Taylor & Francis
Taylor & Francis Group

Expert Review of Anti-infective Therapy

ISSN: 1478-7210 (Print) 1744-8336 (Online) Journal homepage: https://www.tandfonline.com/loi/ierz20

The knowns and unknowns of West Nile virus in Europe: what did we learn from the 2018 outbreak?

Jeremy V Camp & Norbert Nowotny

To cite this article: Jeremy V Camp & Norbert Nowotny (2020): The knowns and unknowns of West Nile virus in Europe: what did we learn from the 2018 outbreak?, Expert Review of Anti-infective Therapy, DOI: 10.1080/14787210.2020.1713751

To link to this article: https://doi.org/10.1080/14787210.2020.1713751

Accepted author version posted online: 08 Jan 2020.
Published online: 14 Jan 2020.

EXPERT REVIEW OF ANTI-INFECTIVE THERAPY
https://doi.org/10.1080/14787210.2020.1713751

Taylor & Francis
Taylor & Francis Group

REVIEW

The knowns and unknowns of West Nile virus in Europe: what did we learn from the 2018 outbreak?

Jeremy V Camp [a] and Norbert Nowotny [a,b]

[a]Viral Zoonoses, Emerging and Vector-Borne Infections Group, Institute of Virology, University of Veterinary Medicine Vienna, Vienna, Austria; [b]Department of Basic Medical Sciences, College of Medicine, Mohammed Bin Rashid University of Medicine and Health Sciences, Dubai, United Arab Emirates

ABSTRACT

Introduction: West Nile virus (WNV) is a mosquito-borne human and animal pathogen with nearly worldwide distribution. In Europe, the virus is endemic with seasonal regional outbreaks that have increased in frequency over the last 10 years. A massive outbreak occurred across southern and central Europe in 2018 with the number of confirmed human cases increasing up to 7.2-fold from the previous year, and expanding to include previously virus-free regions.

Areas covered: This review focuses on potential causes that may explain the 2018 European WNV outbreak. We discuss the role genetic, ecological, and environmental aspects may have played in the increased activity during the 2018 transmission season, summarizing the latest epidemiological and virological publications.

Expert opinion: Optimal environmental conditions, specifically increased temperature, were most likely responsible for the observed outbreak. Other factors cannot be ruled out due to limited available information, including factors that may influence host/vector abundance and contact. Europe will likely experience even larger-scale outbreaks in the coming years. Increased surveillance efforts should be implemented with a focus on early-warning detection methods, and large-scale host and vector surveys should continue to fill gaps in knowledge.

ARTICLE HISTORY
Received 28 October 2019
Accepted 7 January 2020

KEYWORDS
West Nile virus; West Nile fever; risk factors; disease outbreaks; temperature

1. Introduction

West Nile virus ('WNV', Family *Flaviviridae*) is a remarkable virus. First isolated from a febrile patient in Uganda (1937) and later from mosquitoes, birds, and human patients in Egypt (1950s), its success as a 'generalist' arbovirus has since been well documented [1,2,3]. Perhaps owing to the large number of organisms the virus can infect – mosquitoes (Diptera: Culicidae), hard and soft ticks (Acari: Ixodidae and Argasidae), birds, reptiles, amphibians, and mammals – the virus has spread throughout the globe [1]. After sporadic outbreaks in Africa and Eurasia in the 60 years following its first isolation, the last 20 years have seen the (re)emergence of WNV on every continent except Antarctica and the increased frequency of outbreaks in humans, birds, and horses [2].

Whereas WNV is associated with a febrile illness in humans ('West Nile fever', WNF), two successful genetic lineages – lineage 1 in North America and lineages 1 and 2 in southern, central, and eastern Europe, respectively – are associated with a neuroinvasive disease ('West Nile neuroinvasive disease', WNND) in humans, horses, and birds [4, 5]. In general, it is thought that 80% of human infections are asymptomatic, with only 20% resulting in mild febrile illness [6]. Epidemiological data from Europe reported by the European Centre for Disease Prevention and Control (ECDC) over the last 3 years suggest that approximately 65–73% of reported WNF cases will develop WNND, although this figure likely underestimates the number of unreported WNF cases, and therefore overestimates the proportion of infections which develop WNND, as revealed through blood-donor screening in endemic countries [7,8,9]. Of those patients who develop WNND, the case fatality ratio is approximately 10% [10]. Thus, for every fatal case of WNND, there are at least 40 total cases, with only 10 displaying signs of disease. As the risk groups for WNND include people over 50 years old, and immunocompromised patients (e.g. organ transplant recipients), these asymptomatic cases pose a threat to the donor organ and blood supply [7, 11, 12].

The natural maintenance of WNV (i.e. enzootic cycles of transmission) relies on the virus infecting wild birds and mosquitoes; humans and horses are dead-end hosts. Transmission of the virus occurs when uninfected mosquitoes feed on viremic birds and when virus-competent mosquitoes transmit the virus to naïve hosts. Efficient transmission relies on competent mosquitoes selecting competent avian hosts (i.e. hosts which develop high viremia). Although the virus may infect a large number of animals, vector and host competence vary by species. For example, the virus causes high viremia in many species of birds, making them excellent hosts [13]; however in some bird species, for example North American corvids (American crows, Black-billed magpies) and European accipiters (Northern goshawk), virus infection may cause death [5, 14,15,16]. Similarly, the virus may infect many species of mosquitoes (e.g. *Coquillettidia* spp., *Aedes*

CONTACT Jeremy V Camp Jeremy.Camp@vetmeduni.ac.at; Norbert Nowotny Norbert.Nowotny@vetmeduni.ac.at; Norbert.Nowotny@mbru.ac.ae Viral Zoonoses, Emerging and Vector-Borne Infections Group, Institute of Virology, University of Veterinary Medicine Vienna, Veterinaerplatz 1, Vienna 1210, Austria

Article highlights

- In 2018, Europe experienced the largest outbreak of West Nile virus (WNV) ever recorded
- Increased temperature and precipitation conditions (wet spring followed by drought) provide the most likely explanation for the observed increase in WNV activity.
- There are clear gaps in knowledge about the transmission ecology of WNV in Europe, and future efforts should focus on better defining the enzootic cycle of transmission.

vexans), but *Culex* species are considered the most important vectors, capable of transmitting the virus via saliva [1]. The differences in efficient use of competent vertebrate hosts and competent vectors can be attributed partly to virus genetics, and specific virus mutations that increase the efficiency of virus replication in certain hosts have been demonstrated.

In southern and central Europe and throughout North America, the virus undergoes cycles of annual amplification, reflecting a seasonal enzootic transmission cycle involving birds and mosquitoes. This pattern of limited seasonal outbreaks, which also involve asymptomatic cases in humans and horses, can be traced to the establishment of endemic transmission cycles via the successful overwintering of the virus in these temperate areas [11, 17]. New virus introductions, likely via migratory birds, are more rare – Europe has experienced only two successful WNV introductions in the past 20 years, once in central Europe [4] and once in eastern Europe [18]. Thus, the maintenance of WNV relies on complex interactions between virus genetics (i.e. adaptation to arthropod and vertebrate hosts), environmental factors which influence the abundance of mosquito vectors and the efficiency of virus replication, as well as ecological factors which affect the rate of contact between avian hosts, mosquito vectors, and spillover into humans and other mammals.

In 2018, Europe experienced the largest outbreak of WNV in recorded history, both in the number of cases and in geographic extent, with more cases (n = 2083) than the total of the seven previous years combined (n= 1832), and a 7.2-fold increase from the previous year (Figure 1). Considering only human cases, Hungary, Greece, and France had 14.7-, 13.7-, and 12.0-fold increases, respectively, compared to the previous year; however, Italy (n = 576), Serbia (n= 365), and Romania (n= 262) comprised nearly 75% of all human cases (Figure 2). In total, 181 deaths from WNND were reported in 2018 [10]. WNV is considered endemic throughout most of Europe, based on seroconversion in wild birds or WNV-positive mosquito pools, and human cases have been reported seasonally in the following countries over the last four years: Austria, Bulgaria, Croatia, Cyprus, Czech Republic, France, Greece, Hungary, Italy, Portugal, Romania, Serbia, Slovenia, Spain, Turkey (Figures 2 and 3) [10].

While volumes have been researched and written on virological and ecological aspects of WNV, this review will focus on discussing the most likely cause(s) of the 2018 European outbreak. Based on what is known about general arbovirus transmission and maintenance, and specifically about WNV, we consider the following explanations:

(1) Genetic changes in the virus that affected replication efficiency, host/vector competence, host pathogenicity, etc.
(2) Ecological changes which increased opportunities for the virus to efficiently expand in the environment (e.g. host abundance, vector-host contact, habitat, herd immunity).
(3) Environmental changes which influenced the efficiency of replication and vector abundance (e.g. temperature, precipitation).

2. Historical perspective leading to the 2018 outbreak

Following the related outbreaks in Egypt and Israel in the 1950s, the first major outbreak of WNV in Europe occurred in France (1962–1965) and was focused around the Camargue wetlands. Indications of an outbreak began in the summer 1962, with both wild and domestic horses showing signs of a neurologic disorder (case fatality rate of 25–30%) [19, 20].

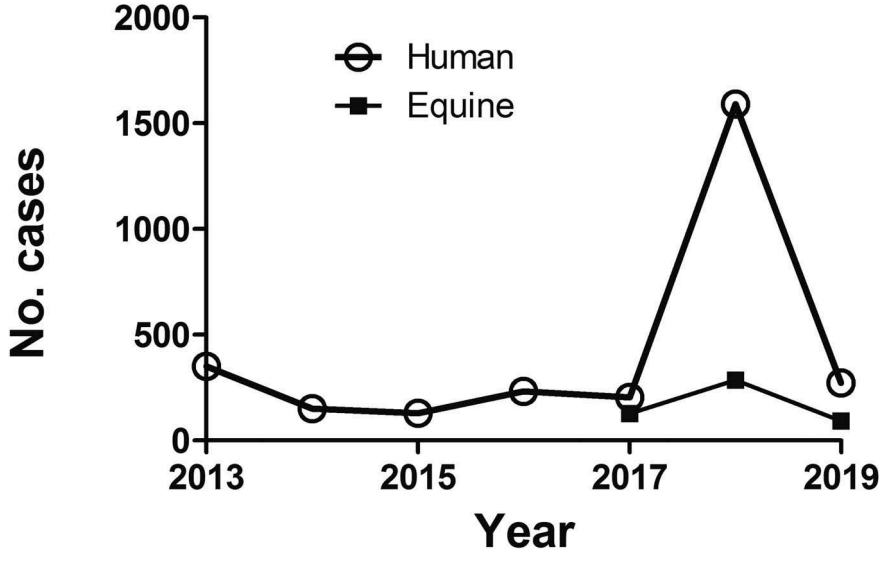

Figure 1. Total human and equine cases of West Nile virus per year in Europe. Data obtained from European Centre for disease prevention and control, available at https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical. *N.B.* Equine cases have only been reported since 2016.

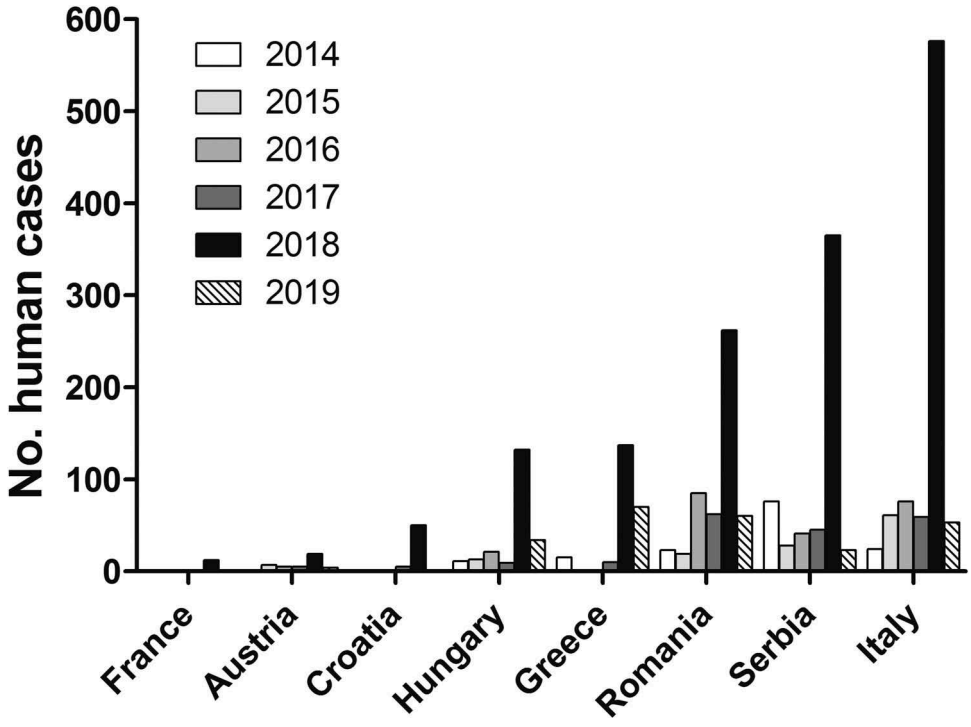

Figure 2. Total human cases of West Nile virus per year by country, showing countries that reported at least 10 cases in 2018. Data obtained from European Centre for Disease Prevention and Control, available at https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical.

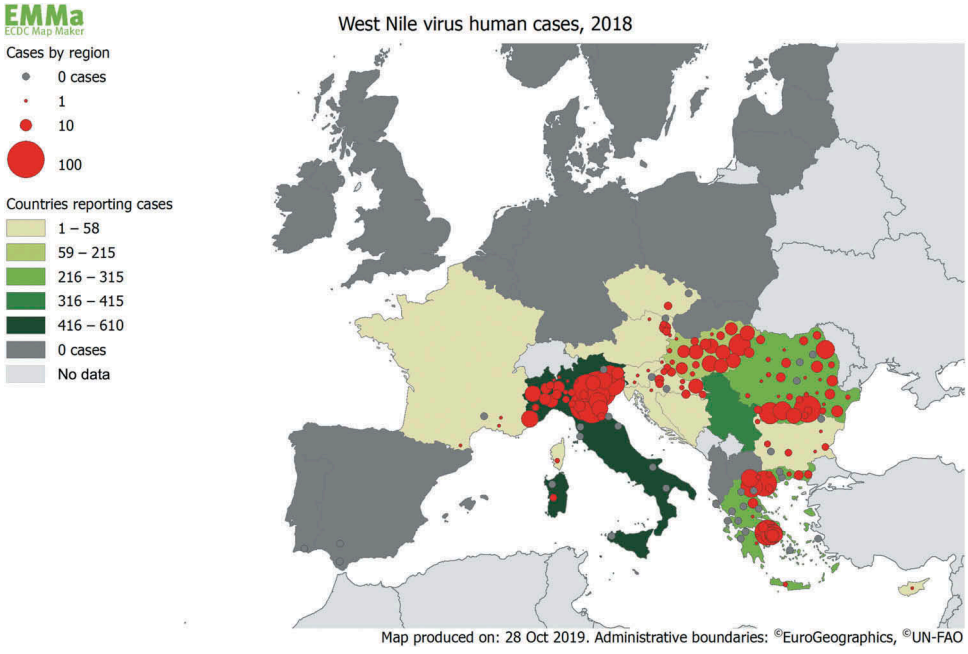

Figure 3. Geographic location of European countries reporting human cases of West Nile virus in 2018 (green color range) and approximate total number of human cases by NUTS3 region (red circles). Map prepared 25 October 2019 using European Centre for disease prevention and control map maker and data from https://www.ecdc.europa.eu/en/west-nile-fever/surveillance-and-disease-data/historical. *N.B.* Regional case counts not available for Serbia or Bosnia and Herzegovina.

Subsequent epidemiological surveys revealed seroconversion in humans, and finally, the virus was isolated from *Culex modestus* mosquitoes and from the blood of two humans [21]. This outbreak continued until 1965, with more fatal horse cases and isolations of the virus from *Cx. modestus*. Although no further WNV cases were identified in France until 2000, serosurveillance from 1975 to 1979 suggested that the virus was still present and circulating in the region at a very low level.

The next major outbreak in Europe was in Romania (1996–2000), and the pattern of continued seasonal transmission in Europe can be traced back to this initial event. For the first time in Europe, the outbreak was associated with severe WNND in humans, with at least 393 cases and

17 deaths [22, 23]. The virus remained in circulation in Romania in the years following the outbreak, and 39 cases (5 deaths) were recorded from 1997 to 2000 [24, 25]. Around the same time, another human outbreak began in Volgograd and the Volga delta region of Russia in 1999, resulting in approximately 1000 human cases and 40 deaths [26, 27]. The above 'historic' European outbreaks were attributed to a lineage 1 WNV.

Subsequent scientific studies of these outbreaks and others have highlighted several notable and generalizable features of WNV in Europe: (i) WNV has likely been present in Europe since the 1960s, with many countries reporting low-level seroconversion in birds and/or virus isolations from mosquitoes; (ii) migratory birds are thought to be the source of introductions of new virus strains into new regions; (iii) the subsequent establishment of enzootic circulation most likely involves *Culex* mosquitoes and native resident birds; (iv) the virus is capable of overwintering in hibernating adult *Culex* mosquitoes; (v) and epizootic transmission is due to mammal-feeding by *Culex* mosquitoes.

3. Genetics of WNV and the 2018 outbreak

Genetically, the virus in central Europe was a lineage 1 WNV until 2004, when a lineage 2 WNV was first isolated from a dead goshawk in Hungary [4] and another lineage 2 WNV isolated from human serum in Russia [18]. The 'Hungarian' lineage 2 virus spread rapidly throughout central and southern Europe and is now responsible for the majority of seasonal outbreaks in Europe. In contrast, the 'Russian' lineage 2 WNV is still circulating in eastern Europe, and only limited isolations of lineage 1 WNV have been reported in southern and western Europe in recent years. According to several phylogeographic models, the lineage 2 WNV introduced to Hungary spread first to Austria, then northward to the Czech Republic and south to Italy (Clade A), while another group spread south and east through the Balkans (Clade B) [5, 28,29,30,31]. So far, published sequences show that the 2018 viruses (Austria, Hungary, Greece, Italy, Slovakia) were lineage 2 WNV from multiple subclades of Clade A and Clade B [9, 28, 30, 32, 33].

One potential hypothesis in explaining the dramatic increase in WNV cases in Europe in 2018 is that a genetic change in the virus resulted in increased replication efficiency, transmission efficiency, and/or pathogenicity. For example, the introduction of WNV to New York in 1999 and the spread across the US was marked by an abrupt change in the virus genome in 2002. Viruses with this genetic change (the 'WN02' genotype, signified by a positively selected valine to alanine substitution at residue 159 of the envelope protein) rapidly replaced the previously circulating genotype ('NY99') [34, 35]. It was demonstrated that WN02 was associated with increased rate of replication and dissemination in laboratory experiments with *Culex pipiens* [36], but not in similar experiments with *Culex salinarius* nor in *Culex tarsalis* [37, 38]. Another viral variant (a proline at position 249 in the NS3 protein) has been associated with increased viremia in American crows experimentally infected with lineage 1 WNV, and viruses with this same substitution (a histidine to proline) were found in lineage 2 WNV during the 2010–2013 outbreak in Greece [29, 39]. However, experimental data suggested that this residue (a proline at NS3-249) is not associated with increased virulence or replication of lineage 2 WNV in native European birds [40, 41], and this variant seems to be restricted to the southern Balkan region [28]. Similar to the mosquito competence studies, the interaction between the virus and avian hosts is likely species specific. More research should be done to understand host competency for European bird species, and whether specific European strains have differential virulence in native birds, as has been done with North American species [13].

Much of what is known about the evolution of WNV comes from studies of lineage 1 WNV in the US, where the virus accumulates approximately 10^{-4} nucleotide substitutions per site per year [28, 42] and the same rate is seen in lineage 2 WNV in Europe [28, 30, 31]. These changes are driven by negative/purifying selection, and this relatively low rate of evolution can be attributed to the host-switching hypothesis as the virus must remain infectious to both arthropod and vertebrate hosts [43, 44]. Recent phylogeographic analyses of European lineage 2 WNV that include 2018 viruses from Italy and Greece detected a high degree of spatial correlation, with viral variants belonging to multiple subclades of either Clades A or B [28, 30]. Thus, there is no evidence that a novel viral variant could explain the increased activity of WNV in Europe during 2018. A similar situation – a large outbreak in a WNV-endemic area without evidence of genetic basis for the increase – has been previously recorded from the US [45]. However, the lack of complete WNV genomes from the 2018 outbreak in Europe makes this conclusion difficult to support.

4. Ecology of WNV transmission

Other considerations which may explain the increase in WNV cases in 2018 lie in the complex ecology of virus transmission and maintenance. Prospective scientific research of hosts and vectors as well as retrospective modeling of WNV past epidemics indicate that there are at least three components to understanding the dynamics of WNV abundance: wild bird hosts, mosquito vectors, and the interaction between them (i.e. host feeding preference by competent vectors) (see excellent reviews in [35, 46,47,48]). Specifically, with respect to the 2018 transmission season, here we consider: (i) changes which may have occurred in the avian population; (ii) changes which may have occurred in the mosquito population; (iii) changes which may have affected the interaction between vector and hosts.

The majority of research into the role of wild birds in the transmission of WNV in Europe consistently identifies two main factors that predict exposure to the virus: body size and migratory status. However, it should first be noted that some potential factors are routinely excluded as predictors of virus exposure: in Europe, there seems to be no correlation between seropositivity in birds and urban/suburban habitat preference nor with sociality [49,50,51]. This is in contrast to the situation in the US, where the primary amplifying hosts of WNV are thought to be resident birds which form large foraging/roosting communities in suburban habitats [47, 52]. This discrepancy between WNV ecology in the US and Europe may in part be explained by the scope of these analyses, as the association between urban/rural habitats and WNV transmission (both enzootic and epizootic) is region-specific in

Europe [53,54,55]. This may suggest that avian species that utilize both urban and suburban habitats, frequently traveling between them over short distances, hold the key to understanding the risk of spillover in Europe.

Based on avian serosurveys, the body size is positively correlated with seropositivity [49, 50, 56]. This may be explained by larger birds being better 'targets' for questing mosquitoes (e.g. larger surface area, more CO_2), or because larger birds tend to be longer-lived. Second, migratory status is frequently associated with increased likelihood of WNV exposure: European summer-resident migrants tend to have higher seropositivity than resident birds [50, 51, 56] but not all models support this [49] and migrants are rarely infected with WNV in the US compared to residents [57]. Although migratory status seems to be an important determinant of WNV exposure in European birds, and is likely the principal way in which WNV is introduced into a new region [1, 58], it should be noted that virus phylogenetic analyses suggest that transmission and spread of WNV are very localized. This is true for both North American lineage 1 WNV [59,60,61,62] as well as in Europe [30, 31, 63, 64]. Indeed, nearly identical strains can be found in the same area across multiple seasons [9, 30, 31, 63, 65, 66]. Therefore, the role of resident species in WNV maintenance in Europe requires further study, and must consider whether aspects of their specific ecology (foraging behavior, range, habitat utilization) predict the risk of virus exposure.

The second major component of WNV ecology is the vector population. Mosquito exposure is the single largest risk factor for epizootic transmission. Depending on the region, WNV nucleic acid can be detected from a large number of mosquito species (reviewed in [1]). However, in Europe, *Culex pipiens* has a high vector competence for WNV [67,68,69], and *Culex modestus* and *Culex torrentium* are also competent vectors [67, 70,71,72]. These three species differ in their large-scale geographic distribution in Europe, with *Culex torrentium* occupying more northern latitudes than *Culex pipiens*, and with *Culex modestus* having a relatively recent range expansion northward [73,74,75,76]. These species overwinter as adults, and infected adults are capable of transmitting WNV when leaving diapause [17, 77,78,79]. In some regions, it is thought that *Culex modestus* is the major amplifying vector, particularly in 'sylvatic' cycles, whereas spillover into mammals is performed by *Culex pipiens*, particularly in urban settings [3, 23, 80].

The third component of WNV ecology relies on the relationship between vectors and hosts. Analysis of blood meals and host attraction studies clearly demonstrate that *Cx. pipiens* and *Cx. modestus* feed on both birds and mammals, and the relative proportion depends on host abundance which is related to habitat [55, 81–88]. This suggests that people living in urban and suburban habitats (i.e. higher population densities) are at higher risk for being bitten by these species and becoming infected with WNV [22, 46, 55, 80, 89], but efficient virus amplification and maintenance may occur elsewhere, where there is a high population density of highly competent amplifying hosts or a higher ratio of highly competent to less-competent hosts (i.e. rural and/or sylvatic habitats). Furthermore, several studies have attempted to relate WNV abundance to vector and/or host abundance, often taking an epidemiological approach to identify ecological factors that explain variance in the abundance of virus (estimated by the ratio of infected hosts or vectors) [22, 46, 89, 90]. These studies suggest that increased vector abundance correlates with increased WNV seroconversion in avian hosts, and increases in avian species diversity also correlate with increased WNV seroconversion (i.e. an amplification effect, not a dilution effect) [80, 91]; however, the same eco-epidemiological models demonstrated that this is dependent on the presence of competent vectors and heterogeneous host competence [80].

With these three components in mind, a recent phylodynamic analysis of lineage 2 WNV evolution in Italy, which included 2018 viruses, discovered a sharp increase in the size of the viral population beginning in 2016 [30]. This suggests that there was a significant increase in available hosts during this time (i.e. naïve birds or competent vectors). This increase in the viral population led to an increase in the effective reproductive rate ($R_e > 1$) beginning 2017, conditions which were ideal for the observed increase of spillover of WNV into humans and horses in 2018 [47]. Indeed, the authors of this analysis state that increasing numbers of specific resident (non-migrating) bird species had been exposed to the virus in the years prior to 2018, based on a country-wide systematic serosurveillance program [30]. Italy, where WNV has caused seasonal epidemics since 2008, is one of the few countries in Europe which performs systematic monitoring of mosquito populations and surveillance of both mosquitoes and birds for WNV [11, 92]. In contrast, Germany has reported WNV-seropositive migratory birds since at least 2005 [56, 93]. Of interest, 2018 was the first year in which viral RNA was detected in resident birds in Germany [94], and subsequently, the first autochthonous (equine) case of WNV was discovered late in 2019 [95]. This highlights that resident birds are important indicators of WNV activity, and that equine cases often precede human cases [3, 54].

It is unknown whether populations of naïve resident birds increased in 2017–2018 in other locations throughout the affected regions in Europe. Furthermore, the phylodynamic analysis using data from Italy that revealed increasing exposure of birds to WNV in years preceding the 2018 outbreak suggests that avian seroprevalence (i.e. herd/flock immunity) did not dampen WNV amplification, as expected based on data from the US [96, 97]. Future retrospective studies, based on systematic bird count data, and prospective studies, based on serosurveillance, are desperately needed in order to resolve some of these gaps. Furthermore, both the ECDC and the World Health Organization (WHO) have called for harmonized mosquito monitoring and routine dissemination of these data, as these would provide a valuable tool for retrospective and predictive analyses of outbreaks. To date, empirical data on mosquito abundance in affected areas before and during the 2018 outbreak have not been published, and the understanding of the relationship between avian seroprevalence (flock immunity) and host competence requires more research. Thus, it is unclear whether changes in ecological conditions that favored spillover were responsible for the 2018 outbreak; however, it seems likely that there was increased contact between highly competent naïve hosts and competent mosquito vectors, which may be due to a larger vector population.

5. Environmental aspects

The link between increased temperature and increased WNV activity has long been established and involves changes to the

vector population, vector competence, and virus replication [90, 98–100]. This is, in part, related to the fact that the ectothermic vector populations increase in abundance during warmer temperatures (reduced gonotrophic cycle) [80, 101–103]. Perhaps more importantly, WNV replication and vector competence are both temperature dependent, with warmer temperatures leading to increased replication, shorter extrinsic incubation period, and an increased rate of virus transmission [67–69, 72, 104–106]. Additionally, WNV activity is associated with precipitation [90, 98, 100, 107]. While vector populations may benefit from increased rainfall, increased WNV activity is rather related to drought conditions, which may concentrate vectors and hosts at water sources [108–112], in addition to drought being typically associated with elevated temperatures.

In Europe, 2018 was one of the four hottest years ever recorded after 2016, 2015, and 2017 [113] (Figure 4). Second, 2018 was characterized by a 'wet spring' followed by summer drought [113]. Therefore, it is likely that early expansion of the vector population led to increased transmission of the virus, which was exacerbated by the increased temperatures. As a result, cases of WNV were reported at least 2 weeks earlier than in years prior [114]. Indeed, some models have suggested that early-season temperature is a reliable predictor of the typically late transmission season, which peaks in August–September [12]. Global climate change (particularly global warming) has wide-ranging effects on the transmission ecology of WNV and other arboviruses [115–118].

6. Conclusion

We considered potential factors that may have contributed to the observed increase in WNV cases in Europe during the 2018 outbreak. It is likely that virus genetics can be excluded as a cause, but more data are required to fully support this claim. While empirical data from 2018 are yet to be published, it seems clear that increased temperatures and particular precipitation patterns had the most direct effect on the increased transmission and spillover of WNV in Europe during 2018. Specifically, a 'wet spring' likely increased the vector population early in the season, and the summer droughts combined with increased temperatures led to the massive outbreak in humans and horses. Although 2019 had similarly high temperatures (Figure 4), the number of human and equine cases dropped to pre-2018 levels (Figures 1 and 2); therefore factors apart from optimal weather conditions (e.g. avian flock immunity) are important for explaining WNV outbreaks. In this review, we highlight gaps in current knowledge in the transmission ecology of WNV in Europe, which should be considered to better predict future outbreaks.

7. Expert opinion

WNV is a highly successful arbovirus with a complicated transmission ecology. The ability to efficiently use multiple hosts and vectors to maintain transmission has allowed the virus to expand throughout the globe. As an ecology-dependent arbovirus, the finer-points of virus maintenance vary on the scales of continents as well as within individual biogeographic regions. Efforts to understand the cycle of transmission should focus on broad 'universal' factors, but it is likely that there are key differences on smaller geographic scales which are as yet unknown, unappreciated, and/or under-studied. For example, past outbreaks have shown that the risk of human or horse spillover is region specific in Europe [53, 98], similar to the US,

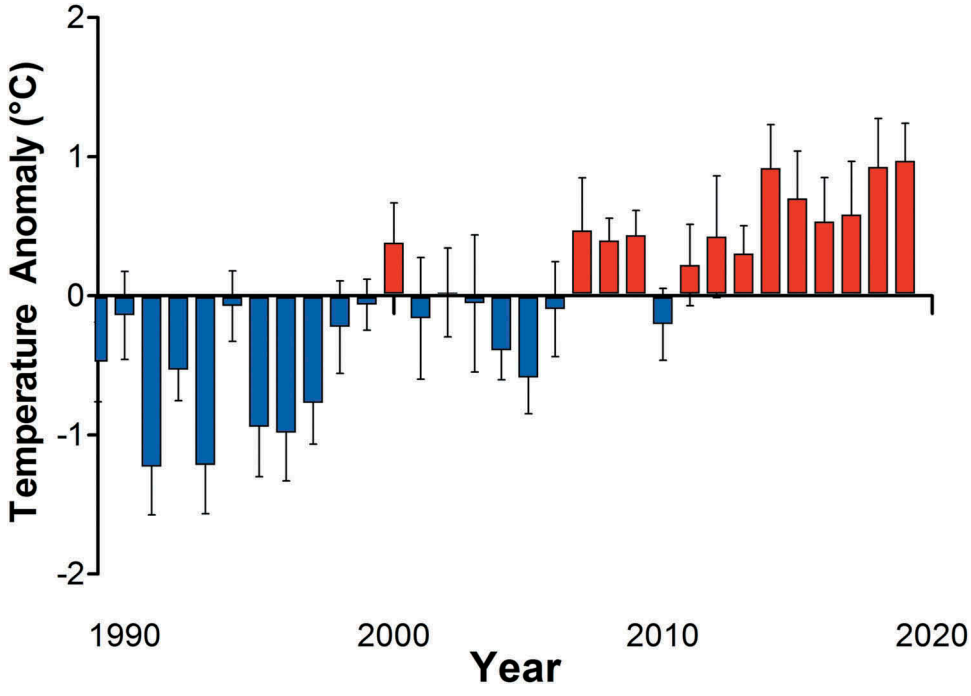

Figure 4. Average temperature anomaly (with standard error bars) over the last 25 years. Daily data were taken from one weather station per country in the 10 European countries which have reported WNV activity in the last 5 years (https://www.ncdc.noaa.gov/cdo-web/accessed 20 December 2019) as listed in the text. Monthly averages were compared to 25-year monthly average to calculate temperature anomaly (°C), and the yearly averages are displayed from 1989 to 2019.

where the correlation between WNV activity and environmental conditions was dependent on region during the 2012 outbreak [119]. In these examples, it was clear that temperature could be directly correlated with vector populations and human cases on small geographic scales, but only when accounting for the timing of temperature (warm winters or short time lags) [98, 119]. Optimal temperature seems to be the most likely direct explanation of the 2018 outbreak in Europe, but multifactor retrospective analyses should consider regional effects.

While the important vectors are relatively well defined in Europe, the importance of the various natural avian hosts remains poorly defined. Recent studies have made significant advances, beyond simply identifying the hosts of vector mosquito species, and provide insight into the relationship between vector and host abundance on community levels. A key focus should be to define urban/suburban/sylvatic amplifying hosts and link these data to known host-feeding patterns of vector mosquitoes. In addition to virus surveys of avian populations, experimental infection data are lacking for European bird species, although some candidate amplifying hosts have been identified [40, 120]. Serosurveillance of bird populations for WNV is also important for years preceding and following outbreaks, as in Europe in 2019 and in the US following the 2012 outbreak the number of human cases dropped to pre-outbreak levels. A potential explanation may be an increase in avian herd immunity, as theoretically there should have been a larger number of virus-infected overwintering mosquitoes in the years after the outbreak. This may also explain the interesting delay following introduction until the first large epidemic in both the US (lineage 1 WNV introduced in 1999, epidemics in 2002 and 2003) and Europe (lineage 2 WNV introduced around 2004, first explosive spread in 2008), and a second large-scale outbreak on both continents 10 years later (2012 and 2018, respectively). Ecological models are helpful tools in guiding future research, and epidemiological models suggest that the relationships between environmental conditions, habitat, and host/vector populations are key elements to describe yearly variation in WNV abundance. These models are further supported by bioinformatics analyses of virus evolution, and therefore efforts should be made to expand the collection of virus genomes from the 2018 season for future analyses.

In Europe, WNV remains a public health threat in many countries, and the 2018 season clearly demonstrated that the risk is not trivial. The ultimate goal of future research should be to provide clear predictions of WNV outbreaks. The 2018 season showed that existing models were largely supported, but should perhaps consider additional factors. Ideally, the research should inform public health policies and practices. Some countries have enacted measures for routine monitoring of mosquito and bird populations, but these practices are not harmonized across countries, and many countries have yet to enact these measures. Future efforts should be made to promote standardized, routine monitoring of mosquito and bird populations, with an emphasis on transnational cooperations in sharing data and best practices. Although there are clear differences from the situation in Europe, the US approach may offer a blueprint to enact a coordinated European mosquito control system. Such a system may allow opportunities for scientists (entomologists, virologists, epidemiologists) to collaborate and answer important questions about WNV in a European context: are current vector control measures (reactive and proactive) effective for reducing the severity of WNV outbreaks?; what are the best practices for predicting outbreaks?; are sentinel birds effective or are there more cost-effective methods?; are large-scale mosquito monitoring and WNV-surveillance operations effective (despite the cost and specially trained personnel requirements)?

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

ORCID

Jeremy V Camp http://orcid.org/0000-0002-9040-5786
Norbert Nowotny http://orcid.org/0000-0002-3548-571X

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Hubálek Z, Halouzka J. West Nile fever–a reemerging mosquito-borne viral disease in Europe. Emerg Infect Dis. 1999;5:643–650.
2. May FJ, Davis CT, Tesh RB, et al. Phylogeography of West Nile virus: from the cradle of evolution in Africa to Eurasia, Australia, and the Americas. J Virol. 2011;85:2964–2974.
3. Hubálek Z. European experience with the West Nile virus ecology and epidemiology: could it be relevant for the New World? Viral Immunol. 2000;13:415–426.
4. Bakonyi T, Ivanics E, Erdélyi K, et al. Lineage 1 and 2 strains of encephalitic West Nile virus, central Europe. Emerg Infect Dis. 2006;12:618–623.
   • **First report of the emergence of the contemporary lineage 2 WNV circulating in Central and Southern Europe.**
5. Bakonyi T, Ferenczi E, Erdélyi K, et al. Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. Vet Microbiol. 2013;165:61–70.
6. World Health Organization. West Nile virus; 2017 [cited 2019 Oct 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/west-nile-virus
7. Domanović D, Gossner CM, Lieshout-Krikke R, et al. West Nile and Usutu virus infections and challenges to blood safety in the European Union. Emerg Infect Dis. 2019;25:1050–1057.
8. Pisani G, Cristiano K, Pupella S, et al. West Nile virus in Europe and safety of blood transfusion. Transfus Med Hemother. 2016;43:158–167.
9. Aberle SW, Kolodziejek J, Jungbauer C, et al. Increase in human West Nile and Usutu virus infections, Austria, 2018. Eurosurveillance. 2018;23:1800545.

10. ECDC. Epidemiological update: West Nile virus transmission season in Europe, 2018. Eur Centre Disease Prev Control. 2018 [cited 2019 Oct 24]. Available from: https://www.ecdc.europa.eu/en/news-events/epidemiological-update-west-nile-virus-transmission-season-europe-2018
11. Gossner CM, Marrama L, Carson M, et al. West Nile virus surveillance in Europe: moving towards an integrated animal-human-vector approach. Eurosurveillance. 2017;22:30526.
12. Semenza JC, Tran A, Espinosa L, et al. Climate change projections of West Nile virus infections in Europe: implications for blood safety practices. Environ Health. 2016;15:S28.
13. Komar N, Langevin S, Hinten S, et al. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis. 2003;9:311–322.
• **Comparison of host competence for multiple species of North American birds.**
14. LaDeau SL, Calder CA, Doran PJ, et al. West Nile virus impacts in American crow populations are associated with human land use and climate. Ecol Res. 2011;26:909–916.
15. Kilpatrick AM, LaDeau SL, Marra PP. Ecology of West Nile virus transmission and its impact on birds in the western hemisphere. Auk. 2007;124:1121–1136.
16. Wheeler SS, Barker CM, Fang Y, et al. Differential impact of West Nile virus on California birds. Condor. 2009;111:1–20.
17. Rudolf I, Betášová L, Blažejová H, et al. West Nile virus in over-wintering mosquitoes, central Europe. Parasit Vectors. 2017;10:452.
18. Platonov AE, Karan LS, Shopenskaia TA, et al. Genotyping of West Nile fever virus strains circulating in southern Russia as an epidemiological investigation method: principles and results. Zh Mikrobiol Epidemiol Immunobiol. 2011;2:29–37.
19. Joubert L, Oudar J, Hannoun C, et al. [Epidemiology of the West Nile virus: study of a focus in Camargue. IV. Meningo-encephalomyelitis of the horse]. Ann Inst Pasteur. 1970;118:239–247.
20. Pantheir R, Hannoun C, Oudar J, et al. Isolation of West Nile virus in a Camarge horse with encephalomyelitis. C R Hebd Seances Acad Sci Ser Sci Nat. 1966;262:1308–1310.
21. Murgue B, Murri S, Zientara S, et al. West Nile outbreak in horses in southern France, 2000: the return after 35 years. Emerg Infect Dis. 2001;7:692–696.
22. Han LL, Popovici F, Alexander JP Jr, et al. Risk factors for West Nile virus infection and meningoencephalitis, Romania, 1996. J Infect Dis. 1999;179:230–233.
23. Tsai TF, Popovici F, Cernescu C, et al. West Nile encephalitis epidemic in southeastern Romania. Lancet. 1998;352:767–771.
24. Ceianu CS, Ungureanu A, Nicolescu G, et al. West Nile virus surveillance in Romania: 1997–2000. Viral Immunol. 2001;14:251–262.
25. Dinu S, Cotar AI, Păunculescu-Gătej IR, et al. West Nile virus circulation in south-eastern Romania, 2011 to 2013. Euro Surveill. 2015;20:21130.
26. Platonov AE, Shipulin GA, Shipulina OY, et al. Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. Emerg Infect Dis. 2001;7:128–132.
27. Platonov AE, Fedorova MV, Karan LS, et al. Epidemiology of West Nile infection in Volgograd, Russia, in relation to climate change and mosquito (Diptera: culicidae) bionomics. Parasitol Res. 2008;103:45–53.
28. Chaintoutis SC, Papa A, Pervanidou D, et al. Evolutionary dynamics of lineage 2 West Nile virus in Europe, 2004–2018: phylogeny, selection pressure and phylogeography. Mol Phylogenet Evol. 2019;141:106617.
•• **Phylogeographic analysis of WNV which includes isolates from 2018.**
29. Papa A, Bakonyi T, Xanthopoulou K, et al. Genetic characterization of West Nile virus lineage 2, Greece, 2010. Emerg Infect Dis. 2011;17:920–922.
30. Veo C, Della Ventura C, Moreno A, et al. Evolutionary dynamics of the lineage 2 West Nile virus that caused the largest European epidemic: Italy 2011–2018. Viruses. 2019;11:814.
•• **Phylogeographic analysis of WNV which includes isolates from 2018.**
31. Zehender G, Veo C, Ebranati 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:e0179679.
32. Čabanová V, Šikutová S, Straková P, et al. Co-circulation of West Nile and Usutu flaviviruses in mosquitoes in Slovakia, 2018. Viruses. 2019;11:639.
•• **Published description of the 2018 European WNV outbreak**
33. Nagy A, Mezei E, Nagy O, et al. Extraordinary increase in West Nile virus cases and first confirmed human Usutu virus infection in Hungary, 2018. Eurosurveillance. 2019;24:1900038.
•• **Published description of the 2018 European WNV outbreak**
34. Davis CT, Ebel GD, Lanciotti RS, et al. Phylogenetic analysis of North American West Nile virus isolates, 2001–2004: evidence for the emergence of a dominant genotype. Virology. 2005;342:252–265.
35. Duggal NK, Langwig KE, Ebel GD, et al. On the fly: interactions between birds, mosquitoes, and environment that have molded West Nile virus genomic structure over two decades. J Med Entomol. 2019;56(6):1467–1474.
36. Kilpatrick AM, Meola MA, Moudy RM, et al. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. PLoS Pathog. 2008;4:e1000092.
37. Danforth ME, Reisen WK, Barker CM. Extrinsic incubation rate is not accelerated in recent California strains of West Nile virus in *Culex tarsalis* (Diptera: Culicidae). J Med Entomol. 2015;52:1083–1089.
38. Anderson JF, Main AJ, Cheng G, et al. Horizontal and vertical transmission of West Nile virus genotype NY99 by *Culex salinarius* and genotypes NY99 and WN02 by Culex tarsalis. Am J Trop Med Hyg. 2012;86:134–139.
39. Brault AC, Huang CY-H, Langevin SA, et al. A single positively selected West Nile viral mutation confers increased virogenesis in American crows. Nat Genet. 2007;39:1162–1166.
40. Del Amo J, Llorente F, Figuerola J, et al. Experimental infection of house sparrows (*Passer domesticus*) with West Nile virus isolates of Euro-Mediterranean and North American origins. Vet Res. 2014;45:33.
41. Sotelo E, Gutierrez-Guzmán AV, Del Amo J, et al. Pathogenicity of two recent Western Mediterranean West Nile virus isolates in a wild bird species indigenous to Southern Europe: the red-legged partridge. Vet Res. 2011;42:11.
42. Di Giallonardo F, Geoghegan JL, Docherty DE, et al. Fluid spatial dynamics of West Nile virus in the United States: rapid spread in a permissive host environment. J Virol. 2016;90:862–872.
43. Deardorff ER, Fitzpatrick KA, Jerzak GVS, et al. West Nile virus experimental evolution in vivo and the trade-off hypothesis. PLoS Pathog. 2011;7:e1002335.
44. Jerzak GVS, Brown I, Shi P-Y, et al. Genetic diversity and purifying selection in West Nile virus populations are maintained during host switching. Virology. 2008;374:256–260.
45. Duggal NK, D'Anton M, Xiang J, et al. Sequence analyses of 2012 West Nile virus isolates from Texas fail to associate viral genetic factors with outbreak magnitude. Am J Trop Med Hyg. 2013;89:205–210.
46. Chevalier V, Tran A, Durand B. Predictive modeling of West Nile virus transmission risk in the Mediterranean Basin: how far from landing? Int J Environ Res Public Health. 2014;11:67–90.
47. Kilpatrick AM. Globalization, land use, and the invasion of West Nile virus. Science. 2011;334:323–327.
48. Reisen WK, Fang Y, Martinez VM. Avian host and mosquito (Diptera: Culicidae) vector competence determine the efficiency of West Nile and St. Louis encephalitis virus transmission. J Med Entomol. 2005;42:367–375.
49. Durand B, Tran A, Balança G, et al. Geographic variations of the bird-borne structural risk of West Nile virus circulation in Europe. Plos One. 2017;12:e0185962.
50. Figuerola J, Jiménez-Clavero MA, López G, et al. Size matters: West Nile virus neutralizing antibodies in resident and migratory birds in Spain. Vet Microbiol. 2008;132:39–46.
51. López G, Jiménez-Clavero MA, Tejedor CG, et al. Prevalence of West Nile virus neutralizing antibodies in Spain is related to the behavior of migratory birds. Vector Borne Zoonotic Dis. 2008;8:615–621.

52. Lothrop HD, Reisen WK. Landscape affects the host-seeking patterns of *Culex tarsalis* (Diptera: Culicidae) in the Coachella Valley of California. J Med Entomol. 2001;38:325–332.
53. Chaskopoulou A, L'Ambert G, Petric D, et al. Ecology of West Nile virus across four European countries: review of weather profiles, vector population dynamics and vector control response. Parasit Vectors. 2016;9:482.
54. Calistri P, Giovannini A, Hubalek Z, et al. Epidemiology of West Nile in Europe and in the Mediterranean basin. Open Virol J. 2010;4:29–37.
55. Martínez-de la Puente J, Ferraguti M, Ruiz S, et al. *Culex pipiens* forms and urbanization: effects on blood feeding sources and transmission of avian *Plasmodium*. Malar J. 2016;15:589.
56. Michel F, Fischer D, Eiden M, et al. West Nile virus and Usutu virus monitoring of wild birds in Germany. Int J Environ Res Public Health. 2018;15:171.
57. Reisen WK, Wheeler SS. Surveys for antibodies against mosquito-borne encephalitis viruses in California birds, 1996–2013. Vector-Borne Zoonotic Dis. 2016;16:264–282.
58. Rappole JH, Hubálek Z. Migratory birds and West Nile virus. J Appl Microbiol. 2003;94(Suppl):47S–58S.
59. Armstrong PM, Vossbrinck CR, Andreadis TG, et al. Molecular evolution of West Nile virus in a northern temperate region: Connecticut, USA 1999–2008. Virology. 2011;417:203–210.
60. Bertolotti L, Kitron UD, Walker ED, et al. Fine-scale genetic variation and evolution of West Nile Virus in a transmission "hot spot" in suburban Chicago, USA. Virology. 2008;374:381–389.
61. Grinev A, Chancey C, Volkova E, et al. Genetic variability of West Nile virus in U.S. blood donors from the 2012 epidemic season. PLoS Negl Trop Dis. 2016;10:e0004717.
62. McMullen AR, Albayrak H, May FJ, et al. Molecular evolution of lineage 2 West Nile virus. J Gen Virol. 2013;94:318–325.
63. Barzon L, Papa A, Lavezzo E, et al. Phylogenetic characterization of Central/Southern European lineage 2 West Nile virus: analysis of human outbreaks in Italy and Greece, 2013–2014. Clin Microbiol Infect 2015;21:1122.e1–10.
64. Charrel RN, Brault AC, Gallian P, et al. Evolutionary relationship between Old World West Nile virus strains: evidence for viral gene flow between Africa, the Middle East, and Europe. Virology. 2003;315:381–388.
65. Kolodziejek J, Jungbauer C, Aberle SW, et al. Integrated analysis of human-animal-vector surveillance: West Nile virus infections in Austria, 2015–2016. Emerg Microbes Infect. 2018;7:25.
66. Zehender G, Ebranati E, Bernini F, et al. Phylogeography and epidemiological history of West Nile virus genotype 1a in Europe and the Mediterranean basin. Infect Genet Evol. 2011;11:646–653.
67. Leggewie M, Badusche M, Rudolf M, et al. *Culex pipiens* and *Culex torrentium* populations from Central Europe are susceptible to West Nile virus infection. One Health. 2016;2:88–94.
68. Vogels CBF, Fros JJ, Göertz GP, et al. Vector competence of northern European *Culex pipiens* biotypes and hybrids for West Nile virus is differentially affected by temperature. Parasit Vectors. 2016;9:393.
69. Vogels CBF, Göertz GP, Pijlman GP, et al. Vector competence of northern and southern European *Culex pipiens pipiens* mosquitoes for West Nile virus across a gradient of temperatures. Med Vet Entomol. 2017;31:358–364.
70. Balenghien T, Vazeille M, Reiter P, et al. Evidence of laboratory vector competence of *Culex modestus* for West Nile virus. J Am Mosq Control Assoc. 2007;23:233–236.
71. Balenghien T, Vazeille M, Grandadam M, et al. Vector competence of some French *Culex* and *Aedes* mosquitoes for West Nile virus. Vector Borne Zoonotic Dis. 2008;8:589–595.
72. Jansen S, Heitmann A, Lühken R, et al. *Culex torrentium*: a potent vector for the transmission of West Nile virus in Central Europe. Viruses. 2019;11:492.
73. Golding N, Nunn MA, Medlock JM, et al. West Nile virus vector *Culex modestus* established in southern England. Parasit Vectors. 2012;5:32.
74. Hesson JC, Rettich F, Merdić E, et al. The arbovirus vector *Culex torrentium* is more prevalent than *Culex pipiens* in northern and central Europe. Med Vet Entomol. 2014;28:179–186.
75. Votýpka J, Šeblová V, Rádrová J. Spread of the West Nile virus vector *Culex modestus* and the potential malaria vector *Anopheles hyrcanus* in central Europe. J Vector Ecol. 2008;33:269–277.
76. Weitzel T, Braun K, Collado A, et al. Distribution and frequency of *Culex pipiens* and *Culex torrentium* (Culicidae) in Europe and diagnostic allozyme markers. Eur Mosq Bull. 2011;29:22–37.
77. Koenraadt CJM, Möhlmann TWR, Verhulst NO, et al. Effect of overwintering on survival and vector competence of the West Nile virus vector *Culex pipiens*. Parasit Vectors. 2019;12:147.
• **Field experiment combining overwintering survival and vector competence.**
78. Vogels CBF, van de Peppel LJJ, van Vliet AJH, et al. Winter activity and aboveground hybridization between the two biotypes of the West Nile virus vector *Culex pipiens*. Vector-Borne Zoonotic Dis. 2015;15:619–626.
79. Nelms BM, Kothera L, Thiemann T, et al. Phenotypic variation among *Culex pipiens* Complex (Diptera: Culicidae) populations from the Sacramento Valley, California: horizontal and vertical transmission of West Nile virus, diapause potential, autogeny, and host selection. Am J Trop Med Hyg. 2013;89:1168–1178.
80. Tran A, L'Ambert G, Balança G, et al. An integrative eco-epidemiological analysis of West Nile virus transmission. EcoHealth. 2017;14:474–489.
81. Alcaide M, Rico C, Ruiz S, et al. Disentangling vector-borne transmission networks: a universal DNA barcoding method to identify vertebrate hosts from arthropod bloodmeals. PLoS ONE. 2009;4:e7092.
82. Börstler J, Jöst H, Garms R, et al. Host-feeding patterns of mosquito species in Germany. Parasit Vectors. 2016;9:318.
83. Gomes B, Sousa CA, Vicente JL, et al. Feeding patterns of molestus and pipiens forms of *Culex pipiens* (Diptera: Culicidae) in a region of high hybridization. Parasit Vectors. 2013;6:93.
84. Muñoz J, Eritja R, Alcaide M, et al. Host-feeding patterns of native *Culex pipiens* and invasive *Aedes albopictus* mosquitoes (Diptera: culicidae) in urban zones from Barcelona, Spain. J Med Entomol. 2011;48:956–960.
85. Muñoz J, Ruiz S, Soriguer R, et al. Feeding patterns of potential West Nile virus vectors in south-west Spain. Plos One. 2012;7:e39549.
86. Osório HC, Zé-Zé L, Alves MJ. Host-feeding patterns of *Culex pipiens* and other potential mosquito vectors (Diptera: Culicidae) of West Nile virus (Flaviviridae) collected in Portugal. J Med Entomol. 2012;49:717–721.
87. Rizzoli A, Bolzoni L, Chadwick EA, et al. Understanding West Nile virus ecology in Europe: *Culex pipiens* host feeding preference in a hotspot of virus emergence. Parasit Vectors. 2015;8:213.
88. Roiz D, Vazquez A, Rosà R, et al. Blood meal analysis, flavivirus screening, and influence of meteorological variables on the dynamics of potential mosquito vectors of West Nile virus in northern Italy. J Vector Ecol. 2012;37:20–28.
89. Kwan JL, Park BK, Carpenter TE, et al. Comparison of enzootic risk measures for predicting West Nile disease, Los Angeles, California, USA, 2004–2010. Emerg Infect Dis. 2012;18:1298–1306.
90. Tran A, Sudre B, Paz S, et al. Environmental predictors of West Nile fever risk in Europe. Int J Health Geogr. 2014;13:26.
91. Levine RS, Hedeen DL, Hedeen MW, et al. Avian species diversity and transmission of West Nile virus in Atlanta, Georgia. Parasit Vectors. 2017;10:62.
92. Monaco F, Lelli R, Teodori L, et al. Re-emergence of West Nile virus in Italy. Zoonoses Public Health. 2010;57:476–486.
93. Seidowski D, Ziegler U, von Rönn JAC, et al. West Nile virus monitoring of migratory and resident birds in Germany. Vector-Borne Zoonotic Dis. 2010;10:639–647.
94. Ziegler U, Lühken R, Keller M, et al. West Nile virus epizootic in Germany, 2018. Antiviral Res. 2019;162:39–43.
•• **Published description of the 2018 European WNV outbreak**

95. ProMED-mail. West Nile virus: europe (Germany) horse, OIE. *ProMED-mail*; 2019 [cited 2019 Oct 23]. Available from: http://www.promedmail.org
96. Kwan JL, Kluh S, Reisen WK. Antecedent avian immunity limits tangential transmission of West Nile virus to humans. Plos One. 2012;7:e34127.
97. Hayes EB, Komar N, Nasci RS, et al. Epidemiology and transmission dynamics of West Nile virus disease. Emerg Infect Dis. 2005;11:1167–1173.
98. Groen TA, L'Ambert G, Bellini R, et al. Ecology of West Nile virus across four European countries: empirical modelling of the Culex pipiens abundance dynamics as a function of weather. Parasit Vectors. 2017;10:524.
99. Hartley DM, Barker CM, Le Menach A, et al. Effects of temperature on emergence and seasonality of West Nile virus in California. Am J Trop Med Hyg. 2012;86:884–894.
100. Paz S, Malkinson D, Green MS, et al. Permissive summer temperatures of the 2010 European West Nile fever upsurge. Plos One. 2013;8:e56398.
101. Meyer RP, Hardy JL, Reisen WK. Diel changes in adult mosquito microhabitat temperatures and their relationship to the extrinsic incubation of arboviruses in mosquitoes in Kern County, California. J Med Entomol. 1990;27:607–614.
102. Paz S, Albersheim I. Influence of warming tendency on *Culex pipiens* population abundance and on the probability of West Nile fever outbreaks (Israeli case study: 2001–2005). EcoHealth. 2008;5:40–48.
103. Ruiz MO, Chaves LF, Hamer GL, et al. Local impact of temperature and precipitation on West Nile virus infection in *Culex* species mosquitoes in northeast Illinois, USA. Parasit Vectors. 2010;3:19.
104. Danforth ME, Reisen WK, Barker CM. The impact of cycling temperature on the transmission of West Nile virus. J Med Entomol. 2016;53:681–686.
105. Fros JJ, Miesen P, Vogels CB, et al. Comparative Usutu and West Nile virus transmission potential by local *Culex pipiens* mosquitoes in north-western Europe. One Health. 2015;1:31–36.
106. Reisen WK, Fang Y, Martinez VM. Effects of temperature on the transmission of west nile virus by *Culex tarsalis* (Diptera: Culicidae). J Med Entomol. 2006;43:309–317.
107. Marcantonio M, Rizzoli A, Metz M, et al. Identifying the environmental conditions favouring West Nile virus outbreaks in Europe. Plos One. 2015;10:e0121158.
108. Epstein PR. West Nile virus and the climate. J Urban Health. 2001;78:367–371.
109. Johnson BJ, Sukhdeo MVK. Drought-induced amplification of local and regional West Nile virus infection rates in New Jersey. J Med Entomol. 2013;50:195–204.
110. Paull SH, Horton DE, Ashfaq M, et al. Drought and immunity determine the intensity of West Nile virus epidemics and climate change impacts. Proc R Soc B Biol Sci. 2017;284:20162078.
111. Shaman J, Day JF, Stieglitz M. Drought-induced amplification and epidemic transmission of West Nile virus in southern Florida. J Med Entomol. 2005;42:134–141.
112. Wang G, Minnis RB, Belant JL, et al. Dry weather induces outbreaks of human West Nile virus infections. BMC Infect Dis. 2010;10:38.
113. European Environment Agency. Global and European temperature. Eur Environ Agency. 2019 [cited 2019 Oct 24]. Available from: https://www.eea.europa.eu/ds_resolveuid/IND-4-en
114. Haussig JM, Young JJ, Gossner CM, et al. Early start of the West Nile fever transmission season 2018 in Europe. Eurosurveillance. 2018;23:1800428.

•• **Published description of the 2018 European WNV outbreak**

115. Day JF, Tabachnick WJ, Smartt CT. Factors that influence the transmission of West Nile virus in Florida. J Med Entomol. 2015;52:743–754.
116. Morin CW, Comrie AC. Regional and seasonal response of a West Nile virus vector to climate change. Proc Natl Acad Sci. 2013;110:15620–15625.
117. Soverow JE, Wellenius GA, Fisman DN, et al. Infectious disease in a warming world: how weather influenced West Nile virus in the United States (2001–2005). Environ Health Perspect. 2009;117:1049–1052.
118. Tabachnick WJ. Challenges in predicting climate and environmental effects on vector-borne disease episystems in a changing world. J Exp Biol. 2010;213:946–954.
119. DeGroote JP, Sugumaran R, Ecker M. Landscape, demographic and climatic associations with human West Nile virus occurrence regionally in 2012 in the United States of America. Geospat Health. 2014;9:153–168.
120. Jiménez de Oya N, Camacho M-C, Blázquez A-B, et al. High susceptibility of magpie (*Pica pica*) to experimental infection with lineage 1 and 2 West Nile virus. PLoS Negl Trop Dis. 2018;12. Epub ahead of print 10 April 2018. doi:10.1371/journal.pntd.0006394