subsequently detected in many African countries but rarely associated with disease in humans [16]. In Europe, Usutu virus was isolated for the first time in 2001 from dead blackbirds (*Turdus merula*) during an epizootic in Austria. A retrospective analysis attributed the high mortality of blackbirds in Tuscany (Italy) in 1996 to USUV [17]. The virus has since been detected in many western, southern, and central European countries [15,18], predominantly in birds and mosquitoes, but it has also been found in different mammalian species, including humans. Most USUV infections in humans remain asymptomatic, however sporadic neuroinvasive disease cases have been reported in Europe, especially in immunocompromised and elderly patients [19,20].

Phylogenetic analyses grouped USUV strains into at least eight distinct lineages [21]. The Europe 2 lineage is the most commonly detected USUV lineage in European countries; but USUVs of other genetic lineages also circulate on the continent. Experimental infection in animal models suggests that African lineages are more virulent than European USUV lineages [22].

Infections in humans

Most often, humans get infected through the bite of an infected mosquito. Transmission may also occur through donations of blood and blood components or transplantation of cells, tissues, and organs [23]. Vertical transmission (transplacental mother-to-child transmission) and transmission via breastfeeding are extremely rare [24].

Most WNV infections in humans remain asymptomatic, while 20 to 30% of infected individuals develop influenza-like illness, defined as West Nile fever. Less than 1% of infected individuals, mainly the elderly, the immunocompromised, and those with pre-existing medical conditions, develop West Nile neuroinvasive disease (WNND), characterised by encephalitis, meningitis, acute flaccid paralysis, or polyradiculoneuritis.

The case fatality ratio among patients with WNND can be up to 20% [25,26] and severe sequelae persist in 20–40% of survivors [27]. No specific antiviral drugs are available to prevent or treat the disease in humans [28,29].

Most USUV infections in humans are asymptomatic. Only a few cases of USUV infection have so far been described; these cases presented with meningoencephalitis, encephalitis, polyneuritis, or facial paralysis [19,20,30-33] and, less frequently, with febrile illness [16,33]. Risk factors for developing symptomatic USUV infection include being immunocompromised and advanced age [19,20,33].

There are no specific antiviral drugs to prevent or treat disease related to USUV infections in humans.

Infections in birds and equids

For many avian species, WNV infection causes no evident signs while some birds, such as corvids and raptors, often succumb to fatal systemic illness [34]. Avian mass mortality events may occur and are influenced by geographical and environmental factors, as well as by genetic characteristics of WNV strains [35].

West Nile virus infections in equids are usually asymptomatic and only approximately 10% may show neurological signs arising from viral-induced encephalitis or encephalomyelitis, which can range from mild ataxia to total recumbency [36].

Regarding bird species in Africa, no increase in mortality following USUV infection has been reported, nevertheless in Europe, USUV has been highly pathogenic and fatal for several bird species, especially blackbirds (*Turdus merula*) and great grey owls (*Strix nebulosa*) [18]. USUV infections in birds are characterised by seasonal mass mortality events among wild and captive birds during the summer months. Rarely observed are clinical manifestations among infected blackbirds included lethargy, weakness, ataxia, and seizures.

Antibodies against USUV have been detected in horses, but no signs or symptoms have ever been reported in equids [18].

There are no specific antiviral drugs to prevent or treat USUV infections in animals.

Infection in other mammals

West Nile virus and USUV and/or antibodies against WNV/USUV have been detected in companion animals, livestock, rodents, wild ungulates and carnivores as well as zoo mammals [37-44].

Infection in mosquitoes

To date, six European mosquito species, *Aedes albopictus, Ae. detritus, Ae. japonicus*, *Culex pipiens* (the forms *Cx. p. pipiens* and *Cx. p. molestus*), *Cx. modestus* and *Cx. torrentium* have been demonstrated to be competent to transmit WNV [45-47]. *Cx. p. pipiens* is the principal vector for WNV in Europe [48]. Mosquitoes within the genus *Culex* show a marked preference for feeding on birds and are the primary enzootic maintenance and bridge vectors of WNV in nature [49]. However, the *Cx. p. molestus* form is characterised by biting humans and other mammals indoors. Hybrids between the two forms (*Cx. p. pipiens* and *Cx. p. molestus*) are expected to play a role as bridge vectors [50]. Mosquitoes of the Aedes genus exhibit generalist feeding behaviour, allowing them to act as bridge vectors between different vertebrate hosts.

Culex mosquitoes are also the main vectors for USUV, although the virus has been detected in mosquitoes from other genera within the family of Culicidae (i.e., *Cx. p. pipiens, Cx. p. modestus, Cx. torrentium, Ae. japonicus* and *Ae. albopictus*) [51-54].

Diagnosis of West Nile virus and Usutu virus infections

Diagnosis in humans

Laboratory diagnosis of WNV infection is based on WNV isolation or detection of viral RNA in bodily fluids and the demonstration of a specific antibody response against WNV in serum or CSF. Immunostaining of WNV antigens can be applied to the brain and other tissues for post-mortem diagnosis.

West Nile virus is a risk group 3 pathogen, therefore virus propagation should be performed in Biosafety Level 3 conditions. Both mammalian cells (Vero E6, BHK-21) and mosquito cells (C6/36) are typically used to isolate and propagate the virus [55].

West Nile virus RNA is mainly amplified from clinical specimen using reverse-transcription polymerase chain reaction (RT-PCR). Viral RNA can be detected from whole blood, plasma, cerebrospinal fluid (CSF), urine and infected tissues, depending on the host species and stage of infection. Real-time RT-PCR assays are frequently used, because they are more appropriate for large-scale screening of samples, provide results quicker, and in some systems the specificity of the test is high due to nucleic acid hybridisation step (e.g., using specific probes in

TaqMan assays). Additionally, real time RT-PCR systems can be used for quantification of the viral RNA in the sample (quantitative reverse-transcription polymerase chain reaction, RT-qPCR). In humans, the length of viraemia is approximately eight to ten days [56], and the virus titre in the blood is low, which limits the applicability of the direct demonstration of viral RNA in blood [24]. Plasma samples of asymptomatic blood and organ donors are tested for WNV infection with automated, high-throughput Nucleic Acid Tests (NATs) specifically licensed for donor screening [57]. WNV NATs can also detect genetically related flaviviruses, such as USUV and Japanese encephalitis virus (JEV).

Due to the potential cross-reactivity in certain molecular assays (e.g., NATs, pan-flavi RT-PCRs) the identification of the nucleotide sequence of the amplification product is a frequently used method for result validation. The sequence analysis can also identify to which genetic lineage the detected WNV belongs to and might allow estimations on the origin of the infection. While next-generation sequencing (NGS) is increasingly used for WNV genome sequencing in clinical specimens after target sequence enrichment [58], the application of a metagenomic approach to the diagnosis of infectious diseases is still limited. Promising results have been shown for NGS of CSF samples in the diagnosis of encephalitis and meningitis [59,60].

The majority of human WNV infections are diagnosed by serological methods. IgM antibodies against WNV become detectable in serum from day 3-9 after infection, which is followed by WNV IgG from day 4-16 [61]. In patients with neuroinvasive disease, IgM antibodies are detectable in the CSF in about 60-70% of cases at the onset of neurological symptoms [62]. Due to similarities in the antigen structures among flaviviruses, antibodies cross-react, particularly between flaviviruses belonging to the same serocomplex [63]. Therefore, simultaneous virus neutralisation tests/plaque reduction assays should be performed with related flaviviruses, to identify the highest neutralisation reactivity of the tested serum and hence the specificity of the antibodies. In patients with existing immunity against a flavivirus (due to previous infection or vaccination), infection with an antigenically related, other flavivirus might trigger new antibody production against the previous virus too. Therefore, simultaneous neutralisation assays may be inconclusive to confirm the recent infection [25,64].

IgM antibodies do not cross the blood-brain barrier, hence their presence in the CSF indicates local production by infiltrating lymphocytes. Therefore, detection of IgM antibodies against flaviviruses in CSF is a reliable method to diagnose acute infection of the central nervous system.

Laboratory diagnosis of USUV infection in humans is based on virus isolation in cell culture from body fluids, detection of USUV nucleic acids, or demonstration of a specific antibody response against the virus. USUV can be isolated and propagated in vitro e.g. in Vero, BHK-21, and C6/36 cell cultures. The presence of USUV RNA has been demonstrated in blood, urine, and CSF of patients with acute infection [19,20,30-33]. Follow-up investigation demonstrated persistence of USUV RNA in whole blood and urine for about one month in three patients with neuroinvasive disease or fever [33]. Due to the limited availability of commercial serological and molecular assays for diagnosis in humans, in-house developed methods are generally used. Molecular methods include USUV-specific real-time RT-PCR assays, either as a single-target or multiplex test, and broad-range flavivirus RT-PCR followed by amplicon sequencing [65-67]. WNV NATs validated for donor screening can also detect USUV with high sensitivity [67]. NGS-based methods for detecting and sequencing USUV have been set up for use during outbreaks [68]. Detection of antibodies against USUV by enzyme and immunofluorescence assays shows cross-reactivity with heterologous flaviviruses, including WNV [69,70]. Plaque reduction neutralisation tests or microneutralisation assays are used to confirm positive serology results, but, as reported above for WNV diagnosis, USUV-neutralising antibodies may cross-react with WNV antibodies [25,64,69].

Diagnosis in birds and equids

In live animals, WNV antibodies can be identified in serum by IgM and IgG ELISA, VNT/PRNT and haemagglutination inhibition test [71]. In some serological assays, there may be antibody cross-reactions with related flaviviruses, as St. Louis encephalitis virus, USUV, JEV, or TBEV. The direct detection of WNV in living animals (e.g. from swabs, peripheral blood mononuclear cells (PBMCs)) is possible by virus isolation or molecular detection methods, however it is less frequently used than serological assays.

The virus can be identified by molecular detection or viral isolation. The preferred tissues for virus isolation from horses are brain and spinal cord while kidney, heart, brain, liver or intestine tissues can be used to isolate the virus from birds. Viral nucleic acid can be demonstrated in tissues of infected animals by RT-PCR and viral antigens can be detected by immunohistochemistry [71,72].

No international protocols (e.g. World organisation for Animal Health (WOAH) manual diagnostic tests) are available for the diagnosis of USUV infections. Several tests used for WNV also detect USUV, except those with specific WNV antibodies in serology or specific primers in molecular testing. In samples positive for antibodies against flaviviruses or RNA, WNV and USUV may be distinguished from each other using VNT or specific RT-PCR tests. In dead birds, the pathological lesions of WNV and USUV are similar, but can be differentiated using USUV specific antibody in immunochemistry [73].

Diagnosis in mosquitoes

The detection of WNV in mosquito samples is challenged by the relatively low number of WNV-infected individuals in a pool of mosquitoes, and the risk of viral RNA decomposition during trapping, transport, storage and entomological identification of the specimen. In recent years, new diagnostic techniques, such as the analysis of mosquito saliva through Flinders Technology Associates cards, are being developed with promising results [74].

Virological detection can be performed using different molecular techniques: pan-flavivirus RT-qPCR and positive samples are validated by sequencing of the amplification products or specific RT-qPCRs for WNV lineage 1, and 2 and USUV [6,65,66,75,76]. The viruses can also be isolated from mosquitoes in cell culture, using for instance mosquito cells C6/36 from *Aedes albopictus* [55,77].

Surveillance of West Nile virus and Usutu virus infections

West Nile virus infection in humans is a notifiable disease at the EU/EEA level and human cases should be reported to ECDC following the EU case definition outlined in Decision (EU) 2018/945 [78]. Beyond the usual, annual reporting of indicator-based surveillance data to ECDC, EU/EEA Member States are requested to report human cases of West Nile virus infections on a weekly basis during the WNV transmission season in Europe (i.e., from the beginning of June until one month after the onset of the clinical signs of the last reported case). The seasonal surveillance is aimed to identify risk areas of locally acquired WNV, to support EU/EEA countries for the implementation of temporary deferral or testing of allogeneic blood donations from returning traveller donors.

Usutu virus infection in humans is not a mandatory notifiable disease at the EU/EEA level.

An outbreak of WNV in animals is defined as one or more equids/birds infected with WNV within a certain geographical area and time frame that constitute an epidemiological unit. Equine or bird cases are defined according to the Terrestrial Animal Health Code of the WOAH [79].

Outbreaks of WNV in animals are notified to the Animal Disease Information System (ADIS) of the European Commission. At the EU/EEA level it is mandatory to report equine encephalomyelitis due to WNV infection and West Nile virus infections among birds in accordance with Commission Implementing Regulation (EU) 2018/1882 [80]. In addition, data from passive and active surveillance activities including outbreaks are collected by EFSA,

The data collected from ECDC and EFSA both in human and animal cases and surveillance activities are analysed and published in the annual European Union One Health Zoonoses Report [81].

Prevention and control of West Nile virus and Usutu virus infections

Interventions can focus on the reduction of vectorial or non-vectorial transmission of WNV and USUV infections to vertebrate hosts, and to facilitate immune protection against these viruses. In humans, the most frequent non-vectorial transmission route is through substances of human origin, particularly transfusion of contaminated blood products. In animals, oral transmission (e.g. infection of raptors through eating infected prey birds) may play a role, which can only be prevented for captive birds.

Prevention of mosquito-borne transmission

The principal vectors of both WNV and USUV are *Culex spp*. mosquitoes, hence control measures against these species may have joint effect on the vectorial transmission of both viruses. Mosquito control measures can be applied preventively (e.g., larval and adult control before and during the transmission season); however, there is little evidence on the direct effect of preventive mosquito control actions on the intensity of WNV and USUV outbreaks later in the season [82]. Reactive mosquito control measures (e.g., adulticide treatments in affected areas during outbreaks) is a widely applied method, although the implementation (geographic coverage, timing, repetitions) greatly vary in different countries, depending on the legislation of the use of biocides, available resources, as well as the public and animal health impacts of the outbreaks.

The use of individual protective measures to prevent mosquito bites (e.g. repellents, nets) and the reduction of mosquito breeding sites (e.g. stagnant water around households) can be facilitated by public awareness campaigns at the start of and during the transmission season. The protection against mosquito bites of sensitive mammals and birds (e.g. equids, poultry and captive wild birds) is challenging, as repellents may have short-term effects,

and the animals are usually kept outdoors in the transmission season. However, mosquito control in stables (e.g. screens on the windows, indoor adulticide treatment) may reduce the chances of vectorial transmission.

Safety of substances of human origin

There are approximately 15 million blood donors in EU countries, who donate 20 million units of blood each year [83]. Technological advances have led to a progressive increase in the application of tissue- and cell-based medical treatments in the EU, with about 390 000 of non-reproductive tissue and cells units distributed for transplantation per year. Kidney is the most frequently transplanted organ (16 890 in 2020), followed by liver (6 917 in 2020), heart (2 081 in 2020) and lung (1 740 in 2020).

The legal framework defining the quality and safety standards for SoHO is set out in the following EU Directives:

- blood and its components: Directive 2002/98/EC, also referred to as the European Blood Directive [84].
- tissues and cells: Directive 2004/23/EC, also referred to as the European Tissues and Cells Directive [85].
- organ transplantation: Directive 2010/45/EU, also referred to as the European Organs Directive [86].

Following an evaluation of these directives, the European Commission is planning to issue a Regulation on standards of quality and safety for substances of human origin intended for human application; this regulation will replace Directive 2002/98/EC and Directive 2004/23/EC [84,85].

The exchange of alerts of cross-border relevance on SoHO among national competent authorities is done though the rapid alert system for blood and the rapid alert system for tissues and cells, which are managed by the European Commission.

West Nile virus may be transmitted to SoHO recipients through blood, cells, tissue, and organ transfusion and/or transplantation. Viraemia occurs one to three days after infection, and usually lasts for eight to ten days, but in some cases may last for over a month [25]. Although individuals who have symptoms of WNV infection are excluded from donating, individuals who remain asymptomatic or who are in the incubation period may unwittingly provide infectious SoHO donations. In order to improve the safety of blood donations, the European Directive 2004/33/EC [87] requires deferral for 28 days of travellers returning from an area with ongoing transmission of WNV in humans. Alternatively, WNV ID-NAT can be carried out in place of deferral [88].

Complementary information on areas at risk, and on blood and blood component safety can be found in the document 'West Nile Virus and Blood Safety: Introduction to a Preparedness Plan in Europe' published by the European Commission in 2012 [89].

Immunisation

Most flaviviruses induce strong immune reaction in their respective hosts, which result in long-term (usually lifelong) immunity after wild-type virus infection and can provide considerable maternal immunity (including yolk immunity) in their offspring for a few weeks or months. Active immunisations against flaviviruses are applied as preventive measures for several diseases, including yellow fewer, tick-borne encephalitis, Japanese encephalitis, and recently dengue.

Vaccines against WNV have only been authorised and marketed for equids in the EU. Those are either inactivated, whole virus vaccines, or inactivated chimeric Yellow fever virus construct expressing the prM and E proteins of WNV, or attenuated, recombinant canarypox-based vaccine expressing the prM and E proteins of the virus [90]. These vaccines are proven to cross-protect against both WNV lineage 1 and 2 infection induced diseases in horses. Primary immunisation of foals can be started usually from the age of five to six months and annual boosters are recommended (optimally prior to the start of the transmission season). There are no WNV vaccines authorised for birds in the EU. Experimental, off-label use of equine vaccines in captive birds revealed no adverse effects, increase of neutralising antibody titres and no clinical disease of vaccinated birds [91,92]; however, a controlled clinical trial in falcons showed limited efficacy of two equine vaccines [93]. Canarypox-based, recombinant live vaccines might induce cutaneous avian pox in birds.

Several vaccines have been developed against WNV for humans, however none of them have been assessed in phase III clinical trials yet [94,95]. The development and approval of human WNV vaccines is hindered by safety concerns (e.g. the potential risk of antibody-dependent enhancement [96,97]), limitations of clinical trial set-ups and economic considerations (e.g. cost-efficacy of the authorisation process), although the use of vaccines is expected to be a useful tool to reduce West Nile virus disease burden in endemic areas [95].

There are no authorised vaccines against USUV infections. A study described the protective effect of a recombinant DNA vaccine against lethal challenge with USUV in alpha/beta interferon receptor deficient mouse model [98]. Another study reported the protective effect of an attenuated WNV - dengue virus 2 chimeric vaccine against USUV in the same mouse model. However, the efficacy and safety of these vaccine candidates in natural hosts (birds) has not been evaluated. Considering the rarity of USUV associated disease in mammals, the use of immunoprophylaxis in them is not considered to be justified.

Methods

Survey

A survey was developed to collect structured data from national representatives from the public health, animal health, and SoHO sectors in all 30 EU/EEA countries. The survey was conducted online using the EU survey tool and was sent out on 9 July 2021.

The questionnaire included 135 questions that were mainly closed-ended (with single, multiple choice, and Yes/No questions); complementary information was collected though open-ended questions. The questionnaire was divided into four sections:

- Public health: WNV and USUV epidemiological situation, surveillance of humans, and laboratory diagnosis of human cases;
- Animal health: WNV and USUV epidemiological situation, surveillance of animal hosts and mosquitoes, and laboratory diagnosis of animal cases;
- SoHO: WNV and USUV epidemiological situation, preventive measures to secure SoHO supply upon detection of an autochthonous WNV or USUV infection, preventive measures for returning travellers and laboratory testing;
- EU/EEA and global perspective and guidance.

The questionnaire ended with the possibility of providing concluding remarks.

Experts were invited to complete one or more of the first three sections of the questionnaire according to their sector of expertise; in addition, all experts were invited to answer the EU and global perspective and guidance section and/or provide concluding remarks. In the case of contrasting data reported by respondents from the same countries and/or between survey respondents and the literature review, a follow-up clarification was ensured.

Survey results were analysed through univariate and bivariate statistical techniques using Statistical Package for Social Science (SPSS) software (version 25.0.0.0) for Windows (SPSS Inc., Chicago, Illinois). Maps were created with the ECDC Map Maker (EMMa). Administrative boundaries and names shown on the maps do not imply official endorsement or acceptance by the European Union.

Literature review

The aim of the literature review was to complete and complement the information collected through the survey, i.e. for countries/sectors that did not reply to the survey or areas not addressed by the survey. Five online databases were searched: PubMed, Web of Science, Scopus, Embase and CAB abstracts, using two separate search strings (Annex 1). The search was restricted to papers published in English between 1 January 2012 and 31 December 2021. No restriction on the type of document or study was applied.

After removing duplicates, an eligibility assessment was performed, which consisted of validating that the paper matched the scope of the review.

Expert meeting

An online expert meeting was organised on 18 and 19 January 2022 (two half-days), with the aim of discussing the preliminary results of the questionnaire and the literature review, exchange information and practices about surveillance, prevention, and control of WNV and USUV infection and identify possible gaps in knowledge, and finally, promote intersectoral networking within and between EU/EEA countries. The minutes of the meeting and the summary reports of the chairs of breakout sessions have been used for resolving conflicting information from the survey and literature review, collect further details and updates from the country representatives, to improve the informative value and accuracy of the report.

Representatives from national veterinary, public health, and SoHO institutes and/or authorities from 13 EU/EEA countries (Austria, Croatia, Cyprus, France, Germany, Greece, Hungary, Italy, the Netherlands, Portugal, Romania, Slovenia and Spain) attended the meeting. In addition, representatives from ECDC/EFSA funded networks (VectorNet covering the entomological aspects and EVD-LabNet covering the laboratory diagnostic aspects) and the EU Commission were present. The list of institutes/authorities participating to the meeting is provided in Annex 2.

Results

General results

This report presents the data and information provided by the countries through the survey, retrieved by the literature review and discussed during the expert meeting as well as their analysis. Data were collected and analysed in 2021-2022.

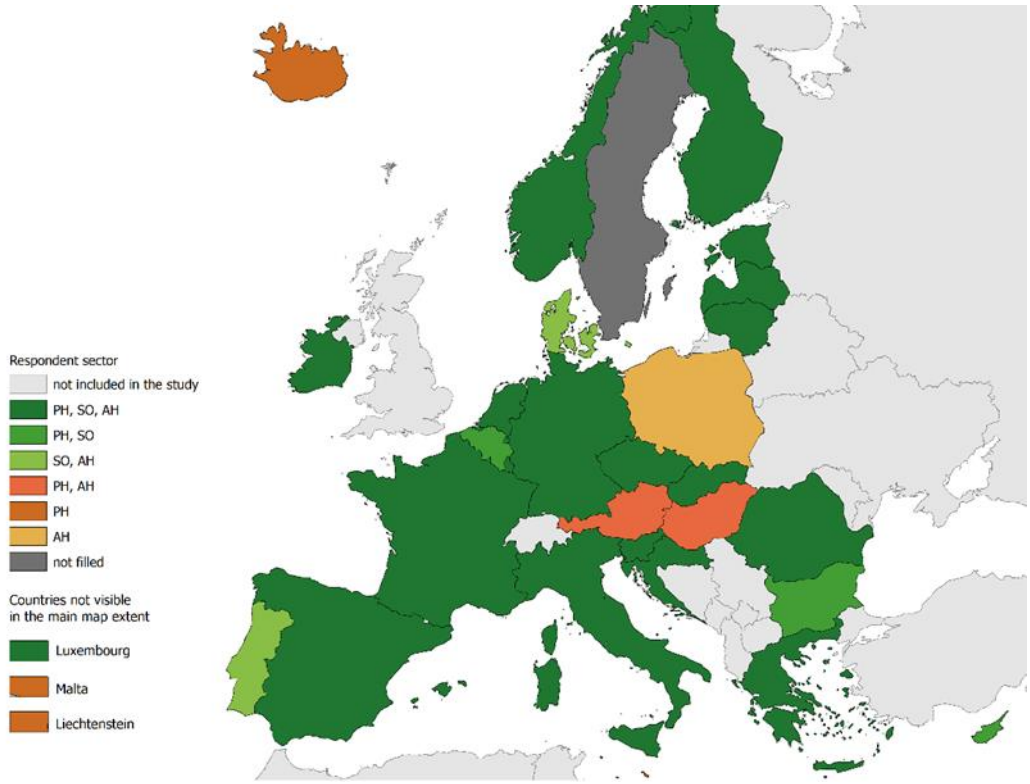
Data collected through the literature review is marked with an asterisk (*) and the reference is provided. The remaining data were collected through the survey and were amended or updated according to the input from the participants of the expert meeting.

Results of the survey

Thirty countries were invited to complete the survey, 29 provided a reply from at least one sector (Figure 1). There were 68 respondents in total: 24 respondents to the public health section, 24 respondents to the animal health section, 26 respondents to the SoHO safety section and 67 respondents to the EU and global perspective and guidance section.

The list of institutes and/or authorities that responded to the survey, per sector and per country, is available in Annex 3.

Figure 1. Geographical distribution of survey respondents per sector, 2022

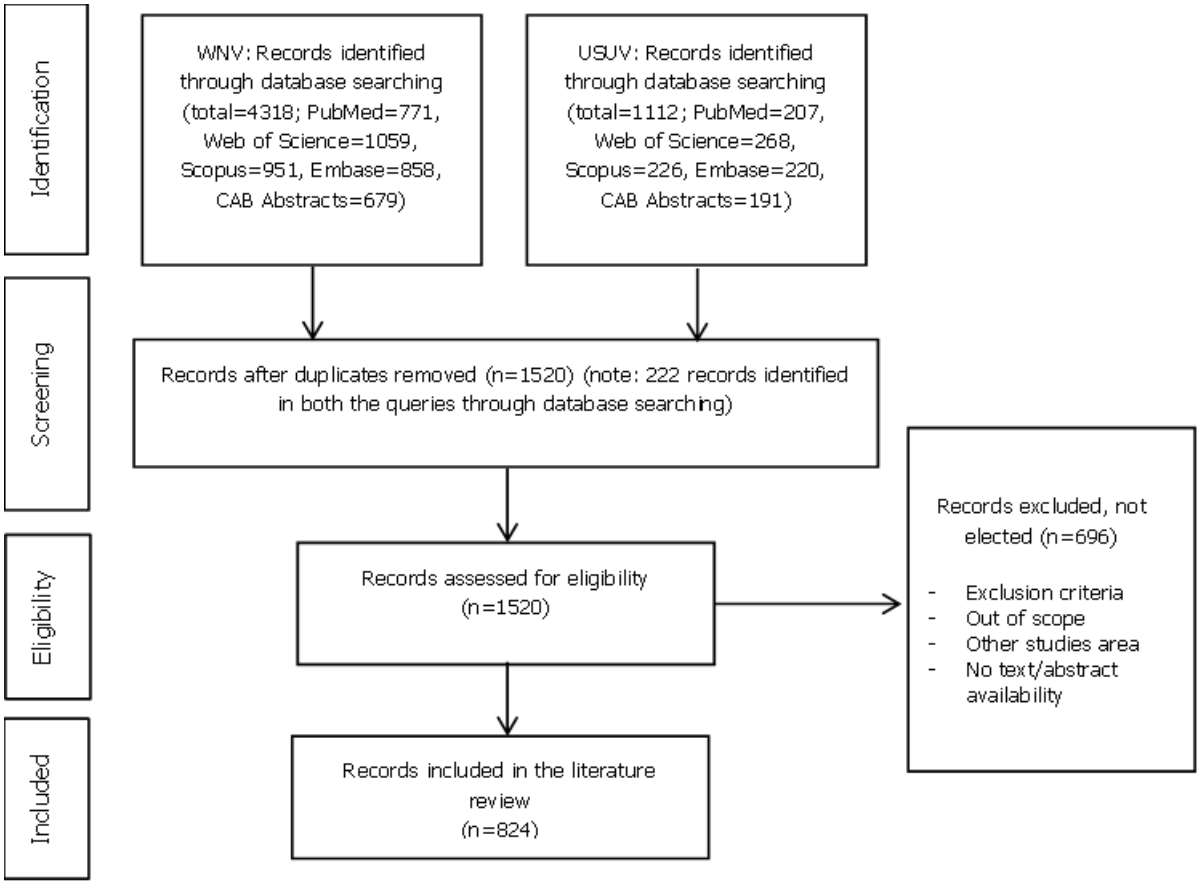


Note: PH=Public Health, SO=SoHO safety, AH=Animal Health

Results of the literature review

There were 5 430 papers identified (WNV = 4 318; USUV = 1 112). After removing duplicates and articles without an abstract or full-text, 1 520 papers were screened and assessed against the eligibility criteria. The PRISMA flow chart is provided in Figure 2. As a result, data from 824 papers (abstract or full-text) were extracted and used for this study.

Figure 2. PRISMA flow chart of the literature review



Most of the selected articles were about WNV (579 papers); 23 were related to USUV and 222 papers were about WNV and USUV. Two hundred and twenty-nine out of 824 articles contained general background information on WNV and USUV (i.e. biology, ecology, transmission, epidemiology, diagnosis, disease, therapy, vaccine, role of animals, and mosquitoes) and 595 papers referred specifically to one or more of the EU/EEA countries; 91 papers presented the European perspective and the other papers presented a country-specific perspective: Italy (n=144; 24.2%), Greece (n=81; 13.6%), Spain (n=53, 8.9%), Germany (n=51, 8.6%), Romania (n=30, 5.0%), France (n=29, 4.9%), Croatia (n=27, 4.5%), Hungary (n=27, 4.5%), Austria (n=26, 443%), the Netherlands (n=21, 3.5%), Czechia (n=19, 3.2%), Belgium (n=15, 2.5%), Poland (n=11, 1.8%), Portugal (n=11, 1.8%), Slovakia (n=11, 1.8%), Bulgaria (n=10, 1.7%), Slovenia (n=9), Cyprus (n=6), Denmark (n=2), Ireland (n=2), Iceland, Luxembourg and Sweden (n=1 each). No article was found regarding Finland, Estonia, Latvia, Liechtenstein, Lithuania, Malta or Norway.

Results of the expert meeting

The first and the second day of the meeting were attended by 77 and 69 participants, respectively.

The main topics discussed during the meeting were: i) EU case definitions; ii) Surveillance plans; iii) Disease notification and reporting; iv) Laboratory diagnosis. Both WNV and USUV were explored, and these topics were discussed in ad hoc breakout rooms dedicated to infection in humans, animals, and mosquitoes. Participants of the meeting were informed on the results of the survey and the literature review. During the second day a plenary discussion was held on SoHO safety and selected experts from France, Hungary, and Italy presented the experiences in their respective countries. A final session dedicated to knowledge gaps and proposals was also organised. Information gathered during the meeting was considered in this technical report.

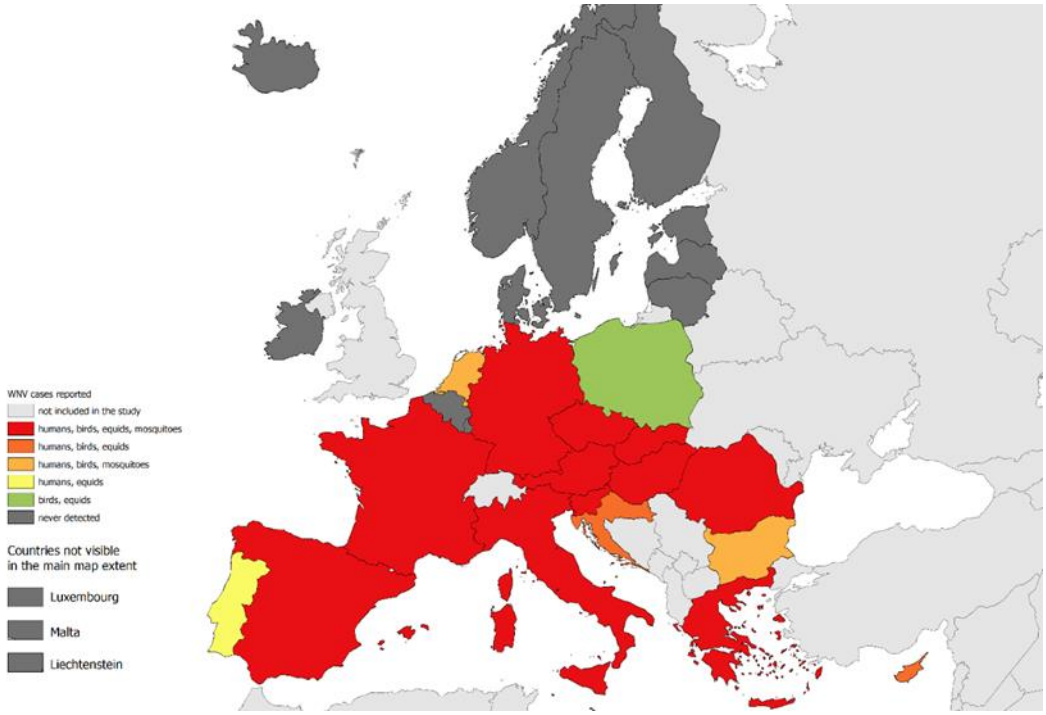
Epidemiological situation in the EU/EEA

Epidemiological situation of West Nile virus infection

In humans

During the preceding decade (2012-2021), sixteen EU/EEA countries reported a total of 3 632 cases of WNV infection, with most infections reported by Italy and Greece, representing 62% of all cases (Italy: n=1 201 and Greece: n=1 058) [99] (Figure 3). Austria, Croatia, Czechia, Germany, Hungary, Romania and Spain reported autochthonous human cases of WNV infection in consecutive years, and/or annual incidence ≥0.1/100 000 population in the affected Nomenclature of Territorial Units for Statistics (NUTS)-2/3 areas in some years. Sporadic WNV infections occurred in Bulgaria* [99], Cyprus* [99], France, the Netherlands, Portugal* [99], Slovakia and Slovenia, where few autochthonous human cases of WNV infection have been reported so far, with annual rates lower than 0.1/100 000 population in the affected areas (NUTS-2/3). A sharp increase in WNV infections was reported in the EU/EEA in 2018 (n=1 551 cases, compared to the average annual 169 autochthonous human cases between 2012 and 2017). Autochthonous human cases of WNV infection have not yet been diagnosed in Belgium, Denmark, Estonia, Finland, Iceland, Ireland, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Norway, Poland* [100 101] and Sweden. In affected countries, human WNV infections occurred in rural and urban areas, except in Slovakia and Slovenia where infections occurred in rural areas only, and in Austria where infections occurred only in urban areas.

Figure 3. West Nile virus infections in humans, animals (birds-equids) and mosquitoes in the EU/EEA, 2012-2021



In animals

During the same decade (2012-2021), seventeen EU/EEA countries (Austria, Bulgaria* [102], Croatia, Cyprus, Czechia, France, Germany, Greece, Hungary, Italy, the Netherlands, Poland* [100,101], Portugal, Romania, Slovakia, Slovenia and Spain) reported animal cases of WNV (equids and/or birds) (Figure 3). Fourteen countries reported WNV in equids (i.e., Austria, Croatia, Cyprus, Czechia, France, Germany, Greece, Hungary, Italy, Portugal, Romania, Slovakia, Slovenia and Spain). Fourteen countries (i.e., Austria, Croatia, Cyprus, Czechia, France, Germany, Greece, Hungary, Italy, the Netherlands, Poland* [103,104], Romania, Slovakia and Spain) detected WNV in resident/captive wild birds, eight countries (i.e., France, Germany, Hungary, Italy, the Netherlands, Romania, Slovenia and Spain) in migratory birds, and three countries (i.e. Croatia, France and Spain) in sentinel birds. Two WNV outbreaks in birds in 2020 were notified by Bulgaria to ADIS [102].

Among birds, WNV cases have been most frequently detected in wild or captive raptors (e.g., eagles, goshawks, sparrow hawk, Harris hawks, Gyrfalcons, Red-footed falcons, owls) scavengers (e.g., magpies) aquatic birds (e.g., coots, geese, storks) and songbirds. Occasionally WNV was also detected in several other bird species, including peacocks and canaries in Greece and in zoo birds in France*, Germany* and Slovenia* (i.e. orange-winged

amazons, marabou stork, great grey owl, guineafowl, Eurasian eagle-owls, barn and snowy owls and pelicans) (Figure 3) [37,105-107].

In mammalian animals WNV infections have most frequently been diagnosed in equids, however the virus and/or antibodies against it have been detected in cats and dogs (Croatia, Greece, Italy* [108], France* [40], Spain* [109] and Slovenia* [44]); cattle (Croatia, Greece* [110]); in different rodents [111-113]; wild boars, red foxes and Iberian pigs (Italy*, Poland*, Romania* and Spain* [114-117]), brown bears (Slovakia* [118]) and fallow, red and roe deer, red sheep, European hares, and mouflons (Czechia* and Spain* [119,120]). WNV antibodies have been detected in zoo animals in Spain* (i.e. African elephants, Barbary macaques, giant pandas, plains zebras and Thomson's gazelles [43]), France* (i.e. dama gazelles) [37] and Slovenia* (i.e. guinea pigs, rabbits, grey and north-western wolves, wild boars and red foxes) [105].

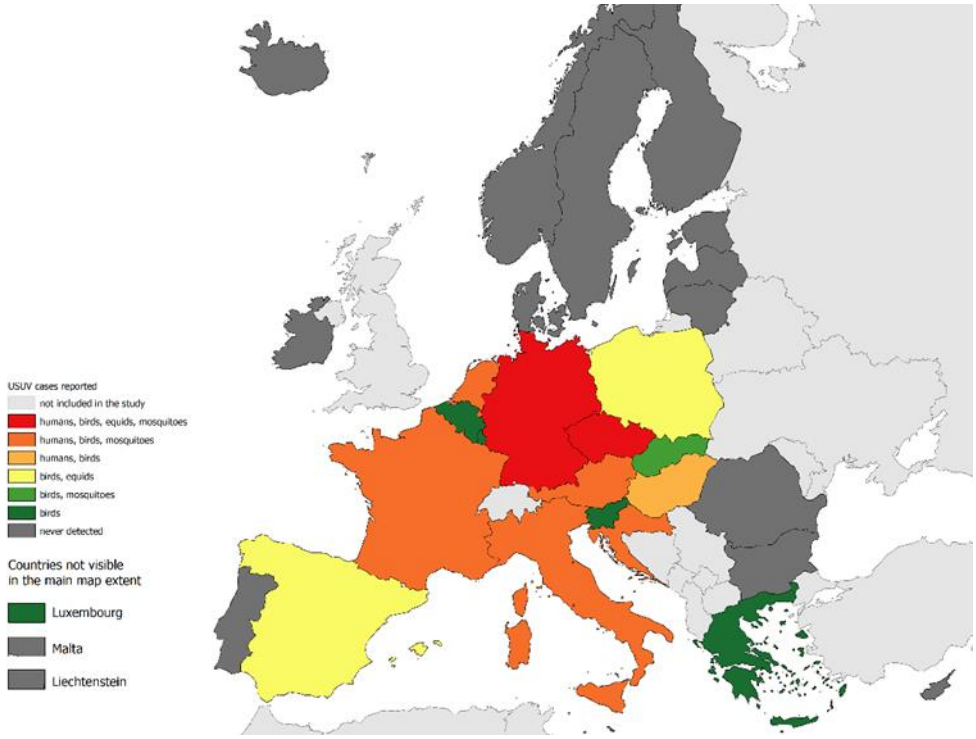
West Nile virus has been detected in at least nine mosquito species in thirteen countries, including Austria*, Bulgaria*, Czechia*, France*, Germany*, Greece*, Hungary*, Italy*, the Netherlands*, Romania*, Slovakia*, Slovenia* and Spain* (Figure 3). Most frequently *Culex pipiens s.l.* was found positive for WNV, however the virus was also detected in *Culex modestus, Culex perexiguus, Anopheles maculipennis s.l., Ochlerotatus cantans, Ochlerotatus caspius, Ochlerotatus excrucians, Aedes vexans* and *Coquillettidia richardii* mosquitoes [44,121-133].

Epidemiological situation of Usutu virus infection

In humans

From 2012 through 2021, autochthonous human cases of USUV infection have been detected in Austria, Croatia, Czechia* [134], France, Germany* [135,136], Hungary [137], Italy, and the Netherlands (Figure 4). These eight countries reported a total of 104 cases of acute USUV infection, most of which were reported by Italy (n=56, 54%), Austria (n=26, 25%) and the Netherlands (n=11, 11%). Among the 104 detected, 11 cases (11%) had neuroinvasive symptoms (reported by Croatia*, Czechia*, France*, Italy* and Hungary* [30,32,33,134,137,138]), and the remaining cases were either asymptomatic blood donors, or individuals with unspecific symptoms (e.g. fever) or no information about the cases was available.

Figure 4. Usutu virus infections in humans, animals (birds-equids) and mosquitoes in the EU/EEA, 2012-2021



In animals

Fifteen out of 30 EU/EEA countries reported USUV circulation among animals (Austria, Belgium* [139 140], Croatia, Czechia, France, Germany, Greece* [141], Hungary, Italy, Luxembourg, the Netherlands, Poland* [104], Slovakia, Slovenia and Spain* [142,143]) (Figure 4). USUV has been detected mainly in birds; in resident/captive wild birds in 11 countries (Austria, Belgium* [140], Croatia, Czechia, France, Germany, Greece* [141], Italy, Luxembourg, the Netherlands and Spain* [142-144], and in migratory birds in 10 countries (Austria, France, Germany, Hungary, Italy, the Netherlands, Poland, Slovenia, Slovakia and Spain* [142]). Czechia, Germany, Poland* [104,145] and Spain* [146,147] reported USUV antibodies in equids.

In birds, the most affected species is the Eurasian blackbird; however, USUV infections of different owl species, sparrows, and several other songbirds were also reported. Occasionally, USUV was detected in zoo birds in Germany* (i.e. marabou stock, ruddy shellducks, red-breasted geese, Humboldt penguins, laughing kookaburras, steamer ducks, greater flamingos, snowy owls, Ural owls, white storks, Egyptian vultures, and Eurasian eagle owls), France* (i.e. Abyssinian ground hornbills, common peafowls, emus, scarlet ibis, and greater rheas) and Slovenia* (i.e. pelicans, Eurasian eagle-owls, barn and snowy owls) [37,38,105,148].

In non-avian hosts, USUV (or antibodies against it) has been detected in several species, including dogs (Italy* [42] and Slovenia* [44]); squirrels (Italy* [111]); bats (Belgium*[139], Germany* [39]); green lizards (Slovakia; wild boars, roe and red deer, and zoo mammals (i.e. Asian lions, maned wolves, Iberian wolves, grey and northwestern wolves, African wild dogs, chimpanzees, common elands, giant pandas, Malayan tapirs, white rhinoceros, guinea pigs, rabbits, and red foxes) (France*, Spain* and Slovenia* [37,38,41,43,105,119]).

Usutu virus has also been detected in mosquitoes in Austria, Croatia, Czechia* [149,150], France, Germany, Italy, the Netherlands and Slovakia.

Laboratory diagnosis

Laboratory diagnosis of West Nile virus infection

In humans

Laboratory diagnosis of WNV infection was conducted in all age groups in all countries except Romania, where testing was indicated only for persons aged over 15 years. Clinical criteria for testing during the surveillance period were unexplained fever, encephalitis, meningitis, acute flaccid paralysis, and polyradiculoneuritis (similar to Guillain-Barré syndrome). In Germany rash was included among the criteria for WNV testing; in Croatia fever was not a criterion for testing.

EU/EEA countries had the laboratory capability to diagnose WNV infections; this was done not only by the National Reference Laboratories (NRLs), but also by regional laboratories, local laboratories, hospital laboratories, research laboratories and laboratories associated with blood donation centres. Laboratory confirmation of WNV infection was done by the NRL in all EU/EEA countries. Regional laboratories were also performing confirmation testing for WNV infection diagnosis in Italy, the Netherlands and Romania.

Laboratory methods for diagnosing WNV infection included viral isolation from biological samples, viral nucleic acid detection in biological samples, and detection of WNV-specific antibodies in serum and/or CSF. Due to cross-reactivity of antibodies induced by WNV infection with other flaviviruses, a positive serology test result requires confirmation by neutralisation assays. Most EU/EEA countries (Austria, Belgium, Croatia, Czechia, France, Greece, Hungary, Ireland, Italy, Latvia, Liechtenstein, the Netherlands, Slovenia, Spain) routinely performed virus neutralisation assays to rule out possible serological cross-reactions with other flaviviruses. In France, Greece, the Netherlands and Spain, neutralisation assays were done only to confirm the first probable cases of WNV in the region (NUTS-2/3) but not to confirm any subsequent case. Besides WNV, TBEV and/or USUV were commonly included in the simultaneous neutralisation tests.

Most EU/EEA countries (67%) routinely determined WNV lineage in positive biological samples, comprising human, animal and mosquito samples (43% of countries), if the applied diagnostic method allowed (i.e., molecular detection). Information about WNV lineage was generally included in case notification reports.

In animals

West Nile virus diagnosis in animals was made by NRL in 21 countries. In addition to the NRLs, regional laboratories were involved in WNV diagnosis in France, Germany, Italy and Spain, while in Greece, Luxembourg and Slovakia local laboratories contributed to WNV diagnosis as well. Austria, Germany and Slovakia indicated involvement of private laboratories or other public institutes, while Norway relied on the European Union Reference Laboratory in France or the WOAH WNV reference laboratory in Italy.

Detection of WNV nucleic acid in blood, tissue, and CSF was performed by 21 and 20 countries for birds and equids, respectively. IgM indirect ELISA was used (n=21) to identify WNV antibody response in alive equids. Flavivirus antibody response was detected using IgG competitive ELISA by 14 countries for birds and by 18 countries for equids.

West Nile virus case confirmation was mostly done using WNV-specific PCR and sequencing. WNV neutralising antibody titres determination, simultaneous neutralization tests with different flaviviruses, or four-fold or greater increase in virus-specific quantitative antibody titres in paired sera were also performed as confirmatory tests by Austria, Croatia, Czechia, France, Germany, Italy, Luxembourg, and Spain. In some countries other tests were reported to be specifically used (competitive ELISA to detect anti-pr-E IgG antibodies in Greece and Italy; simultaneous seroneutralisation testing to discriminate WNV and USUV infections in poultry in Italy).

Estonia, Finland, Ireland, Norway, and Romania specified that they did not perform confirmatory laboratory tests to detect WNV in animals. Denmark and Ireland sent the positive sample to the EU Reference Laboratory (The French Agency for Food, Environmental and Occupational Health & Safety (ANSES), France) for confirmation.

West Nile virus infections in mosquitoes were tested by molecular methods in 19 countries, sequencing in 17 countries, and viral isolation in three countries (Estonia, Italy and Lithuania).

Laboratory diagnosis of Usutu virus infection

In humans

Usutu virus infection can be diagnosed by virus isolation, detection of viral RNA in biological samples, or detection of USUV-specific antibodies (confirmed by neutralisation assay).

In contrast to laboratory diagnosis of WNV infections, for which several in vitro diagnostic assays with approved CE marking [151] were commercially available for both molecular and serology testing, the diagnosis of USUV infections largely relied on in-house assays developed by NRL. The laboratory tests used in EU/EEA countries to detect USUV included pan-flavivirus PCR and sequencing in blood, tissue, CSF, and other body fluids (eight countries; Czechia, Germany, Greece, Italy, Liechtenstein, the Netherlands, Slovenia, and Spain), USUV-specific PCR (six countries; Croatia, France, Hungary, Italy, Liechtenstein, and the Netherlands), detection of antibody response (ten countries; Croatia, France, Germany, Greece, Hungary, Italy, Latvia, Liechtenstein, the Netherlands, and Spain), and virus isolation from biological samples (three countries; Hungary, Italy, and Liechtenstein). Usutu virus-specific PCR and viral genome sequencing (Croatia, Czechia, France, Germany, Greece, Hungary, Italy, Liechtenstein, the Netherlands, and Spain), and simultaneous neutralisation tests with different flaviviruses (Croatia, Czechia, France, Germany, Hungary, Italy, Liechtenstein, the Netherlands, and Spain) were used as second line tests to confirm positive results obtained with first line broad-range or cross-reactive tests. In countries where patients were not tested for USUV, like Romania and Finland, human cases of USUV could be diagnosed in the laboratory via cross-reactive PCR tests and serological tests used for other flavivirus infections (i.e., WNV, TBEV). Usutu virus infection was diagnosed and confirmed by NRLs in most countries. In Italy and Lithuania, regional laboratories were also in charge of confirming USUV infections. Usutu virus could also be diagnosed by other laboratories (i.e., academic, research and private laboratories) in Estonia, Greece, and Norway.

In animals

Laboratory diagnosis of USUV in animals was performed differently among countries. As in the case of WNV, most countries (n=18) indicated that the NRLs were in charge of USUV diagnosis. However, in Italy and the Netherlands, NRLs were supported by regional reference laboratories, while in Austria and Slovakia, local or private laboratories were also involved. In Germany, national, regional and local laboratories were all responsible for USUV diagnosis. In Poland and Luxembourg, only local laboratories were in charge of USUV diagnosis. Estonia, Finland, Greece and Norway reported the absence of laboratories for USUV detection.

Similarly to WNV, the laboratory tests used in EU/EEA countries to identify USUV infection in animals included the detection of USUV-specific nucleic acid in blood, tissue and/or CSF (n=13 countries), and the detection of flavivirus-specific nucleic acid in blood, tissue and/or CSF (n=11 countries).

In animals, USUV-specific PCR and sequencing was performed by Austria, Croatia, France, Germany, Portugal, Slovakia, and Slovenia; only USUV-specific PCR by Italy, Lithuania, Luxembourg and, the Netherlands while sequencing of pan-flavi RT-PCR products by Hungary, Latvia, Poland, and Spain. Viral isolation was used only by France, Portugal, Slovakia, and Spain as confirmation method.

Usutu virus neutralizing antibody titres were determined in Croatia, France, Germany, and Slovakia, while simultaneous neutralisation tests involving related flaviviruses (e.g., WNV and TBEV) were performed for confirmation in animal samples in Croatia, Czechia, France, Germany (only in equids), and Italy (not TBEV).

Cyprus, Estonia, Finland, Greece, Norway, and Romania specified that they did not perform confirmatory laboratory tests to detect USUV in animals while Denmark and Ireland send the positive sample to the EU Reference Laboratory (ANSES, France) for confirmation.

Surveillance

Surveillance of West Nile virus infection

In humans

Human WNV infection is one of the diseases notifiable at the EU/EEA level during the period covered in this study. Sixteen EU/EEA countries used the EU case definition for WNV infection in humans; while 13 countries (i.e., Czechia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, the Netherlands, Norway, Romania, Slovenia and Spain) have developed a different WNV case definition as detailed in Annex 4. The main differences with respect to the EU case definition was the use of urine and other biological samples for WNV isolation and nucleic acid detection for laboratory case confirmation in the WNV case definition applied in some countries (Italy, Greece, Germany, the Netherlands).

All EU/EEA countries conducted passive surveillance, except Czechia, Greece and Spain that conducted active surveillance. In passive surveillance, information on WNV cases was reported from hospitals and reference laboratories. In active surveillance, healthcare facilities were regularly contacted to actively collect information on newly diagnosed cases [7]. WNV surveillance was conducted all year round, covering the entire national territory, except in Romania, where WNV surveillance was conducted seasonally, from May to October. In Italy and France, the national territory was divided into various areas for which the surveillance plan was adapted [152] according to the WNV risk classification of the respective area. In Croatia, Czechia, France, Germany and Italy the surveillance of WNV was part of the surveillance plan for arboviruses.

Cases were reported at time of the recognition or diagnosis in the majority of the countries (n=17; Austria, Croatia, Czechia, Finland, France, Germany, Greece, Hungary, Italy, Liechtenstein, Lithuania, Luxembourg the Netherlands, Norway, Romania, Slovakia and Spain), weekly in Ireland and Slovenia, monthly in Malta, and yearly in Belgium. The information reported at the time of notification of human cases of WNV infection included laboratory data (19 countries) and/or epidemiological data (15 countries) and/or clinical data (15 countries).

In Austria, Croatia, Czechia, France, Germany, Greece, Italy, the Netherlands and Spain, surveillance in humans was integrated with surveillance in animal hosts and/or mosquitoes.

In animals

WNF is one of the diseases notifiable in equids and birds during the period covered by this study, and must be reported according to the Terrestrial Code of the WOAH and EU Regulation 2018/1882 of 3 December 2018 [80].

In 24 out of 30 EU/EEA countries, the criteria defined in the Terrestrial Code of the WOAH for West Nile Fever occurrence was used in both birds and equids. Twenty-one out of the 30 EU/EEA countries had a surveillance system in place to detect WNV infections in animals; there was no surveillance system in place in Lithuania, Norway and Poland. No information was available for six countries (Belgium, Bulgaria, Iceland, Liechtenstein, Malta and Sweden).

Twenty-one countries carried out passive surveillance in animals, capturing symptomatic (neurological signs) or dead animals; of these, 14 countries conducted active surveillance in equids and in birds. A few countries performed active case finding: Croatia, Cyprus and Spain in both sentinel equids and poultry; Czechia, Greece and Romania only in sentinel equids; while Denmark, Germany and Italy in sentinel poultry. Besides sentinel poultry, different kinds of birds were investigated during surveillance: breeding birds (e.g., ducks, poultry, pheasants, geese, and ostriches); wild (non-domesticated) and captive birds (i.e. from zoos, wildlife parks and/or rehabilitation centres). In Czechia, Estonia, Ireland and Latvia, birds were not included in the surveillance system. Moreover, equids were tested for WNV in response to human or other animal cases in Czechia, Denmark, Germany, Italy, Portugal, Romania and Slovenia. In the Netherlands information on WNV in birds was collected through project-related monitoring.

Surveillance of Usutu virus infection

In humans

Human USUV infection was not a notifiable disease at the EU level. However, aseptic (viral) encephalitis without accurate aetiological diagnosis was notifiable in several EU countries, e.g. in Greece.

Four countries (Greece, Germany, Italy and Norway) developed a case definition for USUV infection in humans, as specified in Annex 5. Italy established a specific surveillance system for USUV with a view to identify conditions that may favourable the risk of USUV transmission and activate appropriate control measures; USUV surveillance was aiming at detecting viral circulation among target bird species and mosquitoes and monitoring the impact of USUV infection in humans [153]. USUV surveillance was in place all year round and is strengthened between May and November in areas classified as ‘at risk’.

In animals

Animal USUV infection was not a notifiable disease at the EU level.

Seven out of 30 EU/EEA countries implemented a USUV surveillance system in animals (Denmark, France, Germany, Hungary, Italy, Luxembourg and Spain). France and Luxembourg conduct USUV surveillance on wild birds, and Denmark, Germany and Italy perform surveillance on domestic and wild birds. In Finland and the Netherlands, USUV surveillance was carried out through specific research projects.

In Luxembourg and Germany all birds testing positive for USUV by RT-PCR were considered as animal USUV cases. Hungary was in the process of developing a case definition. Italy had a specific surveillance case definition of USUV infection in birds. USUV cases included positive ELISA samples confirmed by the National Reference Laboratory using serum neutralisation testing in free-ranging or rural poultry farms, in animals under six months of age. The definition also included positive RT-PCR tests in mosquito pools or organs/blood samples of birds (captured or found dead) detected by the competent territorial authority.

Viral antigen or nucleic acid detection of USUV was performed in encephalitic/dead birds in the seven countries conducting USUV surveillance, and in encephalitic/dead equids in France and Spain. Serosurveillance was carried out in birds in Denmark, France and Germany; in wild boars, roe deer and cattle in France; and in equids in Italy.

Austria, Croatia, Czechia, Poland, Slovakia and Slovenia did not carry out specific surveillance activities in animals related to USUV, although USUV was detected in these countries. Cyprus, Estonia, Greece, Ireland, Latvia, Lithuania, Norway, Portugal and Romania did not carry out specific surveillance activities related to USUV and no cases were reported from these countries. No information on surveillance activities was available from the remaining six countries.

Surveillance of West Nile virus and Usutu virus in mosquitoes

Eight out of the 30 EU/EEA countries had national surveillance activities in place to detect WNV and/or Usutu virus in mosquito populations (Austria, Croatia, Denmark, Germany, Greece, Italy, the Netherlands and Spain). In France, Slovakia and Slovenia the surveillance activities for WNV/USUV detection in mosquitos was conducted through recurring research projects, while Bulgaria*, Cyprus, Czechia*, Finland, Hungary*, Portugal*, Romania* and Sweden did so through occasional research projects [122,123,127,130,150,154-157]. Fourteen countries tested mosquitoes for both viruses while Czechia, Romania and Spain test only for WNV.

Eleven countries (Belgium, Estonia, Iceland, Ireland, Latvia, Liechtenstein, Lithuania, Luxembourg, Malta, Norway and Poland*) [104] did not collect data on WNV/USUV infection in mosquitoes.

Molecular detection, sequencing, and viral isolation to detect WNV/USUV infection in mosquitoes were performed in 19, 16 and four EU/EEA countries, respectively. WNV lineage in positive mosquito pools was routinely determined in Austria, Croatia, Czechia, France, Germany, Greece, Italy, the Netherlands, Slovakia, Slovenia and Spain.

In mosquito surveillance, all captured specimens of adult female mosquitoes were usually analysed (Austria, Denmark, Germany, the Netherlands, Romania, Slovakia, Slovenia and Spain). In France and Greece only *Culex pipiens* females were routinely analysed, while in Croatia, Czechia and Italy, *Culex pipiens* and certain other species were screened. Specifically, in Croatia *Aedes albopictus*, in Czechia *Culex modestus*, and in Italy *Aedes albopictus*, *Culex modestus*, *Ochlerotatus caspius*, *Culex spp.*, *Anopheles maculipennis s.l., Culiseta annulata* and *Ochlerotatus detritus* were also tested.

Integrated surveillance system

An integrated surveillance system for WNV surveillance, defined as a surveillance system for the early detection of viral circulation through targeted surveillance of humans, animals, and mosquitoes, was implemented in Austria, Croatia, Czechia, France, Germany, Greece, Italy, the Netherlands and Spain. Surveillance plans in Germany and Spain were partially integrated since they include surveillance in humans and animal hosts, but not in mosquitoes. France implemented mosquito surveillance only in the surroundings of positive WNV cases. Some countries also included additional vector-borne viruses into their integrated WNV surveillance: e.g., USUV in Croatia, Germany and Italy; TBEV in Croatia, Czechia, France and Italy; Toscana virus in France and Italy; yellow fever virus, JEV and Rift Valley fever virus in Italy.

Prevention and control of West Nile virus and Usutu virus infections

Prevention and control measures for SoHO supplies

West Nile virus seasonal NAT screening of blood was used in most EU/EEA countries (Austria, Croatia, Cyprus, Czechia, France, Germany, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal, Romania, Slovakia, Slovenia and Spain).

From 2016 through 2020, the following countries reported WNV-positive blood donations: Austria (four cases in 2016, one in 2017, and six in 2018), Croatia (three cases in 2018), Germany (nine cases in 2020), Greece (11 cases in 2018, five in 2019, and seven in 2020), Italy (31 cases in 2016, 25 in 2017, 96 in 2018, eight in 2019, and 37 in 2020), Romania (one case in 2016, one in 2017, and two in 2019) and Spain (five cases in 2020) (Table 2). Among the countries that specified the number of NAT performed on blood donations, on average, 6% of donations were screened. Croatia was screening the highest proportion of blood donations (23%), followed by Italy (15%), Cyprus (14%) and Slovenia (13%).

Table 2. West Nile virus- and USUV-positive blood donations identified from 2016 through 2020

Country (2016-2020)	Collected donations	Screened donations (%)	WNV positive donations	USUV positive donations
Croatia	957 579	217 047 (23%)	3	Not available
Cyprus	211 885	29 850 (14%)	4	Not available
France	14 635 993	90 223 (1%)	0	0
Greece	2 641 081	215 910 (8%)	23	0
Ireland	700 058	12 705 (2%)	0	Not available
Italy	14 924 494	2 241 555 (15%)	197	6
Luxembourg	113 338	3 920 (3%)	0	Not available
The Netherlands	3 635 827	42 040 (1%)	0	10
Romania	2 008 110	65 146 (3%)	4	0
Slovakia	1 113 021	12 500 (1%)	0	Not available
Slovenia	437 379	56 581 (13%)	0	Not available
Spain	8 384 749	42 321 (1%)	5	Not available
Sub-Total	**49 763 514**	**3 029 798 (6%)**	**236**	**16**
Austria	2 752 026	Not available	11	29
Czechia	5 871 323	Not available	0	0
Germany	32 531 069	Not available	9	Not available
Portugal	1 570 435	Not available	Not available	Not available
Total	**92 488 367**	**-**	**256**	**45**

Data source: Survey questionnaire

Three of the responding countries also reported the detection of USUV in donations (Table 2). Overall, 45 USUV positive blood donations have been reported in EU/EEA countries (of which 29 in Austria, with a peak of 20 in 2018, six in Italy in 2018, and ten in the Netherlands, with eight in 2018).

Lithuania used WNV serology testing to screen cells, tissues, and organs. France screened organs using WNV NAT and WNV IgM testing in the sera of donors. France, Luxembourg, Portugal, Slovakia and Spain specified that they applied pathogen inactivation procedures for platelets and plasma.

Detailed information on the donations tested for WNV per type of donations and countries is provided in Figure 5.

Figure 5. Overview of the countries performing WNV screening per SoHO component

Donors of blood and blood components:

- *Blood, plasma and other blood components*: Austria, Belgium, Czechia, Germany, Ireland, Luxembourg, the Netherlands, Portugal and Romania.
- *Blood, plasma and other blood components including platelets*: Croatia, Cyprus, France, Greece, Ireland, Italy, Slovakia, Slovenia and Spain.

Donors of stem cells:

- *Bone marrow stem cells*: Austria, Belgium, Croatia, France, Germany, Italy, Slovenia and Spain.
- *Peripheral blood stem cells*: Austria, Belgium, Croatia, France, Germany, Italy, Slovenia and Spain.
- *Cord blood stem cells*: Belgium, Croatia, France, Germany, Ireland, Italy, Slovenia and Spain.

Donors of tissues:

- *Bone*: Belgium, Croatia, France, Italy, Slovenia and Spain.
- *Tendons*: Belgium, Croatia, France, Slovenia and Spain
- *Corneas*: Belgium, Croatia, France, Slovenia and Spain.
- *Skin*: Belgium, Croatia, France, Ireland, Slovenia and Spain
- *Heart valves*: Belgium, Croatia, France, Ireland and Spain

Organ donors:

- *Deceased organ donors*: Croatia, France, Italy, Portugal, Slovenia and Spain
- *Living organ donors*: Croatia, France, Italy, Slovenia and Spain

Donors of reproductive tissues:

- Donors of reproductive cells are not screened for WNV in EU/EEA countries.

The differential diagnosis between WNV and other flavivirus infections (e.g. USUV) was part of routine practice in WNV NAT-positive SoHO donors in seven countries (Austria, Czechia, France, Germany, Italy, the Netherlands and Slovakia). Differential diagnosis was performed through virus-specific PCR and, for some countries also through sequencing of amplification products.

None of the EU/EEA countries implemented specific SoHO safety measures for USUV infection, except the deferral of proven flavivirus positive donations. However, measures applied to secure the safety of blood and other SoHO donations in relation to the risk of WNV infection, such as deferral of potentially exposed blood donors, pathogen inactivation procedures, haemovigilance measures (post–transfusion monitoring and reporting of transfusion-transmitted infections), and WNV NAT testing, could mitigate the likelihood of donor-derived USUV transmission, at least in the areas of co-circulation of the two viruses.

In countries with WNV transmission, SoHO prevention measures were either initiated on a fixed date (e.g. 1 June) in Austria, Bulgaria, Croatia, Germany, and the Netherlands, or were triggered by an epidemiological finding: In Czechia, France, Greece, Italy, Luxembourg, Portugal, Romania, Slovakia, Slovenia and Spain, the trigger was the detection of a laboratory-confirmed human case of WNV infection; in addition, in Italy and Slovenia, the detection of a confirmed equine case with positive IgM antibodies and/or a positive molecular test, a confirmed case in a bird (any species, resident, migratory or sentinel), or virus detection in mosquitoes would also triggered the implementation of SoHO measures.

In most countries, preventive measures (WNV NAT screening or donor deferral) were applied until a pre-defined date. Dates mentioned by countries were 31 October or 30 November. The duration of implementation can be prolonged in case of evidence of continued WNV circulation. In Czechia, Greece, Romania and Slovakia, the end date was not pre-defined but was based on indicators providing evidence of virus circulation.

In terms of travellers returning from WNV affected areas, Austria, Cyprus, Germany, Ireland, Italy, Luxembourg, the Netherlands, Romania and Spain specified that they applied WNV blood screening according to the Commission Directive 2014/110/EU [88]. Other EU/EEA countries applied deferral of donations.

EU/EEA countries used ECDC weekly epidemiological updates/maps to define a risk area in other EU/EEA countries and enlargement countries. Some EU/EEA counties also produced national risk maps e.g., Germany, Greece, Italy, the Netherlands and Slovakia. EU/EEA countries (n=15) also used maps/sources for countries outside the EU/EEA (e.g., Center for Disease Control (CDC), Health Canada, Pan American Health Organization (PAHO), Fitfortravel UK).

Mosquito control measures and other preventive actions

Mosquito control strategies were highly diverse among the EU/EEA countries. Eleven EU/EEA countries performed mosquito control actions and/or citizen education programmes on mosquito control. Among these, Croatia, Czechia, France, Germany, Greece, Italy, Slovakia and Spain performed both. Malta focused only on citizen education programmes, and Cyprus, Hungary and Romania solely on control actions. Citizen education programmes provided information on mosquitoes, bite prevention, and breeding. In some countries (e.g. Germany, Italy, Spain) tools/apps were available to enable citizens to report the presence of mosquitoes and biting events.

Croatia, Cyprus, Italy and Romania specified that the application of vector control measures around human cases was mandatory.

In Cyprus, control measures were under the responsibility of the public health authorities and measures were implemented routinely (as a preventive measure) and intensified in case of viral circulation in humans.

In Greece, mosquito control measures were implemented by the regional and/or municipal authorities, routinely (breeding sites management and larviciding) and intensified (adulticiding) as a response measure to the detection of WNV circulation in humans, animals or mosquitoes.

In Italy, mosquito control actions were mandatory following the detection of a cluster of human cases in urban areas (more than two cases in the same area within two weeks). In particular, larvicidal products were used when the breeding sites could not be removed, while adulticidal treatment was required only in specific areas (i.e., hospitals, recreational areas, public parks) or during outdoor social events.

In Spain, mosquito control measures were performed every time a WNV case was detected in humans. National public health authorities provided national recommendations but the regions were responsible for implementing the actions.

Discussion

Using a questionnaire addressed to public health, veterinary and SoHO competent authorities in the EU/EEA, a literature review, and an expert meeting, we have described the WNV and USUV epidemiological situations, the laboratory diagnostic methods used by EU/EEA countries, the surveillance activities in place, and the preventive measures applied to ensure the safety of SoHO supplies.

The strength of this study is that it collated information from multiple sectors, including the public health and veterinary sectors, but also the SoHO sector and the entomological sector. The overall response rate to the questionnaire was considered very good (80% for the public health, 77% for veterinary and 80% for the SoHO sectors) considering the length of the questionnaire and the fact that it was launched during the summer. In the countries (e.g., Greece and Italy) most affected by WNV and/or USUV, all three sectors (public health, veterinary and SoHO) replied, which highlights the importance given to the topic in countries.

In July 2021, when the questionnaire was sent out, ECDC did not have networks of national focal points for blood safety and for organ, tissues and cells. Therefore, we contacted instead DG SANTE's network of the National Competent Authorities for Blood. Since December 2022 ECDC has NFP and those will be the target audience for future surveys.

A large number of papers were screened (>1 500 papers) and a large amount of data were extracted to complete the information obtained through the questionnaire and the expert meeting. By combining several sources of information, we could obtain a comprehensive overview of the situation. The comparison of the information obtained from the questionnaire survey and the literature review indicates that the respondents at the national public health and veterinary authorities might not be aware of the published results of scientific studies on the WNV and USUV epidemiological situation in their countries.

Epidemiology of West Nile virus and Usutu virus

West Nile virus has been identified in humans, animals and/or mosquitoes in 17 EU/EEA countries; 16 countries reported a total of 3 632 WNV infections in humans from 2012 to 2021. WNV infection was considered endemic in several EU/EEA countries and was expanding its geographical range to northern areas. After the peak incidence of cases recorded in 2018, human cases of WNV infection have been reported for the first time in Slovakia [155], Germany [107], and the Netherlands [158], while an outbreak with an unprecedented number of cases occurred in Spain in 2020 [159]. In 2022 (outside the study period), a high number of cases (n=965) were reported, mostly from Italy (61%, n=586) and Greece (29%, n=284), highlighting the high level of endemicity of the disease in the EU [160]. WNV infection is an outbreak prone disease that is spreading and there is a need for prevention, preparedness and surveillance activities with regards to WNV, even in countries that have not reported cases to date.

There were no clear annual trends regarding the number of cases in the EU/EEA. There were years with large outbreaks in many EU countries (2018), years with large outbreaks in few countries (2022) and years with few cases all over the EU. The intensity of WNV activity in a particular season could be influenced by several factors, including environmental drivers (e.g., ambient temperature, precipitation), abundance of vectors, infected and susceptible avian hosts, etc. The environmental conditions have effects on WNV circulation and exposure of humans to WNV infection over multiple pathways (e.g., the effects of temperature and weather on mosquito propagation, extrinsic cycle of WNV in vectors, bird migration and reproduction, human-animal host-vector interfaces). Only short-term (e.g., within-season) forecasting of the WNV intensity can be performed with sufficient fidelity.

In newly affected countries, the spread of WNV transmission has often progressed relatively slowly after introduction, e.g. in Germany, or even halted in the season after the first detection of cases e.g. the Netherlands.

Although the natural cycle of WNV relies on wild bird hosts and mosquito vectors, human and mammalian cases have been detected both in rural and urban areas. Certain urban settings (e.g., suburbs, zoological gardens, city parks) appeared to be favourable for local circulation of the virus.

There were multiple genetic lineages of WNV circulating in Europe. Lineage 2 strains (with a recent common ancestor) were well established and became predominant over lineage 1 strains, but lineage 1 strains still caused outbreaks (Spain 2021) or sporadic cases (Italy 2022) [161]. However, there was no evidence on differences in the virulence of the circulating, wild-type, lineage 1 and 2 strains. In some areas, simultaneous circulation of different genetic lineage WNV strains was reported.

Among the affected countries, only Poland reported cases in animals only (birds and equids) and no human cases. However, the reports in Poland relied on serological data and most positive samples were obtained from migratory birds. The findings might indicate that the ecological conditions were not favourable for high intensity of transmission and the establishment of sustained cycle. However, as the climatic conditions change (e.g., warmer summers), they might become favourable for WNV multiplication and transmission. Therefore, the surveillance data justifies vigilance and targeted preparedness for WNV infections. All other affected countries had human cases in addition to cases in animal and, sometimes virus detection also vectors. No country reported only human cases without animal cases. As humans are accidental hosts of WNV, detection of human cases usually requires high level of circulation in the natural hosts (birds) and vectors. Although equids are also accidental hosts, their exposure to WNV infection (through mosquito bites) is usually higher than that of humans. Detection of human cases in a country triggers targeted investigations of animal hosts and vectors. Those studies so far always revealed infections and/or cases in animals. In general, countries which reported human cases for the first time during the study period, at the time of reporting already had animal cases or they were bordering with a country (or countries) with cases in humans and animals.

A large variety of bird species can be used for surveillance, including resident/captive wild birds, migratory birds, sentinel birds (mainly domestic species). Certain species (e.g. birds of prey) are more vulnerable to infection so finding the virus in them is more likely than in others. In birds of prey and scavengers, non-vectorial transmission (i.e. eating infected prey or carcasses) can also play an important role in transmission, and the high dose of virus uptake (compared to infection via mosquito bite) might result in more severe infection in those bird species. The choice of bird species for surveillance is also influenced by the accessibility to wild bird samples (e.g., abundance, protected species). Beyond equids, several mammalian animal species are also susceptible to WNV infection, including dogs, cats, mice, rabbits, elephants, etc.; however, mammalians are considered as dead-end hosts of WNV, so principally, they do not play a role in the transmission of the WNV. Nevertheless, mammalian species might be used for serosurveillance, particularly if sera collected for other purposes are available for WNV testing.

The majority of WNV infections in natural wild bird hosts remain asymptomatic and infected birds develop life-long immunity. This can limit the intensity of the virus circulation in the ecosystems. However, according to the experiences from the past decades, WNV persisted in affected (enzootic) areas in Europe. The results of phylogenetic studies indicate overwintering of the virus rather than repeated introduction to the same area. The herd immunity in wild birds does not seem to reach general protection level, as susceptible individuals (e.g. nestlings, fledglings) are always present. Therefore, it is very likely that WNV remains endemic/enzootic in several EU countries and outbreaks in animals and in humans are expected in the future. Hence, having in place surveillance activities and maintaining a high level of awareness and preparedness is important to prevent the spread of WNV.

From 2012 through 2021, 104 sporadic human cases of USUV infections were reported in eight EU countries, with 79% of the cases reported in Italy and Austria; infections in animals were identified in these same eight countries plus seven additional countries. The wider presence of USUV in animals compared to humans could be explained by the fact that surveillance of USUV is mostly research based and predominantly covering the animal sector.

Comparison of the geographical expansion of USUV and WNV would indicate that WNV was more widely spread than USUV; however, this should be taken with caution as USUV infections in human and animals were not notifiable and therefore less systematically reported than WNV infections.

As for WNV infections, the majority of the human USUV infections are asymptomatic or present as a mild disease, and therefore might not be diagnosed. The limited number of USUV infections detected did not allow us to ascertain with confidence the proportion of asymptomatic infections. Additionally, considering that most serological diagnostic methods and NAT testing would not differentiate infections with WNV or USUV viruses, it cannot be excluded that some of the USUV infections were diagnosed as WNV infections, the latter being more common and better known than the former. This would apply to infection both in humans and in animals. However, despite the uncertainties, the data collected suggest that the public health and animal health impact of USUV in the EU/EEA was limited and far less than the public health impact of WNV.

The impact of the co-circulation of WNV and USUV is currently unknown and should be further investigated. Considering that both viruses are closely genetically related, co-circulation could raise questions around co-infections and host immune response in humans and animals including the potential for cross protection and co-infection.

Gaps and avenues to address these gaps:

The proportion of asymptomatic WNV and USUV human cases should be further ascertained through seroprevalence studies in the general population. Seroprevalence studies would inform about the general exposure rates to flaviviruses in the EU/EEA. In addition, individuals with neurological symptoms compatible with WNV or USUV infection should be tested for these infections as part of the differential diagnosis.

The impact of co-circulation of WNV and USUV on humans and animals should be further investigated through experimental studies exploring cross protection and potential interactions between the two virus infections. Research projects could also focus on the differential diagnosis of WNV and USUV infections to ascertain the number of USUV infection mis-diagnosed as WNV infection. In terms of public health, this distinction is of limited value i) for the management of the cases, as the treatment is symptomatic for both infections and ii) for SoHO measure implementations, as NAT screening would detect both viruses and positive donations are discarded, hence ensuring a SoHO safety. The differentiation between infections would however be relevant to identify which virus is circulating in an area, since it is only for WNV that systematic donation deferral or testing may be considered necessary.

Surveillance of WNV and USUV in birds mostly focused on species that were found dead or were relatively easy to capture and monitor. However, the role of the different bird species in the maintenance of the epidemiological cycle and transmission to humans of these two viruses remained largely unknown. Similarly, the role of the different mosquito species was not well defined. Increased coordinated research and surveillance in affected countries could provide new evidence supporting the targeting of the bird and mosquito species to focus on early detection and monitoring of WNV and USUV.

It was unknown how climate change impacts the epidemiology of WNV and USUV infection, particularly considering that the weather can influence bird movements, vector abundance and virus replication in the vectors. Further ecological studies are required to get an understanding of the relationship between climate variables and human case incidence and to refine models or predictions of outbreak occurrences. Modelling the epidemiology of WNV throughout Europe and suggesting possible scenarios under different climate changes could help authorities to prepare emergence of the disease and change in epidemiology.

Laboratory diagnostics

The diagnosis and surveillance of WNV and USUV infections in humans and animals relied on the laboratory testing capabilities of EU/EEA countries. National Reference Laboratories and, in some countries, regional reference laboratories were in charge of diagnosing and confirming WNV and USUV infections. Similar laboratory methods were applied for the detection of both WNV and USUV in humans and in animals. For the direct detection of the infection, predominantly molecular methods (e.g., RT-PCR, RT-qPCR) were used. Due to the nucleotide sequence similarities of WNV and USUV genomes, certain assays could simultaneously detect both viral RNAs. This also applies to the NAT assays for the testing of blood donations. The relatively short viraemic periods, particularly in humans, could limit the applicability of direct diagnostic methods. Therefore, the serological detection of acute infections (e.g., detection of IgM antibodies, seroconversion, titre-increase and/or low-avidity IgG) were frequently used. As both WNV and USUV are in the JEV-serocomplex, serological cross-reactions hinder the differential diagnosis of WNV and USUV infections. Additionally, in some assays, cross reactivity with antibodies raised against further flaviviruses (e.g. TBEV, dengue virus, Zika virus, JEV, yellow fever virus) might occur. Simultaneous virus neutralisation tests were applied in several laboratories to identify the specificity of antibodies and differentiate between infections. However, the technical requirements (e.g., BSL-3 facilities for WNV neutralisation tests, appropriate cell lines and reference viruses), the time-consuming and labour-demanding nature of the simultaneous virus neutralisation assay limited its applicability or posed capacity challenges, particularly for large-scale testing.

Several NRLs of WNV infections were members of the Emerging Viral Diseases-Expert Laboratory Network (EVD-LabNet). This multidisciplinary network of expert laboratories aimed to strengthen Europe's laboratory capacity and capability to respond to emerging, re-emerging, and vector-borne viral disease threats. An external quality assessment (EQA), organised by EVD-LabNet in 2017, focused on neurotropic vector-borne viruses, such as WNV, USUV, Toscana virus, and TBEV. Within this EQA, the WNV detection capability was highest, while USUV diagnostic capability was lowest amongst the four viruses [162]. To improve the sensitivity and specificity of laboratory diagnosis of WNV and USUV infection in humans, several EU/EEA countries included urine and other biological specimens for molecular testing for confirmation of cases, especially in later phases of infection, when viral RNA is no longer detectable in blood [163]. Molecular testing in whole blood and urine samples allows WNV infection to be confirmed in many cases, reducing the need to perform time-consuming neutralisation assays [24,66,75,76,163]. This is particularly relevant in areas where WNV, USUV and other flaviviruses are co-circulating, because the cross-reactivity of neutralising antibodies represents a challenge for the differential diagnosis based on serology [25,64,70].

An important obstacle to face in the diagnosis of USUV infection in humans was the limited availability of validated commercial tests. In EU/EEA countries, diagnosis was generally done by in-house assays at the NRLs [82–84,200]. These tests were applied for the differential diagnosis of probable WNV cases, to confirm WNV NAT-positive blood donors, and, less frequently, for the routine diagnosis of patients with neurological symptoms or suspected arbovirus infection. Some EU/EEA countries declared to perform a differential diagnosis between WNV and other flaviviruses using neutralisation assays only to confirm the first probable case in a region, but not to confirm any subsequent cases. Due to the limited diagnostic capacity and difficulties in the differentiation between USUV and WNV infections, it is conceivable that several human USUV infections remained undiagnosed or were misdiagnosed as WNV infection.

Gaps and avenues to address these gaps:

The small diagnostic window for the detection of human WNV infection could be broadened by molecular testing of urine samples; however, shipment and storage conditions are very important for virus detection. The applicability of urine testing for WNV infection in animals and for USUV infection both in humans and animals requires further studies.

If generic molecular methods (e.g., generic flavivirus PCRs, blood NAT) are used, subsequent specific assays (e.g., virus-specific RT-qPCRs) or determination and analysis of the nucleotide sequence of the amplification products should be performed to differentiate between viruses and validate the diagnosis.

To increase the specificity of serological diagnosis, the capabilities and capacities of (reference) laboratories for simultaneous virus neutralisation assays should be established, maintained and/or improved, for instance through EQA and trainings. Simultaneously, research should focus on the development of specific serological tests for the reliable differentiation between flavivirus infections.

Validated commercial tests were limitedly available for WNV and USUV infections in certain hosts. The validation of both commercial and in-house developed laboratory methods should be done in line with the In Vitro Diagnostic Medical Devices Regulation (EU) 2017/746 [164].

Surveillance

West Nile virus surveillance was well established in most EU/EEA countries especially those that experienced outbreaks very often. Surveillance and timely reporting of detected cases was important to assure appropriate implementation of measures, for example regarding safety of blood donations.

It should be noted, that according to the EU case definition, any person meeting the laboratory criteria for case confirmation is considered a confirmed case, irrespective of symptoms, and therefore also asymptomatic blood donors fulfilling the respective laboratory criteria should be reported to ECDC.

Besides birds, equids or mosquitoes, a wide range of animals were found positive for WNV and/or USUV or with antibodies against them (more than 30 different species of birds and mammals including pets, rodents, ruminants, and other domestic and wild mammals). Therefore, testing captive or wild susceptible animals (e.g. zoo, natural park and wild-life, rehabilitation centre) could be considered as a complement to human, equids and/or bird surveillance. Before including additional animal species into surveillance systems for WNV and USUV, countries should conduct a cost-effectiveness assessment to estimate the benefits of such inclusion. Furthermore, it should be noted that for virus or antibody detections in migratory birds it might not be possible to assign the place of infection.

Based on the acquired experience in the diagnosis of WNV infection in humans, some EU/EEA countries developed national case definitions for confirmation of WNV infection in humans that differ from the current EU case definition, mainly to allow the inclusion of any biological sample as valuable for molecular testing and virus isolation.

Surveillance of WNV in mosquitoes was performed in some EU/EEA countries and could be integrated in further countries. This data could also be included in the EU/EEA level surveillance as detection of WNV in mosquito is often an early indicator of WNV circulation [165,166].

No specific surveillance was in place for human USUV infections in EU/EEA countries, except for Italy. However, the majority of the countries collected information on USUV infections through differential diagnosis of suspected cases of WNV infection in humans and animals. Due to the difficulties in the differentiation between USUV and WNV infections, it is likely that some human USUV infections were misdiagnosed as WNV infection. For blood safety measures, however, it is advisable, in case of doubt, to report those cases promptly as WNV infections if differential diagnosis cannot be provided in a timely way. In case an USUV infection is confirmed at a later stage the case then can be discarded retrospectively.

Gaps and avenues to address these gaps:

The data collection and the data included in the reports and notification systems both for humans and for animals were not harmonised among countries and between humans and animals. This made the comparison between surveillance activities and outbreaks in humans and animals in different countries and the implementation of more detailed epidemiological analysis difficult or even impossible. There was a variability in the temporal variables used to report the outbreaks, cases or surveillance activities e.g. day of sampling, day of confirmation, day of testing, day of infection and day of reporting. In addition, variability was observed in geographical variables that provide information of the location where the outbreaks /cases are identified or where the surveillance activities are conducted, ranging from higher resolution such as points (x,y coordinates) to lower resolution such as polygons (regions or countries).

Setting some common rules for data collection that may allow a better comparison among surveillance activities and outbreaks both in human and animal population would support detailed epidemiological analysis. For example, for the temporal distribution of the cases in humans and animals a common more objective temporal variable should be used, and this could be the time of sampling which is available, and it is not subjected to any interpretation. For the location the coordinates could be the preferable geographical variables for animals, but it is understandable for the protection of personal data could not be applicable for the humans.

The EU case definition for confirmed human WNV infections did not include urine as a possible sample, which was used in several EU countries. However, the proposed revised EU Case Definition includes all biological specimens as samples for confirmed cases which would allow to also include those cases in EU/EEA level surveillance in the future.

Currently, evidence about the public health impact of USUV does not indicate that the establishment of USUV-specific surveillance at EU/EEA level would have significant benefits for human health. However, the well-established WNV surveillance, if it includes testing for differential diagnosis including USUV infection, could provide useful information about USUV epidemiology and clinical manifestations. To this end, information on USUV infections acquired through differential diagnosis could be collected systematically to estimate the public health and animal health impact of USUV infections without establishing an USUV-specific surveillance system.

Surveillance of WNV in mosquitoes was established in some EU countries and can be useful for example to detect an early start of WNV transmission season, which in turn could be used to timely raise awareness among healthcare professionals and the general population about the risk of WNV infections. The cost-benefit of surveillance of WNV in mosquitoes was however likely not positive in all countries and therefore should be analysed in the respective context, which will depend e.g. of the epidemiology of WNV in the respective country with a higher benefit for highly endemic countries. For EU/EEA countries with already established surveillance of WNV in mosquitoes, this data could be integrated in the seasonal EU/EEA level WNV surveillance in addition to the data on WNV infections in humans, equids and birds as a further early warning sign of WNV circulation.

Prevention and control

Answers to the survey questionnaire indicate that a relatively small fraction (1 to 23%) of the total blood donations were submitted to NAT in the countries between 2016 and 2020. It can be explained by the differences in the WNV epidemiological situation in the countries (e.g. number of risk areas, number of donations at risk areas, timing of the deferral period) as well as differences in deferral/testing policies. WNV and USUV have been sporadically found in tested blood donations. The data we collected did not allow us to define whether positive donations were from people residing in WNV affected areas or travellers returning from affected areas. Also, the timing of the detection (e.g., month) was not provided so we could not provide conclusions regarding the exposure of the positive donors.

Not all countries that performed WNV NAT differentiated WNV from USUV infections. While from a surveillance and research perspective it would have been relevant to distinguish the two viruses, from a SoHO safety perspective the added value remains limited, since USUV-positive donations should be discarded as a precautionary measure.

Countries with autochthonous WNV infections have defined their trigger(s) for the initiation of deferral or testing of SoHO donations. The difference of approach can be explained by the epidemiological situation of the country and the associated risk of virus transmission via SoHO, but also the risk related to SoHO security.

The Commission Directive 2004/33/EC [87] established a deferral period for 28 days after leaving an area with ongoing transmission of WNV to humans. The Commission Directive 2014/110/EU amended Directive 2004/33/EC that the deferral period is 28 days after leaving a risk area of locally acquired West Nile Virus unless an individual Nucleic Acid Test (NAT) is negative [88]. While some countries applied prevention measures based on viral detection in animals and vectors or in detection of recent infections in animals for their locally acquired cases, there is no indication that these triggers would increase the safety of SoHO donations regarding returning travellers.

As there was no vaccine for humans and very limited vector control possibilities, prevention of WNV human cases focused on the application of SoHO safety measures and avoidance of mosquito bites in areas where the virus was known to be present. Prevention of cases of WNV-related equine encephalitis focused on vaccination of equids and avoidance of mosquito bites.

Source reduction, through environmental management, and larviciding interventions allow a reduction of vector populations to low levels [167]. However, there was no evidence of the efficacy of larviciding interventions in the control of WNV outbreaks. Aerial ultra-low volume adulticiding was the only method proven to reduce WNV circulation in natural-wetland environments and in urban settings. A survey conducted in 2019 through VectorNet highlighted the limited availability of insecticidal active substances, the lack of long-term registration for products/methods, a complex regulatory framework for the use of biocidal products, and the lack of EU-wide technical guidelines as significant barriers to effective vector control operations [167].

Gaps and avenues to address these gaps:

The control measures options, including during WNV outbreaks, were limited, with prevention measures focusing on SoHO measures and avoidance of mosquito bites. Having a human vaccine could support the prevention of severe cases. Research should be supported to develop such vaccine.

Additional studies are needed on the impact of vector control measures to better inform public health policy decisions on strategies for WNV management. The development of news tools and concepts for eco-friendly mosquito population control is required.

Conclusions and potential implications

West Nile virus infection is considered endemic in several EU/EEA countries and has been expanding its geographical range to northern areas. WNV has major implications on blood safety and security. Seasonal surveillance should be maintained for early detection of WNV activity and emergence of WNV in new areas or countries, to inform SoHO safety authorities. Prevention of WNV human cases has been focusing on the application of blood safety measures and avoidance of mosquito bites while prevention of equine cases has been focusing on vaccination of equids and avoidance of mosquito bites. Research, public health and/or veterinary activities should gather data that will help understanding better the epidemiology of the disease in humans and animals, including the various animal and vector species implicated in the transmission cycle. Strengthening laboratory capability to diagnose human and animal cases, enhancing, where relevant, virus surveillance in vectors, understanding the impact of climate on the occurrence of the disease, developing a vaccine against disease due to WNV infections in humans and, developing sustainable and eco-friendly vector control measures could improve prevention and control activities and reduce the risk of human disease.

Despite some uncertainties, we conclude that the public health and animal health impact of USUV in the EU/EEA is limited and far less than the public health impact of WNV. The information currently available does not support the implementation of USUV targeted surveillance systems, or USUV-specific regional deferral as a SoHO safety measure, in the EU/EEA countries or at EU level. However, it is worth inviting EU/EEA countries to enhance diagnostic capacity and differential diagnosis both for humans and animals, to better understand the epidemiology of USUV infection, and monitor the possible emergence of new USUV strains, which may show enhanced pathogenicity for humans. It is worth mentioning that any epidemiological or microbiological change that would substantially increase the public health risk should be reported to the EU level through EpiPulse [168], which is the event-based surveillance system managed by ECDC. This review allowed us to have a detailed overview of the recent and current situation and we suggest performing continuous monitoring of the reported human USUV infections, was well as reviewing the situation in 5 to 10 years to assess any possible change. If significant increase in the number of human USUV infections, increased severity of the human cases or transmission of USUV via SoHO were reported, the assessment in this report should be revisited and adopted to the given situation. Continued research could reveal new information on the USUV strains circulating in Europe, potential molecular markers of host specificity and virulence, environmental drivers of transmission patterns and epidemiological processes. Revealing the similarities and differences between the ecology and epidemiology of WNV and USUV infections, as well as the implications of their co-circulation in the same host and vector populations might improve our diagnostic, response, prevention and control activities of the two diseases, both in humans and in animals.

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Annex 1

Literature search strings for the WNV and the USUV searches

Database	Query WNV	Query USUV
PubMed	("west nile"[Title/Abstract] AND ("Austria"[Title/Abstract] OR "Belgium"[Title/Abstract] OR "Bulgaria"[Title/Abstract] OR "Croatia"[Title/Abstract] OR "czech*"[Title/Abstract] OR "Denmark"[Title/Abstract] OR "Estonia"[Title/Abstract] OR "Finland"[Title/Abstract] OR "France"[Title/Abstract] OR "Germany"[Title/Abstract] OR "Greece"[Title/Abstract] OR "Hungary"[Title/Abstract] OR "Iceland"[Title/Abstract] OR "Ireland"[Title/Abstract] OR "Italy"[Title/Abstract] OR "Latvia"[Title/Abstract] OR "Liechtenstein"[Title/Abstract] OR "Lithuania"[Title/Abstract] OR "Luxembourg"[Title/Abstract] OR "Malta"[Title/Abstract] OR "Netherlands"[Title/Abstract] OR "Norway"[Title/Abstract] OR "Poland"[Title/Abstract] OR "Portugal"[Title/Abstract] OR "Cyprus"[Title/Abstract] OR "Romania"[Title/Abstract] OR "Slovakia"[Title/Abstract] OR "Slovenia"[Title/Abstract] OR "Spain"[Title/Abstract] OR "Sweden"[Title/Abstract] OR "europe*"[Title/Abstract]))	("usutu"[Title/Abstract] AND ("Austria"[Title/Abstract] OR "Belgium"[Title/Abstract] OR "Bulgaria"[Title/Abstract] OR "Croatia"[Title/Abstract] OR "czech*"[Title/Abstract] OR "Denmark"[Title/Abstract] OR "Estonia"[Title/Abstract] OR "Finland"[Title/Abstract] OR "France"[Title/Abstract] OR "Germany"[Title/Abstract] OR "Greece"[Title/Abstract] OR "Hungary"[Title/Abstract] OR "Iceland"[Title/Abstract] OR "Ireland"[Title/Abstract] OR "Italy"[Title/Abstract] OR "Latvia"[Title/Abstract] OR "Liechtenstein"[Title/Abstract] OR "Lithuania"[Title/Abstract] OR "Luxembourg"[Title/Abstract] OR "Malta"[Title/Abstract] OR "Netherlands"[Title/Abstract] OR "Norway"[Title/Abstract] OR "Poland"[Title/Abstract] OR "Portugal"[Title/Abstract] OR "Cyprus"[Title/Abstract] OR "Romania"[Title/Abstract] OR "Slovakia"[Title/Abstract] OR "Slovenia"[Title/Abstract] OR "Spain"[Title/Abstract] OR "Sweden"[Title/Abstract] OR "europe*"[Title/Abstract]))
Web of Science	(TS=(west nile) AND TS=(Austria OR Belgium OR Bulgaria OR Croatia OR Czech OR Denmark OR Estonia OR Finland OR France OR Germany OR Greece OR Hungary OR Iceland OR Ireland OR Italy OR Latvia OR Liechtenstein OR Lithuania OR Luxembourg OR Malta OR Netherlands OR Norway OR Poland OR Portugal OR Cyprus OR Romania OR Slovakia OR Slovenia OR Spain OR Sweden OR Europe))	(TS=(usutu) AND TS=(Austria OR Belgium OR Bulgaria OR Croatia OR Czech OR Denmark OR Estonia OR Finland OR France OR Germany OR Greece OR Hungary OR Iceland OR Ireland OR Italy OR Latvia OR Liechtenstein OR Lithuania OR Luxembourg OR Malta OR Netherlands OR Norway OR Poland OR Portugal OR Cyprus OR Romania OR Slovakia OR Slovenia OR Spain OR Sweden OR Europe))
Scopus	(TITLE-ABS-KEY (austria OR belgium OR bulgaria OR croatia OR czech OR denmark OR estonia OR finland OR france OR germany OR greece OR hungary OR iceland OR ireland OR italy OR latvia OR liechtenstein OR lithuania OR luxembourg OR malta OR netherlands OR norway OR poland OR portugal OR cyprus OR romania OR slovakia OR slovenia OR spain OR sweden OR europe)) AND (TITLE-ABS-KEY (west AND nile))	(TITLE-ABS-KEY (austria OR belgium OR bulgaria OR croatia OR czech OR denmark OR estonia OR finland OR france OR germany OR greece OR hungary OR iceland OR ireland OR italy OR latvia OR liechtenstein OR lithuania OR luxembourg OR malta OR netherlands OR norway OR poland OR portugal OR cyprus OR romania OR slovakia OR slovenia OR spain OR sweden OR europe)) AND (TITLE-ABS-KEY (usutu))
Embase	'west nile':ab,ti AND ('austria':ab,ti OR 'belgium':ab,ti OR 'bulgaria':ab,ti OR 'croatia':ab,ti OR 'cyprus':ab,ti OR 'czech republic':ab,ti OR 'denmark':ab,ti OR 'estonia':ab,ti OR 'finland':ab,ti OR 'france':ab,ti OR 'germany':ab,ti OR 'greece':ab,ti OR 'hungary':ab,ti OR 'iceland':ab,ti OR 'ireland':ab,ti OR 'italy':ab,ti OR 'latvia':ab,ti OR 'liechtenstein':ab,ti OR 'lithuania':ab,ti OR 'luxembourg':ab,ti OR 'malta':ab,ti OR 'netherlands':ab,ti OR 'norway':ab,ti OR 'poland':ab,ti OR 'portugal':ab,ti OR 'romania':ab,ti OR 'slovakia':ab,ti OR 'slovenia':ab,ti OR 'spain':ab,ti OR 'sweden':ab,ti OR 'europe':ab,ti)	'usutu':ab,ti AND ('austria':ab,ti OR 'belgium':ab,ti OR 'bulgaria':ab,ti OR 'croatia':ab,ti OR 'cyprus':ab,ti OR 'czech republic':ab,ti OR 'denmark':ab,ti OR 'estonia':ab,ti OR 'finland':ab,ti OR 'france':ab,ti OR 'germany':ab,ti OR 'greece':ab,ti OR 'hungary':ab,ti OR 'iceland':ab,ti OR 'ireland':ab,ti OR 'italy':ab,ti OR 'latvia':ab,ti OR 'liechtenstein':ab,ti OR 'lithuania':ab,ti OR 'luxembourg':ab,ti OR 'malta':ab,ti OR 'netherlands':ab,ti OR 'norway':ab,ti OR 'poland':ab,ti OR 'portugal':ab,ti OR 'romania':ab,ti OR 'slovakia':ab,ti OR 'slovenia':ab,ti OR 'spain':ab,ti OR 'sweden':ab,ti OR 'europe':ab,ti)
CAB abstracts	((ab:(west nile) AND ab:((Austria) OR (Belgium) OR (Bulgaria) OR (Croatia) OR (Czech) OR (Denmark) OR (Estonia) OR (Finland) OR (France) OR (Germany) OR (Greece) OR (Hungary) OR (Iceland) OR (Ireland) OR (Italy) OR (Latvia) OR (Liechtenstein) OR (Lithuania) OR (Luxembourg) OR (Malta) OR (Netherlands) OR (Norway) OR (Poland) OR (Portugal) OR (Cyprus) OR (Romania) OR (Slovakia) OR (Slovenia) OR (Spain) OR (Sweden) OR (Europe)))	((ab:(usutu) AND ab:((Austria) OR (Belgium) OR (Bulgaria) OR (Croatia) OR (Czech) OR (Denmark) OR (Estonia) OR (Finland) OR (France) OR (Germany) OR (Greece) OR (Hungary) OR (Iceland) OR (Ireland) OR (Italy) OR (Latvia) OR (Liechtenstein) OR (Lithuania) OR (Luxembourg) OR (Malta) OR (Netherlands) OR (Norway) OR (Poland) OR (Portugal) OR (Cyprus) OR (Romania) OR (Slovakia) OR (Slovenia) OR (Spain) OR (Sweden) OR (Europe)))

Annex 2

Institutes and authorities that participated to the expert meeting on 18-19 January 2022

COUNTRY	INSTITUTION
Austria	Ministry of Social Affairs, Health, Care and Consumer Protection
	Austrian Agency for Food and Health Safety (AGES)
	Federal Office for Safety in Healthcare
	Medical University of Vienna
	University of Veterinary Medicine, Vienna
Croatia	Faculty of Veterinary Medicine, University of Zagreb
	Ministry of Health
Cyprus	Cyprus Blood Establishment
	Ministry of Health
	Veterinary Services
France	French Public Health Agency (SPF)
	The French Agency for Food, Environmental and Occupational Health and Safety (ANSES)
	The French National Agency for Medicines and Health Products Safety (ANSM)
Germany	Robert Koch Institute (Federal Institute for Public Health)
	Federal Armed Force, Medical Service Headquarters
	Friedrich Loeffler Institute (Federal Institute for Animal Health)
Greece	Hellenic National Public Health Organization
	Aristotle University of Thessaloniki
	Ministry of Rural Development and Food
Hungary	National Blood Transfusion Service
	National Food Chain Safety Office
	National Public Health Centre
Italy	Italian National Institute of Health (ISS)
	Italian National Blood Centre (CNS)
	Ministry of Health
	University of Milan, Department of Veterinary Medicine
	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise
Netherlands	Dutch National Institute of Public Health and the Environment
	Netherlands Food and Consumer Product Safety Authority
Portugal	Portuguese Blood and Transplantation Institute
	Ministry of Agriculture, Directorate General for Food and Veterinary
Romania	Regional Blood Transfusion Centre
	National Public Health Institute
Slovenia	Blood Transfusion Centre
	National Institute of Public Health
	Agency for Medicinal Products and Medical Devices
	Administration for Food Safety, Veterinary Sector and Plant Protection
Spain	Carlos III Health Institute
	Andalusian Network of Transfusion Medicine, Tissues and Cells
European Union	European Commission, Directorate for Health and Food Safety, Animal Health unit
	European Commission, Directorate for Health and Food Safety, Health Security unit
	ECDC, Disease Programmes Unit
	EFSA, Biological Hazards & Animal Health and Welfare Unit

Annex 3

Institutes and authorities that responded to the survey, by sector and by country

Country	Institute or authority name	Public health	Animal health	SoHO safety
Austria	Austrian Health Agency for Health and Food Safety (AGES)	✓	✓	✓
	Ministry of Social Affairs, Health, Care and Consumer Protection, Pharmaceuticals, Medical Devices, Blood, Tissue and Transplantation			✓
	Austrian Federal Office for Safety in Health Care (BASG)			✓
Belgium	Sciensano, Epidemiology of Infectious diseases	✓		✓
Bulgaria	Bulgarian Drug Agency, Department for Control of the Blood transfusion system			✓
Croatia	Croatian Institute of Public Health, Department of Virology	✓		
	Faculty of Veterinary Medicine, University of Zagreb, Department of Microbiology and Infectious Diseases with Clinic		✓	
	Croatian Institute of Transfusion Medicine, Medical Department			✓
Cyprus	Veterinary Services of Cyprus, Laboratory for Animal Health		✓	
	Cyprus Blood Establishment			✓
Czechia	Ministry of Health; National Institute of Public Health, Prague, Centre for Epidemiology and Microbiology; Public Health Institute in Ostrava, National Reference Laboratory for Arboviruses	✓		✓
	State Veterinary Administration of the Czech Republic, Department of Animal Health and Animal Welfare Protection		✓	
Denmark	Danish Veterinary and Food Administration, Division of Animal Health		✓	
	Danish Patient Safety Authority			✓
Estonia	Health Board, CD Department	✓		
	Agriculture and Food Board, Animal Health		✓	
	State Agency of Medicines, Department of Biologicals			✓
Finland	Finnish Institute for Health and Welfare, Infectious Disease Control and Vaccinations Unit, Department of Health Security	✓		
	Finnish Food Authority		✓	
	Finnish Medicines Agency			✓
France	Ministry of Solidarity and Health, General Directorate for Health	✓		✓
	The French Agency for Food, Environmental and Occupational Health and Safety (ANSES)		✓	
	The French National Agency for Medicines and Health Products Safety (ANSM)			✓
Germany	Robert Koch Institute (Federal Institute for Public Health)	✓		
	Friedrich Loeffler Institute (Federal Institute for Animal Health)		✓	
	Paul Ehrlich Institute (Federal Institute for Vaccines and Biomedicine)			✓
Greece	Hellenic National Public Health Organization, National Reference Laboratory for Arboviruses, Aristotle University of Thessaloniki, Dept. of Microbiology	✓		
	Ministry of Rural Development and Food		✓	
	Hellenic National Blood Transfusion Centre			✓
Hungary	National Public Health Centre	✓		
	National Food Chain Safety Office		✓	
Iceland	Directorate of Health, Centre for Health Security and Communicable Disease Control	✓		
Ireland	The Health Protection Surveillance Centre (HPSC)	✓		
	Department of Agriculture, Food and the Marine		✓	
	Health Products Regulatory Authority			✓
Italy	Italian National Institute of Health (ISS)	✓		✓
	Ministry of Health		✓	
Latvia	Riga East University Hospital, National Microbiology Reference Laboratory	✓		
	Institute of Food Safety, Animal Health and Environment		✓	
	State Agency of Medicines			✓
Liechtenstein	Office of Public Health	✓		
Lithuania	Ministry of Health, National Public Health Centre	✓		
	The State Food and Veterinary Service		✓	
	National Blood Centre			✓
	National Bureau of Transportation			✓
Luxembourg	Luxembourg Health Directorate	✓		✓
	Veterinary Services Administration, State Veterinary Laboratory		✓	
Malta	Ministry of Health, Infectious Disease Prevention and Control Unit	✓		
Netherlands	National Institute for Public Health and the Environment	✓	✓	✓

Country	Institute or authority name	Public health	Animal health	SoHO safety
Norway	Zoonotic, Food and Waterborne Infections	✓		
	Norwegian Veterinary Institute		✓	
	Directorate of Health Norway			✓
Poland	National Veterinary Research Institute		✓	
Portugal	Ministry of Agriculture, Directorate General for Food and Veterinary		✓	
	Portuguese Blood and Transplantation Institute			✓
Romania	National Public Health Institutes	✓		
	Institute for Diagnosis and Animal Health		✓	
	Regional Blood Transfusion Centre of Constanta			✓
Slovakia	Regional Authority of Public Health in Banská Bystrica	✓		
	University of Veterinary Medicine, Microbiology and Immunology and Pharmacy in Košice		✓	
	National Transfusion Service of Slovak Republic			✓
Slovenia	Centre for Communicable Diseases	✓		
	Administration for Food Safety, Veterinary Sector and Plant Protection		✓	
	Agency for Medicinal Products and Medical Devices			✓
	Blood Transfusion Centre			✓
Spain	National Centre for Epidemiology	✓		
	Ministry of Agriculture, Fisheries and Food		✓	
	Ministry of Health			✓

Annex 4

Definition of a WNV infection human case among EU/EEA countries that are not using the EU case definition

Country	WNV case definition
Czechia	473/2008 Sb. DECREE of the Ministry of Health of 17 December 2008 on the system of epidemiological vigilance for selected infections, as amended by Decree No. 275/2010 Coll. and No. 233/2011 Coll. Annex No. 15 System of epidemiological vigilance of diseases caused by the West Nile virus (hereinafter referred to as "WNV") Art. 1 Clinical definition of the disease 1. Clinical picture corresponding to a febrile illness with neurological symptoms, ranging from severe headache and muscle pain to aseptic meningitis or encephalitis, with an incubation period of 2 to 6 days, in a maximum range of 2 - 15 days, after exposure which is caused by mosquito bites, rarely by sucking of a tick of the genus *Hyalomma*, or by human-to-human transmission by transplantation, transfusion or transplacentally. ..... **Art. 2 Laboratory diagnostics** 1. Demonstration of specific antibody response (serum, cerebrospinal fluid [CSF]). 2. Detection of nucleic acid in blood or CSF. **Laboratory criteria for a probable case:** 1. Determination of IgM antibodies against WNV in serum by ELISA test. 2. Determination of IgG antibodies against WNV in serum by ELISA test. 3. Determination of anti-WNV antibodies in serum by Haemagglutination Inhibition Test. **Laboratory criteria for a confirmed case:** 1. Demonstration of the presence of specific IgM antibodies against WNV in the CSF. 2. Isolation of WNV from blood or CSF. 3. Detection of WNV nucleic acid in blood or CSF. 4. Positive virus neutralisation test. The collected biological material (serum or CSF) will be sent by the relevant medical facility to the National Reference Laboratory for Arboviruses. Laboratory results must always be interpreted according to the state of possible vaccination against some infections caused by other flaviviruses, or to exclude recent diseases with these infections (tick-borne encephalitis, yellow fever, Japanese encephalitis, dengue). **Art. 3 Epidemiological criteria:** At least one of the following epidemiological contexts: 1. Animal-to-human transmission (stay, visit or exposure to mosquito bites in areas with endemic WNV in horses and birds, or places with extreme mosquito overgrowth, especially in connection with floods, exceptionally tick-borne transmission) 2. Human-to-human transmission (transplantation, blood transfusion, or transplacentally). **Art. 4 Case classification** A. Possible: Not applicable. B. Probable: Any person meeting the clinical criteria and with at least one of the following two conditions: 1. epidemiological link, 2. at least one of the laboratory criteria for the probable case. C. Confirmed: Any person meeting the clinical criteria and at least one of the laboratory criteria for the confirmed case.
Finland	Laboratory-based surveillance
France	**Laboratory Definition:** **Confirmed case:** - Detection of WNV genome in biological sample - Isolation of WNV from biological sample; - Detection of WNV IgM from CSF - Seroconversion or 4-fold increase in IgG level confirmed by neutralisation **Probable case:** - Detection of WNV IgM in serum by ELISA - Seroconversion or 4-fold increase in IgG level in 2 consecutive samplings
Germany	Detection of WNV RNA or WNV-specific antibodies (confirmatory testing required) in patients with any symptoms
Greece	The EU case definition of WNV infection is used with slight modifications, mainly in that only laboratory – and not epidemiological – criteria are used to define probable cases, and testing in urine is also used for confirmation. **Case definition:** **Clinical criteria:** Any person with neurological manifestations (e.g., encephalitis, meningitis, myelitis/ acute flaccid paralysis, or radiculoneuritis), or fever or other non-neurological compatible symptoms. **Laboratory criteria for case confirmation:**

Country	WNV case definition
	At least one of the following: - isolation of WNV from a clinical specimen (blood or cerebrospinal fluid (CSF) or urine), - detection of WNV nucleic acid in blood or CSF or urine, - WNV specific IgM antibody response in CSF, - serum WNV IgM high titre AND detection of WNV IgG, AND confirmation by neutralisation. **Laboratory criteria for a probable case:** WNV specific IgM antibody response in serum. **Case classification:** - Probable case: Any person meeting clinical criteria and laboratory criteria for a probable case. - Confirmed case: Any person meeting laboratory criteria for case confirmation (asymptomatic infections are also included).
Hungary	**Clinical criteria:** Any person with fever and swollen lymph nodes or muscle/joint pain or at least one of the following two syndromes: a) encephalitis; b) meningitis. **Epidemiological criteria:** At least one of the following two epidemiological links: - animal-to-human transmission (stay in or visit to an area where WNV mosquito bites are endemic for birds and horses) - human-to-human transmission (vertical transmission, blood transfusion, transplantation). **Laboratory criteria (confirmed case):** at least one of the following four conditions: - isolation of the virus from blood, urine or cerebrospinal fluid, - detection of viral nucleic acid in blood or cerebrospinal fluid, - detection of specific IgM-type antibodies against the virus in the CSF, - detection of specific IgM-type antibodies against the virus at high titres AND detection and confirmation of WNV IgG by neutralisation from a blood sample. **Laboratory criteria (probable case):** detection of a specific antibody against the virus in a blood sample. Laboratory findings should consider flavivirus vaccination status or previous flavivirus infection, disease (i.e. tick-borne encephalitis, dengue fever) **Case classification:** - Suspect case: one of the clinical conditions is met. - Probable case: one of the clinical conditions is met and at least one of the following two conditions is met: - epidemiological link, - the laboratory condition of the probable case. - Confirmed case: one of the clinical conditions and the laboratory conditions of the confirmed case are met.
Ireland	**Clinical criteria:** Any person with fever OR at least one of the following two: - Encephalitis - Meningitis **Laboratory criteria (case confirmation):** at least one of the following four: - 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 Laboratory criteria (probable case): WNV specific antibody response in serum Laboratory results need to be interpreted according to flavivirus vaccination status **Epidemiological criteria:** At least one of the following two: - Animal to human transmission (residing, having visited or having been exposed to mosquito bites in an area where WNV is endemic in horses or birds) - Human to human transmission (vertical transmission, blood transfusion, transplants) **Case classification:** - Probable case: Any person meeting the clinical criteria AND with at least one of the following two: a) an epidemiological link b) a laboratory test for a probable case - Confirmed case: Any person meeting the laboratory criteria for case confirmation

Country	WNV case definition
Italy	National Plan for prevention, surveillance and response for Arbovirosis – 2020-2025 (Link: https://www.salute.gov.it/imgs/C_17_pubblicazioni_2955_allegato.pdf) **Clinical criteria:** Any person with fever or at least one of the following clinical manifestations: encephalitis; meningitis with clear CSF; polyradiculoneuritis (similar to Guillain-Barré); acute flaccid paralysis. **Laboratory criteria:** **Laboratory tests for a probable case:** - WNV-specific IgM antibody response in serum. **Laboratory tests for a confirmed case** (at least one of the following): - isolation of WNV in serum, urine, and/or CSF; - identification of the WNV nucleic acid in blood, urine, and/or CSF; - specific antibody response to WNV (IgM) in CSF; - high IgM WNV titre and identification of WNV IgG in serum and confirmation by neutralisation. **Case classification:** - Possible: Not applicable; - Probable: Any person meeting clinical criteria and laboratory criteria for a probable case; - Confirmed: Any person meeting at least one laboratory criterion for a confirmed case.
Netherlands	Notification obligation. WNV infection is a notifiable group C disease. The attending physician and the laboratory report a case of WNV infection to the GGD within 1 working day after the diagnosis has been made. The GGD reports anonymously to the CIb in accordance with the Public Health Act (if an infection has been contracted in the Netherlands, the GGD reports within 24 hours) and provides data for the national surveillance of notifiable diseases. **Notification criteria:** Significant WNV-specific antibody response (single high titres or significant titre increase) in serum in combination with fever; and/or neurological signs (meningitis or encephalitis); and/or mild flu-like symptoms (such as rash, headache, muscle aches); OR at least one of the following two laboratory confirmations: i) Demonstration of WNV RNA in, or isolation of WNV from, a clinical sample (blood, CSF, urine); ii) intrathecal WNV-specific antibody response (IgM).
Norway	EU case definition https://www.fhi.no/nettpub/smittevernveiledere n/sykdommer-a-a/vestnilfeber---veileder-for-helsepe/#meldings-og-varslingsplikt Laboratory detection of: - WNV in CSF or serum by isolation or nucleic acid test or - specific IgM antibodies to WNV IgM in CSF or - high titre of specific IgM antibodies to WNV and concomitant detection of specific IgG antibody to WNV.
Romania	**A probable/confirmed case** is defined as a person meeting the clinical and lab criteria according the EU case definition. **Case definition of suspected cases:** patients aged 15 years and older, with fever and neurological symptoms such as encephalitis, meningitis, meningo-encephalitis with clear CSF.
Slovenia	EU case definition WEST NILE FEVER * (West Nile Virus, UN) A92.3 **Clinical criteria** Any person with fever OR at least one of the following two signs: - encephalitis, meningitis. **Laboratory criteria for case confirmation:** At least one of the following four laboratory tests: - Isolation of WNV from blood or CSF, - Detection of WNV nucleic acid in blood or CSF, - Increase in specific antibodies (IgM) to WNV in CSF, - High titre of IgM to WNV antibodies AND detection of IgG antibodies against WNV AND confirmation by virus neutralisation. **Laboratory tests for a probable case:** An increase in specific antibodies against WNV in serum. The results of laboratory tests should be interpreted according to the vaccine status against flaviviruses. **Epidemiological criteria:** At least one of the following two epidemiological links: - transmission from animals to humans (resident, visited or exposed to mosquito bites in an area where WNV is endemic to horses or birds), - human-to-human transmission (vertical transmission, blood transfusions, transplants). **Case classification** A. Possible case: Not applicable. B. Probable case: Any person meeting the clinical criteria AND who is subject to at least one of the following two findings: - epidemiological link, - laboratory test for probable case. C. Confirmed case Any person who meets the laboratory criteria for confirmation of a case. Application: a probable or confirmed case is reported Epidemiological survey * 27.9.2012 EN Official Journal of the European Union L 262/1

Country	WNV case definition
	Link: https://www.nijz.si/sites/www.nijz.si/files/uploaded/definicije_eu_noneu_2020_december.pdf
Spain	**Clinical criteria:** person with at least one of: encephalitis, meningitis, acute flaccid paralysis, Guillain-Barré Syndrome WITH OR WITHOUT fever **Laboratory criteria:** Confirmed case if at least one of: a) virus isolation, b) viral nucleic acid detection, c) specific IgM specific antibodies detection in CSF, d) high IgM antibodies detection AND IgG AND neutralisation confirmation. Probable case if: IgM specific antibodies detection **Epidemiological criteria:** to live or to have visited areas with known WNV circulation. Person to person transmission: mother to child (newborn from infected mother); transfusion or transplant in the absence of other transmission mechanism. **Case classification** - Probable case: clinical criteria and laboratory criteria (IgM alone) - Confirmed case: laboratory criteria **Link:** https://www.isciii.es/QueHacemos/Servicios/VigilanciaSaludPublicaRENAVE/EnfermedadesTransmisibles/Documents/PROTOCOLOS/Protocolo%20vigilancia%20fiebre%20Nilo%20occidental_RENAVE.pdf

Annex 5

Definition of a USUV infection human case in EU/EEA countries

Country	Case definition for USUV infection
Germany	Detection of USUV RNA or USUV-specific antibodies (confirmatory testing required) in patients with any symptoms
Greece	"Arboviral encephalitis" is mandatory notifiable; the Arboviral encephalitis' case definition includes clinical criteria (encephalitis symptoms, i.e., fever, headache, altered consciousness, neurological signs) and laboratory criteria: i) for case confirmation (increased titre of virus specific antibodies in serum, or detection of IgM antibodies in CSF, or isolation of virus from a clinical specimen, or detection of virus's nucleic acid in a clinical specimen), and ii) for a probable case (detection of IgM antibodies in serum). Case classification includes: "confirmed" case (clinical criteria and laboratory criteria for case confirmation), and "probable" case (clinical criteria and laboratory criteria for a probable case).
Italy	Available online at page 85 of the 5-year arboviral disease plan (2020-2025) (Link: https://www.salute.gov.it/imgs/C_17_pubblicazioni_2955_allegato.pdf) **Clinical criteria:** Any person with fever or at least one of the following clinical manifestations: encephalitis; meningitis with clear CSF; polyradiculoneuritis (similar to Guillain-Barré); acute flaccid paralysis. **Laboratory criteria*:** Laboratory tests for a probable case: - USUV-specific IgM antibody response in serum. Laboratory tests for a confirmed case (at least one of the following): - isolation of USUV in serum, urine, and/or CSF; - identification of USUV nucleic acid in blood, urine, and/or CSF; - specific antibody response to USUV (IgM) in CSF; - high IgM USUV titre and identification of USUV IgG in serum and confirmation by neutralisation. **Case classification:** - Possible: Not applicable; - Probable: Any person meeting clinical criteria and laboratory criteria for a probable case; - Confirmed: Any person meeting at least one laboratory criteria for a confirmed case. * Molecular and IgM specific tests for USUV diagnosis are not available on the market: samples should be sent to Reference Laboratories for testing with in-house methods, if available. - Laboratory results should be interpreted according to the vaccination status against flavivirus.
Norway	It is mandatory to report viral infections of the central nervous system, which include USUV infection. Criteria for notification are laboratory detection of virus in CSF by isolation or nucleic acid detection or detection of specific antibody response in serum and / or CSF.

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