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West Nile virus in the vertebrate world

Brief Review

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Summary. West Nile virus (WNV), an arthropod-borne virus belonging to the family *Flaviviridae*, had been recognized in Africa, Asia and the south of Europe for many decades. Only recently, it has been associated with an increasing number of outbreaks of encephalitis in humans and equines as well as an increasing number of infections in vertebrates of a wide variety of species. In this article, the data available on the incidence of WNV in vertebrates are reviewed. Moreover, the role of vertebrates in the transmission of WNV, the control of WNV infections in veterinary medicine as well as future perspectives are discussed. A wide variety of vertebrates, including more than 150 bird species and at least 30 other vertebrate species, are susceptible to WNV infection. The outcome of infection depends on the species, the age of the animal, its immune status and the pathogenicity of the WNV isolate. WNV infection of various birds, especially passeriforms, but also of young chickens and domestic geese, results in high-titred viremia that allows arthropod-borne transmission. For other vertebrate species, only lemurs, lake frogs and hamsters develop suitable viremia levels to support arthropod-borne transmission. The role of vertebrates in direct, non-arthropod-borne transmission, such as via virus-contaminated organs, tissues or excretions is less well characterized. Even though direct transmission can occur among vertebrates of several species, data are lacking on the exact amounts of infectious virus needed. Finally, the increased importance of WNV infections has led to the development of killed, live-attenuated, DNA-recombinant and chimeric veterinary vaccines.

Introduction

West Nile virus (WNV) infections have been recognized for many decades. The virus was first isolated in 1937 from the blood of a woman with fever in the

West Nile province of Uganda [94]. Soon after, the virus became recognized as one of the most widespread flaviviruses in humans, birds and mosquitoes with geographic distribution in Africa, the Middle East and Southern Europe. Even though infections with WNV were common in these regions, they were usually mild or subclinical. Since the early 1990's, however, the frequency and severity of WNV infections in humans increased, as did the number of reports of WNV in a variety of vertebrates including pets, farm animals and wildlife. Moreover, WNV infections were recognized in previously unaffected areas. The most striking example is the introduction of the virus in New York City in 1999 and its spread in the subsequent three years to humans and to a variety of vertebrates throughout North America. The increased prevalence of WNV in the last decade resulted in extensive research in human medicine and, to a lesser extent, in veterinary medicine.

The purpose of this article is to review WNV research in the field of veterinary medicine. Hereby, we will focus on the incidence of the virus in various vertebrate species, as well as on the possible role of infected vertebrates in transmission of WNV to other vertebrates, including humans. Finally, we will briefly discuss the current strategies of control as well as the perspectives. References to WNV in humans are held to a minimum, since such reports have been recently reviewed [11, 15].

West Nile virus

WNV is a member of the Japanese encephalitis virus complex within the genus *Flavivirus*, family *Flaviviridae* [35]. Other members include Japanese encephalitis virus, Saint Louis encephalitis virus and Murray Valley encephalitis virus.

The typical WNV virion is approximately 50 nm in diameter and contains a nucleocapsid core with a single stranded, positive-sense RNA genome surrounded by an envelope. Embedded in the viral envelope are the viral envelope (E) proteins and membrane (M) proteins, which are responsible for many important properties of the virus such as host range, virulence, tissue tropism, replication and induction of immune responses.

Phylogenetic analysis of the genomes of a number of WNV strains has revealed two distinct lineages of the virus. Lineage 1 viruses have been isolated from the northeastern United States, Europe, Israel, Africa, India and Russia. Lineage 2 viruses have been isolated only in sub-Saharan Africa and Madagascar [52].

Incidence and importance of WNV in vertebrates

WNV infections have been described in a wide variety of vertebrates. Most important are birds, especially wild birds, since they are the principal hosts of the virus. The high and long-term viremia observed in wild birds allows transmission of WNV to mosquitoes and spring migrations of wild birds are instrumental in the introduction of WNV into previously unaffected areas. Beside birds, a broad range of other vertebrate species is susceptible to WNV infection as well,

but naturally acquired disease is rare. Equines are an exception. They regularly develop encephalitis as a result of natural WNV infection. This part of the review successively describes the incidence and importance of WNV infections in birds, equines and other vertebrates.

Birds

WNV has been detected in more than 150 species of wild and domestic birds, worldwide. The susceptibilities of birds to WNV infection differ. Species of the order Passeriformes are most susceptible [46, 61, 110]. They develop the highest viremia levels and shed the highest quantity of virus in oral and cloacal fluids. Moreover, in passeriforms, infection may result in severe neurological signs and high mortality rates [43, 46]. Also, bird species belonging to the order Chadriiformes [46] as well as domestic geese (order Anseriformes) [5, 6, 75] are highly susceptible to infection and disease. In other birds, viremia and virus shedding are generally more restricted and disease or death is rarely observed. Psittacine and gallinaceous birds are least susceptible [46, 61].

In addition to the genetics of the bird, other factors may influence the susceptibility to WNV infection and disease as well. Recently, it was suggested that there have evolved genetic variants of WNV with greater pathogenicity than earlier isolates [60]. Indeed, severe disease and deaths were not reported in natural WNV infections of birds until 1998. Then, an outbreak occurred in Israel, during which 160 of 400 domestic goslings developed fatal encephalitis [74]. One year later, an outbreak of WNV in North America resulted in thousands of deaths in a variety of native and exotic birds. Genomic analysis revealed that the North American and the Israelian WNV isolate were essentially identical [51], but differed from strains isolated earlier.

Also, the age of birds plays a role in susceptibility. When 1- to 11-day old chickens were experimentally infected by allowing infected mosquitoes to feed on them, they developed viremia levels of more than 10^5 PFU/ml. On the other hand, when chickens 3 weeks or older were infected with the same strain of WNV, they developed much lower viremia levels [38, 63]. The role of age on the outcome of infection is also illustrated in a recent outbreak in domestic geese in Manitoba, Canada. There, WNV most severely affected the 6-week old cohort with a mortality of 25%, while the cohorts of 15-month old and 5-year old geese showed only seroconversion without clinical disease [5]. A similar observation was made during the WNV outbreak in geese in Israel [74].

If disease occurs in birds, it is characterized by neurological signs, including ataxia, paralysis, somersaulting, paddling, torticollis, opisthotonus, and incoordination. Also non-neurological signs, such as depression, lethargy, ruffled feathers, weight loss and myocarditis may be observed [33, 46, 99]. Mortality rates are high. During natural infections in geese, 25–40% of the birds died [5, 74] and following experimental infection mortality rates as high as 50–75% have been observed [6, 99]. In the susceptible passerine and chadriiform species described by Komar et al. [46], most birds that showed clinical signs died within 24 hours.

Equines

Serological evidence of WNV infections in equines had been found in 1956 in Egypt [100] and in 1960 in Israel [1]. Three years later, Schmidt and El Mansoury [90] isolated WNV from a mare with nervous system disorders. Since then, WNV-induced neurological disease has been reported in horses in France [40, 68, 77, http://www.uky.edu/Agriculture/VetScience/q_jan04/q_jan04.htm], Portugal [26], Morocco [101], Italy [16] and Israel [96]. Since its introduction in 1999, WNV also caused over 15000 cases of neurological disease in horses in North America, where the disease became officially endemic in 2003. Serological surveys demonstrated the presence of WNV in horses in Mexico [12, 56] and in Austria [109].

WNV infection in horses often passes without presentation of clinical illness. Only 10–12% of the infections result in clinical disease [14, 78], which is also referred to as Near Eastern equine encephalitis or lourdige. Disease is mainly characterized by weakness, ataxia and recumbency as a result of nerve damage in the spinal cord. Furthermore, abnormal behaviour, cranial nerve deficits and teeth grinding may be observed, the latter symptoms being the result of brain damage. In addition, fever and anorexia may occur. Clinical signs differ between outbreaks. During the outbreak in Italy and France, ataxia and recumbency were the main clinical signs [16, 68]. In Israel and the USA, however, clinical signs were also indicative of brain damage [95, 96]. Such differences may be explained by differences in virus isolates, as suggested by Steinman et al. [96]. The mortality rate in equines varies from 28% [68] to 45% [101].

Other vertebrates

Beside birds and equines, a wide range of other vertebrate species is susceptible to WNV infection, as summarized in Table 1.

Antibodies against WNV have been detected in at least 30 different vertebrate species, including farm animals, pets and wild life (Table 1). The seroprevalence appears to be higher in regions where the virus has already circulated for many decades. For example, in dogs examined in South Africa, the seroprevalence varied between 8% [93] and 37% [10]. In dogs in Missouri [13] and New York [44], where WNV has only recently been introduced, antibodies were detected in 2.4% and 5%, respectively. Similarly, in a bear population in Croatia, 36% were seropositive [58], whereas in a bear population in New Jersey, 6% had antibodies against WNV [24]. The number of seropositive animals also depends on the vertebrate species. Low percentages of seropositive animals were found in wild small rodents and insectivores in Morocco (0.8%) [19], in wild rabbits in France (0.37%) [54], in domestic pigs in India (2.6 to 9.8%) [29, 82] and in domestic cattle in Mexico (1.5%) [108] and Nigeria (6%) [75]. On the other hand, a high seroprevalence was found among wild lemurs in Madagascar [85], in camels (26%), sheep (20%) and goats (18%) in Nigeria [75], among captive non-human primates in Louisiana (36%) [83] and among a group of crocodiles on a commercial farm in Israel (70%) [97]. The difference in seroprevalence may be related to the

Table 1. WNV infections in vertebrates other than birds and equines

Vertebrate species	Indications for susceptibility to WNV infections [reference]			
	Detection of antibodies	Detection of virus	Cases of disease	Experimental inoculation
Alligators	nd	[66]	[66]	nd
Alpacas	nd	[112]	[23, 112]	nd
American bullfrogs	nd	nd	nd	[42]
Baboon	[83]	nd	nd	nd
Bats	http://www.cdc.gov/mmwr/PDF/wk/mm4946.pdf	nd	nd	nd
Black bears	[24]	nd	nd	nd
Brown bears	[58]	nd	nd	nd
Camels	[75]	nd	nd	nd
Cats	http://www.cdc.gov/mmwr/PDF/wk/mm4946.pdf	nd	nd	[4]
Cattle	[75, 108]	nd	nd	nd
Crocodile	[97]	nd	nd	nd
Crocodile monitor	http://www.cdc.gov/ncidod/dvbid/westnile/conf/ppt/1a-travis.ppt	nd	nd	nd
Dogs	[10, 13, 44, 93]	[13, 55]	[13, 55, 93]	[10]
Garter snakes	nd	nd	nd	[42]
Goats	[75]	nd	nd	nd
Green iguanas	nd	nd	nd	[42]
Insectivores	[19]	nd	nd	nd
Lake frogs	nd	[48]	nd	[48]
Lemurs	[85]	nd	nd	[86]
Mice	nd	nd	nd	[9, 72]
Pigs	[29, 82]	nd	nd	[39]
Pigtail macaques	[83]	nd	nd	nd
Rabbits	[54] http://www.cdc.gov/mmwr/PDF/wk/mm4946.pdf	nd	nd	nd
Raccoon	http://www.cdc.gov/mmwr/PDF/wk/mm4946.pdf	nd	nd	nd
Rhesus macaques	[83]	nd	nd	[79, 84]
Reindeer	nd	[76]	[76]	nd
Rodents	[19] http://www.cdc.gov/mmwr/PDF/wk/mm4946.pdf	nd	nd	[111]
Sheep	[75]	[107, 112]	[107, 112]	[8]
Skunk	http://www.cdc.gov/mmwr/PDF/wk/mm4946.pdf	nd	nd	nd
Green iguanas	nd	nd	nd	[42]
Turtle	[71]	nd	nd	nd
Wolf	nd	[55]	[55]	nd

nd: not determined

susceptibility of the vertebrate species, to environmental conditions (exposure to mosquitoes) and/or to other unknown factors involved in the pathogenesis of WNV infection.

In most vertebrate species other than equines and birds, WNV is not a principal cause of neurological disease. However, in farmed alligators in the USA, infection resulted in two severe outbreaks of neurological disease during the fall of 2001 and 2002 [66]. The affected alligators appeared to "star-gaze" in the water, stranded in dry parts of the pen and showed neck spasms and loss of leg control. Of the >10.000 alligators housed at the farm, 250 died in 2001 and more than 1.000 died in 2002. Young animals were more severely affected than were adults. The latter could be due to the fact that immune competence in young alligators is dependent on the temperature [27]. Interestingly, WNV-contaminated horsemeat fed to the alligators was the presumed source of the outbreak. The importance of such oral transmission routes will be discussed below.

Individual cases of WNV-induced disease were observed in sheep [107, 112], alpacas [23, 112], dogs [13, 55, 93] and in a wolf [55]. Disease was always characterized by progressing neurological signs including weakness, ataxia, convulsions, paralysis, torticollis, hyperesthesia, recumbency and altered mentation. In the wolf, blindness was also observed. Non-neurological signs were observed in dogs and comprised anorexia, fever and myocarditis. In all diseased animals, death (or euthanasia) followed after 8 hours [112] to 7 days [55, 107]. In the diseased dog described by Lichtensteiger et al. [55], numerous other clinical signs were observed, such as polydipsia, nasal and ocular discharge, diarrhoea, abdominal pain, ptyalism and dyspnoea. The authors suggested that this dog was more severely affected by WNV, due to a concurrent immune-mediated disease. A correlation between the outcome of infection and the immune status of dogs was also observed by Blackburn et al. [10], who experimentally inoculated three dogs. Only one developed viremia and this particular dog had Cushing's disease, typified by immune suppression. In alligators, an age-related immune-competence appeared to affect the outcome of WNV infection, as mentioned previously [66].

The list of vertebrates susceptible to WNV infection based on serological surveys and cases of naturally acquired disease is extended by experimental inoculation studies, which confirmed susceptibility of American bullfrogs, garter snakes, green iguanas [42], lake frogs [48] and mice [9, 72].

Transmission of West Nile virus

Like all members of the Japanese encephalitis virus complex, WNV is maintained in nature by an arthropod-borne transmission cycle. Mosquitoes are the main vectors, although virus isolations from soft and hard ticks also were reported (reviewed in [37]). Wild birds are the major hosts of WNV. They develop a high, long-term viremia sufficient to infect vectors. Transmission of WNV between arthropods and birds is called the rural or sylvatic transmission cycle. As mentioned previously, many other vertebrates are susceptible to WNV infection as well.

Transmission of WNV between arthropods and other vertebrates is called urban transmission. The urban transmission cycle of WNV is less well characterized than is the rural transmission cycle. Beside arthropod-borne transmission, direct transmission without the involvement of arthropods can play a role in maintaining the virus in nature. This part of the review subsequently describes the role of vertebrates in different routes of transmission.

Arthropod transmission

Role of arthropods

Arthropod transmission of WNV includes uptake of virus by a vector during feeding on a viremic host, subsequent replication and dissemination of virus in the vector [30] and, finally, transmission of virus from the vector during feeding on an uninfected and susceptible host.

The competence of a vector to transmit WNV varies widely between different species. Worldwide, *Culex* spp. are considered as primary vectors [41, 88, 105]. The principal vectors of WNV are *Culex univitattus* in Africa and in the Middle East [62, 71], members of the *Culex vishnui* complex in Asia [2] and members of the *Culex pipiens* complex in North America [103]. Under field conditions, WNV has also been isolated from mosquitoes of other genera, including *Ochlerotatus*, *Aedes* and *Culiseta* [17, 18, 36, 69] and experimental studies demonstrate high laboratory vector competence by *Ochlerotatus japonicus japonicus* [87] and by several *Aedes albopictus* strains [89, 104].

Within mosquito species, the vector competence is affected by the exposure dose, as demonstrated by Goddard et al. [31]. Exposure of various *Culex, Ochlerotatus*, *Aedes* and *Culiseta* species to a dose of $10^{7.1}$ PFU/ml of blood resulted in a higher percentage of infected mosquitoes, than exposure to a dose of $10^{4.9}$ PFU. The increase in the percentage of infected mosquitoes using the higher infection dose was dependent on the species. Some of the species were only infected at the higher infection dose, but not at the lower dose. Also, the proportion of mosquitoes able to transmit the virus was considerably higher upon exposure to $10^{7.1}$ PFU than upon exposure to $10^{4.9}$ PFU, with mosquitoes of many species not transmitting virus at the lower infection dose [31]. Comparable dose-dependent infection and transmission rates were reported for *Culex* spp. by Sardelis et al. [88] and Turell et al. [105].

The vector competence of mosquitoes also depends on the time interval between their exposure to WNV and host feeding [31, 89]. The proportions of infected mosquitoes were generally higher after a time interval of 7 days, whereas the proportions of mosquitoes able to transmit virus were highest after 14 days.

Finally, other factors such as sex of the mosquitoes, host preference, feeding behaviour, longevity, temperature, humidity, seasonal activity and whether the mosquitoes have already fed or not, will influence the vector capacities. Also, population density of the host and housing (inside or outside) of the host will affect the capacity of the mosquitoes to transmit WNV.

Role of birds

Birds can play a role in the transmission cycle if they develop viremia levels sufficient to infect vectors that feed on them. Generally, viremia levels higher than $10^{6.0}$ PFU/ml of blood are considered to be infectious for the majority of *Culex* mosquitoes [31, 88, 103, 105] and *Culiseta inornata* mosquitoes [31]. Although infection and transmission rates may be lower, it is very likely that *Aedes* and *Ochlerotatus* spp. also become infected when feeding during such viremia [31, 87, 89, 104]. An extensive study by Komar et al. [46] including 25 bird species representing a wide range of avian orders showed that viremia levels in birds often exceed $10^{6.0}$ PFU/ml of blood. Passeriforms, the most susceptible birds, even exhibited viremic titres as high as $10^{12.1}$ PFU/ml. Swayne et al. [99] reported peak viremia titres of $10^{6.5}$ to $10^{7.5}$ $TCID_{50}$/ml of blood in 2-week old geese experimentally inoculated with WNV. These high levels of viremia, taken together with the broad range of susceptible mosquitoes (both ornithophilic and mammalophilic) and the high risk of exposure of birds to mosquito bites, it is generally accepted that birds, especially passeriforms, play a principal role in the transmission of WNV.

Gallinaceous and psittacine birds form an exception since they are, of all birds, least susceptible to WNV infection [46, 61]. Experimental infection of 17- to 60-week old chickens resulted in mean virus titres of less than 10^4 PFU/ml of blood, which were deemed insufficient to infect mosquitoes [53]. Similarly, 3-week old turkeys developed insufficient viremia titres [98]. On the other hand, young chickens developed higher levels of viremia. Senne et al. [92] reported titres of $10^{5.0}$ PFU/ml of blood in 7-week old chickens and several other studies described viremia titres of more than $10^{6.3}$ PFU/ml of blood in 2- to 3-day old chickens, the latter being sufficient to infect susceptible mosquitoes at high rates [88, 105]. The high viremia levels in young chickens may be attributed to the age-related susceptibility of birds, as described before.

The probability that WNV can be transmitted from an infected bird to a mosquito increases the longer infectious virus persists in the blood of the bird. Such persistence was demonstrated in experimentally infected ducks [25] and grey pigeons [91], in which WNV could be isolated from the blood for up to 101 and 100 days, respectively. Komar et al. [46] detected high titres of infectious virus (up to $10^{6.9}$ PFU/0.5 cm^3) in skin samples collected from dead birds at 14 days after experimental inoculation and suggested that such skin infection could allow transmission of WNV to vectors during feeding, even if viremia is no longer sufficient to do so.

Role of other vertebrates

Like birds, other vertebrates can play a role in the arthropod-borne virus transmission cycle if they develop sufficient viremia levels. Several studies have been performed to quantitate viremia in other vertebrates and, subsequently, to predict their role in transmission (Table 2). Occassionally, experimental transmission studies have been carried out.

Table 2. WNV transmission in vertebrates other than birds

Vertebrate species	Detection of infectious WNV [reference]			Transmission	
	Viremia	Organs/tissues	Excretion	Arthropod	Direct
Alligators	nd	multiple organs, especially liver [66]	nd	not known	not known
Alpacas	nd	brain [112]	nd	not known	possibly
American bullfrogs	$10^{2.2}$ PFU /ml [42]	heart (20 PFU/0.5 cm^3 tissue) [42]	oral/cloacal (5 PFU/swab) [42]	unlikely	possibly
Cats	$10^{4.0}$ PFU /ml [4]	nd	nd	unlikely	yes
Dogs	$10^{2.8}$ PFU/ml [10]	multiple organs, especially kidney [13, 55]	nd	unlikely	not known
Garter snakes	no [42]	spleen (400 PFU/0.5 cm^3) [42]	no [42]	unlikely	possibly
Green iguanas	$10^{3.2}$ PFU/ml [42]	spleen, small intestine (20–23 PFU/0.5 cm^3 tissue) [42]	oral/cloacal (5 PFU/swab) [42]	unlikely	possibly
Hamsters	10^5 $TCID_{50}$/ml [111]	brain, spinal cord ($10^{6.5}$ in 10% brain suspension) [111]	nd	possibly	possibly
Horses	$10^{3.0}$ PFU /ml [14]	brain, spinal cord ($10^{6.8}$/tissue) [14]	nd	unlikely	yes
Lake frogs	yes [48]	yes [47]	nd	yes	possibly
Lemurs	yes [86]	nd	nd	possibly	not known
Pigs	$10^{2.7}$ LD_{50}/ml [39]	nd	nd	unlikely	not known
Rhesus macaques	100 $TCID_{50}$/ml [84]	brain [79]	nd	unlikely	possibly
Sheep	yes [107]	brain ($10^{4.0}$/gr tissue) [107]	nd	not known	possibly
Wolf	nd	brain, myocardium, adrenal gland [112]	nd	not known	not known

nd: not determined
PFU: plaque forming units
TCID: tissue culture infectious dose
LD: lethal dose

Only in lemurs [86], lake frogs [48] and hamsters [111], were virus titres in the blood high enough, suggesting that these vertebrates are potential sources of virus for mosquitoes. Interestingly, a more recent study in American bullfrogs revealed much lower viremia titres compared to lake frogs [42], indicating that a possible role of frogs in transmission cannot be generalized.

Other studied vertebrates are unlikely to have a role in arthropod-borne virus transmission (Table 2). Low levels of virus were observed in the blood of dogs [10], sheep [8], pigs [39], equines [90, Lubroth, personal communication], American bullfrogs, green iguanas [42] and rhesus macaques [84]. No viremia was detected in experimentally inoculated garter snakes [42]. A recent transmission study performed by Bunning et al. [14] strengthened the idea that equines are not important in arthropod-borne WNV transmission. The authors inoculated *Aedes albopictus* mosquitoes intracoelomically, which resulted in virus titres of $10^{6.6}$ to $10^{7.9}$ Vero cell PFU per mosquito. Subsequently, the WNV-infected mosquitoes were allowed to feed on horses. The horses developed viremia levels of $10^{1.0}$ to $10^{3.0}$ Vero cell PFU/ml of blood, as determined by a plaque assay on Vero cells. WNV was, however, not transmitted to uninfected mosquitoes fed on the viremic horses.

Transmission without involvement of arthropod vectors or "direct" transmission

In mid-winter New York, 2000, a dead hawk infected with WNV was found in the absence of mosquito activity [28]. This suggested either persistent infection, as previously mentioned for ducks [25] and grey pigeons [91], or transmission without the involvement of arthropod vectors. Such "direct" transmission [49] can occur when infectious virus is present in or shed by the infected host.

Direct transmission via virus-contaminated tissues and organs

Presence of infectious WNV in tissues or organs of an infected host may contribute to direct oral transmission to an uninfected host. This was experimentally demonstrated in birds that became infected upon ingesting WNV-infected mice [46, 64] or a WNV-infected house sparrow carcass [46]. In nature, direct oral transmission was the cause of a severe outbreak of neurological disease in farmed alligators in the USA [66]. Alligators had been fed raw horsemeat in which WNV subsequently was demonstrated by RT-PCR. Also, the infection and subsequent death of the red-tailed hawk in New York was most likely the result of consumption of a WNV-contaminated prey [28]. Presence of virus in the skin of geese could lead to direct oral transmission via cannibalism and feather-picking [6].

It is possible that infected mosquitoes are a source of infection when consumed by insectivores. In this context, Komar et al. [46] demonstrated viremia in a house finch that ate a WNV-infected mosquito. Infection via consumption of infected

mosquitoes was also demonstrated for other arboviruses, including Rift Valley fever virus [73] and Japanese encephalitis virus [50]. The quantity of virus in vertebrate or mosquito tissues needed to induce oral transmission remains the subject of future research.

Direct transmission via virus-contaminated excretions and secretions

Shedding of WNV by an infected bird may contribute to direct transmission to an uninfected bird. Komar et al. [46] experimentally infected 78 birds comprising 24 different species and demonstrated viral shedding via cloacal and oral fluids in 59% and 69% of the birds, respectively. No or only very low amounts of virus were shed by psittaciforms, piciforms and galliforms, birds considered to be poorly susceptible to WNV. The highest titres were generally found in passeriforms, the order including the most susceptible birds (maximum $10^{6.0}$ PFU per cloacal swab; maximum $10^{5.7}$ PFU per oral swab). In another study by the same research group, titres in passeriforms reached $10^{6.9}$ PFU and $10^{7.3}$ PFU in cloacal and oral swabs, respectively [45]. Subsequently, Komar et al. [46] demonstrated that infected birds of three passeriform species and one charadriiform species directly transmitted virus to uninfected cage mates. Direct contact transmission was also demonstrated in experimentally inoculated geese [6, 99] and chickens [53], even though the amount of virus shed did not exceed $10^{2.5}$ $TCID_{50}$/ml in geese [99] and 200 infectious virus particles per swab in chickens [53]. In a study by Senne et al. [92], no direct transmission between experimentally infected chickens was demonstrated, despite the detection of comparable low titres of virus in cloacal swabs. Also, in experimentally inoculated turkeys, no direct contact transmission was observed [98].

Given the presence of infectious virus in cloacal and oral fluids together with close cloacal and oral contact occurring during the breeding season, it is possible that direct contact transmission occurs between birds in nature. Since the infectivity of WNV in avian faecal material outside the host is dramatically reduced after 24 hours, the risk of transmission via infected faeces decreases as the time outside the host increases [53].

Besides birds, American bullfrogs and green iguanas shed virus via oral and cloacal fluids, but the amounts were extremely low (fewer than 5 PFU/swab) and, therefore, unlikely to be a source of infection [42]. Virus shedding by other vertebrates and their possible role in direct transmission has not (yet) been examined.

Vertical transmission

Vertical transmission requires that virus is passed from parent to progeny. For vectors, such transmission has been clearly demonstrated in culicine and aedine mosquitoes [7, 22, 32, 65, 104] and could potentially serve as a mechanism for the virus to overwinter in regions with a temperate climate. Until now, the role of vertical transmission in vertebrates has been unclear.

Control of West Nile virus infections in veterinary medicine

Until 5 years ago, mosquito control was the only practical strategy to prevent and control WNV infection. However, the increased frequency and severity of infection observed during the last decade initiated the development of various WNV vaccines. These include inactivated, live-attenuated, and recombinant vaccines for use in both human and veterinary medicine. The main purpose of vaccination in veterinary medicine is to protect highly susceptible vertebrate species such as equines, geese and other economically important birds. Another purpose of vaccination is the reduction of viremia, principally in birds, in order to reduce the probability of the host-mosquito transmission cycle to occur.

For equines, several vaccines have been developed that significantly protect against viremia and disease. Upon vaccination with a formalin-inactivated cell culture vaccine (Fort Dodge Animal Health, Overland Park, KS, USA) and subsequent challenge, 1 of 19 (5.3%) vaccinates developed viremia, as opposed to 9 of 11 (81.8%) unvaccinated control horses that did [70]. Vaccination with a recombinant DNA vaccine consisting of the WNV pre-membrane and envelope genes inserted into a plasmid [21] protected all 4 horses against viremia and disease upon challenge, while 7 of 8 (87.5%) of the non-vaccinated horses became viremic and 1 developed fever and severe neurological signs. A canarypoxvirus-based recombinant vaccine expressing the pre-membrane and envelope genes, designed by Merial Animal Health Ltd (UK), protected 100% and 90% of horses against viremia when challenged 2 weeks and 1 year after vaccination, respectively, while 80% of non-vaccinated horses became viremic [67]. Neither the vaccinated nor the control horses developed clinical signs upon challenge.

Malkinson et al. [59] investigated protection rates against the neurological form of WNV in young geese. They administered an attenuated, commercial flavivirus vaccine derived from the Israel turkey meningoencephalitis virus (TME) or an inactivated TME vaccine or an inactivated WNV vaccine and challenged the geese with WNV by the intracerebral route. Both TME vaccines protected against neurological disease in 39% of geese vaccinated at commercial farms and 72% of geese vaccinated under controlled laboratory conditions. Using the WNV vaccine, they found similar levels of 52 and 80%, respectively. The lower levels of protection in geese vaccinated in farms were attributed to flocks being affected with intercurrent infections at the time of vaccination. In all cases, the level of protection was higher after two vaccinations than after a single vaccination.

Vaccination of fish crows to protect against viremia and disease was examined by Turell et al. [106]. An intramuscular vaccination with a DNA vaccine reduced the number of viremic crows (67% viremic) as well as the peak viremia ($10^{2.9}$ PFU/ml of blood) upon challenge, as compared to non-vaccinated controls (100% viremic; $10^{4.3}$ PFU/ml of blood). Moreover, vaccination prevented death upon subsequent challenge. Oral administration of the vaccine elicited no immune response and no protection against lethal infection. The latter suggests that the

route of administration is important for the ability of this DNA vaccine to provide protection.

Two other potential veterinary vaccines have been described. Lustig et al. [57] described a live-attenuated full length WNV isolate derived from empirical passage of a *wild-type* isolate in *Aedes aegypti* mosquito cells. One dose of attenuated virus induced complete protection against subsequent intracerebral challenge in mice and geese. One may, however, question the safety of such a vaccine, considering the possibility of a spontaneous virulent reversion owing to the relatively high mutation rate of RNA viruses. A molecularly engineered live-attenuated chimeric WNV vaccine (ChimeriVax^{TM}-WN(vet), Acambis Inc., Cambridge, MA, USA), containing the pre-membrane and envelope genes from wild type WNV-NY99 [51] in a backbone of the yellow fever 17D vaccine virus was described by Arroyo et al. [3]. In hamsters, a single intramuscular administration of this ChimeriVax^{TM}-WN protected 17 of 18 (94%) of the animals against viremia upon subsequent challenge [102]. A virus titre of $10^{0.7}$ $TCID_{50}$/ml of blood, as determined by titration of blood samples on *Aedes albopictus* cells, was reported in the single viremic hamster [102]. This was significantly lower than previously observed in non-vaccinated animals (10^5 $TCID_{50}$/ml) [111]. Moreover, all vaccinated hamsters were protected against disease and death [102]. Comparatively, in non-vaccinated hamsters, infection resulted in a mortality rate of up to 70% [111]. The protective properties of ChimeriVax^{TM}-WN have not yet been examined in other vertebrate species.

Future perspectives on West Nile virus infections in veterinary medicine

Predicting the future impact of WNV on veterinary medicine is a difficult if not an unachievable task. Many players are involved in determining the course and spread of WNV infections. These include the mosquito as a vector, the bird as a principal host, other vertebrate species as additional hosts and, finally, humans, which have the capacity to intervene with the role of each of the key players. Additionally, factors such as the evolution of WNV and the presence of heterologous flavivirus antibodies may affect the outcome of infection. Of help in predicting the future impact of WNV for veterinary medicine are answers to questions such as when and where WNV will be introduced in previously unaffected areas, which vertebrates with veterinary importance are susceptible to WNV and what the consequences of WNV infection in these vertebrates will be in terms of disease and possible transmission.

Migratory birds have an important role in the introduction of WNV into previously unaffected areas [80, 81]. During migration, they can carry virus from endemically infected wintering grounds to stopover sites in unaffected regions. In order to identify possible sites of virus introduction, studying migratory routes of birds is, therefore, indispensable. However, in addition to normal migration, also less predictable and controllable routes such as accidental displacement by storms and (il)legal importation of birds can contribute to the introduction of virus into

unaffected areas [80, 81], making it difficult to predict whether, where and when WNV will be introduced. Strict control of international trade of birds (quarantine, serological and virological examination) as well as monitoring abnormally high die-offs indicative of WNV infection can be helpful. Also, mosquitoes can contribute to the introduction of WNV into unaffected areas. They often ride on intercontinental flights in overhead bins and, if an infected mosquito is among them, introduction of virus may occur after landing and taking a blood meal from a local bird. Monitoring infection rates of potential vector mosquitoes, worldwide, may help in the surveillance for WNV.

Even if an infected bird or mosquito enters an unaffected area, WNV is only introduced when certain conditions are fulfilled. These include the presence of numerous potential vectors, lack of heterologous flavivirus antibody in local host populations, amplification of WNV in local host populations, and suitable environmental conditions [34]. As such, virus outbreaks often occur near wetlands and in urban regions where introductory host, vector, amplifying host and additional host are all present at the same location. However, environmental variations such as heavy rains, floods and increase of temperature can occur in previously unsuitable locations, subsequently allowing the introduction of virus. Moreover, human activities such as irrigation, landscape alteration, landscape destruction and pollution can substantially contribute to environmental changes, which may affect vector and/or host populations and, subsequently, allow the emergence of WNV into new geographic locations [20].

As summarized in this review, many vertebrate species are susceptible to WNV infection, but clinical disease appears to be a rather uncommon event, except for passerine birds, equines, geese and humans. Since most data collected on susceptibility are from serological surveys performed during a relatively short period of 5 years following the extensive outbreak of WNV in the USA, additional data on susceptibility to and clinical impact of WNV infections in vertebrates of veterinary importance have to be collected. These additional data should not only be collected from worldwide serological surveys in a wide variety of vertebrates, but also from experimental inoculation studies, in order to obtain more insights in the pathogenesis of WNV infection. Points of interest are potential routes of infection, principal vectors, amount of WNV needed for infection, amount and duration of viremia, amount and duration of virus shedding, routes of virus shedding, clinical outcome of infection as well as possible occurrence of vertical transmission. The suggestion that susceptibility to and outcome of WNV infection varies between WNV isolates [51, 96], indicates the need for comparative studies using different isolates.

Susceptibility of vertebrates not only determines the outcome of infection, but also the possible role of the vertebrates in transmission of virus to other vertebrates. Susceptible birds are involved in both arthropod-borne transmission and direct transmission. Most other vertebrates are unlikely to play a role in arthropod-borne transmission. Their role in direct transmission of WNV is much less apparent. Therefore, it is crucial to perform studies that quantify the amount of infectious WNV present in organs and tissues of infected vertebrates as well as the

amount and routes of virus shedding. Subsequently, it should be examined whether this infectious virus can directly be transmitted to non-infected and susceptible vertebrates.

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