Awaiting the First Locally-Acquired Human West Nile Virus Infection in Belgium

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Abstract

West Nile virus is an arthropod-borne *Flavivirus* transmitted by *Culex* mosquitoes. Birds are the primary hosts. However, the virus can be transmitted to humans through mosquito bites. Human infection is mostly asymptomatic, but 1 in 5 may develop illness: West Nile fever or severe West Nile neuroinvasive disease. Although the virus and disease are spreading in Europe, no locally acquired infections have been reported in Belgium. However, there is a real risk that West Nile virus infections will occur in Belgium in the near future. Because children can become infected and ill, pediatricians must be aware of the disease. In this manuscript, we describe the symptoms and epidemiology of West Nile virus disease.

Introduction

The West Nile virus (WNV) is a single-stranded RNA virus of the family *Flaviviridae*, genus *Flavivirus*. It is an arthropod-borne virus ("arbovirus") transmitted by mosquitoes, mainly of the genus *Culex*. Birds, both migratory and non-migratory, are the primary hosts of WNV. The virus is maintained in nature in an enzootic bird-mosquito-bird transmission cycle. The virus can be transmitted to mammalian species through mosquito bites. In particular, humans and horses can develop disease. However, they are considered dead-end hosts because they do not contribute to the transmission cycle (Figure 1) (1).

Human WNV infection was first detected in a woman in the West Nile district of Uganda in 1937 (2). WNV is the most widely distributed arbovirus in the world (3). It is widespread in Africa, the Middle East and western Asia. Serological surveys have demonstrated WNV circulation in Europe since the 1950s. Human disease affects southern, eastern and western Europe, and human cases have increased in recent decades (4, 5). The virus emerged in the Americas in 1999. After its initial detection in New York, the virus spread dramatically and rapidly across the continent. Today, WNV is the leading mosquito-borne viral infection and the most common cause of viral encephalitis in the United States (6). This rapid spread and the potential for serious health problems are reasons for careful vigilance.

Human WNV infection

Human infection can result in 3 scenarios: asymptomatic infection, febrile illness (West Nile fever (WNF)), or severe disease affecting the central nervous system (West Nile neuroinvasive disease (WNND)). Asymptomatic infection occurs in approximately 80% of infected individuals, WNF in approximately 20%, and WNND in ≤ 1% (1, 7). In terms of incidence, pediatric cases account for 4% of all WNND cases, while 96% of WNND occurs in adults (8, 9).

After an incubation period of 2 to 15 days (up to 21 days in immunocompromised individuals), WNF in children presents as a relatively mild illness with fever (sometimes high), headache, muscle weakness, muscle and joint aches, and fatigue (10). In 50-80% of cases, a maculopapular rash develops on the chest, back, and arms. Other possible symptoms include vomiting and diarrhea, eye pain, and lymphadenopathy. Acute symptoms last 3 to 10 days, but full recovery, especially from fatigue, may take up to 60 days (11, 12).

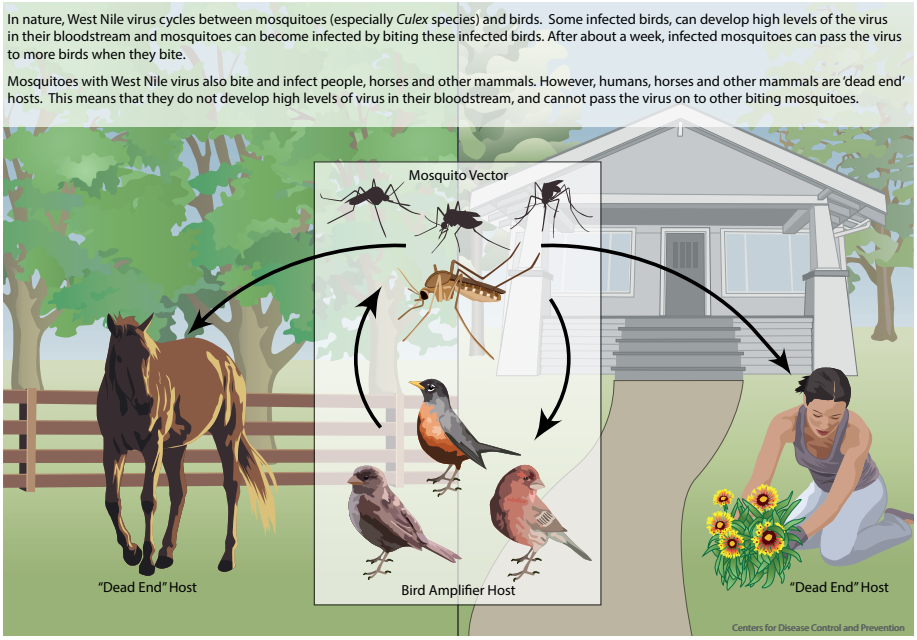
The 3 most common presentations of WNND in children are meningitis, encephalitis, and acute flaccid paralysis (AFP). In contrast to adults, meningitis is a more likely presentation in children than encephalitis. Meningitis is characterized by nuchal rigidity, headache, and other classic meningitis symptoms. Besides headache, encephalitis can present with altered consciousness, lethargy, personality changes, focal neural deficits, seizures, and other movement disorders. AFP has been reported in 1% of children with WNND and may occur with or without encephalitis. As in poliomyelitis, WNV-associated AFP is caused by invasion of the anterior horn cells, resulting in progressive asymmetric flaccid paralysis without sensory abnormalities, sometimes requiring mechanical ventilation (9, 11, 12). Brain magnetic resonance imaging often appears normal, but signal abnormalities may be observed in the basal ganglia, thalamus, and brainstem in cases of WNV encephalitis, and in the spinal cord in cases of WNV acute flaccid paralysis (10). Recovery from WNND takes weeks to months and may result in long-term sequelae, mainly fatigue and apathy, in 50% of cases. Although children with WNND have a better prognosis than older, they remain at risk for serious neurological sequelae or death, with a mortality rate of 1% compared to 14% in adults (13). WNV infections can also trigger Guillain-Barré syndrome (10). Finally, WNV infections can rarely cause cardiac dysrhythmias, myocarditis, rhabdomyolysis, optic neuritis, uveitis, chorioretinitis, pancreatitis, hepatitis, and orchitis.

Transmission of the virus is primarily through a mosquito bite and occurs when mosquitoes are most active, which is from June to November in Europe (14). Therefore, WNV is primarily a seasonal disease.

Transmission by blood transfusion or organ donation has been described, but can be prevented by screening blood and organ donors in areas of WNV activity.

In 2002, a case of intrauterine transmission was first described in a child born to a mother infected with WNV at 27 weeks' gestation who developed neuroinvasive disease. The infant had severe central

Figure 1: West Nile virus transmission cycle (CDC).



nervous system abnormalities and chorioretinitis at birth, with positive markers for WNV infection in blood and cerebrospinal fluid (both anti-WNV IgM positive) and in placental and umbilical cord tissue (both WNV PCR positive) (15). Subsequently, several studies were conducted on the occurrence of intrauterine transmission (16-19). In a total of 120 pregnant women with WNV infection, there were 3 newborns with possible congenital infection: 1 infant with WNV meningitis at 10 days of age, 1 infant born with rash (and bicuspid aortic valve and aortic coarctation), and 1 infant with fatal WNV encephalitis. The problem is that congenital intrauterine infection could not be diagnosed with certainty due to the lack of umbilical cord blood and serum from the newborns. Intrauterine transmission is possible, but apparently very rare.

A first case of transmission through breastfeeding was also described in 2002. A breastfeeding mother had contracted WNV infection from a postpartum transfusion and developed WNND. WNV RNA and specific IgM antibodies were detected in the breast milk. The infant remained healthy, but at 25 days of age, serum specific IgM antibodies turned positive (20). Hinckley et al. reported on 6 infants breastfed by mothers with WNV infection. 5 of the 6 infants developed no clinical or biological signs of infection. 1 infant developed a rash 11 days after the onset of maternal infection, but was not tested (21). Analysis of a total of 46 breast milk samples from mothers with WNV infection revealed specific IgM antibodies in 15/46 (33%) (18, 21). In conclusion, mother-to-child transmission through breastfeeding is possible, but remains rare. The Centers for Disease Control and Prevention (CDC) recommends continued breastfeeding during maternal WNV infection because the risk of WNV transmission does not outweigh the benefits of breastfeeding (22).

Laboratory diagnosis is accomplished by the detection of anti-WNV IgM (and IgG) antibodies in the blood or cerebrospinal fluid (CSF) or by the detection of viral RNA by PCR in the blood or CSF (1, 7, 11). CSF pleocytosis is generally lymphocytic, but can be neutrophilic in the beginning (10).

Anti-WNV IgM antibodies typically become detectable 3 to 9 days after the onset of symptoms and can persist for 30 to 90 days, sometimes up to a year. Therefore, a positive IgM test result may not always indicate acute infection. Anti-WNV IgG can be detected as early as 8 days following illness onset (it generally appears shortly after IgM) and persists for years (10). Diagnosis based on IgG requires collection of an acute and convalescent sample (2 to 3 weeks apart) to demonstrate seroconversion or at least a 4-fold increase in titer. Diagnosis based on antibodies is complicated by significant cross-reactivity with antibodies to other viruses of the genus *Flavivirus*, e.g. after infection with tick-borne encephalitis virus or dengue virus, but also after vaccination against yellow fever or Japanese encephalitis. Positive results should be confirmed by a virus neutralization assay. IgM detection in CSF indicates central nervous system infection and is typically detectable 1 to 8 days after the onset of neurologic illness.

Diagnosis can also be confirmed by detection of viral RNA by PCR in blood or CSF. However, as in most arboviruses, viremia is low, and the viremic period is short, making the probability of detecting WNV infection by molecular testing relatively low. Viral RNA can be detected in blood from 2 to 18 days post-infection and up to 5 days post onset of symptoms. The sensitivity in a whole blood sample is 86.8%, and it is lower in CSF. WNV is excreted in the urine during acute infection and remains detectable for a longer period than in the blood. Therefore, urine may be a useful non-invasive specimen to detect WNV. According to the European Centre for Disease Control and Prevention (ECDC), whole blood is the preferred sample for testing.

To date, there is no specific treatment for WNV disease (7). Treatment is supportive. Treatment with intravenous immunoglobulin (IVIG) in 2 cases of AFP has been described, but it is unclear whether it has any effect (11). Research into specific IVIG with high anti-WNV titers, monoclonal antibodies, antivirals, and the potential benefits of corticosteroids in aiding recovery is still in its early stages (23).

Unlike in horses, there is no vaccine available for humans. However, a number of human vaccine candidates is in preclinical development (24, 25).

After recovery, immunity to WNV is thought to be lifelong (12).

Epidemiology of human WNV disease in European countries

Geographic distribution

The ECDC has reported a total of 825 human cases of West Nile Virus (WNV) infection across the European Union (EU), European Economic Area (EEA), and European Enlargement countries in 2023 (14). This is the third highest number of cases after the peak years of 2018 (1549 cases) and 2022 (1116 cases). The geographic distribution is shown in Figure 2. There were 65 deaths (0.8%).

804 cases were due to locally acquired infection. 21 cases were travel-related (13 of which were in another European country). Most cases occurred between July and September.

Figure 2: Number of cases in 2023 by country.

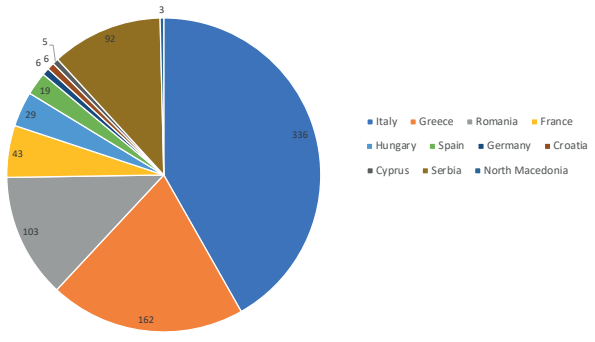
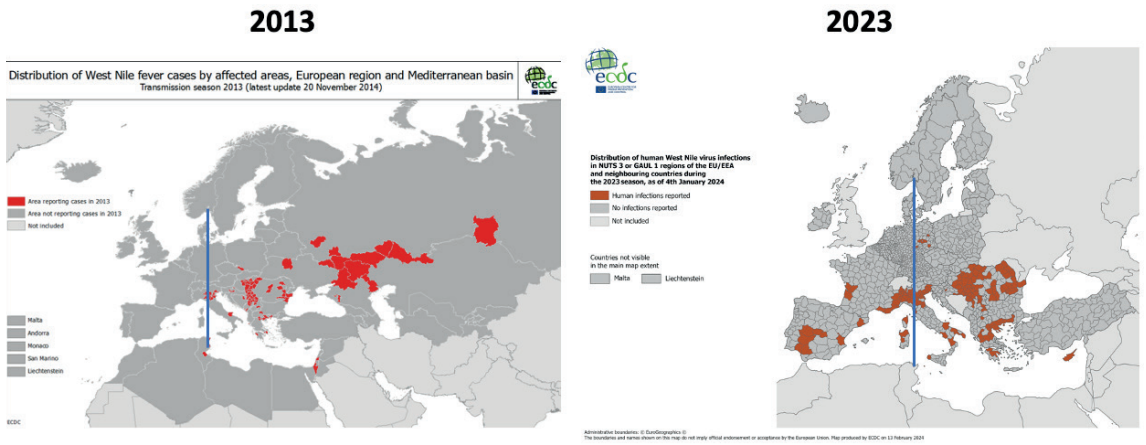


Figure 3: Distribution of WNV cases by affected areas, comparison 2013-2023.



Importantly, although there were fewer cases than in 2022, the number of affected regions increased by 31%, indicating a geographic expansion of the virus. This expansion is illustrated in Figure 3, a comparison between the years 2013 and 2023, in which a westward expansion can be observed.

In 2023, there were no cases of WNV reported in Belgium. However, between 2012 and 2022, 7 cases were recorded, all of which were travel-associated (26).

Dynamics of infection

The co-occurrence of WNV, mosquito vectors, primary avian hosts, and susceptible humans is necessary for the emergence and spread of an epidemic. In addition, there is the influence of climate and environment. All these elements influence the dynamics of infection.

In Europe, human WNV infections are mainly caused by WNV lineages 1a and 2. Historically, lineage 1a has been the most important. However, in 2004, lineage 2 emerged in Hungary and became responsible for most outbreaks between 2010 and 2020 (27). This lineage originated from South Africa and was probably introduced into Hungary by migratory birds between 1996 and 2004 (27, 28). The spread of WNV lineage 2 eventually resulted in a major outbreak in Europe in 2018, marking the highest number of human cases ever recorded in the EU/EEA. Similar to other viruses, WNV lineages undergo mutations in the genome. This genetic plasticity poses a constant risk of the emergence of genotypes with increased virulence (29). Shifts in lineage and/or virulence have been associated with regional spread of the virus. Shifts may occur locally or, more importantly, as a result of the reintroduction of variant virus clades by migratory birds. Such reintroductions are common in Europe. Genomic analyses have identified at least 13 reintroductions with multi-year persistence (27). This is illustrated by the emergence of a new, more pathogenic WNV 1a variant in northern Italy in 2021 (30).

Mosquitoes of the genus *Culex* (*Cx*) are a major vector of WNV (31). Mosquitoes become infected with WNV when they suck blood from a sick bird. After development, the virus ends up in their salivary glands and is transmitted during the next blood meal. The virus does not harm the mosquitoes, which can carry the virus for life (32). In addition, vertical transmission from infected female mosquitoes to their offspring has been described, which contributes to the survival of WNV (33, 34). In Belgium *Cx pipiens* and *Cx torrentium* are the most prevalent *Cx* species (35, 36). There are 2 biotypes of *Cx pipiens*: *Cx pipiens pipiens* and *Cx pipiens molestus*, as well as hybrid forms that combine characteristics of both biotypes. *Cx pipiens pipiens* is an ornithophilic mosquito that mainly bites birds and occasionally humans. It plays an important role in the enzootic mosquito-bird-mosquito cycle. *Cx pipiens molestus* and the hybrid forms are more anthropophilic and act as bridging vectors to humans and other mammals. *Cx torrentium* is also an ornithophilic species but also considered a bridging vector (37). Another *Culex* species, *Cx modestus*, is increasingly found in Europe, including Belgium. This species is anthropophilic and may be more competent at transmitting WNV to humans than *Cx pipiens* (38, 39).

Birds are natural hosts and reservoirs for WNV. The mosquito-bird-mosquito cycle maintains the circulation of the virus. Migratory birds are considered important introducers of WNV into new regions (5, 40). Outbreaks of WNV infection often occur in late summer and early fall, coinciding with the arrival of large numbers of migratory birds. Infection of migratory birds has been documented by virus isolation and antibody detection. Domestic birds can also become infected.

Disease in birds is characterized by loss of coordination, head tilt, tremors, weakness, and apparent loss of vision (32). The susceptibility of birds varies, with corvids (crows, jays, ravens, magpies) being most likely to die from the disease. Birds of prey (owls, falcons, hawks, etc.) are also particularly susceptible, potentially because they prey on infected animals (41).

Both humans and horses typically do not reach a level or duration of viremia adequate to transmit the virus to mosquitoes, making them dead-end hosts for WNV. As in humans, infection in horses is usually asymptomatic. 10% of infected horses show neurological signs of disease; mortality in horses with clinical signs is approximately 33% (7, 32). Presumptive diagnosis is made on the basis of specific IgM antibodies. Vaccination against WNV is available for horses.

Surveillance of mosquitoes, sick birds and horses by public health officials is essential for the detection of WNV.

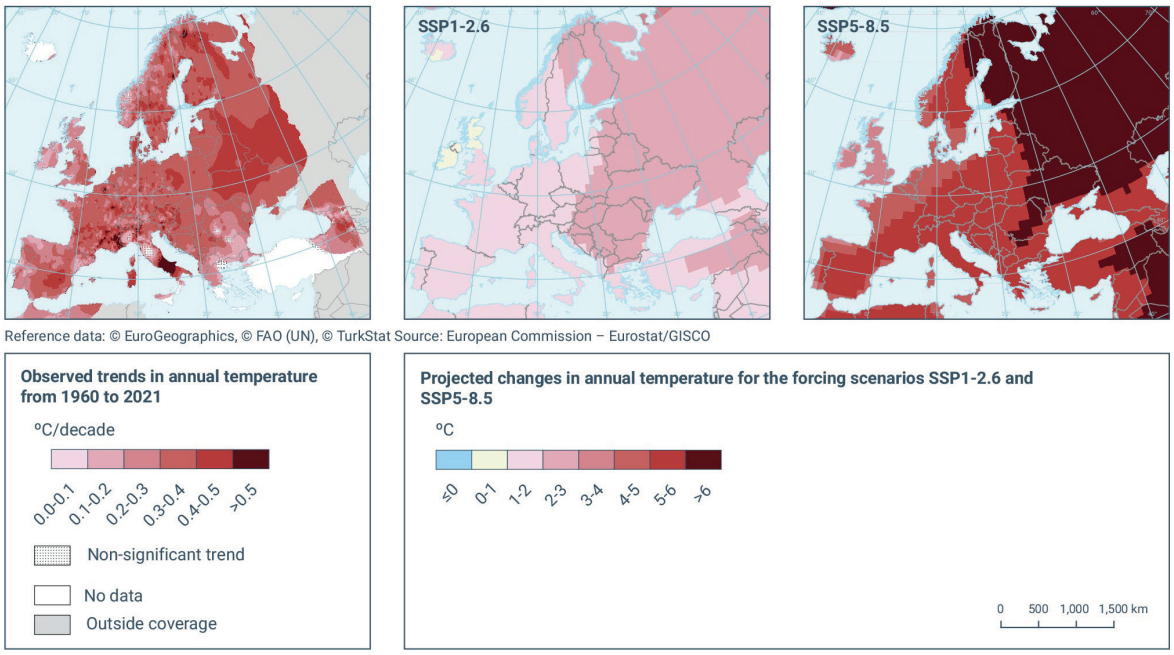
To initiate and sustain a human epidemic, there must be regular interactions between infected mosquitoes and humans. These interactions are significantly shaped by environmental factors such as temperature, rainfall, wetlands, vegetation, proximity to migratory bird routes, periurban environment, and human and mammalian density. While understanding the complexities of these relationships on a global scale can be challenging, ongoing research is revealing some clear connections (5, 42). There is a positive correlation between higher mean temperature and mosquito abundance and activity, increased circulation of WNV and transmission risk in birds and occurrence of WNV infection in humans and mammals. The temperature during the warmest quarter of the previous year appears to be the main driver of WNV outbreaks in Europe (43). Above-average spring temperatures may also be a precursor to an outbreak in the second half of the year. Warmer temperatures also influence human behavior by increasing outdoor activities and the risk of exposure to mosquitoes. *Cx.* vectors are present in both rural and urban environments, where human population density may also play a role in the risk of infection.

The presence of wetlands is positively associated with the abundance of mosquitoes and birds and the transmission of WNV to humans and mammals. Proximity to migratory bird flyways is also important.

Climate change could profoundly influence environmental factors.

Europe is getting warmer (Figure 4). Heat waves are becoming more frequent and severe, and summers are getting longer and warmer. This

Figure 4: European Environment Agency, Projected temperature changes under the best and worst greenhouse gas emission scenarios.



is anticipated to lead to the expansion of viral vectors and hosts, thereby facilitating the virus's spread to new areas, particularly in western and central Europe. It has been calculated that, depending on the CO_2 release scenario, the risk of WNV infection could increase by a factor of 2.5 to 5 (3, 44, 45). There is also an overall trend toward less precipitation in southern and western Europe. Drought conditions with stagnant pools of water increase the interaction between vectors and hosts, increasing the likelihood of virus transmission and disease. But there is also an increased risk of exceptional rainfall and flooding, which can bring more standing water for mosquito breeding.

Migratory birds play an important role in introducing the virus to new areas (40, 45). They bring the virus to Europe from southern stopovers in Africa or the Middle East. The spread of WNV to more northern parts of Europe carries the risk of the virus spreading not only from the south in the spring, but also from the north during fall migration. Climate change may also lead to changes in migratory patterns, which may also contribute to the spread of WNV to new regions (46).

What about Belgium?

Epidemiologic surveillance of WNV in Belgium is carried out by the National Reference Centre for Arboviruses at the Institute of Tropical Medicine in Antwerp. Between 2012 and 2022, 7 travel-associated human infections were identified. The travelers were all adults and came from Djibouti (2), Greece, the Democratic Republic of Congo, South Sudan, Serbia, and Kosovo. No autochthonous human infections and no infections in birds or horses were identified (26).

However, there is a clear risk of WNV infection in Belgium. The vectors *Cx. pipiens pipiens*, *Cx. pipiens molestus*, their hybrids and *Cx. torrentium* are widespread and the presence of *Cx. modestus* has been demonstrated (38, 47). There is a wide variety of native bird species, and Belgium is located on the East Atlantic Flyway for migratory birds. Numerous habitats foster mosquito-bird interactions, such as nature reserves, wetlands, vegetated areas near ponds or lakes, and human-made small bodies of stagnant fresh water in periurban regions, like buckets, bottles, gutters, and water storage tanks. Belgium has a high human population density, along with having the highest number of horses per capita among EU countries (48). Additionally, West Nile Virus transmission is ongoing in neighboring countries such as France, Germany, and the Netherlands. Considering the impact of climate change, it is highly likely that the virus will emerge in Belgium in the near future. Therefore, it is worrisome that active surveillance of birds and horses was halted in 2017 (26).

Although WNV is notifiable throughout Belgium, only cases acquired in Europe need to be reported.

Conclusion

There is a genuine risk of West Nile Virus (WNV) infections in Belgium in the near future. It is crucial for pediatricians to give serious consideration to WNV when assessing children with unexplained fever, rash, meningitis, and/or encephalitis, as well as acute flaccid paralysis during the mosquito season.

Conflict of interest disclosure

The authors have no conflicts of interest to declare with regard to the topic discussed in this manuscript.

REFERENCES

1. Centers_for_Disease_Control_and_Prevention. West Nile Virus. Atlanta, Georgia, USA: CDC; 2023 [cited 2024 February 24]. Available from: https://www.cdc.gov/westnile/index.html.
2. World_Health_Organisation. West Nile Virus. Geneva, Switzerland: WHO; 2017 [cited 2024 February 24]. Available from: www.who.int/news-room/fact-sheets/detail/west-nile-virus.
3. Paz S. Climate change impacts on West Nile virus transmission in a global context. Philosophical Transactions of the Royal Society B: Biological Sciences. 2015;370(1665):20130561.
4. Zehender G, Veo C, Ebranati E, Carta V, Rovida F, Percivalle E, et al. Reconstructing the recent West Nile virus lineage 2 epidemic in Europe and Italy using discrete and continuous phylogeography. PLoS One. 2017;12(7):e0179679.
5. Giesen C, Herrador Z, Fernandez-Martinez B, Figuerola J, Gangoso L, Vazquez A, et al. A systematic review of environmental factors related to WNV circulation in European and Mediterranean countries. One Health. 2023;16:100478.
6. Hadfield J, Brito AF, Swetnam DM, Vogels CBF, Tokarz RE, Andersen KG, et al. Twenty years of West Nile virus spread and evolution in the Americas visualized by Nextstrain. PLoS Pathog. 2019;15(10):e1008042.
7. European_Centre_for_Disease_Prevention_and_Control. Factsheet about West Nile virus infection. Stockholm, Sweden: ECDC; 2021 [cited 2024 February 24]. Available from: https://www.ecdc.europa.eu/en/west-nile-fever/facts.
8. Fraley CE, Pettersson DR, Nolt D. Encephalitis in Previously Healthy Children. Pediatr Rev. 2021;42(2):68-77.

9. Herring R, Desai N, Parnes M, Jarjour I. Pediatric West Nile Virus-Associated Neuroinvasive Disease: A Review of the Literature. Pediatr Neurol. 2019;92:16-25.
10. Committee on Infectious Diseases AAoP, Kimberlin DW, Barnett ED, Lynfield R, Sawyer MH. Red Book: 2021–2024 Report of the Committee on Infectious Diseases: American Academy of Pediatrics; 2021.
11. Barzon L, Pacenti M, Sinigaglia A, Berto A, Trevisan M, Palù G. West Nile virus infection in children. Expert Rev Anti Infect Ther. 2015;13(11):1373-86.
12. Rizzo C, Esposito S, Azzari C, Bartolozzi G, Fara GM, Lo Giudice M, et al. West Nile Virus infections in children: a disease pediatricians should think about. Pediatr Infect Dis J. 2011;30(1):65-6.
13. Lindsey NP, Hayes EB, Staples JE, Fischer M. West Nile virus disease in children, United States, 1999-2007. Pediatrics. 2009;123(6):e1084-9.
14. European_Centre_for_Disease_Prevention_and_Control. Epidemiological update: West Nile virus transmision season iin Europe, 2023. Stockholm, Sweden: ECDC; 2024 [cited 2024 February 26]. Available from: https://www.ecdc.europa.eu/en/news-events/epidemiological-update-west-nile-virus-transmission-season-europe-2023-0.
15. Centers_for_Disease_Control_and_Prevention. Intrauterine West Nile Virus Infection --- New York, 2002 MMWR [Internet]. 2002 [cited 2024 February 25]; 51(50):[1135-6 pp.]. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5150a3.htm.
16. Hayes EB, Komar N, Nasci RS, Montgomery SP, O'Leary DR, Campbell GL. Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis. 2005;11(8):1167-73.
17. O'Leary DR, Kuhn S, Kniss KL, Hinckley AF, Rasmussen SA, Pape WJ, et al. Birth outcomes following West Nile Virus infection of pregnant women in the United States: 2003-2004. Pediatrics. 2006;117(3):e537-45.
18. Paisley JE, Hinckley AF, O'Leary DR, Kramer WC, Lanciotti RS, Campbell GL, et al. West Nile virus infection among pregnant women in a northern Colorado community, 2003 to 2004. Pediatrics. 2006;117(3):814-20.
19. Pridjian G, Sirois PA, McRae S, Hinckley AF, Rasmussen SA, Kissinger P, et al. Prospective study of pregnancy and newborn outcomes in mothers with West nile illness during pregnancy. Birth Defects Res A Clin Mol Teratol. 2016;106(8):716-23.
20. Stobierski D, Stoltman G, Downes F, Smith K. Possible West Nile Virus Transmission to an Infant Through Breast-Feeding—Michigan, 2002. Morbidity and Mortality Weekly Report [Internet]. 2002 [cited 2024 February 25]; 51(39):[877-8 pp.]. Available from: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5139a1.htm.
21. Hinckley AF, O'Leary DR, Hayes EB. Transmission of West Nile virus through human breast milk seems to be rare. Pediatrics. 2007;119(3):e666-71.
22. Centers_for_Disease_Control_and_Prevention. Mother to Baby during Pregnancy, Delivery, or Breast Feeding. Atlanta, Georgia, USA: CDC; 2021 [cited 2024 February 25]. Available from: https://www.cdc.gov/westnile/transmission/pregnancy.html.
23. Ormundo LF, Barreto CT, Tsuruta LR. Development of Therapeutic Monoclonal Antibodies for Emerging Arbovirus Infections. Viruses. 2023;15(11).
24. Ronca SE, Ruff JC, Murray KO. A 20-year historical review of West Nile virus since its initial emergence in North America: Has West Nile virus become a neglected tropical disease? PLoS Negl Trop Dis. 2021;15(5):e0009190.
25. Wu B, Qi Z, Qian X. Recent Advancements in Mosquito-Borne Flavivirus Vaccine Development. Viruses. 2023;15(4).
26. Stefani G, Rebolledo J, Van Esbroeck M. Epidemiologische surveillance van Westnijl koorts Westnijl virus (WNV) - 2022. 2023 [cited 2024 February 24]:[2 p.]. Available from: https://www.sciensano.be/en/biblio/epidemiologische-surveillance-van-westijlekoorts-2022.
27. Koch RT, Erazo D, Folly AJ, Johnson N, Dellicour S, Grubaugh ND, et al. Genomic epidemiology of West Nile virus in Europe. One Health. 2024;18:100664.
28. Srihi H, Chatti N, Ben Mhadheb M, Gharbi J, Abid N. Phylodynamic and phylogeographic analysis of the complete genome of the West Nile virus lineage 2 (WNV-2) in the Mediterranean basin. BMC Ecol Evol. 2021;21(1):183.
29. Donadieu E, Bahuon C, Lowenski S, Zientara S, Coulpier M, Lecollinet S. Differential virulence and pathogenesis of West Nile viruses. Viruses. 2013;5(11):2856-80.
30. Barzon L, Pacenti M, Montarsi F, Fornasiero D, Gobbo F, Quaranta E, et al. Rapid spread of a new West Nile virus lineage 1 associated with increased risk of neuroinvasive disease during a large outbreak in northern Italy, 2022: One Health analysis. J Travel Med. 2022.
31. European_Centre_for_Disease_Prevention_and_Control. Culex pipiens - Factsheet for experts Stockholm, Sweden: ECDC; 2020 [cited 2024 February 2028]. Available from: https://www.ecdc.europa.eu/en/infectious-disease-topics/related-public-health-topics/disease-vectors/facts/mosquito-factsheets/culex-pipiens.
32. 32. Cornell_Wildlife_Health_Center. West Nile Virus Ithaca, NY, USA: Cornell University; 2023 [cited 2024 February 29]. Available from: https://cwhl.vet.cornell.edu/disease/west-nile-virus.
33. Baqar S, Hayes CG, Murphy JR, Watts DM. Vertical transmission of West Nile virus by Culex and Aedes species mosquitoes. Am J Trop Med Hyg. 1993;48(6):757-62.
34. Nelms BM, Fechter-Leggett E, Carroll BD, Macedo P, Kluh S, Reisen WK. Experimental and Natural Vertical Transmission of West Nile Virus by California Culex (Diptera: Culicidae) Mosquitoes. Journal of Medical Entomology. 2013;50(2):371-8.
35. Vanderheyden A, Smitz N, De Wolf K, Deblauwe I, Dekoninck W, Meganck K, et al. DNA Identification and Diversity of the Vector Mosquitoes Culex pipiens ss and Culex torrentium in Belgium (Diptera: Culicidae). Diversity 2022, 14, 486. s Note: MDPI stays neutral with regard to jurisdictional claims in published …; 2022.
36. Versteirt V, Boyer S, Damiens D, De Clercq EM, Dekoninck W, Ducheyne E, et al. Nationwide inventory of mosquito biodiversity (Diptera: Culicidae) in Belgium, Europe. Bulletin of Entomological Research. 2013;103(2):193-203.
37. Jansen S, Heitmann A, Lühken R, Leggewie M, Helms M, Badusche M, et al. Culex torrentium: A Potent Vector for the Transmission of West Nile Virus in Central Europe. Viruses. 2019;11(6):492.
38. Soto A, Delang L. Culex modestus: the overlooked mosquito vector. Parasit Vectors. 2023;16(1):373.
39. Wang L, Rosales Rosas AL, De Coninck L, Shi C, Bouckaert J, Matthijnssens J, et al. Establishment of Culex modestus in Belgium and a Glance into the Virome of Belgian Mosquito Species. mSphere. 2021;6(2).
40. Rappole JH, Derrickson SR, Hubálek Z. Migratory birds and spread of West Nile virus in the Western Hemisphere. Emerg Infect Dis. 2000;6(4):319-28.
41. Vidaña B, Busquets N, Napp S, Pérez-Ramírez E, Jiménez-Clavero MÁ, Johnson N. The role of birds of prey in West Nile virus epidemiology. Vaccines. 2020;8(3):550.
42. Tran A, Sudre B, Paz S, Rossi M, Desbrosse A, Chevalier V, et al. Environmental predictors of West Nile fever risk in Europe. Int J Health Geogr. 2014;13:26.
43. Farooq Z, Rocklöv J, Wallin J, Abiri N, Sewe MO, Sjödin H, et al. Artificial intelligence to predict West Nile virus outbreaks with eco-climatic drivers. The Lancet Regional Health - Europe. 2022;17:100370.
44. Farooq Z, Sjödin H, Semenza JC, Tozan Y, Sewe MO, Wallin J, et al. European projections of West Nile virus transmission under climate change scenarios. One Health. 2023;16:100509.
45. Heidecke J, Lavarello Schettini A, Rocklöv J. West Nile virus eco-epidemiology and climate change. PLOS Climate. 2023;2(5):e0000129.
46. Dufour P, de Franceschi C, Doniol-Valcroze P, Jiguet F, Guéguen M, Renaud J, et al. A new westward migration route in an Asian passerine bird. Current Biology. 2021;31(24):5590-6. e4.
47. Vanderheyden A, Smitz N, De Wolf K, Deblauwe I, Dekoninck W, Meganck K, et al. DNA Identification and Diversity of the Vector Mosquitoes Culex pipiens ss and Culex torrentium in Belgium (Diptera: Culicidae). Diversity. 2022;14(6):486.
48. Arnett G. How many horses are there in the European Union? London, UK: The Guardian; 2015 [cited 2024 March 5]. Available from: https://www.theguardian.com/news/datablog/2015/jun/12/how-many-horses-european-union-eu-equine-census-population.