

WEST NILE VIRUS IN EUROPE: Epidemiology, drivers of transmission and an integrated approach to surveillance and control.



WRITING ASSIGNMENT
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Layman summary

West Nile Virus (WNV) is a mosquito borne virus that is maintained in nature in a bird-mosquito-bird transmission cycle. Mosquitos can occasionally infect humans and other vertebrate animals like horses and crocodiles. Most West Nile virus infections are characterized by subclinical signs like fever, headache, and weakness; however, infections can also progress to more severe neurological manifestations and death.

It was initially discovered in the West Nile province of Uganda and the virus is widely distributed in Africa, Europe, Australia, and Asia. In recent years, sporadic cases and major outbreaks have increased in Europe, especially in countries around the Mediterranean Sea, the South-Eastern region, and Central Europe. In addition, recent pattern also shows the geographical spread of the virus towards the Northern part of Europe. The increased geographical spread and occurrence of WNV infections in Europe makes it a public health concern. Therefore, this literature review provides an overview of West Nile Virus infection in general, evaluating the spread across Europe and factors that influenced the distribution.

The spread of the virus can be influenced by the migratory behavior of birds. Migratory birds can cause re-introduction of the virus in an area and the host competence among resident birds can also have an impact on the continued circulation of the virus. The *Culex* species of mosquito is the most ubiquitous species in Europe, and it plays a significant role in the transmission of WNV. It has two different biotypes which differ in their physiological and behavioral capacity affecting transmission dynamics in different regions. Also, climate change is an important environmental driver for WNV transmission. Higher temperatures play an important role in the growth rate of mosquito population, viral replication rate and contact rate between host and mosquitos. Precipitation creates favorable conditions for interaction between mosquito and hosts and affects the larval stage of the mosquitos.

The interaction between environmental drivers, reservoir host and mosquito vector are complex. Hence, an integrated approach to surveillance, prevention and control can help mitigate the impact of the virus within Europe and this is further discussed in the review. Vector surveillance of mosquitos can help early identification of WNV circulation. Dead bird and active bird surveillance can provide information about the potential risk of introduction of WNV within an area. Also, active, and passive surveillance of horses can help monitor the virus circulation and ensure prompt identification of outbreaks within the equine and human population. Human syndromic surveillance and routine screening of blood transfusion and organ transplantation is also an effective strategy for WNV surveillance. Regarding control, vector management of both larva and adult mosquito population are efficient control measures. Vaccinations are only currently available for horses, hence more preventive methods against WNV are advised.

To conclude, for the different surveillance, prevention, and control strategies to work. It needs to be an integrated approach that is tailored to each country epidemiological situation to optimize efficiency and rational use of resources. This would involve a multisectoral collaboration between veterinarians, epidemiologist, public health workers, public institutions, and other allied professionals to ensure early detection of the virus, appropriate control, and an efficient response during an outbreak.

Introduction

West Nile virus is a zoonotic mosquito-borne virus, it is classified within the family Flaviviridae, genus Flavivirus (Petersen et al., 2013). It is serologically classified within the Japanese encephalitis serocomplex which also includes the Japanese encephalitis and Saint Louis encephalitis virus (May et al., 2011). West Nile virus (WNV) is classified into 5 distinct phylogenetic lineages. WNV lineage 1 and WNV lineage 2 have been associated with human epidemics. Lineage 1 has been geographically linked to outbreaks across Europe, Africa, Middle East, Russia, and the Americas. Lineage 2 is predominantly found within Sub-Saharan Africa. However, in recent times, WNV lineage 2 has also been detected in outbreaks across Europe (Ciccozzi et al., 2013; Hernandez-Triana et al., 2014). The virus was first discovered in December 1937 when it was found in the blood of a febrile woman from the West Nile district of Uganda and since that discovery, both sporadic cases and small outbreaks have been documented across Europe (Smithburn et al., 1940; Zeller & Schuffenecker, 2004).

In recent years, WNV has been endemic in some regions in Europe. There has also been an increasing number of reported outbreaks across Europe, the expansion of the geographical distribution of WNV is a source of public health concern. This may be due to several factors ranging from migration of reservoirs, increase in vector density or climatic changes (Young et al., 2021). This literature review provides an overview of West Nile Virus infection in general, assessing its spread across Europe and factors that influenced the spread. Furthermore, an integrated approach to surveillance, prevention and control which highlights existing knowledge and gaps is discussed.

WNV infections in Europe

West Nile virus lineage 1 was first reported in Europe in 1962, in the Camargue, France. It caused sporadic outbreak in different European countries after its initial detection. After several decades in which no cases were reported, the virus re-emerged in 1996 in Bucharest, Romania. It was the largest outbreak in Europe, characterized by a high fatality rate with 393 reported cases, including 17 fatalities (Tsai et al., 1998). Subsequently, there have been sporadic new cases reported in eastern, western, and southern Europe since this major outbreak in Romania (García-Carrasco et al., 2021).

The other predominant virus lineage known as West Nile virus lineage 2 was first detected within Europe in 2004. It was found in a northern goshawk (*Accipiter gentilis*) in Hungary. This lineage caused important outbreaks in Hungary and neighboring country Austria between 2008 and 2009 (Bakonyi et al., 2006). The virus then spread from Hungary to western and southern European countries, causing large-scale human outbreaks in places like Greece and Italy (Pacenti et al.,

2020). In 2010, the second largest outbreak of West Nile virus occurred within Europe in Greece. It was characterized by a high number of neuro-invasive disease, out of 262 cases, 197 patients were reported to have West Nile neuroinvasive disease (WNND) and it led to 33 deaths (Bouzas et al., 2016; de Filette et al., 2012).

In 2018, Europe experienced its most severe WNV outbreak, both in terms of the number of cases and the geographic scope. The total number of cases (2083) that year was higher than the total number of cases (1832) for the previous seven years (Camp & Nowotny, 2020; Kampen et al., 2020). During this outbreak and onwards, many countries in the Northern part of Europe began reporting their first cases, for example Germany and Netherlands reported the first autochthonous (i.e., acquired by local transmission) case in their country in 2018 and 2020 respectively (Vlaskamp et al., 2020; Ziegler et al., 2020).

Besides the spread of WNV virus in humans, clinical outbreaks have been described in horses in some countries. The most affected countries involving equine outbreaks are Italy and France. The first reported outbreak in Italy occurred in Tuscany in 1998. It re-appeared in 2008 and it has been recurrently detected in horses in the country (di Sabatino et al., 2014; ECDC, 2022; Monaco et al., 2011). For France, the first reported outbreak occurred in 1962, and there have been subsequent major outbreaks in the year 2000, 2015 and 2018 (Beck et al., 2020; Murgue et al., 2001). Although these two countries were the most significantly affected by equine infections. Less severe WNV infections in horses have been reported in countries across Europe. For example, in Portugal, in 2016, an outbreak in horses was reported in the Algrave municipality of Portugal, 10 clinical cases were identified with a case fatality of 50% (Barros et al., 2017). In that same year also, Austria also reported its first equine case (de Heus et al., 2021).

Total number of reported WNV human infections from 2010-2019

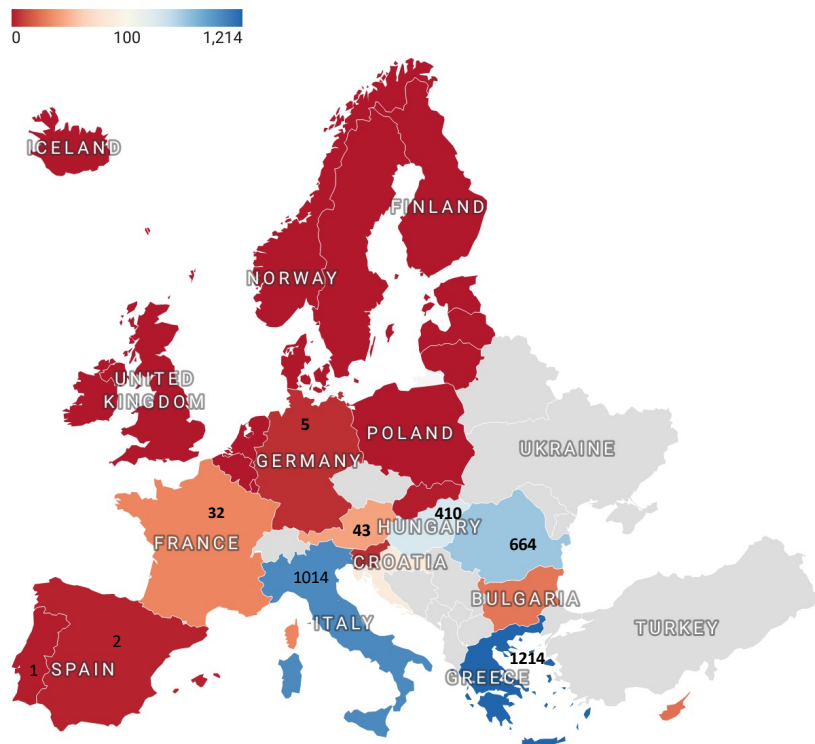


Fig 1: showing the number of human WNV infections from 2010-2019

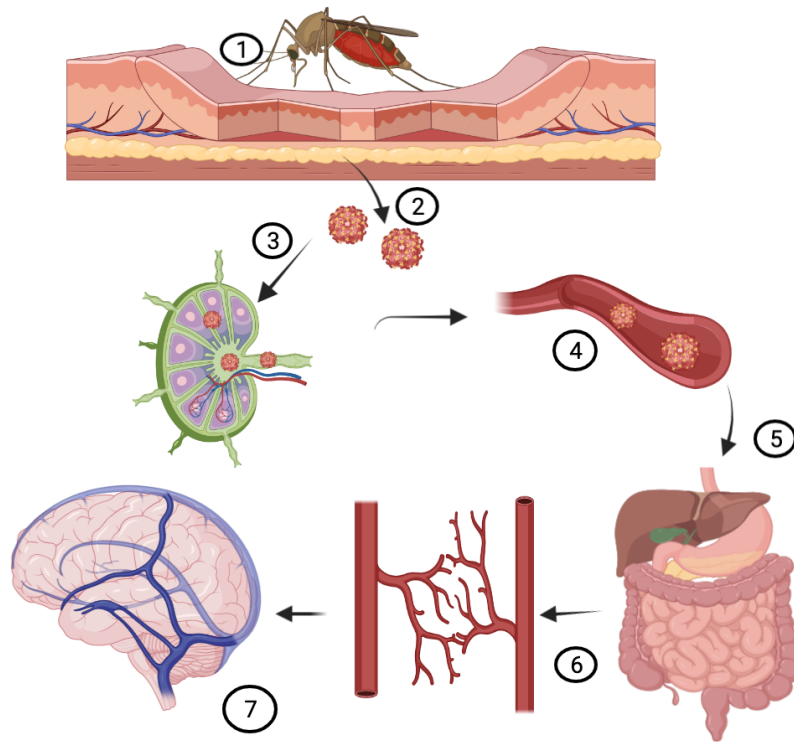
Data sourced from European center for disease control, Annual Epidemiological report- West Nile fever (ECDC, 2022)

(<https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/annual-epidemiological-report>) (see Appendix 1)

Virology, Pathogenesis, and Clinical Features

The genome of the West Nile virus is a positive sense single-stranded RNA. The genome encodes a single polyprotein which is cleaved by the host cell and viral proteases to produce three structural and seven non-structural proteins (Dubrau et al., 2017). The three structural proteins are the capsid (C), pre-membrane (prM/M) and envelope (E) proteins while the seven non-structural proteins are (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5) (Brinton, 2002). The structural proteins are involved in viral particle formation while the non-structural proteins are involved in viral replication, virion assembly and induction of host immune response system (Habarugira et al., 2020; Kramer et al., 2007; van der Meulen et al., 2005).

The pathogenesis of WNV has been studied in several animal models. Mosquitoes become infected with WNV after biting a bird with high level viremia. The virus infects the mid-gut of the mosquito and subsequently it diffuses in the hemolymph to other organs especially to the salivary gland. Mosquitoes may transmit the virus to a host following a blood meal from that host (Fiacre et al., 2020). In the host, after the subcutaneous inoculation of the virus through the mosquito bite, the virus replicates in keratinocytes and Langerhans cells, it then spreads to the lymph nodes resulting in a primary viremia (Lim et al., 2011). Subsequently it spreads to peripheral tissues like spleen and kidney producing a high titer viremia. Following the high viremia, the virus might spread to other visceral organs and potentially disseminate to the central nervous system (CNS) (Petersen et al., 2013). The neurons are the main target cells of the virus in the CNS, with preference for the cerebellar Purkinje cells, anterior horn cells in the spinal cord and neurons of the thalamus and basal ganglia (see Fig 2). The mechanism by which the virus crosses the blood brain barrier into the CNS and the selective tropism among neuronal population remain to be elucidated (Diamond et al., 2003; Shrestha et al., 2003).



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Fig 2: Showing the pathogenesis of WNV infection.

(1) Mosquito transmitting the virus during a blood meal (2) Primary viremia in the lymph nodes (3) migration to peripheral lymph nodes (4) High viremia in the blood (5) Spread of the virus to visceral organs (6) spread of the virus via the blood brain barrier (7) WNV infection within the central nervous system

WNV is maintained in nature in a transmission cycle between mosquitoes and birds. The virus can also infect humans and other vertebrate animals like horses and crocodile leading to severe illness and death. In humans, the majority (80%) of WNV infections is mostly asymptomatic, however about 20% of infected cases develop a febrile illness known as West Nile fever (WNF). WNF symptoms include fever, fatigue, headache, muscle pain, vomiting, diarrhea, rash, and neck-pain (Campbell et al., 2002; Hayes & Gubler, 2006). Also, a small number of infected cases (< 1%) progress to the neuroinvasive form of WNV infection known as West Nile Neuroinvasive disease (WNND) (Fall et al., 2017). WNND is characterized by ataxia, in-coordination, meningitis, encephalitis and polio-myelitis-like flaccid paralysis (Davis et al., 2006). Also in animals, the clinical manifestations are similar ranging from non-neurological signs like, fever, fatigue, lethargy, and weight loss to neurological signs like ataxia, recumbency, paralysis and seizures (Byas & Ebel, 2020; Erdélyi et al., 2007; Komar et al., 2003; Miller et al., 2003).

Transmission Cycle

Mosquitoes are the principal vectors of WNV, especially the species of the genus *Aedes* and *Culex* (García-Carrasco et al., 2021). However, the efficiency of the species as vectors varies depending on geographical location. For example, *Culex pipiens* and *Cx. quinquefasciatus* have been found to be efficient vectors of WNV in the Northern and Southern region of the United States respectively (Petersen et al., 2013). While *Cx. univittatus* has been identified as an important vector in South Africa (Jupp, 2006). The distribution of these mosquito vectors could be dependent on several factors such as mosquito feeding behavior, temperature, rainfall patterns, or density of susceptible host populations (Epstein, 2001). Also, the virus has been isolated from arthropods like ticks (*Hyalomma marginatum*, *Rhipicephalus sanguineus*, *Argas Hermannii*) and mites (*Ornithonyssus sylviarum*), although their role in the transmission cycle needs to be further investigated (Hubálek & Halouzka, 1999; Mumcuoglu et al., 2005).

With regards to birds, they are the natural reservoir hosts for WNV. They not only act as a reservoir but also amplify the virus (Campbell et al., 2001). Birds of some species, especially those belonging to the order Passeriformes (songbirds), are the most susceptible to WNV; they develop high viremia, become ill and die after infection (McLean et al., 2006). Other birds, like common grackles (*Quiscalus quiscula*) and house sparrows (*Passer domesticus*), develop sufficient levels of viremia capable of infection but have lower mortality rates (Komar et al., 2003). Humans and horses are dead-end hosts for WNV. Dead-end hosts are hosts susceptible to infection by the virus but the concentration of the virus (i.e., viremia) within them is not sufficient to infect a mosquito and maintain the virus in circulation (Sule et al., 2018).

WNV is primarily transmitted by mosquitoes to the host. WNV can also be transmitted when susceptible hosts consume infected animals or are bitten by mosquitoes (van der Meulen et al., 2005). Many avian species also transmit the virus from bird-to-bird or bird-to-human via contact. This might be due to shedding of large levels of the virus in their feces or oral secretions (Kipp et al., 2006). In addition, transmission has been reported to occur in humans via blood transfusions and organ transplants, see Figure 3 (Iwamoto et al., 2003; Pealer et al., 2003).

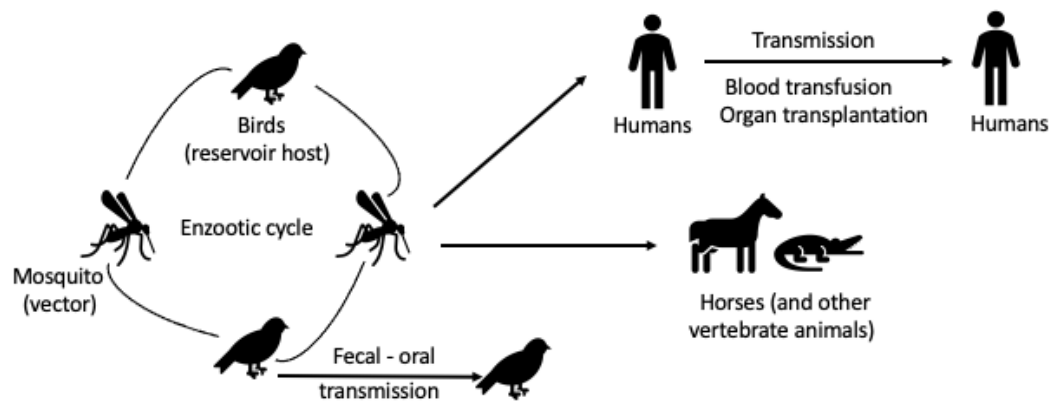


Fig 3: Transmission cycle of West Nile Virus

Factors influencing WNV transmission

As previously stated, West Nile virus is maintained in nature by an enzootic cycle between birds and mosquitoes. It could also cause spill-over infections in horses and humans which are dead end hosts. WNV has a complex interaction between its vector, reservoir host and the environment and the interaction between these factors leads to varied transmission dynamics across the continent. The factors influencing WNV transmission are discussed below.

i) Avian host

Birds have the physical ability to travel through continents crossing ecological barriers like seas and mountains. During their migratory phase, they can act as vehicles in transmitting the virus to a wider geographical range other than their established region (Mancuso et al., 2022). In Europe, it has been hypothesized that the introduction of the WNV lineage 2, a lineage responsible for the recent outbreaks in the continent, was because of migratory birds travelling to Europe from Africa during spring migration (Calistri et al., 2010; Rappole & Hubálek, 2003). It was first detected in Hungary and since then, it appeared to have been locally spread throughout Hungary, and it was also reported to be isolated from birds in Austria. Austria is a neighboring country and phylogenetic analysis showed that the lineage identified was similar to the one detected in Hungary (Hernandez-Triana et al., 2014; Wodak et al., 2011). Subsequently, WNV lineage 2 has been dispersed across central and south-eastern Europe, presumably by birds (di Sabatino et al., 2014).

Their role regarding possible introduction into the environment could be in terms of developing sufficient viremia, enough to establish the virus within the mosquito population in the area. This draws attention to the fact that host competence varies between birds, and this might affect the transmission dynamics of WNV. For example, a highly susceptible host may not develop sufficient viremia to infect mosquito, or they can also become too ill to fly and migrate and this can make them incompetent reservoir host. Also, the feeding preference of the mosquito vector can be dependent on the type of bird. Research has also shown that the most preferred host for mosquitoes regarding WNV virus transmission are house sparrows, American robin, and various corvid species. Host competence and vector preference vary temporally and spatially within Europe (Hamer et al., 2009; Rizzoli et al., 2015; Simpson et al., 2012).

ii) Mosquito vectors

In Europe, the principal vector of WNV belongs to the *Cx. pipiens* complex and it is the most ubiquitous mosquito species. The *Cx. pipiens* complex contains two biotypes: *pipiens* and *molestus*. They are morphologically similar but differ in physiology and behavior (Harbach, 2012; Reusken et al., 2010). This difference can be seen in the vectorial capacities of members within

the complex which is based on their different feeding preferences. The biotype *pipiens* is described as ornithophilic (bird-host biting preference), anautogenous (requires blood meal to lay eggs) and eurygamous (unable to mate in confined spaces). The biotype *modestus* is mammophilic (mammalian-host biting preference), autogenous (lays eggs without a blood meal) and stenogamous (mates in confined spaces) (Fonseca et al., 2004; Zittra et al., 2016). The differences in vectorial capacity of the *Culex* biotypes influences transmission dynamics of WNV, for example *Cx pipiens* which has a feeding preference for birds would play an important role in maintaining the enzootic transmission of WNV (Vogels et al., 2017).

These two biotypes could also form hybrids which have been shown to exhibit opportunistic feeding behavior resulting in bridge vectors that could feed on both on humans and birds. So far, these hybrids have been occasionally observed in some part of Europe like the Netherlands and Portugal (Osório et al., 2014; Reusken et al., 2010). An increase in the presence of hybrid vectors can create a higher risk of WNV outbreaks within human population (Vogels et al., 2016). However, hybridization events for both biotypes are rare due to selective and exclusive habitat use, but habitat suitability in Europe especially in the South is changing due to climatic changes (Zittra et al., 2016).

Apart from different vectorial capacities, another major distinction between the two biotypes is the peculiarity of *Cx.pipiens*. Females of this species can enter a period of arrested development known as diapause (Denlinger & Armbruster, 2014). Diapause offers a mechanism to bridge unfavorable conditions during winter, hormonal pathways program physiological changes such as building up fat reserves and these pathways are triggered by lower temperature and reduced daylength (Denlinger & Armbruster, 2014; Sim & Denlinger, 2008). This process is commonly known as overwintering. Some studies have detected WNV in populations of overwintering mosquitoes. This means that during warmer months, the overwintered mosquitoes are capable of initiating virus circulation. This might suggest that the virus is also maintained in the environment via this process rather than only re-introduction through migratory birds. (Rudolf et al., 2017).

iii) Environmental factors

Climate change is an important environmental driver for WNV transmission. Higher temperature is a favorable climatic condition that can lead to extensive spread of West Nile virus infection. Warm temperature plays a role in viral replications by increasing the number of replication cycles of the virus in mosquitoes. This shortens the incubation time of the virus within the mosquitoes and leads to increased rate of virus transmission (Camp & Nowotny, 2020). Higher temperature has also been found to affect the mosquito vector by prolonging the breeding season, increasing the growth rate of its population, and decreasing the interval between blood meals on host (Paz et al., 2013; Paz & Semenza, 2013). The influence of temperature on the reservoir bird host is also an important point to consider. A higher temperature creates a favorable condition for migratory

breeding bird species. Migratory birds infected with WNV can possibly carry the virus to their breeding sites in Europe during the spring migration (Bhowmick et al., 2020).

Additionally, WNV activity can also be associated with precipitation. This interaction is complex and varies depending on geographical region and other climatic conditions. To illustrate, a high amount of precipitation may lead to large areas of surface waters which may favor vector abundance (Leblond et al., 2007; Tran et al., 2014). This is because the larvae stage of mosquitoes requires standing surface water to complete their lifecycle (Becker et al., 2010; Chevalier et al., 2013). On the other hand, in times of drought, the reduced number of water sources may lead to the aggregation of birds and mosquitoes in the same area creating more interaction for WNV transmission between host and vector. Also, the organic matter in water bodies can become more concentrated during seasons of low levels of precipitation and this can create a conducive environment for the adult mosquitoes to proliferate (Camp & Nowotny, 2020; Chase & Knight, 2003).

While the environmental and ecological drivers of WNV outbreaks are complex, the occurrence and geographical expansion in Europe persists. This highlights the need to have a strategic approach to its surveillance, prevention, and control, this would help mitigate the impact of the virus within Europe.

Discussion

Since WNV has a lifecycle which involves complex interactions between its mosquito vectors, reservoir hosts, dead-end hosts which all interact in a dynamic environment, an integrated approach would be effective in its surveillance and control of the virus.

Entomological surveillance of adult mosquitoes could help identify if WNV virus is currently in high circulation especially in endemic areas. This could impact preparedness and the implementation of appropriate and timely measures to reduce outbreaks. For example, Italy has an established entomological surveillance, and in 2013, mosquitoes were the first to signal WNV circulation almost a month before the first reported case of Human West Nile neuroinvasive disease (Bellini et al., 2014). There could be some limitations to mosquito surveillance because to validate and increase the probability of detection, large number pools of mosquitoes need to be tested. Also, accurate identification of mosquito species and rapid detection of WNV is necessary. This is time consuming and costly (Engler et al., 2013). To help ensure that effective mosquito surveillance can be implemented, factors such as ecological condition, geographical location should be considered during surveillance. The surveillance of mosquitoes could also help provide evidence-based information to aid communication channels about creating awareness in humans. Such awareness information could involve adoption of appropriate protective measures to reduce interaction with mosquitoes, for example, wearing protective clothing and precautionary behavior like avoiding mosquito dense areas or residential reduction of surface water around.

Horses can also be used as sentinels in WN virus infection surveillance (Dauphin et al., 2004). This could be by active or passive surveillance of horses. Passive surveillance can involve early identification of a potential outbreak based on classical clinical signs exhibited by the horses and/or a diagnostic laboratory confirmation (Faverjon et al., 2016). The efficacy of passive surveillance of horses would be dependent on the amount of morbidity caused by WNV strains and how this information is passed on to the appropriate authorities (Dauphin et al., 2004). This may not be effective in early detection of virus circulation within horses, but it could be a useful approach to monitor the risk of an outbreak in human populations within the area (Cito et al., 2013; Leblond et al., 2007). Active surveillance would involve scheduled regular serological surveillance and this is particularly useful in high risks area or places where previous outbreaks have occurred (Costa et al., 2020). The sensitivity of active surveillance can be influenced by the use of equine vaccines among owners. This concern is limited in Europe because equine vaccination is rarely conducted in Europe (Beck 2013).

Additionally, birds could also be used to monitor WNV since the virus is maintained in nature within birds. The surveillance of birds has been used to detect WNV circulation in several

European countries. (Bakonyi et al., 2013; Balança et al., 2009; Calistri et al., 2010). Increased mortality in wild birds could be an indicator of WNV circulation. However, a limitation of this is that unless mortality is high, the circulation of the virus is not detected early. Active surveillance of live wild birds in combination with passive surveillance of dead birds could be a more effective strategy (Lauriano et al., 2021). However, the active surveillance of WNV using birds has been reported to not be effective for early detection of the virus but it can be used as an indicator for areas at risk of WNV introduction and understanding WNV epidemiology within the surveillance area (Alba et al., 2014; Petrović et al., 2018).

Also, for mosquitos, birds, and horses' surveillance to be effective, it would require a close multi-sectoral collaboration. Veterinary public health organization would need to work in close collaboration with human health services to ensure that the pipeline of such notification is effective, and this information can be used to effectively improve surveillance within the human populations. In humans, a syndromic surveillance of patients presenting neurological signs like meningitis or meningoencephalitis can be used to detect outbreaks of WNV in an area. Also considering that WNV can also be transmitted by blood transfusion and organ transplantation, routine screening for WNV would be effective for surveillance. Already, nucleic acid testing of blood supplies has been implemented in some European countries where WNV is endemic (Napoli et al., 2013).

In terms of control, it consists of vector control, control of the disease in horses via vaccination, and developing vaccine for humans and other susceptible species. For vector control, the reduction of the larval population of mosquitoes could help reduce the population density of adult mosquitoes to a level where it does not pose a public health risk. Larval control can involve the use of biological, microbial, chemical, or environmental method. Biological larva control involves the introduction of biological agents or predators such as fish and copepods into water bodies. This approach needs to be assessed in terms of environmental risk and potential biodiversity loss (Bellini et al., 2014). Microbial larval control involves the use of industrially produced bacteria such as *Bacillus thuringiensis israelensis* and *Bacillus sphaericus* to control larval population. The use of these products is also permitted in natural protected areas; however, it has a short-lasting activity, requires repeated application and resistance after repeated use as been reported (Bellini et al., 2014; Mulla et al., 2003). Chemical larva control involves using chemical larvicides on targeted breeding sites. Environmental management method can involve reduction of standing water, control of vegetations in wetlands (Knight et al., 2003).

In addition, adult control of mosquitoes can be used during an outbreak situation to promptly reduce vector density within outbreak area. The use of adulticides is the most common method and it is highly regulated due to the development of resistance, its impact on the environment and the effect on non-target organisms. During vector control activities, effective surveillance must be maintained to determine if and when re-treatment is required to keep vector populations suppressed

(CDC, 2013). There is currently no threshold for the degree of vector population suppression that must be achieved or the length of time that this suppression must be maintained to reduce human disease transmission (CDC, 2013)

In terms of vaccination, currently there are 4 WNV veterinary vaccines licensed for use in horses. The first three are whole inactivated virus and the fourth is a chimeric recombinant canarypoxvirus vaccine (de Filette et al., 2012; el Garch et al., 2008; Kaiser & Barrett, 2019). These vaccines require multiple primary doses and annual boosters to induce protection. In Europe, the vaccines are sparingly used, this may be due to the annual vaccination requirement and the cost of vaccination is usually incurred by the owner (Young et al., 2019). In addition, there is currently no commercially approved vaccine for human use but there is current research undergoing, therefore other preventive methods previously mentioned are advised.

To conclude, with the current trend of globalization and changes in climatic condition, WNV infections have become a public health concern. The complex dynamics of WNV epidemiological situation in Europe means that surveillance strategies should be tailored to each country epidemiological, climatic, and geographic situation. Integrating an interdisciplinary approach which would involve collaboration from medical practitioners, veterinary public health services, environmentalists, human public health services would help improve surveillance and monitoring efficiency. This would ensure rapid detection of virus circulation and efficient response to mitigate the impact of WNV disease.

APPENDIX

APPENDIX 1

Table showing Human West Nile Infections in Europe from 2010 -2019 (ECDC 2022)

Country	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	Total
Austria	0	0	0	0	1	6	5	6	21	4	43
Belgium	0	0	0	0	0	0	0	0	0	0	0
Bulgaria	.	.	2	0	0	2	2	1	15	5	27
Croatia	.	.	5	16	.	1	2	5	58	0	87
Cyprus	0	0	0	0	0	0	1	0	1	23	25
Czech Republic	0	0	0	1	0	0	0	0	5	1	7
Denmark	0
Estonia	0	0	0	0	0	0	0	0	0	0	0
Finland	0	0	0	0	0	0	0	0	0	0	0
France	0	0	0	0	0	1	0	2	27	2	32
Germany	5	5
Greece	262	100	161	86	15	0	0	48	315	227	1214
Hungary	18	0	17	31	11	18	44	20	215	36	410
Iceland	0	0	0	0
Ireland	0	0	0	0	0	0	0	0	0	0	0
Italy	3	14	50	69	24	61	76	53	610	54	1014
Latvia	0	0	0	0	0	0	0	0	0	0	0
Liechtenstein	0
Lithuania	0	0	0	0	0	0	0	0	0	0	0
Luxembourg	0	0	0	0	0	0	0	.	0	0	0
Malta	0	0	0	0	0	0	0	0	0	0	0
Netherlands	0	0	0	0	0	0	0	0	0	0	0
Norway	0	0	0	0	0	0	0	0	0	0	0
Poland	0	0	0	0	0	0	0	0	0	0	0
Portugal	1	0	0	0	0	1
Romania	57	11	14	24	23	32	93	66	277	67	664
Slovakia	0	0	0	0	0	0	0	0	0	1	1
Slovenia	0	0	0	1	0	0	0	0	4	0	5
Spain	2	0	0	0	0	0	0	0	0	0	2
Sweden	0	0	0	0	0	0	0	0	0	0	0
United Kingdom	0	0	0	0	0	0	0	0	0	0	0

APPENDIX 2: Search strategy for Literature review

A literature search was conducted across PubMed. A combination of the search terms “West Nile Virus” and “Europe” was used. The hit results were screened by their title and abstract. A set of qualifier words were used to screen the articles (geographical location: [(Europe)], [(humans)], [(animals)], [(epidemiology)], [(surveillance)], [(virology)], [(ecology)], [(control)], [(review)], [(vaccine)], [(treatment)], [(prevention)], [(environment)]). These qualifier words were used as the inclusion criteria for the literature search. The exclusion criteria for this literature review were: i) articles focusing on one specific case ii) articles focused on experimental studies iii) studies reporting exclusively on laboratory studies of West Nile virus iv) articles that are part of a textbook. v) articles focused solely on seroprevalence. Selected articles were written in English and no date limit was considered. Additional literature was identified using specific keyword search on Google scholar.

Search engine	Search terms
PubMed	("west nile virus"[MeSH Terms] OR ("west"[All Fields] AND "nile"[All Fields] AND "virus"[All Fields]) OR "west nile virus"[All Fields]) AND ("europe"[MeSH Terms] OR "europe"[All Fields] OR "europes"[All Fields] OR "europes"[All Fields])
Google scholar	“West Nile virus” and “surveillance” and “mosquitoes” and “migratory birds” and “vaccine”

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