ÉPIDÉMIOLOGIE

West Nile virus in Europe and Africa: still minor pathogen, or potential threat to public health?

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Résumé : **Le virus West Nile en Europe et en Afrique : vieille connaissance ou menace potentielle pour la santé publique ?**

Jusqu'en 1999, année marquant l'émergence soudaine et inattendue du virus West Nile (WN) dans le « nouveau monde », le WN n'était connu que dans « l'ancien monde », Europe, Afrique, Asie, Moyen-Orient, où son activité était associée à quelques cas sporadiques ou épidémies de faible ampleur chez les populations humaines ou les équidés. L'extension massive et rapide du WN à l'ensemble de l'Amérique du Nord, moins de 4 ans après son émergence à New York en 1999, a conduit à une « prise de conscience », de la part des autorités sanitaires de différents pays, du risque potentiel associé à des virus jusque là considérés comme d'importance mineure en terme de santé publique.
La revue présente une synthèse des données illustrant la situation épidémiologique du WN en Europe et en Afrique au cours des 50 dernières années. La ré-émergence récente du WN dans certains pays européens sera notamment discutée au regard de la situation épidémiologique de ce virus sur le continent américain.

arbovirose
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Afrique
Amérique

Summary :
Until 1999 the West Nile virus had been reported only in the "Old world" and particularly in Europe, Africa, Middle East and Asia where it was responsible only for sporadic or size-and-time-limited outbreaks in humans and equines. The sudden and unexpected emergence of WN in New York in 1999, followed by a rapid and huge extension to the whole North America in less than four years, made health authorities aware of the potential of previously forgotten viruses to become a threat to public health.
The present review will focus on the epidemiology of West Nile virus in Europe and Africa during the last five decades. The recent re-emergence of WN activity in some European countries will be discussed regarding the current actuality of WN in the Americas.

arbovirose
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emergence
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public health
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Americas

Introduction

Since its first isolation in 1937 in Uganda, from a woman presenting a febrile syndrome, West Nile virus has been associated mainly to sporadic or temporally- and size-limited outbreaks in humans and/or horses in Africa, Europe, Middle East and Asia, thus being considered as a virus from the "Old world" with few if any significant impact for public health. Its recent and unexpected emergence in the Americas where it was first reported in 1999 in New York district, has changed the attention paid to this virus. Indeed within only 6 years, West Nile virus has extended to the whole USA, being responsible for nearly 20 000 clinical infections and hundreds of deaths in human populations and for thousands of infections in equine (16). Afterwards, it has rapidly spread to Canada where it has been responsible for many human cases since 2002. Since that time, WNv has also spread to Mexico and several Caribbean islands where West Nile virus activity has been established thanks to specific surveillance programs based on sentinel and wild animals (16). It should be pointed out that unlike in North America (USA and Canada), only sparse cases of avian, equine and human infections due to WNv have been reported in Central or South America and in Caribbean region since 1999, stressing the role of both ecological, viral and host factors in the differential emergence of WNv in one or another region of the world (26).
Since factors involved in the sudden emergence of West Nile virus in North America are still poorly understood, this unexpected epidemiological situation makes everyone question the possibility that such an event may occur in the Old world.
This review is a synthesis of the epidemiological, entomological and ecological data from studies carried out from the 1950's to nowadays and is aimed at understanding the features of West Nile virus epidemiology in Europe and in Africa. A particular focus will be made on the more recent outbreaks that have occurred since the 1990's underlining the similarities and the specificities associated to outbreaks in the Old and New world.

General presentation

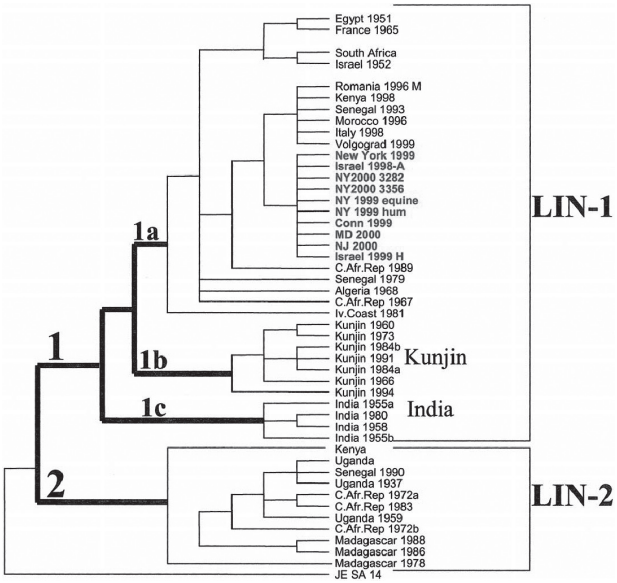
Characteristics of West Nile virus (WNv)

The virus West Nile is a member of the genus *Flavivirus* in the family *Flaviviridae*. It belongs to the "Japanese Encephalitis

Figure 1.

Phylogenetic tree generated by parsimony analysis of aligned nucleotide sequences from the 255bp region of the enveloppe protein of 47 WNv strains and one Japanese encephalitis virus (from ref 28).

Arbre phylogénique produit par analyse de restriction des séquences nucléotidiques à partir de la région 255 pb de l'enveloppe protéinique de 47 souches de WN et d'une souche d'encéphalite japonaise (28)



Virus" complex that includes several arboviruses responsible for encephalitic syndromes of which JEV itself, Saint-Louis encephalitis virus (SLE) and Murray Valley encephalitis virus (MVE), West Nile virus and Kunjin virus (an Australian variant of WNv).

Phylogenetically WNv splits into 2 distinct lineages LIN1 and LIN2 (figure 1), diverging by 25 to 30% nucleotide differences (3, 28).

The former lineage includes all strains associated to human outbreaks in Europe, Africa, Israel, Asia, Australia and Americas and can be subdivided into three clades. Clade 1a includes strains from Europe, Middle East, Asia, Africa and USA, clade 1b includes Kunjin virus strains, and clade 1c includes WNv strains from India. The LIN 2 includes only strains from sub-Saharan Africa and from Madagascar island. Strains from LIN2 are considered less pathogenic to humans since they were mainly isolated from mild febrile cases or incidentally from asymptomatic individuals when searching for other pathogens (28).

WNv transmission cycle

WNv is maintained and transmitted through an enzootic cycle involving birds as main amplifying hosts and ornithophilic mosquitoes of the genus *Culex* as main vectors.

Whereas more than 110 avian species can be infected either naturally or experimentally, viremia intensity and duration as well as pathology can be dramatically different depending on birds species. Indeed, studies carried out to better understand the role of avian species in WNv transmission in the USA, have shown that some birds species, in particular members of the *Corvidae* family (American crows, Blue jay…), are particularly susceptible to WNv infection. Differently others show few if any symptoms despite a significant viremia followed by acquisition of a long-lasting immunity (27).

While in most cases, infections due to WNv are asymptomatic in birds, high avian mortality was a characteristic of outbreaks in Israel in 1998 and in the USA since 1999. This unusual high mortality was used as a predictor factor in the surveillance programs implemented in the USA whereas it has poor if any relevance in Europe and in Africa, where avian mortality due to WNv is low to insignificant.

Regarding the vectors, *Culex* species are generally considered to be the main vectors involved in the transmission of WNv. However in North America, at least 59 mosquito species with diverse behaviour and ecology have been found to be naturally infected by WNv (16), a feature that has likely contributed to the efficiency of WNv dissemination in the USA (13). Another hypothetical explanation could be the existence, in the USA, of an increased proportion of hybrid mosquitoes with mixed behaviour of bird-biters and human-biters in the *Cx. pipiens* complex, that could have represented bridge vectors of WNv between avian and human hosts (11).

In Europe and Africa few information are available regarding the ecology of WNv mosquito vectors. *Cx. pipiens*, *Cx. modestus* and *Aedes spp*. seem to be the main vectors of the virus in Europe (16, 19). In southern France in particular, *Cx. modestus* could represent the main vector for WNv as suggested by repeated isolations of the virus from this mosquito specie in the 1960's (15, 41). In Africa, *Cx. univittatus* represents the principal vector, while different mosquito species from the genera *Culex*, *Aedes spp*. and *Mimomyia spp*. have been found infected by WNv and could play a role in WNv transmission in some regions of Africa (CRORA website; 19). The possible role played by other arthropods, particularly soft and hard ticks, in the transmission of WNv has been suspected but is still controversed (16).

Human and horses are only incidental hosts in the transmission cycle of WNv. Both are dead-end hosts for the virus since the intensity and duration of viraemia are likely insufficient to allow infection of biting mosquitoes. Despite that, infection due to WNv may have dramatic consequences in both human and equine populations, and the recent emergence of WNv in North America has clearly illustrated its possible impact for human public health.

Many other mammals and non-mammals vertebrates like reptiles and amphibians in particular, can be infected either naturally or experimentally by WNv (24, 34). However, only some of them, like alligators in the USA, are able to sustain a significant and durable viraemia, and could represent amplifier hosts for WNv (24, 37) in some regions of the world.

New modes of transmission of WNv

The huge extension of WNv in the Americas has revealed other unexpected modes of transmission of the virus in humans, mainly through blood donation or organ transplantation but also through intra-uterine contamination or sporadic transmission by breast-feeding (13).

Unexpected transmission modes have also been demonstrated experimentally in birds, revealing that transmission may occur between infected and uninfected birds kept in close proximity either through cloacal or oral routes (27, 35). Similar non-mosquitoborne transmission has been reported or strongly suspected in alligators in the USA (24). Finally, vertical (trans-ovarian) transmission has been established in mosquito vectors and may represent a way for the virus to survive through winter in cold regions of North America in particular (44).

Clinical presentations of infections due to WNv in humans

It is currently assumed that more than 80% of infections due to WNv are asymptomatic. In 15 to 20% of cases, infection is associated to a non specific febrile syndrome including fever,

fatigue, arthralgias, myalgias, anorexia, gastro-intestinal symptoms and frequent maculopapular cutaneous rash (17, 32). Some severe clinical manifestations have been more rarely reported including fulminant hepatitis or pancreatitis as well as myocarditis. More recently, different authors have reported ocular manifestations presenting as multifocal chorioretinitis in patients infected by WNv that could be more common than previously expected as suggested by a recent study in Tunisia (1). Age seems to be associated to the risk of developing WNv-related ocular manifestations (25) while such clinical presentation has been reported recently in children (57).
Neurological disease due to WNv occurs in less than 1% of infected people and is most often characterized by encephalitic syndrome or meningo-encephalitis. Polyomyelitis-like asymmetric flaccid paralysis is observed in more than 10% of patients presenting severe neurological WNv-related disease as a consequence of infection of spinal motor neurons by the virus (29, 54). Acute respiratory failure, often requiring endotracheal intubation, is a frequent life-threatening complication associated to WNv-related paralysis syndrome (53). Other neurological signs like altered consciousness, movement disorders including tremors, myoclonus, or parkinsonism are also observed in WNv-related encephalitis (53, 54). Age is definitely the main risk factor for the development of neurological disease during WNv infection, most encephalitic syndromes occurring in the elderly (16, 32). The role of other associated diseases like hypertension, cerebrovascular disease or diabetes as risk factors to develop severe WNv infection has been evoked but is still debated (17).

Treatments and vaccine development

Treatments currently available for WNv-related infections are only supportive. Ribavirin, interferon-α or WNv-specific antibodies have been considered as possible treatments but none of them has been assessed in controlled clinical trials (17, 46).
Several WNv vaccines candidates are under development or at different stages of evaluation both for human and equine. The ChimeriVax-WN02 is an attenuated chimeric vaccine derived from an infectious clone of Yellow Fever Virus 17D (YF17D) in which the pre-membrane and the envelope protein genes of the YF17D have been replaced by the corresponding genes of WNv (2, 39). The envelope gene of WNv has been modified by introducing 3 mutations in sites supposed to support neurovirulence, resulting in a highly attenuated phenotype. This vaccine candidate was shown to protect hamsters and more recently rhesus macaques against intracerebral infectious challenge with wild-type WNv strains (2). Moreover previous studies have shown that mosquitoes are unable to transmit this chimeric attenuated virus (21).
A recent clinical study in healthy subjects receiving a single dose of the ChimeriVax-WN02, indicates that, after a transient viraemia, all vaccinated subjects develop neutralizing antibodies to WNv and a majority of them also develop a specific T lymphocyte response (39). This attenuated vaccine is thus considered as an interesting candidate for prevention of WNv infection in humans. Other candidate vaccines for humans are being studied, including another chimeric vaccine incorporating WNv gene sequences into back-bone genome of an attenuated Dengue serotype 4 virus (6). Recombinant DNA vaccines expressing genes encoding pre-membrane, envelope or capsid proteins of WNv have been assessed in animals (6, 13).
However, the vaccination of humans should be evaluated regarding the economical cost and the benefits relatively to possible risks. Ideally, it should be targeted more specifically to people at higher risk to develop severe WNv-related infection in particular the elderly.
For use in equines, two vaccines are currently available: the one used in the USA, in a large scale since 2002, is a formalin-inactivated WNv which prevents viraemia in 94% of immunized horses, but requires two initial doses followed by annual boosters due to the short-lasting antibody response elicited (13, 45). Another veterinary vaccine against WNv uses a canarypox virus which expresses WNv antigens (38).

WNv activity in Africa and Europe in the 1950's-1990's: a discrete presence

Situation in Africa before the 1990's

The virus West Nile is known to be endemic in many African regions since the 1950's. Sero-epidemiological surveys in humans, mammals and birds as well as entomological studies carried out at that time in Egypt in the upper Nile Delta region, have greatly contributed to improve the knowledge on the transmission and the ecology of WNv in Africa (20, 36, 51). These and other sero-epidemiological studies, conducted further in populations of Egypt, confirmed the endemicity of WNv along the Egyptian Nile (8, 43). Endemicity of WNv has been also established in other regions of African countries like northern Senegal (ZELLER 1989, unpublished, 43), South Africa (14, 22, 43) or Madagascar Island (30, 40).
The continuous presence of WNv in endemic foci within several African regions probably explains, at least partly, the low number of WNv outbreaks reported in human African populations. Indeed, likely due to endemic circulation of the virus, WNv infection is mostly considered as an infection of early childhood in Africa, presenting most often as a self-limited and uncomplicated febrile syndrome.
This general situation of WNv in Africa is however not exclusive, and major human outbreaks have occasionally occurred. The most important outbreak reported occurred in 1974 in South Africa where WNv is also considered to be endemic and maintained through an enzootic cycle between different avian species and *Culex univittatus* mosquitoes (22). Estimations from serosurveys indicate that during this huge outbreak, about 18,000 humans developed febrile illness due to WNv but neither death nor severe case were reported (33, 43).

Epidemiological situation in Europe before the 1990's: a quiet cohabitation…

The situation in Europe regarding the epidemiology of WNv is different from that in Africa where endemic foci of West Nile virus have been clearly established in several African regions. WNv activity has been reported repeatedly in western and central European countries through the last decades but the possibility that WNv is present endemically in some foci or alternatively re-introduced periodically in those regions of Europe, is still debated (18).
Indeed, in the last decades and until the 1990's, countries from western Europe (Italy, France, Portugal, Spain) and central and eastern Europe (Russia, Romania, Chezland, Ukraine, Slovakia) have experienced either sporadic cases or few temporally and size-limited human or equine outbreaks (18, 19, 43). Other evidences of WNv activity in those countries were provided through entomological surveys allowing the isolation of WNv from mosquito vectors or thanks to serological

surveys carried out in humans or animals in the context of surveillance programs (18, 43).

WNv in Africa and in Europe since the 1990's: significance of the re-emergence of WNv activity?

Situation in Africa since the 1990's

Despite the absence of major changes in the epidemiological situation of WNv in Africa after the 1990's, some new events have occurred that should be looked within the context of the epidemiology of WNv in some European countries in the same period of time. Indeed, several size and temporally-limited human outbreaks and/or equine epizootics have been reported in North Africa close to the Mediterranean basin since the 1990's (43). WNv-related human outbreaks associated to a significant incidence of neurological forms were reported in particular in Algeria in 1994, in Tunisia in 1997 (43) and again in Tunisia in 2003 (1), being responsible for some deaths in patients presenting neurological forms. Interestingly, a WNv strain isolated in the context of the 1997 Tunisia outbreak was shown to be phylogenetically very closed to the strains «associated» respectively to the huge bird epizootic in Israel in 1998 and to the first outbreak of WNv in New York in 1999 (7). In the same time Morocco has experienced two equine epizootics, one in 1996 with nearly100 equine cases (55) and a second and more limited one in 2003 (52).
Finally the continuous circulation of WNv in other African countries, Chad, Côte d'Ivoire and Senegal was confirmed recently through sero-epidemiological surveys carried out in populations of horses (4).

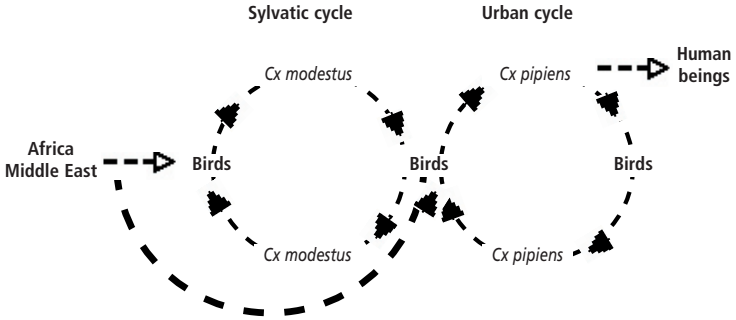
The epidemiological situation of WNv in Europe since the 1990's: facing new interrogations

Despite repeated evidences of WNv activity in the 1950 to 1990's time period, only few and limited outbreaks have been reported through the whole Europe both in humans and in equines. The situation changed significantly after the 1990's particularly with the unexpected occurrence of two major human outbreaks due to WNv, respectively in Romania in 1996 and in Russia in 1999 (19).

The Romania and Russia human outbreaks: major but temporally-limited human outbreaks in urban environments.
The 1996 Romania outbreak occurred during a dry summer in an urban area including the city of Bucharest and nearby areas (14 districts) in the lower Danube valley region (56). The peak of the epidemics was recorded between July and October. Out of 835 patients hospitalised for suspicion of central nervous system infection, 77% were found to have antibodies specific to WNv by serological diagnosis. A feature of this unexpected outbreak was the unusual high incidence of neurological forms: 40% of meningitis, 44% of meningoencephalitis and 16% of encephalitis (56). This resulted in 17 deaths that all occurred in patients older than 50, representing a mean fatality/case ratio of 4.3%.
The attack rate was estimated to 12.4 per 100,000 individuals in the district of Bucharest and to 1.5 per 100,000 in other nearby districts.
Serosurvey showed a low and uniform seroprevalence of antibodies against WNv in Bucharest residents and in people from nearby districts independently of age and sex, indicating that the virus had not been present previously in those populations.
Retrospective studies evaluated the ratio of clinical to sub-clinical infection between 1: 140 and 1: 320 (56). This corresponded for the Bucharest district, to an estimated 43,000 to 96,000 infected people and to an estimated 31,000 infected people in the other districts of the southern Danube valley region.
Increased surveillance implemented in the next 3 years between 1997 and 2000 provided evidences of continuous transmission of WNv in humans in the Danube valley region (5). The annual case incidence rate in the surveillance area was estimated at 0.95 case per million residents in this period of time, thus questioning the possibility of a persistence of WNv in endemic foci or alternatively of periodic reintroductions of WNv in those regions.
During this outbreak, WNv could be isolated only from *Cx. pipiens* mosquitoes. Since WNv is likely re-introduced repeatedly in this area, favourable conditions including the presence of *Cx. modestus* in the Danube delta may have favoured the development of a local enzootic transmission WNv cycle from which the virus could have been transferred to a urban cycle or even directly transmitted to humans (figure 2).
Sequencing of the WNv strain RO97-50, isolated from a pool of *Cx. pipiens pipiens* captured during the outbreak, indicated that this viral strain was identical to the strains isolated from mosquito vectors respectively in Kenya and in Senegal, suggesting that the virus may have been introduced in Romania by birds migrating from sub-Saharan regions to north Africa then to southern Europe (50).
The 1999 Russian outbreak occurred, as for Romania, in an urban area including the cities of Volgograd and Volczkii, respectively located on the west and east banks of the Volga river, with approximately 65% of human cases being reported in Volgograd and 30% in Volczkii (48). The epidemic occurred during a dry and hot summer and most cases being reported between August and September. As for the Romanian outbreak, the Russian one was characterized by an unusual high incidence of neurological forms. Indeed out of 826 patients hospitalised, 480 were diagnosed with neurological signs among whom 394 were retrospectively confirmed to have specific IgM antibodies to WNv. Out of 84 presenting such a neurological syndrome, forty died due to acute aseptic meningoencephalitis; most of them being older than 60 years old. The attack rate of clinical illness in the Russian outbreak was estimated at 40:100,000.
Phylogenetic analyses performed from partial sequences of the virus envelope and NS5 genes indicated that the Russian WNv isolates from seven patients were identical between

Figure 2.

Model of transmission of WNV through local enzootic and urban cycles during the 1996 urban Romania outbreak (from ref 56).
Modèle de transmission du virus WN selon des cycles enzootiques et urbains localisés pendant l'épidémie urbaine de 1996 en Roumanie (56).



each other and were closely related to the mosquito-derived Romania isolate RO97-50 and to WNv strains isolated in Senegal in 1993 and in Kenya in 1998. This suggests that birds migrating in southern Romania and Russia may have been infected by a same type of WNv in Africa.
The retrospective analysis of data collected between 1993 and 1998 has pointed out some putative warning signs that could have alerted to a possible activity of WNv in this region of southern Russia (48). Indeed several meningitis had been diagnosed in patients from the Astrakhan region with an unusual high incidence of fatal cases in the elderly. Moreover in 1997, 112 cases of acute meningitis with unknown etiology had been reported in the Volgograd region and similarly close to 70 human cases of meningitis were reported in 1998, the etiology of which was also undefined. Altogether those data suggest that WNv may have been introduced in southern Russia some years before the 1999 outbreak and may have persisted since then, while conditions for its persistence are unknown.
The common features of both 1996 Romania and 1999 Russia outbreaks are mainly their occurrence in urban environments and the high incidence of neurological forms recorded in symptomatic patients. However, regarding those characteristics and the large number of reported cases within the time of outbreak, few clinical infections were reported in the next following years despite increased surveillance. This is very different from the situation in the USA where the virus rapidly and efficiently spread to the whole North America within few years following its first report in New-York city in 1999. The typical presentation of recent human outbreaks in Europe is thus major epidemic burst in the first year followed by a limited number of cases in the following years without further extension (18).

Re-emergence of WNv in France: come-back after a 35 year period of silence…

Excepted the two major and unexpected urban outbreaks in Romania and Russia, few and very limited outbreaks were recorded in other European countries. However, unexpected events were also observed particularly in France where WNv activity was suddenly reported in 2000 after a 35 years period of silence. Indeed, WNv has been first reported in 1962 in the region of Camargue close to the Rhone delta in southern France, where it has been responsible for some 50 equine infections and 13 severe human cases. First isolations of WNv were carried out between 1964 and 1966 from humans, mosquitoes and horses (15, 47). Serological surveys in human populations from southern regions of France i.e. Camargue, Côte-d'Azur and Corsica-confirmed a high prevalence of antibodies to WNv in residents from those areas. A serosurvey carried out some years later in Camargue in the 1975-1980 time period, showed a much lower prevalence of antibodies to WNv both in humans and in horses standing in this region (49) questioning the mode of transmission of WNv in this particular area. The re-emergence of WNv occurred unexpectedly in 2000 and was associated to an equine epizootic in the region of Camargue, with 76 confirmed cases among which 21 were fatal to horses (42). No human case was reported at that time.
Due to the recent and unexpected emergence of WNv in the USA, a particular attention was paid to the re-emergence of WNv activity in France. Consequently, a surveillance program was implemented in 2001-2002 of which objectives were to detect and to evaluate the activity of WNv in Camargue every year between May and October, the optimal period for WNv transmission. The surveillance program, coordinated by the "*Institut national de veille sanitaire*" (InVS) under the control of the French ministry of health, has been carried out through 4 axes: entomological, avian, equine and human surveillance. Mosquito surveillance was shown to be unable to detect WNv activity in this context and was given up thereafter. A particular identification of the mosquito species responsible for the transmission to horses or humans was not possible. Avian surveillance indicated the existence of a low transmission level of WNv through seroconversion studies in sentinel birds and confirmed that avian mortality due to WNv was not significant. Finally, neither human nor equine infection due to WNv could be confirmed during the time of this surveillance program.
Interestingly a large scale retrospective survey carried out on more than 5,000 equines living in the region of Camargue showed that the prevalence of IgG antibodies to WNv could exceed 30% of horses in some "hot spot areas" corresponding to limited dried foci (9) while the seroprevalence for WNv-specific IgM and IgG antibodies was respectively of 4% and 8% in equines from other areas of the Camargue region. Regarding those data, the question remains whether WNv is periodically re-introduced in this region or maintained in limited foci like small marshy areas.
Since the 2000 epizootic, southern regions of France have experimented other limited human and equine outbreaks, in particular with the occurrence of 7 human cases in the Var departement in 2003 (31). As previously, entomological studies were unable to identify the putative vector responsible for WNv transmission in this area. A serosurvey carried out retrospectively in more than 900 horses living in the same area showed that 34% of horses had IgG antibodies to WNv suggesting a possible continuous circulation of WNv in this south-eastern French department (10).
The confirmation in 2004 of 31 equine infections due to WNv and of seroconversions in sentinel birds living in the Camargue region, indicates that WNv activity is still present in this marshy area where favourable environmental conditions for WNv transmission are present, gathering together mosquito vectors, wild resident and migratory birds and equine populations.
Interestingly, despite intense mosquito proliferation following exceptional rainfalls at the end of the summer 2005, WNv transmission in southern France remains very limited both in humans and animals. However, no one can presently predict if such a situation will be stable and how long it will last.

WNv in Europe: can we predict the future?

The actuality of WNv epidemiology in North America has highlighted the risk of a possible unexpected and massive spreading of a virus previously considered as a minor pathogen. The rapid expansion of WNv in the Americas likely resulted from an association of favourable conditions. In particular introduction of the virus in non-immune populations of humans and birds reservoirs joined to a large diversity of mosquito species able to ensure virus transmission in regions with dramatically different climate conditions, have probably contributed to the efficiency of WNv emergence in the New World. However a better knowledge on the ecology of WNv vectors is required to evaluate more accurately the potential risks of transmission. Recent studies on WNv transmission in north-eastern USA have highlighted the importance of considering quantitative information such as WNv infection

prevalence, vector competence and biting behaviour of most abundant mosquito species even if those are considered as mainly ornitophilic, to accurately assess the risk of transmission to humans from each mosquito species considered (23). This allowed showing that, in north-eastern USA, mosquito species like *Cx. pipiens* L and *Cx. restuans* Theobald, generally considered as primary amplification vectors due to their ornitophilic behavior, may in fact be responsible for more than 80% of transmission levels of WNv to humans in those regions (23).

The circulation of WNv in Europe and Africa since more than 5 decades probably explains the particular epidemiological situation observed in the Old World where no massive extension has been reported even after the major outbreaks of Romania and Russia. Factors limiting the spreading of WNv-related outbreaks from an initial epidemic, include likely the level of pre-existing immunity in human and bird populations in Europe and even more in Africa where WNv is endemic in many African regions.

Regarding ecological conditions, it is remarkable that many outbreaks which occurred in Europe in the last decades took place in areas where several conditions appeared favourable to WNv spreading, while most of those outbreaks remained size- and temporally-limited, without further extension. The example of the recent re-emergence of WNv activity in human and equine in south-eastern France, in the district of Camargue, illustrates this paradigm. Indeed, Camargue district is a marshy area with high mosquito density, wild and resident birds living in close proximity with equine and human populations, where climate conditions are highly favourable to WNv spreading, i.e. frequent abundant rainfalls during spring followed by dry and hot summers. Whereas all conditions seem to be present to allow the transmission and spreading of WNv, few viral activity has been detected in the past years apart from the 2000 and 2003 limited outbreaks despite increased surveillance in both avian, mosquito, equine and human populations.

Predicting the putative risk of spreading and worsening of WNv-related outbreaks in one or another region of Europe in the coming years, requires more knowledge on the ecology of WNv vectors and reservoirs and on the prevalence of WNv-specific immunity in human and animal populations in particular. However, factors such as the evolution of distribution and density of animal reservoirs and vectors, local modification of ecological conditions, introduction or appearance of more virulent WNv strains, remain difficult to predict. Thus, prediction of the risks associated to WNv can be only partial and relies on surveillance systems allowing to detect any change in the activity of the virus.

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