# West Nile Virus Infection

*West Nile Fever,*
*West Nile Neuroinvasive Disease,*
*West Nile Disease,*
*Near Eastern Equine Encephalitis,*
*Lordige*

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## Importance

West Nile virus (WNV) is a mosquito-borne virus that circulates among birds, but can also affect other species, particularly humans and horses. Many WNV strains are thought to be maintained in Africa; however, migrating birds carry these viruses to other continents each year, and some strains have become established outside Africa. At one time, the distribution of WNV was limited to the Eastern Hemisphere, and it was infrequently associated with serious illness. Clinical cases usually occurred sporadically in humans and horses, or as relatively small epidemics in rural areas. Most human infections were asymptomatic, and if symptoms occurred, they were typically mild and flu-like. Severe illnesses, characterized by neurological signs, seemed to be uncommon in most outbreaks. Birds appeared to be unaffected throughout the Eastern Hemisphere, possibly because they had become resistant to the virus through repeated exposure.

Since the 1990s, this picture has changed, and WNV has emerged as a significant human and veterinary pathogen in the Americas, Europe, the Middle East and other areas. Severe outbreaks, with an elevated case fatality rate, were initially reported in Algeria, Romania, Morocco, Tunisia, Italy, Russia and Israel between 1994 and 1999. While approximately 80% of the people infected with these strains were still asymptomatic, 20% had flu-like signs, and a small but significant percentage (<1%) developed neurological disease. One of these virulent viruses entered the U.S. in 1999. Despite control efforts, it became established in much of North America, and spread to Central and South America and the Caribbean. Outbreaks were reported across North America in humans, horses and captive alligators; sporadic cases occurred in less susceptible mammals; and asymptomatic infections were recognized in a number of mammalian and several reptilian species. Although the introduction of an equine vaccine has helped control the disease in horses, there is currently no vaccine for humans, and recent experiences suggest that human outbreaks may continue to occur in North America at unpredictable intervals. Many North American species of birds were also affected, particularly when the virus first entered the continent. While some bird populations seem to have rebounded, the effect of WNV on threatened or endangered avian species continues to be a concern. California condors and greater sage grouse are among the species susceptible to this virus, and WNV introduction into Hawaii could have severe consequences for some native birds. Surprisingly, the effects of virus introduction seem to have been milder in South America, although the reason for this is still uncertain.

The epidemiology of WNV infections may also be changing in Eurasia. A number of outbreaks have been reported recently in Europe, Russia and parts of the Middle East. One virus affected significant numbers of wild and captive raptors in Hungary and Austria. This has led to a re-examination of the potential for West Nile viruses to cause illness or deaths in other European birds. Some viruses, which were not thought to persist from year to year in Europe, seem to have become endemic and are spreading.

## Etiology

West Nile virus is an arbovirus in the *Flavivirus* genus of the family Flaviviridae. It belongs to the Japanese encephalitis virus complex or serogroup. The two most common genetic lineages of WNV are lineage 1, which contains 3 clades (1a, 1b and 1c), and lineage 2. Both lineages contain virulent viruses, as well as strains that usually cause asymptomatic infections or mild disease. Many of the virulent viruses from recent outbreaks belonged to clade 1a, which is widespread. The strain that entered the United States in 1999, called NY99, appears to be related to a lineage 1a virus found in Israel from 1997 to 2000, and is among the most pathogenic strains. NY99 has continued to evolve in the Americas, where it has been replaced by its variants, especially WN02. Clade 1b consists of Kunjin viruses, a subtype of WNV found in Australia, and clade 1c consists of some West Nile viruses found in India.

Several additional WNV lineages also exist or have been proposed. Under some taxonomic schemes, it might be possible to classify this virus into as many as eight

lineages by including Koutango virus, a related virus that circulates in Africa, and elevating clades 1b and 1c to lineages.

## Species Affected

### Birds

Wild birds are the main reservoir hosts for West Nile virus. Passeriformes (perching birds) are important in virus amplification. Some members of other orders including (but not limited to) Charadriiformes (shorebirds), Falconiformes (hawks, eagles, vultures and related species) and Strigiformes (owls) may also transmit the virus to mosquitoes.

#### Birds in the Western hemisphere

Overall, WNV-infections have been documented in more than 320 species of North American birds since 1999. Some species usually carry the virus asymptomatically, while others are more likely to become ill. Clinical cases have been reported in domesticated birds, wild birds, and captive wild species in zoos and collections.

Among domesticated birds, outbreaks have occurred in geese, and symptomatic infections have been reported in a number of psittacine species (although psittacine birds were relatively resistant to disease in one experimental study). Chickens and turkeys (order Galliformes) seroconvert but remain asymptomatic; however, outbreaks have occurred in farmed chukar partridges (*Alectoris chukar*) and Impeyan pheasants (*Lophophorus impeyanus*). Some wild gallinaceous birds have also been affected: greater sage grouse (*Centrocercus urophasianus*) are highly susceptible, and one case was reported in a wild turkey (*Meleagris gallopavo* ssp).

The effects of WNV on wild birds vary with the species. When this virus was first introduced to North America, corvids (e.g., crows, ravens, magpies and jays) were severely affected. Other affected wild birds have included American robins (*Turdus migratorius*), eastern bluebirds (*Sialia sialis*), chickadees (*Poecile* sp.), tufted titmice (*Baeolophus bicolor*), house finches (*Carpodacus mexicanus*), house sparrows (*Passer domesticus*), house wrens (*Troglodytes aedon*) and black-crowned night herons (*Nycticorax nycticorax*). Northern cardinals (*Cardinalis cardinalis*) also seem to be susceptible, although the population as a whole appears to be resilient and did not decline. High mortality rates have been reported in infected Falconiformes, and owls have also been killed. Emus, penguins, pigeons, flamingos, American white pelicans (*Pelecanus erythrorhyncos*) cormorants, gulls, sandhill cranes (*Grus canadensis*) and other species can also be affected. Although ducks are not thought to be highly susceptible, there have been reports of illness in several species, or their young.

Similarly to the pattern of disease in humans, birds in South and Central America, Mexico and the Caribbean seem to be less severely affected than birds in North America. However, this might vary with the viral strain and species of bird. One WNV isolate from Mexico was less virulent for crows, but not house sparrows, compared to a North American virus.

#### Birds in the Eastern Hemisphere

WNV outbreaks have been reported among domesticated geese in the Eastern Hemisphere, but generally there have been only sporadic reports of deaths in individual wild birds. It is uncertain whether this is related to the virulence of the viruses circulating in this region, host susceptibility, reduced transmission/ amplification or lack of surveillance. One recently introduced lineage 2 virus in Central Europe has affected significant numbers of wild and captive raptors. Species known to be susceptible to this isolate include sparrow hawks (*Accipiter nisus*), goshawks (*Accipiter gentilis*) and gyrfalcons (*Falco rusticolus*). The same virus was isolated from a dead collared dove (*Streptopelia decaocto*) in Italy, during an outbreak of mortality in collared doves and other species including blackbirds. Lineage 1a or 2 viruses have also been found occasionally in other sick or dead birds including European robins (*Erithacus rubecula*), a raven (*Corvus corax*), common magpies (*Pica pica*), a Eurasian jay (*Garrulus glandarius*), house sparrows (*Passer domesticus*), a black redstart (*Phoenicurus ochruros*), a sedge warbler (*Acrocephalus schoenobaenus*) and a Savi's warbler (*Locustella luscinioides*).

European birds that became ill when infected experimentally with WNV outbreak strains from the Eastern Hemisphere included falcons that received European lineage 1 or lineage 2 viruses; red legged partridges (*Alectoris rufa*) infected with a European lineage 1 virus; house sparrows infected with European lineage 1 viruses; and wild carrion crows (*Corvus corone*) inoculated with lineage 1 isolates from Europe or Israel. Most European species have not yet been examined for susceptibility.

### Mammals and marsupials

Among mammals, disease occurs mainly in equids (horses, donkeys and mules). Although serious illnesses in horses have mainly been attributed to lineage 1 viruses, lineage 2 isolates in Africa and Hungary also caused severe clinical signs or death.

A few clinical cases have been reported in other domesticated mammals including alpacas, sheep and reindeer (*Rangifer tarandus*). Dogs and cats appear to be readily infected, but rarely become ill. Wild or captive wild species that have been affected include squirrels, harbor seals (*Phoca vitulina*), a killer whale (*Orcinus orca*), Indian rhinoceroses (*Rhinoceros unicornis*), wolf pups, a polar bear (*Ursus maritimus*), a Barbary macaque (*Macaca sylvanus*), mountain goats (*Oreamnos americanus*) and a white-tailed deer (*Odocoileus virginianus*). Experimental infections have been established in a variety of mammals: mice, hamsters, chipmunks, cats and rhesus monkeys

developed mild to severe clinical signs, but rabbits, pigs, guinea pigs, dogs, raccoons and hedgehogs (*Erinaceus europaeus)* remained asymptomatic.

Antibodies to WNV have been found in many mammalian and some marsupial species including cattle, sheep, goats, pigs, camels, wild boar, deer, lemurs, bats, skunks, bears, coyotes, foxes, various big cats (e.g., tiger, lion, cougar), stone marten (*Martes foina*), a civet, raccoons (*Procyon lotor*), opossums, rabbits, non-human primates, killer whales, dolphins, seals, small rodents and insectivores. Due to the limitations of serological testing for WNV, some of these antibodies may represent infections with flaviviruses other than WNV.

Some species of mammals including squirrels (*Sciurus* sp.), eastern chipmunks (*Tamias striatus*) and eastern cottontail rabbits (*Sylvilagus floridanus*) may be capable of transmitting WNV to mosquitoes, although their importance as reservoir hosts is still uncertain. Individual animals of certain other species, such as raccoons (*Procyon lotor*), may develop moderate levels of viremia, even if the species overall is not considered to be of epidemiological significance in infecting mosquitoes.

### Reptiles and amphibians

Among reptiles, clinical signs were mainly reported during outbreaks in alligators, although there is also a report of neurological signs associated with WNV infection in a crocodile monitor (*Varanus salvadori*) lizard. Some infections in garter snakes (*Thamnophis sirtalis*) experimentally inoculated with WNV were also fatal. Green iguanas (*Iguana iguana*) can be infected, and antibodies have been found in turtles, wild and farmed crocodiles, and alligators.

Amphibians including lake frogs (*Rana ridibunda*) and North American bullfrogs (*Rana catesbeiana*) can also be infected with WNV.

Some alligators (e.g., American alligators, *Alligator mississippiensis*) and frogs (e.g., *Rana ridibunda* in Russia) may develop viremia sufficient to infect mosquitoes. As with mammals, their importance as reservoir hosts is still uncertain.

### Zoonotic potential

Humans usually acquire WNV in mosquito bites; however, some species of birds, mammals and reptiles can shed this virus in secretions and excretions. Tissues from infected animals, especially the brain, are also sources of exposure.

## Geographic Distribution

West Nile viruses have been found throughout much of the world including Africa, Europe, Asia, the Middle East, Australia, the Americas and the Caribbean. In some regions, the viruses do not seem to be endemic, but are re-introduced regularly by migratory wild birds. These viruses may either cause outbreaks, or circulate asymptomatically among birds during warm weather, and disappear with the onset of cold temperatures. Relatively little is known about the occurrence of WNV in Asia, where the presence of the closely-related Japanese encephalitis virus (JEV) complicates diagnosis. However, WNV is endemic in India, and it was recently reported from an outbreak in China that was originally thought to be caused by JEV.

### Lineage 1 WNV

In the Eastern Hemisphere, lineage 1a viruses have been found in Africa, the Middle East, Europe and parts of Asia. Whether these viruses circulate in any avian populations in Europe, or are only introduced periodically by wild birds without overwintering, is still under investigation. Currently, it appears that many lineage 1 viruses do not overwinter, while others may persist from year to year in the Mediterranean region, but not in some other countries that have been examined (e.g., the U.K. and Germany). There is also evidence for the continuing endemic circulation of a lineage 1 virus in Romania between 1997 and 2009, after an epidemic in 1996. In the Western Hemisphere, lineage 1a viruses have been endemic in North America since 1999. Since spreading to South and Central America, they have been documented in several countries including Colombia, Argentina, Venezuela and Brazil. WNV has also spread to the Caribbean, but it is not yet present in Hawaii in 2013.

Lineage 1b (Kunjin virus) occurs in Australia, and lineage 1c viruses are found in India.

### Lineage 2 WNV

Lineage 2 viruses have mainly been isolated south of the Sahara desert in Africa, where they co-circulate in some regions with lineage 1 viruses. They also occur in Madagascar. Virulent lineage 2 strains have been endemic in Central Europe (Hungary and Austria) since 2004, and seem to be spreading. Identical or closely related viruses were recently isolated from mosquitoes, a dead indigenous bird and two human patients in Italy, and from mosquitoes in Greece during a human WNV outbreak. Different lineage 2 viruses caused outbreaks in Russia in 2007, and viruses related to this strain were found in Romanian outbreaks in 2010. One genetic analysis suggested that the viruses originally identified in Hungary and Russia might have been introduced in 1999 and 2000, respectively.

## Transmission

West Nile virus is primarily transmitted by mosquitoes. Members of the genus *Culex* are the main vectors worldwide, although other mosquito genera can also be infected. In North America alone, there is evidence of infection in more than 60 mosquito species. Transovarial transmission has been demonstrated in some species of mosquitoes, and is likely to important in overwintering. Dormant mosquitoes that survive the winter may also harbor WNV. Other arthropods might have minor roles in transmission. Infections have been

documented in ticks in Asia, Europe and the Middle East, and soft (argasid) ticks have been shown to transmit WNV in the laboratory. Hipposboscid flies might be able to transmit this virus in North America, and infected lice (*Philopterus* spp.) have been collected from WNV-infected crows.

Birds are the primary vertebrate reservoir hosts for West Nile virus, but the level and duration of viremia varies with the species. In endemic regions, the virus is maintained in an enzootic cycle between culicine mosquitoes and birds. When environmental conditions favor high viral amplification, significant numbers of "bridge vector" mosquitoes (mosquitoes that feed on both birds and mammals) become infected in the late summer, and can transmit the virus to humans, horses and other incidental hosts. In some birds, viremia can persist for more than three months, possibly contributing to the overwintering of the virus. Whether birds harbor sufficient infectious virus to initiate a new cycle in mosquitoes, after the winter, is still under investigation. Migratory birds are thought to be important in introducing WNV into new areas, and can reintroduce viruses into some regions each year.

Some species of birds can shed WNV in oral and cloacal secretions, and may transmit the virus directly. Crows, jays, magpies, gulls, raptors and some other birds, including domesticated chickens and turkeys, are known to excrete WNV for varying periods of time, and evidence for horizontal transmission was reported during an outbreak in domesticated geese. However, not all birds that shed WNV seem to transmit the virus efficiently. Experimentally infected red-legged partridges (*Alectoris rufa*) excreted this virus in oral and cloacal secretions, but there was no evidence of transmission to birds in contact. WNV also occurs in the skin of geese and the blood-feather pulp of crows, possibly contributing to transmission by cannibalism and feather picking. Raptors and crows may become infected when they eat other animals, and insectivorous species might eat infected mosquitoes. WNV does not seem to persist very long in the environment: the infectivity of virus in avian feces decreases dramatically after 24 hours.

Mosquito bites are the usual source of WNV for mammals, reptiles and amphibians. Most animals, including horses, appear to be dead-end hosts that do not transmit WNV to mosquitoes, but a few species have higher levels of viremia, and might be able to act as amplifying or maintenance hosts. In some animals, there is also evidence for transmission by other routes. Carnivorous mammals and reptiles (e.g., cats and alligators) can be infected by eating contaminated tissues. WNV-contaminated horsemeat was implicated in one outbreak in alligators. Direct transmission during close contact has also been reported in alligators, possibly via fecal shedding of virus. Chipmunks, squirrels and raccoons can also shed WNV in feces, oral secretions and/or urine. WNV has been found in the urine of experimentally infected hamsters, and in very small amounts in the oral and/or cloacal fluids of experimentally infected North American bullfrogs (*Rana catesbeiana*) and green iguanas (*Iguana iguana*). Transplacental transmission was reported in experimentally infected sheep and mice, as well as in a horse that was fatally infected with a lineage 1 virus in Africa, and aborted in the final stage of the disease. The epidemiological significance (if any) of mammalian, reptilian and amphibian hosts in the maintenance or amplification of WNV remains to be established.

Humans are usually infected by mosquito bites, but a few cases have been linked to accidental inoculation through breaks in the skin. These cases frequently occurred in people who handled infected tissues (often brains) from various animals. One recent infection occurred in a person who had removed the brain of an infected horse, using only latex gloves for protection. Whether the gloves had an unnoticed small puncture, or there was another source of the virus, is uncertain. An outbreak among workers on a turkey farm may have resulted from fecal-oral transmission, exposure of broken skin or mucous membranes to virus, or exposure to aerosolized virus. Humans do not develop viremia sufficient to transmit WNV to mosquitoes, and do not appear to shed significant levels of infectious virus in secretions or excretions. While WNV RNA is often found in patients' urine, infectious virus was only isolated from one encephalitis patient who had a very high viral load, and other isolation attempts on urine have been unsuccessful. For this reason, a recent article concluded that human urine is probably not a risk for virus transmission. However, WNV can be transmitted between people in blood transfusions and organ transplants. Rare cases of transplacental transmission and probable transmission in breast milk have also been reported.

In mammals, WNV is usually cleared from the body during the illness. A few studies have suggested that this virus or its RNA might persist for up to several months, or perhaps even years, in some mammals including humans. The evidence is currently conflicting, and this issue has not yet been resolved.

## Disinfection

West Nile virus can be destroyed by many disinfectants including sodium hypochlorite solutions (500-5000 ppm available chlorine), 2-3% hydrogen peroxide, 2% glutaraldehyde, 3-8% formaldehyde, ethanol, 1% iodine and phenol iodophors. It is also inactivated by UV light and gamma irradiation, as well as exposure to temperatures of 56-60°C (133-140 °F) for 30 minutes.

## Infections in Animals

## Incubation Period

The incubation period in horses is 3 to 15 days. Infections in other mammals are uncommon, and the incubation period is unknown. Clinical cases are reported to

occur in birds, on average, approximately 5 days after experimental inoculation.

## Clinical Signs

### Birds

Some species of birds carry WNV asymptomatically, while others develop clinical signs. Trauma (e.g., as a consequence of neurological signs) or concurrent bacterial, fungal or viral infections may also complicate the course of the disease.

On poultry or game bird farms, outbreaks have been reported in geese, chukar partridges and Impeyan pheasants. Only young geese were affected during outbreaks in North America and Israel; older birds did not become ill. The clinical signs in goslings included weight loss, decreased activity, depression, and neurological signs such as torticollis, opisthotonos and rhythmic side-to-side head movements. Myocarditis was seen in some birds at necropsy. Many infections were fatal. Illness has also been reported in chukar partridges and Impeyan pheasants. In one outbreak, hundreds of 6-8-week-old chukar partridges were either found dead without previous clinical signs, or displayed incoordination for less than a day before dying. Incoordination and diarrhea, followed by death, were reported in Impeyan pheasants. Naturally or experimentally infected chickens and turkeys are asymptomatic regardless of age.

A variety of clinical signs have been reported in zoo birds, pet psittacines and captive raptors. The predominant signs and course of the disease can vary with the species. Nonspecific signs such as anorexia, rapid weight loss, weakness, lethargy and ruffled feathers are common; some birds display only nonspecific signs before death. Neurological signs also occur in some birds; ataxia, incoordination, paresis or paralysis, disorientation, tremors, nystagmus, impaired vision or blindness, circling and seizures have been reported. Myocarditis is sometimes seen at necropsy. Sudden death also occurs. In contrast, one great horned owl had intermittent, mild clinical signs for more than five months, and a vulture with neurological signs exhibited progressive deterioration over the course of three weeks. In early reports, most clinically affected birds died or were euthanized due to their deteriorating condition. However, some birds, including those with neurological signs, can recover with supportive care. Full recovery can sometimes take longer than 6 months in raptors. Sequelae that have been reported in recovered birds of prey include relapses of neurological signs (e.g., ataxia), abnormal molting and persistently abnormal feathers.

Affected wild birds are usually found dead, and the clinical signs in many species have not been well described. Neurological signs have been reported in some moribund birds, and myocarditis, encephalitis or other lesions are sometimes found at necropsy. Experimentally infected sage grouse developed a profuse, clear, watery oral and nasal discharge. Affected birds ruffled their feathers, shivered, isolated themselves from the group, and showed signs of weakness or lethargy. These signs were followed by drooping wings, ataxia, copious oral and nasal secretions, and labored breathing. The grouse became moribund within hours.

### Mammals

Most horses are infected asymptomatically with WNV. In clinical cases, the illness is characterized by anorexia, depression and neurological signs, which may include ataxia, weakness or paralysis of one or more limbs, teeth grinding, aimless wandering, convulsions and/or circling. Tremors of the face and neck muscles are very common. Some animals have cranial nerve deficits, particularly weakness or paralysis of the face and tongue, which may lead to difficulty in swallowing. Attitudinal changes including somnolence, apprehension, hyperesthesia or periods of hyperexcitability are also common. Some horses with severe depression and facial paralysis may hang their heads; this can result in severe facial edema. Coma, impaired vision and head pressing can be seen, but tend to be less common than in cases of encephalitis caused by alphaviruses. Colic and urinary dysfunction (from mild straining to stranguria) have also been reported. Fever is present in some but not all cases. Fatal hepatitis was seen in a donkey with neurological signs in France. Injuries, pulmonary infections acquired during prolonged recumbency, and other secondary effects can complicate the course of the disease in equids. Some animals die spontaneously, but many severely affected animals are euthanized for humane reasons. Horses that recover usually begin to show improvement within seven days of the onset of clinical signs. Most but not all horses return to full function; approximately 10-20% horses are estimated to have residual defects such as weakness in one or more limbs, decreased exercise tolerance, muscle atrophy or behavioral changes. Studies from outbreaks in Hungary suggest that the lineage 2 virus circulating in Central Europe causes similar clinical signs and mortality as lineage 1a strains in horses.

Clinical cases have also been reported in ruminants and cervids, although they seem to be uncommon. In many cases, only a single animal was affected on a farm. Occasionally, a few other animals became ill around the same time. Most affected sheep, alpacas, reindeer and white-tailed deer have had neurological signs, which were often the first signs observed in the animal. However, a prodromal syndrome of fever, anorexia and depression was reported in one alpaca; the fever disappeared by the time the neurological signs appeared. Sudden death without prior clinical signs was seen in a reindeer. Another reindeer had diarrhea for 1-2 weeks before the onset of neurological signs. Most affected animals have died, but one alpaca recovered from mild head tremors and ataxia. Death often occurs within 1-2 days, particularly in reindeer, but some

animals were ill for several days to a week. Experimentally infected sheep did not develop systemic signs, but some pregnant ewes aborted, had stillborn lambs, or gave birth to lambs that died soon after birth.

Neurological signs, sometimes accompanied by other clinical signs, have been reported from rare clinical cases in dogs and wolf pups. In one dog, the first signs were episodes of uncontrolled rolling, which quickly progressed to generalized tremors, ataxia and intermittent fever. Other neurological signs reported in dogs were decreased conscious proprioception, a stiff gait, neck pain, paresis, depressed mentation, muscle atrophy and head tilt. Fever, inappetence, oculonasal discharge, conjunctivitis, excessive salivation, polydypsia, diarrhea, abdominal pain, myocarditis, dyspnea and polyarthritis have also been seen. Asymptomatic infections appear to be common, and only mild recurrent myopathy was reported in experimentally infected dogs. Oculonasal discharge, vomiting, anorexia, and lethargy, progressing to ataxia, were reported in a 4-month-old wolf cub. This animal died 24 hours after the onset of neurological signs. West Nile virus was also recovered from the brain of a cat with CNS signs. Experimentally infected cats were transiently lethargic and had fluctuating fevers, but neurological signs were not seen.

Cases in other animals have also been characterized mainly by neurological signs or sudden death, with systemic signs in some instances. Some infected squirrels circled, chewed at their feet, were lethargy or ataxic; other squirrels have been found dead. Incoordination, tremors and head tilt were reported in one of ten experimentally infected fox squirrels (*Sciurus niger*); the other nine squirrels remained asymptomatic. Acute paraparesis was the presentation in an aged polar bear at a zoo, while a fatal infection in a harbor seal was characterized by progressive neurological signs, inappetence and weakness, with intermitted diarrhea and vomiting, and labored breathing. Tremors and twitching were seen in another captive seal for four days, but this animal recovered. Sudden death occurred in a captive killer whale that had fulminant secondary bacteremia and septicemia, together with primary WNV encephalitis. West Nile virus was also suspected as the cause of depression, lethargy, partial anorexia and a drooping lip in two Indian rhinoceroses during a WNV outbreak at a zoo. Both animals recovered. Infections in some experimentally infected mice, hamsters and rhesus monkeys were characterized by fatal encephalitis. Experimentally infected pigs remained asymptomatic.

### Reptiles

In alligators, the clinical signs included anorexia, lethargy, weakness and neurological signs including tremors, unresponsiveness, slow reflexes, head tilt, anisocoria and opisthotonos. Some alligators were unable to submerge and stranded in dry parts of the pen, dragged their hind feet, or swam on their sides or in circles. Animals usually died 24–48 hours after the onset of clinical signs. A strong association between WNV infection and lymphohistiocytic proliferative cutaneous lesions has also been reported in this species.

Neurological signs in one naturally infected crocodile monitor were also linked to WNV. Fatal illness was reported in experimentally infected garter snakes. Some snakes died suddenly, while others exhibited unusual aggression and immobility of the caudal part of the body, or weakness and cachexia, which may have been caused by inappetence.

## Communicability

Horizontal transmission can occur in some avian species. Birds from diverse families are known to shed WNV in oral secretions and/or feces; shedding has been demonstrated after natural or experimental infections in corvids, various raptors, domesticated geese, aigamo ducks (hybrids of *Anas platyrhynchos* and *Anas platyrhynchos* var. *domesticus*), ring-billed gulls (*Larus delawarensis*), cliff swallows (*Petrochelidon pyrrhonota*) and red-legged partridges, among others. However, the ability of these birds to transmit the virus efficiently seems to vary. There is evidence of horizontal transmission among corvids, as well as among geese, and an outbreak among workers at an infected turkey farm is suggestive (experimentally infected turkeys and chickens can excrete this virus in feces for a few days). In contrast, experimentally infected red-legged partridges did not transmit WNV to uninfected birds, despite shedding the virus in oral and cloacal secretions.

Certain mammals (e.g., chipmunks, squirrels and raccoons) have been shown to excrete WNV in oral secretions, feces and/or urine, and might be capable of horizontal transmission. Among reptiles, only alligators are known to transmit this virus during close contact, but some other species (e.g., iguanas) can transiently shed very small amounts of virus.

## Post Mortem Lesions

### Birds

A wide variety of gross and microscopic lesions, which are often nonspecific, have been reported in birds. Some birds may be thin or emaciated, but others are in good body condition. The most common macroscopic lesions, in addition to emaciation and dehydration, are multiorgan hemorrhages, petechiae and congestion. Splenomegaly, hepatomegaly, myocardial pallor and pale mottling of the liver, spleen or kidney have also been observed in various species. Several reports described cerebral atrophy and malacia in raptors. Gross lesions seem to be minimal or absent in some infected birds, including some psittacines. No macroscopic lesions appear to be pathognomonic for WNV, and reported lesions are not necessarily consistent between species, even when the birds belong to the same family. The limited number of samples from some species may contribute to the seeming high variability in gross lesions.

Histopathologic lesions have been detected in most organs, but in most families of birds, lesions are usually concentrated in the CNS (e.g., encephalitis), heart, spleen, liver and kidney. Frequently reported findings include lymphoplasmacytic and histiocytic infiltrates, cellular degeneration and necrosis, and hemorrhages. The pattern and severity of microscopic lesions vary with the species of bird and the length of time it has been ill. In birds that die quickly, the lesions may be acute and have minimal inflammatory reactions. Birds that have been ill longer, such as raptors, can have chronic lesions, including lesions in the CNS. Severe CNS lesions are not always found in birds with neurological signs.

### Mammals

Gross lesions are uncommon in horses. If they occur, they are usually limited to small multifocal areas of discoloration and hemorrhage in the spinal cord, brain stem and midbrain. The meninges may be congested in acute cases. Meningeal hemorrhages have also been described. Gross lesions in tissues other than the CNS are uncommon. The histopathologic lesions are characterized by lymphocytic or histiocytic poliomeningoencephalitis with perivascular cuffing of mononuclear cells, neuronal degeneration, neuronophagia and focal gliosis. These lesions are particularly apparent in the lower brain stem and spinal cord, and may also occur in the midbrain. They are less common in the cerebral and cerebellar cortices. Mild nonsuppurative myocarditis, scattered hemorrhages in the renal medulla, and lymphoid depletion of the spleen have been seen in some horses.

Few or no gross lesions have been reported in most other mammals including reindeer, squirrels, sheep and alpacas. In one sheep, multifocal hemorrhagic and malacic foci were found in the lumbar spinal cord. A wolf pup was emaciated, with mucoid nasal exudate, and unclotted blood was found in the small and large intestinal lumen. This animal also had vascular lesions in the kidneys, and to a lesser extent, in the cerebral cortex. The liver, which was mildly enlarged, was yellow and friable; hepatic lipidosis was partly attributed to anorexia. In a harbor seal, gross lesions included decreased blubber thickness and hyperemia of the brainstem and spinal cord blood vessels. In mammals, histological lesions in the CNS have generally resembled those seen in horses. Lymphocytic and necrotizing myocarditis, granulomatous inflammation of the kidneys, renal tubular epithelial cell necrosis, pancreatitis, synovitis (polyarthritis) and acute, diffuse, moderate hepatic necrosis were also reported in affected dogs. Mild to moderate lymphocytic myocarditis, myocardial necrosis and mild focal hepatic necrosis were seen in some squirrels.

### Reptiles

During an outbreak in alligators, moderately sized fat bodies and approximately 3–5 ml of clear yellow fluid were found in the coelomic cavity. The liver was mottled red to yellow, and slightly enlarged with rounded edges. Tan to red mottling was also seen in the spleen and myocardium.

## Diagnostic Tests

WNV infections can be diagnosed by isolating the virus, detecting viral antigens or RNA, or using serological methods. The usefulness of the various techniques varies with the level of virus replication in the host. In horses, viremia is short-lived and low, and clinical cases are usually confirmed by serology, or by detecting WNV in the brain and spinal cord at necropsy. Both serology and tests to detect the virus are useful in live birds, with the caveat that the amount of virus can vary between avian species. There is limited information about other animals; however, virus replication seems to be widespread in affected alligators, while viremia appears to be low in ruminants.

Isolation of West Nile virus can be used for diagnosis, especially when disease is suspected in a species not previously known to be susceptible. WNV is often recovered in African green monkey kidney (Vero) cells or rabbit kidney (RK-13) cells. Mosquito cell lines and embryonating chicken eggs may also be used. The identity of the virus can be confirmed by tests such as immunofluorescence or RT-PCR. In birds, WNV is sometimes found in the blood, and it can often be isolated from the CNS and/or major organs (e.g., heart and liver) at necropsy. This virus was also recovered from plasma and several tissues in affected alligators. In one outbreak, viral titers were reported to be higher in the liver of alligators than the CNS. In horses, WNV is difficult to isolate from the blood, but it can sometimes be found in the brain and spinal cord at necropsy. Disadvantages to virus isolation are that it is time-consuming, requires level 3 biosafety (BL 3) containment, and is not widely available at diagnostic laboratories.

RT-PCR assays are valuable as both antemortem and postmortem tests in birds. In some live birds, this test may be able to detect West Nile virus RNA in oral and cloacal swabs and/or serum samples. Viral RNA was also found in plasma samples from sick alligators. In horses, RT-PCR is most useful with brain and spinal cord samples taken at necropsy. Although viral RNA can sometimes be found in the blood of subclinically infected horses, it has usually disappeared by the time the neurological signs appear. In addition, RT-PCR has been used to diagnose WNV infections in other animal species. Some commercial assays may not detect lineage 2 viruses.

Several tests can be used to detect WNV antigens. Immunohistochemistry is often used on tissue samples collected at necropsy. Equine CNS does not contain large quantities of virus, and immunohistochemistry can detect some, but not all, infected animals. In contrast, this test may detect antigens in multiple organs, as well as the CNS, in alligators and some species of birds. One study found that the spleen, liver, kidney and duodenum contained antigens in almost all crows infected in the wild. Antigen-capture

ELISA tests can also be used to find antigens in avian tissues; however, they are not useful in horses, which have much lower levels of virus. An antigen capture dipstick assay is valuable for rapid testing of oral or cloacal swabs from live birds, and tissue homogenates from dead birds. The antigen-capture ELISA and the antigen-capture dipstick assay are also used for mosquito surveillance. Cross-reactions with closely related flaviviruses can occur in antigen tests.

Serological tests for WNV include various ELISAs, hemagglutination inhibition (HI), virus neutralization assays (the plaque reduction neutralization, or PRN assay) and other tests. Cross-reactive antibodies to closely related flaviviruses are also detected by the ELISAs, HI and some other tests, but can be distinguished with the PRN test. This test is also used to confirm positive or equivocal ELISAs in horses. A disadvantage to the PRN test is that it must be performed in a BL 3 laboratory, and it is not available at all diagnostic laboratories. Serological tests are particularly valuable in live horses, where they are often used to diagnose clinical cases. A four-fold or greater increase in WNV-specific antibodies in serum, the detection of specific IgM in CSF, or the detection of specific IgM in serum confirmed by specific IgG in the same or a later sample are diagnostic. If clinical signs have not been present long enough for IgG to develop, the presence of IgM alone in serum is suggestive. Serology is also valuable in birds, as well as some mammals other than horses; however, it should be noted that some ELISAs can only be used in the species for which they have been standardized. Vaccination history must be considered when interpreting serological tests in horses and in some birds (e.g., geese and California condors).

## Treatment

No specific treatment is available, but animals may recover on their own if they are given supportive care. Supportive treatment has the goal of reducing inflammation in the CNS, preventing self-inflicted injuries and adverse effects from recumbency, and providing supportive nutrition and fluids. Therapy is empiric, and similar to the treatment of other causes of viral encephalomyelitis. Mild cases have sometimes recovered without treatment.

## Prevention

Commercial WNV vaccines are available for horses in the U.S. and other countries, and for geese in Israel. Vaccines are sometimes used "off label" to protect sensitive birds (e.g., endangered California condors) or other species.

Topical repellents can reduce the risk of WNV during the mosquito season. Repellents should be approved for the species; products that are safe in one species (including humans) can sometimes be toxic in others. Housing susceptible species indoors or in screened barns, cages or other screened areas can also decrease mosquito bites. Fans can be helpful in barns, as mosquitoes are not

strong flyers. Insecticides or mosquito traps may also be used. Areas around barns, paddocks and pastures should be kept free of weeds, feces and other organic materials that could shelter adult mosquitoes. Standing or stagnant water should be eliminated to prevent them from breeding. Water tanks and buckets should be cleaned at least weekly, and containers (e.g. flower pots and used tires) should be removed or emptied of water. In some areas, ponds may be stocked with mosquito fish (*Gambusia affinis*), which feed on mosquito larvae. The inconveniences from mosquito control measures such as indoor housing can be weighed against the risk of infection in each species. For example, some species of birds are highly susceptible to WNV, but cases in dogs, cats and sheep are rare. In some areas, agencies conduct mosquito abatement programs using larvicides, adulticides and other measures to reduce mosquito populations.

Other measures, such as quarantines of infected animals, may be helpful in species suspected or known to transmit the virus horizontally. Carnivores and omnivores should not be allowed to eat any meat that might be contaminated with WNV. One outbreak occurred in alligators that had been fed WNV-infected horsemeat. Tissues from some birds are also known to contain high levels of virus.

## Morbidity and Mortality

Clinical cases caused by WNV usually occur seasonally. Birds are mainly affected from summer to late fall, and cases in horses peak in late summer and fall. Occasional outbreaks may be seen when mosquitoes are absent, in species that can transmit the virus horizontally. In the U.S., one outbreak occurred among crows during the winter. WNV isolates differ in their virulence for birds, and only some viruses cause severe illness or death. Different patterns of disease have been reported among avian species in the Eastern and Western Hemispheres.

### Birds in North America

With minor exceptions (e.g., some individual animals in zoos), birds in the Western Hemisphere were first exposed to WNV in 1999. Some North American populations of wild birds experienced high mortality rates. Corvids were severely affected. Overall, the number of crows in the U.S. fell by an estimated 30%, with much greater decreases in some localized areas. Declines were also measured in populations of blue jays (*Cyanocitta cristata*). Greater sage grouse are also highly susceptible to WNV, and some local populations of these birds were severely affected. Nearly all of the breeding birds died in some areas. In the Powder River basin of Montana and Wyoming, the minimum mortality rate from WNV-infection in sage grouse was 2-13% in 2003-2005, and the maximum possible mortality rate was 8-29%. In raptors, one study estimated the annual mortality rate from WNV infections to be 7-15%. Other affected populations included American robins, eastern bluebirds, chickadees, tufted titmice, house sparrows, house wrens, house finches and

black-crowned night herons, with declines either after intense epidemics or over longer periods. In some cases, the number of birds fell across their entire range; in others, the decreases were regional. With time, some species (for example, crows, blue jays and house wrens) have apparently recovered or are recovering; other populations remained smaller than normal. In contrast, the abundance of some birds does not seem to have been affected by WNV. While some of these unaffected species may not be very susceptible to WNV, this is not necessarily true in all cases. For example, a wild population of northern cardinals was shown to be affected by the virus, although this population as a whole appeared to be resilient and did not decline. WNV may also affect some age groups more than others. It appears to cause significant mortality in American white pelican chicks in North America, although adults are not severely affected.

In zoos and rehabilitation centers, WNV has affected a wide variety of avian species. During one outbreak at a New York zoo, the overall morbidity rate among infected birds was estimated to be 14%; it was higher among species found in the Western Hemisphere (20%) than species indigenous to Eastern Hemisphere (5%). In this outbreak, the morbidity rate was high in corvids, owls and penguins, but only 9% of infected gallinaceous birds became ill. Most clinical cases ended in death; the case fatality rate was 69% overall, and in most orders, it reached 100%. A high case fatality rate was also reported during an outbreak at Kansas zoos: only one of 11 affected birds, a sandhill crane, survived. Among raptors, symptomatic infections have been documented in both falconiformes and owls. Widely varying mortality rates have been reported among owls at rehabilitation centers, with some species experiencing mortality rates of greater than 90%, while others suffered no deaths.

Among poultry, young geese seem to be particularly susceptible to WNV, and have been affected in both Western and Eastern Hemispheres. In Israel, disease was reported in 3-8-week-old goslings, with morbidity and mortality rates of approximately 40%. During an outbreak in Canada, the mortality rate was 25% in 6-week-old goslings, but 15-month-old and 5-year-old geese seroconverted with no clinical signs. In experimental infections, up to 50–75% of geese may die. Ducks are not thought to be highly susceptible to WNV; however, an outbreak among captive lesser scaup (*Aythya affinis*) ducklings resulted in 70% mortality. During other outbreaks, the morbidity and mortality rates were 100% in Impeyan pheasants, and the mortality rate was 25% in chukar partridges. Similarly to geese, young partridges and pheasants seem to be more susceptible to disease. In contrast, both young and old chickens and turkeys are infected asymptomatically.

A limited number of bird species have been experimentally infected with North American strains of WNV. These studies demonstrate that susceptibility varies greatly between avian species. Mortality rates as high as 100% have been reported in American crows (*Corvus brachyrhynchos*), black-billed magpies (*Pica hudsonia*),

ring-billed gulls, house finches and greater sage grouse. Mortality was 75% in blue jays (*Cyanocitta cristata*), 53% in fish crows (*Corvus ossifragus*), 16% in house sparrows and 0% in cliff swallow nestlings. In addition to the species, factors that may affect the severity of disease include pre-existing immunity to WNV, co-existing conditions and general health, and possibly the route of exposure.

### Birds in South and Central America

Few detailed studies have been conducted on WNV mortality among birds in South and Central America, Mexico and the Caribbean. Overall, these birds are thought to have been less severely affected than birds in North America.

### Birds in the Eastern Hemisphere

In areas where WNV has been endemic for decades, the prevalence of infection in wild birds ranges from 10% to greater than 50%. While WNV outbreaks have been reported among domesticated geese in the Eastern Hemisphere, there have generally been only sporadic reports of deaths in wild birds, and no major mortality events have been reported. It is uncertain whether this is related to the virulence of the viruses circulating in this region, lower host susceptibility (including immunity from repeated exposure), reduced transmission/ amplification or lack of surveillance. However, one recently introduced lineage 2 virus in Central Europe has affected significant numbers of wild and captive raptors. Species known to be susceptible to this isolate include sparrow hawks (*Accipiter nisus*), goshawks (*Accipiter gentilis*) and gyrfalcons (*Falco rusticolus*). Dead songbirds of various species have also been diagnosed occasionally with WNV in this area. The same virus was isolated from a dead collared dove in Italy, during an outbreak of mortality in collared doves and other species including blackbirds. Other lineage 1a or 2 viruses have also been found occasionally in sick or dead birds. For example, lineage 1a viruses have killed raptors in Spain, and a lineage 1a virus was detected in a dead magpie and a moribund wild house sparrow with torticollis and tremors during a WNV outbreak in France. No unusual mortality was otherwise observed in birds during the latter outbreak, but systematic examinations of bird populations were not carried out.

A few European species have been shown to be susceptible to illness after experimental inoculation of WNV. Red-legged partridges infected with two European lineage 1 viruses had mortality rates of 30% or 70%. Both lineage 1 and lineage 2 European viruses caused illness and some deaths in experimentally infected falcons, with both viruses causing a similar clinical picture. In one study, the illness was fatal in a third of the falcons inoculated with an Austrian lineage 2 WNV strain. In other experiments using European birds, the mortality rates were 0-25% in house sparrows inoculated with WNV isolates from Italy or Spain, 33% in wild carrion crows inoculated with a lineage 1 equine isolate from France, and 100% in wild carrion crows

inoculated with an avian (stork) lineage 1 isolate from Israel.

### Equids

Among domesticated mammals, West Nile outbreaks occur mainly in equids. Many infections in horses are asymptomatic. While seroprevalence rates vary greatly between studies (and cross-reactivity with other flaviviruses can be a concern), up to 90% of horses are reported to be seropositive in some parts of Africa. During outbreaks, 10-43% of infected horses are estimated to develop neurological signs. The reported case fatality rate ranges from 23% to 57%. It is approximately 30-40% in the U.S., and was 30% during a lineage 2 outbreak in Hungary. Although some horses that recover have residual neurological defects, approximately 80-90% (60-100% in individual studies) are estimated to return to full function. As with other species, the impact of WNV on horses seems to have been greater in North America than in other parts of the Americas.

### Ruminants and other livestock

Livestock are uncommonly affected by WNV, although seropositive animals are relatively common in some regions. A few clinical cases have been reported in sheep, alpacas and reindeer, but even in North America, clinical signs have been limited to one to a few animals in the herd. However, approximately 26% of camels, 20% of sheep, 18% of goats and 6% of cattle in Nigeria had antibodies to WNV in the HI test, while 29% of camels in Morocco were confirmed to be seropositive by virus neutralization (44% were seropositive by HI). In the Astrakhan region of Russia, antibodies were confirmed by virus neutralization in small numbers of cattle, sheep, pigs and camels; in all species, the seroprevalence rate was less than 6.5% by HI and less than 5% by virus neutralization. Similarly, less than 1% of cattle in Croatia, <1% to 6% of cattle in Belarus, and 1% of sheep in eastern Slovakia were seropositive. One study reported that 4% of cattle and 1% of sheep in Turkey had WNV antibodies, while a report from northern Turkey found antibodies in 3% of goats, and no cattle, sheep or water buffalo. Symptomatic infections have not been reported in pigs, but approximately 3-10% of domesticated pigs in India, 3% of domesticated pigs in Nepal, 3% of pigs in Spain and 22% of feral pigs in Florida, Georgia, and Texas were seropositive.

### Dogs and cats

Only rare clinical cases have been reported in dogs and cats, and they develop few or no clinical signs after experimental infection. However, asymptomatic infections may be common, as significant numbers of dogs and cats have antibodies to WNV. The seroprevalence among dogs was reported to be 8-37% in South Africa, 38% in Turkey, 5% in Shanghai, China and 2-56% in localized areas of the U.S. In two studies from the U.S. studies, the seroprevalence rates were 9-10% in cats, while another U.S. study detected no antibodies among 12 cats. In Shanghai, China, 15% of cats had antibodies to WNV.

### Wild mammals

Antibodies have also been reported in many species of wild mammals, and infections may be common in the members of some species. In the U.S., up to 63% of striped skunks, up to 46% of raccoons and up to 49% of squirrels in some areas may have antibodies to WNV. In Spain, antibodies to WNV or closely related flaviviruses were detected by ELISA in 13% of wild boars and 20% of red foxes (*Vulpes vulpes*). Only a few of the approximately 650 samples could also be analyzed by virus neutralization, but 43% of 21 samples from wild boars and the one sample analyzed from a fox were found to contain antibodies to WNV. High seroprevalence rates were also reported in a recent study of wild lemurs in Madagascar. The susceptibility of most species to WNV is unknown, but occasional clinical cases have been reported in a wide variety of species in zoos. Squirrels may be especially susceptible. In some regions, sick and dead squirrels have been seen during periods of high WNV activity, and the morbidity rate in experimentally infected fox squirrels was 10%.

### Case fatality rate in mammals other than horses

Once a mammal develops neurological signs, the case fatality rate seems to be high. Most clinically affected animals, which included sheep, alpacas, reindeer, dogs, cats, wolves, deer and a bear, have died, although one alpaca with relatively mild neurological signs recovered. Both rhinoceroses affected in a zoo and one of two seals also recovered.

### Reptiles

Among reptiles, disease has been reported only in alligators, one lizard (a crocodile monitor) and experimentally infected garter snakes. At one U.S. alligator farm with more than 10,000 animals, 250 alligators died in an outbreak one year, and more than 1,000 died the following year. Young alligators were more severely affected than adults.

## Infections in Humans

## Incubation Period

The incubation period is approximately 2 to 14 days. It is reported to be longer in transplant patients than in people who are not immunocompromised.

## Clinical Signs

Human illness has been classified into two forms: West Nile fever, which is a flu-like illness, and West Nile neuroinvasive disease, which encompasses all cases with neurological signs. Many WNV infections are asymptomatic.

West Nile fever is the most common form of the disease. This form resembles influenza, and is characterized

by fever, malaise, weakness, headache and body aches. Anorexia, lymphadenopathy, nausea, diarrhea, vomiting, sore throat and conjunctivitis may be seen in some patients. An erythematous, nonpruritic macular, papular or morbilliform skin rash occasionally develops on the neck, trunk, arms or legs. Most uncomplicated infections resolve in 2 to 6 days, but in some severe cases, persistent fatigue can last for a month or more.

Although many cases of West Nile fever are mild, there have also been reports of severe illness, and death is possible, though rare. In the U.S., fatal West Nile fever has occurred mainly in elderly patients, who frequently had underlying health conditions. In most cases, the illness appears to have exacerbated or precipitated an underlying medical condition, resulting in death from acute myocardial infarction, cardiac arrhythmia, respiratory failure, stroke, cancer or other conditions. Death was often the result of cardiac or respiratory complications.

A few patients with West Nile fever develop West Nile neuroinvasive disease. This form can be severe, and in some cases, it is life-threatening. Three syndromes - encephalitis, meningitis, and acute flaccid paralysis – are seen. Symptoms of more than one syndrome often occur in the same patient. West Nile meningitis is characterized by fever, headache, a stiff neck and photophobia. Patients with West Nile encephalitis have changes in consciousness, disorientation and/or focal neurological signs, which may include ataxia, incoordination, tremors, involuntary movements, and signs that resemble Parkinson's disease (rigidity, postural instability and bradykinesia). Concurrent signs of meningitis are common, and seizures or coma may also occur. Some patients who recover have persistent neurological dysfunction.

Acute flaccid paralysis (sometimes called West Nile poliomyelitis) is seen in some patients. The paralysis, which resembles polio, appears suddenly and progresses rapidly, usually reaching a plateau within hours. It is typically asymmetrical and can affect one or more limbs, often the legs. The weakened limbs become darker than normal at the peak of the paralysis. This syndrome may be accompanied by muscle aches in the lower back and/or abnormalities in bladder and bowel function. Some patients develop respiratory distress, which may require mechanical ventilation. Sensory functions are usually normal or minimally affected. Some patients with flaccid paralysis have prodromal signs of West Nile fever, sometimes with signs of meningitis or encephalitis; however, many patients are asymptomatic before the onset of paralysis. Late in the illness, the muscles may become atrophied. Recovery is highly variable: some patients recover completely within weeks, while others remain paralyzed.

Cranial nerve abnormalities in patients with neuroinvasive disease may result in facial weakness, dizziness, vertigo or nystagmus. Rhabdomyelitis, myositis, polyradiculitis and other syndromes have also been seen. Many individuals complain of blurred or impaired vision and photophobia; ocular syndromes that have been reported include chorioretinitis, uveitis, vitritis and optic neuritis.

Other syndromes have occasionally been reported. Myocarditis, pancreatitis, orchitis and fulminant hepatitis are uncommon, but have been seen in some outbreaks. A life-threatening hemorrhagic syndrome occurred in a few West Nile cases in Africa, and was reported in a patient in the U.S. Acute kidney disease has been seen in some patients with WNV encephalitis. Possible associations between West Nile neurological disease and chronic kidney disease have also been suggested by some authors, but a causative role remains to be confirmed.

## Communicability

Person-to-person transmission does not occur during casual contact; however, WNV can be transmitted in blood transfusions and organ transplants from people without clinical signs. Rare cases of transplacental transmission and probable transmission in breast milk have been reported. Infectious virus has been detected in urine, but this is unusual, and urine is not thought to be a risk for virus transmission.

## Diagnostic Tests

In humans, West Nile virus infections are often diagnosed by serology. Serological tests used in humans include ELISAs, a rapid microsphere-based fluorescence immunoassay (MIA), the plaque reduction neutralization (PRN) test, indirect immunofluorescence (IFA) and hemagglutination inhibition. Diagnostic criteria include a rising titer or the presence of IgM in serum or cerebrospinal fluid (CSF). In the serum, anti-WNV IgM can occasionally persist for more than a year. Serum IgM is thus suggestive of recent infection, but not definitive. This is not a concern for IgM in CSF. As in animals, cross-reactions can occur with closely related flaviviruses (e.g., yellow fever, Japanese encephalitis, St. Louis encephalitis or dengue viruses) in some serological tests. Positive reactions in other serological tests may be confirmed with the PRN test, if available.

Infectious viruses, viral antigens or nucleic acids can sometimes be detected in human tissues, CSF, blood, urine and other body fluids. This virus can usually be found in the blood of patients with West Nile fever during the first few days after the onset of illness. However, viremia usually disappears before the onset of neurological signs in immunocompetent patients, and viral RNA is often absent from the serum of patients with neuroinvasive disease. In these patients, nucleic acids may be detected in the CSF, using RT-PCR tests, during the acute stage of CNS signs. Immunohistochemistry to detect viral antigens is mainly used postmortem in fatal cases. Virus isolation requires level 3 biosafety containment, and is rarely performed.

## Treatment

There is no specific recommended treatment, other than supportive care, at present. Intensive care and

mechanical ventilation may be required in some cases. Various therapies including interferon, antisense nucleotides and intravenous immunoglobulins (passive immunization) are being tested in clinical trials. While a few case reports suggest that some of these treatments may be promising, larger studies are still lacking. Some antiviral drugs were promising *in vitro*, but most have been ineffective when tested in animal models or given to humans with severe disease. Screening for new drugs that may inhibit WNV is underway.

## Prevention

In most cases, WNV infections can be prevented by preventing mosquito bites. Outdoor activities should be limited when mosquitoes are active, particularly during the peak biting times of dusk and dawn. Mosquito repellents should be used when avoidance is impractical. Long pants and long-sleeved shirts are helpful; specialized fine mesh clothing (e.g., mesh head coverings and jackets) is also available. Measures to reduce mosquito populations include rational application of adulticides and larvicides, as well as environmental modifications such as emptying containers that may hold standing water. Surveillance in sentinel birds, dead birds and mosquitoes can help predict human exposure. Dead or sick birds should be reported to health, agriculture or mosquito-control agencies. In some cases, only certain species or groups of birds, such as corvids, may be tested for WNV. Dead animals should never be handled without gloves and sanitary precautions, as feces and body fluids may be infectious in some species (and may also contain pathogens other than WNV).

Veterinarians, wildlife rehabilitators, wildlife biologists, laboratory workers and others should practice good biosecurity and hygiene when handling either tissues or birds, mammals, reptiles and amphibians that may shed WNV in feces, oral secretions or urine. Mucous membranes and skin should be protected from contact with infectious material, such as tissues or secretions and excretions. Under some conditions, respiratory protection might be needed. Protective clothing and gloves should be used when performing necropsies.

How soon a human vaccine might become available is uncertain, but some vaccines have entered or completed clinical trials. Blood products are screened for WNV in some countries, to prevent transfusion-associated cases.

## Morbidity and Mortality

West Nile infections usually occur in humans during warm weather, when mosquitoes are active. Outbreaks of West Nile disease appear to be sporadic, as well as geographically focal in their distribution, with shifts in their locations from year to year. Some of the factors that might affect the occurrence of outbreaks include weather patterns, the number and distribution of mosquitoes, and herd immunity in amplifying hosts; however, the interactions of these factors in producing outbreaks are likely to be complex, and outbreaks are difficult or impossible to predict.

The epidemiology of WNV infections appears to differ between geographic regions, although differences in surveillance and diagnostic testing may also play a part in this perception. In some parts of Africa, there seems to be relatively little mortality in people. Although severe disease can occur, it is thought that people tend to become infected as children, and are immune by they time they become more susceptible to neuroinvasive disease as adults. In many parts of Europe, the usual pattern of WNV epidemiology has been that of occasional, self-limited outbreaks affecting very few people. Larger and more severe outbreaks are also reported occasionally. For example, nearly 200 cases of neuroinvasive disease were documented during an outbreak in Greece in 2010. This and other recent outbreaks, together with evidence that some viruses may have become endemic, has led to the suggestion that the epidemiology of WNV infections may be changing in Eurasia. In North America, large numbers of West Nile fever cases, and much fewer cases of neuroinvasive disease, have been seen: more than 13,000 cases of West Nile fever were reported to the CDC between 2002 and 2006. Based on the pattern of infections in North America so far, including a peak in the number of cases in 2002-2003, followed by declines, then another upsurge in 2012, periodic outbreaks and epidemics may be expected in the future. Surprisingly, far fewer clinical cases or deaths have been reported in Central and South America. The reason for this is not known; however, it might involve protective immunity to cross-reactive flaviviruses, the occurrence of WNV isolates with decreased virulence, decreased surveillance and diagnosis, or other causes. In areas where dengue is present, some West Nile infections could be misdiagnosed as this disease.

Most human infections with WNV are asymptomatic. Approximately 20% of those infected during recent outbreaks in the U.S., Europe and Israel developed West Nile fever, and less than 1% had West Nile neuroinvasive disease. Neuroinvasive disease is more likely to occur in people over 50 years of age and patients who are immunocompromised. In a study from North Dakota, the risk varied from 1 in 54, in people over the age of 65, to 1 in more than 1,200 in young, low risk individuals. A study from Ohio estimated approximately 1 case of neuroinvasive disease for every 4,000 infected children, 154 infected adults under the age of 65 years, or 38 infected adults who were more than 65. Recipients of organ transplants are estimated to have a 40% chance of developing neuroinvasive disease. Underlying diseases such as diabetes and autoimmune syndromes are also associated with more severe clinical signs.

Case fatality rates reported during outbreaks have ranged from 3% to 15%, but the case fatality rate varies with the form of the disease. West Nile fever is typically self-limited, and many cases are mild. However, more

severe illnesses have also been reported, and deaths are possible, though uncommon. In a recent analysis of cases in the U.S., approximately 0.2% of West Nile fever cases reported to the CDC between 2002 and 2006 were fatal. (Milder cases are likely to be underdiagnosed, and are probably underrepresented in this database.) Of the fatal cases, 78% occurred in patients who were older than 70 years of age. Many affected patients had underlying health conditions, and most of these deaths appeared to result from the exacerbation or precipitation of these illnesses, similarly to the effects of influenza on the elderly. For West Nile neuroinvasive disease, the overall case fatality rate is approximately 10%. Death is more likely to occur in older patients; case fatality rates of 15-29% have been seen in people who are more than 70 years old. Some patients with neuroinvasive disease may suffer substantial long-term morbidity after recovery from the acute syndrome. Patients with encephalitis are more likely to have a poor prognosis and long term sequelae than those with meningitis alone.

## Internet Resources

Centers for Disease Control and Prevention (CDC)
http://www.cdc.gov/westnile/index.html

Public Health Agency of Canada. Material Safety Data Sheets
http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/index-eng.php

The Merck Manual
http://www.merckmanuals.com/professional/index.html

The Merck Veterinary Manual
http://www.merckmanuals.com/vet/index.html

United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). Equine West Nile Virus.
http://www.aphis.usda.gov/vs/nahss/equine/wnv/

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

OIE Terrestrial Animal Health Code
http://www.oie.int/international-standard-setting/terrestrial-code/access-online/

## References

Albayrak H, Ozan E. Seroepidemiological study of West Nile virus and Rift Valley fever virus in some of mammalian species (herbivores) in northern Turkey. J Arthropod Borne Dis. 2013;7(1):90-3.

Anderson K. (Nebraska Cooperative Extension). West Nile virus guidelines for horse owners. Nebraska Cooperative Extension; 2002 Aug. Available at: http://www.ianr.unl.edu/pubs/animaldisease/nf542.htm.* Accessed 5 Dec 2002.

Appler KK, Brown AN, Stewart BS, Behr MJ, Demarest VL, Wong SJ, Bernard KA. Persistence of West Nile virus in the central nervous system and periphery of mice. PLoS One. 2010;5(5):e10649.

Ariel E.Viruses in reptiles. Vet Res. 2011;42(1):100.

Austgen LE, Bowen RA, Bunning ML, Davis BS, Mitchell CJ, Chang GJ. Experimental infection of cats and dogs with West Nile virus. Emerg Infect Dis. 2004;10:82-6.

Bagnarelli P, Marinelli K, Trotta D, Monachetti A, Tavio M, Del Gobbo R, Capobianchi MR, Menzo S, Nicoletti L, Magurano F, Varaldo PE. Human case of autochthonous West Nile virus lineage 2 infection in Italy, September 2011. Euro Surveill 2011;16. pii: 20002.

Bakonyi T, Ferenczi E, Erdélyi K, Kutasi O, Csörgő T, Seidel B, Weissenböck H, Brugger K, Bán E, Nowotny N. Explosive spread of a neuroinvasive lineage 2 West Nile virus in Central Europe, 2008/2009. Vet Microbiol. 2013;165(1-2):61-70.

Balança G, Gaidet N, Savini G, Vollot B, Foucart A, Reiter P, Boutonnier A, Lelli R, Monicat F. Low West Nile virus circulation in wild birds in an area of recurring outbreaks in Southern France. Vector Borne Zoonotic Dis. 2009;9(6):737-41.

Barbić L, Listeš E, Katić S, Stevanović V, Madić J, Starešina V, Labrović A, Di Gennaro A, Savini G. Spreading of West Nile virus infection in Croatia. Vet Microbiol. 2012 Oct 12;159(3-4):504-8.

Barzon L, Pacenti M, Cusinato R, Cattai M, Franchin E, Pagni S, Martello T, Bressan S, Squarzon L, Cattelan A, Pellizzer G, Scotton P, Beltrame A, Gobbi F, Bisoffi Z, Russo F, Palu G. Human cases of West Nile virus infection in north-eastern Italy, 15 June to 15 November 2010. Euro Surveill. 2011 Aug 18;16(33).

Barzon L, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M, Cusinato R, Palù G. Excretion of West Nile virus in urine during acute infection. J Infect Dis. 2013 Jul 23. [Epub ahead of print]

Barzon L, Pacenti M, Franchin E, Squarzon L, Lavezzo E, Toppo S, Martello T, Cattai M, Cusinato R, Palù G. Novel West Nile virus lineage 1a full genome sequences from human cases of infection in north-eastern Italy, 2011. Clin Microbiol Infect. 2012;18(12):E541-4.

Barzon L, Pacenti M, Palù G. West Nile virus and kidney disease. Expert Rev Anti Infect Ther. 2013;11(5):479-87.

Beasley DW. Recent advances in the molecular biology of West Nile virus. Curr Mol Med. 2005;5:835-50.

Beasley DW, Barrett AD, Tesh RB. Resurgence of West Nile neurologic disease in the United States in 2012: what happened? What needs to be done? Antiviral Res. 2013;99(1):1-5.

Bentler KT, Hall JS, Root JJ, Klenk K, Schmit B, Blackwell BF, Ramey PC, Clark L. Serologic evidence of West Nile virus exposure in North American mesopredators. Am J Trop Med Hyg. 2007;76:173-9.

Blackburn NK, Reyers F, Berry WL, Shepherd AJ. Susceptibility of dogs to West Nile virus: a survey and pathogenicity trial. J Comp Pathol. 1989;100:59-66.

Blitvich BJ. Transmission dynamics and changing epidemiology of West Nile virus. Anim Health Res Rev. 2008;9:71-86.

Brault AC, Langevin SA, Ramey WN, Fang Y, Beasley DW, Barker CM, Sanders TA, Reisen WK, Barrett AD, Bowen RA. Reduced avian virulence and viremia of West Nile virus isolates from Mexico and Texas. Am J Trop Med Hyg. 2011;85(4):758-67.

Buckweitz S, Kleiboeker S, Marioni K, Ramos-Vara J, Rottinghaus A, Schwabenton B, Johnson G. Serological, reverse transcriptase-polymerase chain reaction, and immunohistochemical detection of West Nile virus in a clinically affected dog. J Vet Diagn Invest. 2003;15(4):324-9.

Bunning ML, Bowen RA, Cropp CB, Sullivan KG, Davis BS, Komar N, Godsey MS, Baker D, Hettler DL, Holmes DA, Biggerstaff BJ, Mitchell CJ. Experimental infection of horses with West Nile virus. Emerg Infect Dis. 2002;8:380-6.

Busch MP, Wright DJ, Custer B, Tobler LH, Stramer SL, Kleinman SH, Prince HE, Bianco C, Foster G, Petersen LR, Nemo G, Glynn SA.. West Nile virus infections projected from blood donor screening data, United States, 2003. Emerg Infect Dis. 2006;12:395–402.

Callan RJ, Van Metre DC. Viral diseases of the ruminant nervous system. Vet Clin North Am Food Anim Pract. 2004;20:327-62, vii.

Calzolari M, Bonilauri P, Bellini R, Albieri A, Defilippo F, Tamba M, Tassinari M, Gelati A, Cordioli P, Angelini P, Dottori M. Usutu virus persistence and West Nile virus inactivity in the Emilia-Romagna region (Italy) in 2011. PLoS One. 2013;8(5):e63978.

Cannon AB, Luff JA, Brault AC, MacLachlan NJ, Case JB, Green EN, Sykes JE. Acute encephalitis, polyarthritis, and myocarditis associated with West Nile virus infection in a dog. J Vet Intern Med. 2006;20:1219-23.

Carboni DA, Nevarez JG, Tully TN Jr, Evans DE. West Nile virus infection in a sun conure (*Aratinga solstitialis*). J Avian Med Surg. 2008;22:240-5.

Carson PJ, Borchardt SM, Custer B, Prince HE, Dunn-Williams J, Winkelman V, Tobler L, Biggerstaff BJ, Lanciotti R, Petersen LR, Busch MP. Neuroinvasive disease and West Nile virus infection, North Dakota, USA, 1999-2008. Emerg Infect Dis. 2012;18(4):684-6.

Castillo-Olivares J, Wood J. West Nile virus infection of horses. Vet Res. 2004;35:467-83.

Ceianu CS, Ungureanu A, Nicolescu G, Cernescu C, Nitescu L, Tardei G, Petrescu A, Pitigoi D, Martin D, Ciulacu-Purcarea V, Vladimirescu A, Savage HM. West Nile virus surveillance in Romania: 1997-2000. Viral Immunol. 2001;14(3):251-62.

Centers for Disease Control and Prevention (CDC). Laboratory-acquired West Nile virus infections--United States, 2002. MMWR Morb Mortal Wkly Rep. 2002;51(50):1133-5.

Centers for Disease Control and Prevention (CDC). West Nile avian mortality database. Available at: http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm/* Accessed 28 Feb 2013.

Chin J, editor. Control of communicable diseases manual. 17th ed. Washington, DC: American Public Health Association; 2000. Arthropod-borne viral fevers; p. 48-50.

Ciccozzi M, Peletto S, Cella E, Giovanetti M, Lai A, Gabanelli E, Acutis PL, Modesto P, Rezza G, Platonov AE, Lo Presti A, Zehender G. Epidemiological history and phylogeography of West Nile virus lineage 2. Infect Genet Evol. 2013:46-50.

Clark L, Hall J, McLean R, Dunbar M, Klenk K, Bowen R, Smeraski CA. Susceptibility of greater sage-grouse to experimental infection with West Nile virus. J Wildl Dis. 2006;42:14-22.

Colpitts TM, Conway MJ, Montgomery RR, Fikrig E. West Nile virus: biology, transmission, and human infection. Clin Microbiol Rev. 2012;25(4):635-48.

D'Agostino JJ, Isaza R. Clinical signs and results of specific diagnostic testing among captive birds housed at zoological institutions and infected with West Nile virus. J Am Vet Med Assoc. 2004;224:1640-3, 1606.

Danis K, Papa A, Papanikolaou E, Dougas G, Terzaki I, Baka A, Vrioni G, Kapsimali V, Tsakris A, Kansouzidou A, Tsiodras S, Vakalis N, Bonovas S, Kremastinou J. Ongoing outbreak of West Nile virus infection in humans, Greece, July to August 2011. Euro Surveill. 2011 25;16(34). pii: 19951.

Danis K, Papa A, Theocharopoulos G, Dougas G, Athanasiou M, Detsis M, Baka A, Lytras T, Mellou K, Bonovas S, Panagiotopoulos T. Outbreak of West Nile virus infection in Greece, 2010. Emerg Infect Dis. 2011;17(10):1868-72.

Dauphin G, Zientara S. West Nile virus: recent trends in diagnosis and vaccine development. Vaccine. 2007;25:5563-76.

Davis LE, DeBiasi R, Goade DE, Haaland KY, Harrington JA, Harnar JB, Pergam SA, King MK, DeMasters BK, Tyler KL. West Nile virus neuroinvasive disease. Ann Neurol. 2006;60:286-300.

Dawson JR, Stone WB, Ebel GD, Young DS, Galinski DS, Pensabene JP, Franke MA, Eidson M, Kramer LD. Crow deaths caused by West Nile virus during winter. Emerg Infect Dis. 2007;13:1912-4.

Dayan GH, Bevilacqua J, Coleman D, Buldo A, Risi G. Phase II, dose ranging study of the safety and immunogenicity of single dose West Nile vaccine in healthy adults ≥50 years of age. Vaccine. 2012;30(47):6656-64.

Del Piero F, Stremme DW, Habecker PL, Cantile C.West Nile flavivirus polioencephalomyelitis in a harbor seal (*Phoca vitulina*). Vet Pathol. 2006;43:58-61.

Dridi M, Vangeluwe D, Lecollinet S, van den Berg T, Lambrecht B. Experimental infection of carrion crows (*Corvus corone*) with two European West Nile virus (WNV) strains. Vet Microbiol. 2013;165(1-2):160-6.

Dutton CJ, Quinnell M, Lindsay R, DeLay J, Barker IK. Paraparesis in a polar bear (*Ursus maritimus*) associated with West Nile virus infection. J Zoo Wildl Med. 2009;40(3):568-71.

Ellis AE, Mead DG, Allison AB, Stallknecht DE, Howerth EW. Pathology and epidemiology of natural West Nile viral infection of raptors in Georgia. J Wildl Dis. 2007;43:214-23.

El-Harrak M, Martin-Folgar R, Llorente F, Fernandez-Pacheco P, Brun A, Figuerola J, Jimenez-Clavero MA. Rift Valley and West Nile virus antibodies in camels, North Africa. Emerg Infect Dis. 2011;17(12):2372-4.

Erdélyi K, Ursu K, Ferenczi E, Szeredi L, Rátz F, Skáre J, Bakonyi T. Clinical and pathologic features of lineage 2 West Nile virus infections in birds of prey in Hungary. Vector Borne Zoonotic Dis. 2007;7:181-8.

Foppa IM, Beard RH, Mendenhall IH. The impact of West Nile virus on the abundance of selected North American birds. BMC Vet Res. 2011;7:43.

Gamino V, Höfle U. Pathology and tissue tropism of natural West Nile virus infection in birds: a review.Vet Res. 2013;44(1):39.

Gancz AY, Barker IK, Lindsay R, Dibernardo A, McKeever K, Hunter B. West Nile virus outbreak in North American owls, Ontario, 2002. Emerg Infect Dis. 2004;10:2135-42.

Gibbs SE, Ellis AE, Mead DG, Allison AB, Moulton JK, Howerth EW, Stallknecht DE. West Nile virus detection in the organs of naturally infected blue jays (*Cyanocitta cristata*). J Wildl Dis. 2005;41:354-62.

Gibbs SE, Marlenee NL, Romines J, Kavanaugh D, Corn JL, Stallknecht DE. Antibodies to West Nile virus in feral swine from Florida, Georgia, and Texas, USA. Vector Borne Zoonotic Dis. 2006;6:261-5.

Gibney KB, Lanciotti RS, Sejvar JJ, Nugent CT, Linnen JM, Delorey MJ, Lehman JA, Boswell EN, Staples JE, Fischer M. West Nile virus RNA not detected in urine of 40 people tested 6 years after acute West Nile virus disease. J Infect Dis. 2011;203(3):344-7.

Gubler DJ. The continuing spread of West Nile virus in the Western Hemisphere. Clin Infect Dis. 2007;45:1039-46.

Guthrie AJ, Howell PG, Gardner IA, Swanepoel RE, Nurton JP, Harper CK, Pardini A, Groenewald D, Visage CW, Hedges JF, Balasuriya UB, Cornel AJ, MacLachlan NJ. West Nile virus infection of Thoroughbred horses in South Africa (2000–2001). Equine Vet J 2003;35:601–5.

Gutiérrez-Guzmán AV, Vicente J, Sobrino R, Perez-Ramírez E, Llorente F, Höfle U. Antibodies to West Nile virus and related flaviviruses in wild boar, red foxes and other mesomammals from Spain. Vet Microbiol. 2012;159(3-4):291-7.

Heinz-Taheny KM, Andrews JJ, Kinsel MJ, Pessier AP, Pinkerton ME, Lemberger KY, Novak RJ, Dizikes GJ, Edwards E, Komar N. West Nile virus infection in free-ranging squirrels in Illinois. J Vet Diagn Invest. 2004;16:186-90.

Himsworth CG, Gurney KE, Neimanis AS, Wobeser GA, Leighton FA. An outbreak of West Nile virus infection in captive lesser scaup (*Aythya affinis*) ducklings. Avian Dis. 2009;53(1):129-34.

Hinckley AF, O'Leary DR, Hayes EB. Transmission of West Nile virus through human breast milk seems to be rare. Pediatrics. 2007;119(3):e666-71.

Hutcheson HJ, Gorham CH, Machain-Williams C, Loron o-Pino MA, James AM, Marlenee NL, Winn B, Beaty BJ, Blair CD. Experimental transmission ofWest Nile virus (Flaviviridae: *Flavivirus*) by *Carios capensis* ticks from North America. Vector Borne Zoonotic Dis. 2005;5:293-5.

Jacobson ER, Ginn PE, Troutman JM, Farina L, Stark L, Klenk K, Burkhalter KL, Komar N. West Nile virus infection in farmed American alligators (*Alligator mississippiensis*) in Florida. J Wildl Dis. 2005;41:96-106.

Janusz KB, Lehman JA, Panella AJ, Fischer M, Staples E. Laboratory testing practices for West Nile virus in the United States. Vector Borne Zoonotic Dis. 2011;11(5):597-9.

Jimenez-Clavero MA, Sotelo E, Fernandez-Pinero J, Llorente F, Blanco JM, Rodriguez-Ramos J, Perez-Ramirez E, Hofle U. West Nile virus in golden eagles, Spain, 2007. Emerg Infect Dis. 2008;14:1489-91

Jourdain E, Schuffenecker I, Korimbocus J, Reynard S, Murri S, Kayser Y, Gauthier-Clerc M, Sabatier P, Zeller HG. West Nile virus in wild resident birds, southern France, 2004. Vector Borne Zoonotic Dis. 2007;7:448-52.

Jourdain E, Zeller HG, Sabatier P, Murri S, Kayser Y, Greenland T, Lafaye M, Gauthier-Clerc M. Prevalence of West Nile virus neutralizing antibodies in wild birds from the Camargue area, southern France. J Wildl Dis. 2008;44(3):766-71.

Juricová Z, Mitterpák J, Prokopic J, Hubálek Z. Circulation of mosquito-borne viruses in large-scale sheep farms in eastern Slovakia [abstract]. Folia Parasitol (Praha). 1986;33(3):285-8.

Kahn CA, Line S, Aiello SE editors. The Merck veterinary manual. 10th ed. Whitehouse Station, NJ: Merck and Co; 2010. Flaviviruses; p. 1194-5 .

Kauffman EB, Franke MA, Wong SJ, Kramer LD. Detection of West Nile virus. Methods Mol Biol. 2011;665:383-413.

Kecskeméti S, Bajmócy E, Bacsadi A, Kiss I, Bakonyi T. Encephalitis due to West Nile virus in a sheep. Vet Rec. 2007;161:568-9.

Kile JC, Panella NA, Komar N, Chow CC, MacNeil A, Robbins B, Bunning ML. Serologic survey of cats and dogs during an epidemic of West Nile virus infection in humans. J Am Vet Med Assoc. 2005;226:1349-53.

Klenk K, Komar N. Poor replication of West Nile virus (New York 1999 strain) in three reptilian and one amphibian species. Am J Trop Med Hyg. 2003;69:260-2.

Komar N, Langevin S, Hinten S, Nemeth N, Edwards E, Hettler D, Davis B, Bowen R, Bunning M. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg Infect Dis. 2003;9:311–322.

Komar N, Panella NA, Boyce E. Exposure of domestic mammals to West Nile virus during an outbreak of human encephalitis, New York City, 1999. Emerg Infect Dis. 2001;7:736-8.

Kramer LD, Li J, Shi PY. West Nile virus. Lancet Neurol. 2007;6:171-81.

Kramer LD, Styer LM, Ebel GD. A global perspective on the epidemiology of West Nile virus. Annu Rev Entomol. 2008;53:61-81.

Kutasi O, Bakonyi T, Lecollinet S, Biksi I, Ferenczi E, Bahuon C, Sardi S, Zientara S, Szenci O. Equine encephalomyelitis outbreak caused by a genetic lineage 2 West Nile virus in Hungary. J Vet Intern Med. 2011;25(3):586-91.

Kutzler MA, Bildfell RJ, Gardner-Graff KK, Baker RJ, Delay JP, Mattson DE. West Nile virus infection in two alpacas. J Am Vet Med Assoc. 2004;225:921-4.

LaDeau SL, Kilpatrick AM, Marra PP. West Nile virus emergence and large-scale declines of North American bird populations. Nature. 2007;447:710-3.

Lan D, Ji W, Yu D, Chu J, Wang C, Yang Z, Hua X.Serological evidence of West Nile virus in dogs and cats in China. Arch Virol. 2011;156(5):893-5.

Langevin SA, Bunning M, DavisB, Komar N. Experimental infection of chickens as candidate sentinels for West Nile virus. Emerg Infect Dis. 2001;7:726-9.

Lanthier I, Hébert M, Tremblay D, Harel J, Dallaire AD, Girard C. Natural West Nile virus infection in a captive juvenile Arctic wolf (*Canis lupus*). J Vet Diagn Invest. 2004;16:326-9.

Leake CJ. Mosquito-borne arboviruses. In: Palmer SR, Soulsby EJL, Simpson DIH, editors. Zoonoses. New York: Oxford University Press; 1998. p. 401-13.

Leis AA, Stokic DS. Neuromuscular manifestations of West Nile virus infection. Front Neurol. 2012;3:37.

Levy JK, Lappin MR, Glaser AL, Birkenheuer AJ, Anderson TC, Edinboro CH. Prevalence of infectious diseases in cats and dogs rescued following Hurricane Katrina. J Am Vet Med Assoc. 2011 Feb 1;238(3):311-7.

Li XL, Fu SH, Liu WB, Wang HY, Lu Z, Tong SX, Li ZX, Nasci RS, Kosoy O, Cui Y, Liang GD. West Nile virus infection in Xinjiang, China. Vector Borne Zoonotic Dis. 2013;13(2):131-3.

Lillibridge KM, Parsons R, Randle Y, Travassos da Rosa AP, Guzman H, Siirin M, Wuithiranyagool T, Hailey C, Higgs S, Bala AA, Pascua R, Meyer T, Vanlandingham DL, Tesh RB. The 2002 introduction of West Nile virus into Harris County, Texas, an area historically endemic for St. Louis encephalitis. Am J Trop Med Hyg. 2004;70(6):676-81.

Lindsey NP, Hayes EB, Staples JE, Fischer M. West Nile virus disease in children, United States, 1999-2007. Pediatrics. 2009;123(6):e1084-9.

Loeb M. Elliott SJ. Gibson B. Fearon M, Nosal R, Drebot M, D'Cuhna C, Harrington D, Smith S, George P, Eyles J. Protective behavior and West Nile virus risk. Emerg Infect Dis. 2005;11:1433–1436.

Lopes H, Redig P, Glaser A, Armien A, Wünschmann A. Clinical findings, lesions, and viral antigen distribution in great gray owls (*Strix nebulosa*) and barred owls (*Strix varia*) with spontaneous West Nile virus infection. Avian Dis. 2007 Mar;51:140-5.

López G, Jiménez-Clavero MA, Tejedor CG, Soriguer R, Figuerola J. Prevalence of West Nile virus neutralizing antibodies in Spain is related to the behavior of migratory birds. Vector Borne Zoonotic Dis. 2008;8(5):615-21.

Ludwig GV, Calle PP, Mangiafico JA, Raphael BL, Danner DK, Hile JA, Clippinger TL, Smith JF, Cook RA, McNamara T. An outbreak of West Nile virus in a New York City captive wildlife population. Am J Trop Med Hyg. 2002;67:67-75.

Machain-Williams C, Padilla-Paz SE, Weber M, Cetina-Trejo R, Juarez-Ordaz JA, Loroño-Pino MA, Ulloa A, Wang C, Garcia-Rejon J, Blitvich BJ. Antibodies to West Nile virus in wild and farmed crocodiles in southeastern Mexico. J Wildl Dis. 2013;49(3):690-3.

Magurano F, Remoli ME, Baggieri M, Fortuna C, Marchi A, Fiorentini C, Bucci P, Benedetti E, Ciufolini MG, Rizzo C, Piga S, Salcuni P, Rezza G, Nicoletti L. Circulation of West Nile virus lineage 1 and 2 during an outbreak in Italy. Clin Microbiol Infect. 2012;18(12):E545-7.

Malik-Peiris JS, Amerasinghe FP. West Nile fever. In: Beran GW, editor. Handbook of Zoonoses. 2nd ed. Boca Raton, FL: CRC Press; 1994. p. 139-48.

Mandalakas AM, Kippes C, Sedransk J, Kile JR, Garg A, McLeod J, Berry RL, Marfin AA. West Nile virus epidemic, northeast Ohio, 2002. Emerg Infect Dis. 2005;11(11):1774-7.

May FJ, Davis CT, Tesh RB, Barrett AD. Phylogeography of West Nile virus: from the cradle of evolution in Africa to Eurasia, Australia, and the Americas. J Virol. 2011;85(6):2964-74.

McMullen A, Albayrak H, May F, Davis CT, Beasley D, Barrett AD. The molecular evolution of lineage 2 West Nile virus. J Gen Virol. 2013;94:318-25

McMullen AR, May FJ, Li L, Guzman H, Bueno R Jr, Dennett JA, Tesh RB, Barrett AD. Evolution of new genotype of West Nile virus in North America. Emerg Infect Dis. 2011;17(5):785-93.

Melandri V, Guimarães AÉ, Komar N, Nogueira ML, Mondini A, Fernandez-Sesma A, Alencar J, Bosch I. Serological detection of West Nile virus in horses and chicken from Pantanal, Brazil. Mem Inst Oswaldo Cruz. 2012;107(8):1073-5.

Miller DL, Radi ZA, Baldwin C, Ingram D. Fatal West Nile virus infection in a white-tailed deer (*Odocoileus virginianus*). J Wildl Dis. 2005;41:246-9.

Monaco F, Savini G, Calistri P, Polci A, Pinoni C, Bruno R, Lelli R. 2009 West Nile disease epidemic in Italy: first evidence of overwintering in Western Europe? Res Vet Sci. 2011;91(2):321-6.

Mumcuoglu KY, Banet-Noach C, Malkinson M, Shalom U, Galun R. Argasid ticks as possible vectors of West Nile virus in Israel. Vector Borne Zoonotic Dis. 2005;5:65-71.

Murgue B, Murri S, Zientara S, Durand B, Durand J-P, Zeller H. West Nile outbreak in horses in southern France, 2000: The return after 35 years. Emerg Infect Dis. 2001;7:692-6.

Murphy TD, Grandpre J, Novick SL, Seys SA, Harris RW, Musgrave K. West Nile virus infection among health-fair participants, Wyoming 2003: assessment of symptoms and risk factors. Vector Borne Zoonotic Dis. 2005;5(3):246-51.

Murray K, Walker C, Herrington E, Lewis JA, McCormick J, Beasley DW, Tesh RB, Fisher-Hoch S. Persistent infection with West Nile virus years after initial infection. J Infect Dis. 2010;201(1):2-4.

Neghina AM, Neghina R. Reemergence of human infections with West Nile virus in Romania, 2010: an epidemiological study and brief review of the past situation. Vector Borne Zoonotic Dis. 2011;11(9):1289-92.

Nemeth N, Gould D, Bowen R, Komar N. Natural and experimental West Nile virus infection in five raptor species. J Wildl Dis. 2006;42:1-13.

Nemeth NM, Hahn DC, Gould DH, Bowen RA. Experimental West Nile virus infection in Eastern screech owls (*Megascops asio*). Avian Dis. 2006;50:252-8.

Nemeth NM, Kratz GE, Bates R, Scherpelz JA, Bowen RA, Komar N. Clinical evaluation and outcomes of naturally acquired West Nile virus infection in raptors. J Zoo Wildl Med. 2009;40(1):51-63.

Nevarez JG, Mitchell MA, Morgan T, Roy A, Johnson A. Association of West Nile virus with lymphohistiocytic proliferative cutaneous lesions in American alligators (*Alligator mississippiensis*) detected by RT-PCR. J Zoo Wildl Med. 2008;39:562-6.

Oesterle PT, Nemeth NM, VanDalen K, Sullivan H, Bentler KT, Young GR, McLean RG, Clark L, Smeraski C, Hall JS. Experimental infection of cliff swallows (*Petrochelidon pyrrhonota*) with varying doses of West Nile virus. Am J Trop Med Hyg. 2009;81(6):1159-64.

Olaleye OD, Omilabu SA, Ilomechina EN, Fagbami AH. A survey for haemagglutination-inhibiting antibody to West Nile virus in human and animal sera in Nigeria. Comp Immunol Microbiol Infect Dis. 1990;13(1):35-9.

Ølberg RA, Barker IK, Crawshaw GJ, Bertelsen MF, Drebot MA, Andonova M. West Nile virus encephalitis in a Barbary macaque (*Macaca sylvanus*). Emerg Infect Dis. 2004;10:712-4.

Ostlund EN, Crom RL, Pedersen DD, Johnson DJ, Williams WO, Schmit BJ. Equine West Nile encephalitis, United States. Emerg Infec. Dis. 2001;7:665-9.

Ozkul A, Yıldırım Y, Pinar D, Akcali A, Yilmaz V, Colak D. Serological evidence of West Nile virus (WNV) in mammalian species in Turkey. Epidemiol Infect. 2006;134:826-9.

Paddock CD, Nicholson WL, Bhatnagar J, Goldsmith CS, Greer PW, Hayes EB, Risko JA, Henderson C, Blackmore CG, Lanciotti RS, Campbell GL, Zaki SR. Fatal hemorrhagic fever caused by West Nile virus in the United States. Clin Infect Dis. 2006;42:1527-35.

Palmer MV, Stoffregen WC, Rogers DG, Hamir AN, Richt JA, Pedersen DD, Waters WR. West Nile virus infection in reindeer (*Rangifer tarandus*). J Vet Diagn Invest. 2004;16:219-22.

Pant GR, Lunt RA, Rootes CL, Daniels PW.Serological evidence for Japanese encephalitis and West Nile viruses in domestic animals of Nepal. Comp Immunol Microbiol Infect Dis. 2006;29(2-3):166-75.

Papa A, Bakonyi T, Xanthopoulou K, Vázquez A, Tenorio A, Nowotny N. Genetic characterization of West Nile virus lineage 2, Greece, 2010. Emerg Infect Dis. 2011;17(5):920-2.

Papa A, Politis C, Tsoukala A, Eglezou A, Bakaloudi V, Hatzitaki M, Tsergouli K. West Nile virus lineage 2 from blood donor, Greece. Emerg Infect Dis. 2012;18(4):688-9.

Papa A, Xanthopoulou K, Gewehr S, Mourelatos S. Detection of West Nile virus lineage 2 in mosquitoes during a human outbreak in Greece. Clin Microbiol Infect. 2011;17(8):1176-80.

Pellegrini-Masini A, Livesey LC. Meningitis and encephalomyelitis in horses.Vet Clin North Am Equine Pract. 2006;22:553-89, x.

Perl S, Fiette L, Lahav D, Sheichat N, Banet C, Orgad U, Stram Y, Malkinson M. West Nile encephalitis in horses in Israel. Isr J Vet Med. 2002;57: 65–9.

Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. Ann Intern Med. 2002;137:173-9.

Phipps LP, Duff JP, Holmes JP, Gough RE, McCracken F, McElhinney LM, Johnson N, Hughes L, Chantrey J, Pennycott T, Murray KO, Brown IH, Fooks AR. Surveillance for West Nile virus in British birds (2001 to 2006). Vet Rec. 2008;162:413-5.

Planitzer CB, Modrof J, Yu MY, Kreil TR. West Nile virus infection in plasma of blood and plasma donors, United States. Emerg Infect Dis. 2009;15(10):1668-70.

Platt KB, Tucker BJ, Halbur PG, Blitvich BJ, Fabiosa FG, Mullin K, Parikh GR, Kitikoon P, Bartholomay LC, Rowley WA. Fox squirrels (*Sciurus niger*) develop West Nile virus viremias sufficient for infecting select mosquito species. Vector Borne Zoonotic Dis. 2008;8:225-33.

Pollock CG. West Nile virus in the Americas. J Avian Med Surg. 2008;22:151-7.

Public Health Agency of Canada (PHAC). Pathogen Safety Data Sheet – West Nile virus. Available at: http://www.phac-aspc.gc.ca/lab-bio/res/psds-ftss/wnv-vno-eng.php. Accessed 28 Feb 2013.

Read RW, Rodriguez DB, Summers BA West Nile virus encephalitis in a dog. Vet Pathol. 2005;42:219-22.

Reed LM, Johansson MA, Panella N, McLean R, Creekmore T, Puelle R, Komar N. Declining mortality in American crow (*Corvus brachyrhynchos*) following natural West Nile virus infection. Avian Dis. 2009;53(3):458-61.

Reisen WK, Padgett K, Fang Y, Woods L, Foss L, Anderson J, Kramer V. Chronic infections of West Nile virus detected in California dead birds. Vector Borne Zoonotic Dis. 2013;13(6):401-5.

Reiter P. West Nile virus in Europe: understanding the present to gauge the future. Euro Surveill. 2010;15(10):19508.

Root JJ. West Nile virus associations in wild mammals: a synthesis. Arch Virol. 2013;158(4):735-52.

Root JJ, Bentler KT, Nemeth NM, Gidlewski T, Spraker TR, Franklin AB. Experimental infection of raccoons (*Procyon lotor*) with West Nile virus. Am J Trop Med Hyg. 2010;83(4):803-7.

Root JJ, Oesterle PT, Nemeth NM, Klenk K, Gould DH, McLean RG, Clark L, Hall JS. Experimental infection of fox squirrels (*Sciurus niger*) with West Nile virus. Am J Trop Med Hyg. 2006;75:697-701.

Rossini G, Carletti F, Bordi L, Cavrini F, Gaibani P, Landini MP, Pierro A, Capobianchi MR, Di Caro A, Sambri V. Phylogenetic analysis of West Nile virus isolates, Italy, 2008-2009. Emerg Infect Dis. 2011;17(5):903-6.

St. Leger J, Wu G, Anderson M, Dalton L, Nilson E, Wang D. West Nile virus infection in killer whale, Texas, USA, 2007. Emerg Infect Dis. 2011;17(8):1531-3.

Sambri V, Capobianchi M, Charrel R, Fyodorova M, Gaibani P, Gould E, Niedrig M, Papa A, Pierro A, Rossini G, Varani S, Vocale C, Landini MP. West Nile virus in Europe: emergence, epidemiology, diagnosis, treatment, and prevention. Clin Microbiol Infect. 2013;19(8):699-704.

Samoilova TI, Votiakov VI, Titov LP. Virologic and serologic investigations of West Nile virus circulation in Belarus. Cent Eur J Public Health. 2003;11(2):55-62.

Sandhu TS, Sidhu DS, Dhillon MS. Antigenic distribution of West Nile virus in various organs of wildly infected American crows (*Corvus brachyrhynchos*). J Glob Infect Dis. 2011;3(2):138-42.

Savini G, Capelli G, Monaco F, Polci A, Russo F, Di Gennaro A, Marini V, Teodori L, Montarsi F, Pinoni C, Pisciella M, Terregino C, Marangon S, Capua I, Lelli R. Evidence of West Nile virus lineage 2 circulation in Northern Italy. Vet Microbiol. 2012;158(3-4):267-73.

Savini G, Monaco F, Calistri P, Lelli R. Phylogenetic analysis of West Nile virus isolated in Italy in 2008. Euro Surveill. 2008;13(48). pii: 19048.

Schweitzer BK, Kramer WL, Sambol AR, Meza JL, Hinrichs SH, Iwen PC. Geographic factors contributing to a high seroprevalence of West Nile virus-specific antibodies in humans following an epidemic. Clin Vaccine Immunol. 2006;13(3):314-8.

Seidowski D, Ziegler U, von Rönn JA, Müller K, Hüppop K, Müller T, Freuling C, Mühle RU, Nowotny N, Ulrich RG, Niedrig M, Groschup MH. West Nile virus monitoring of migratory and resident birds in Germany. Vector Borne Zoonotic Dis. 2010;10(7):639-47.

Sejvar JJ, Lindsey NP, Campbell GL. Primary causes of death in reported cases of fatal West Nile fever, United States, 2002-2006. Vector Borne Zoonotic Dis. 2011;11(2):161-4.

Shirafuji H, Kanehira K, Kubo M, Shibahara T, Kamio T. Experimental West Nile virus infection in aigamo ducks, a cross between wild ducks (*Anas platyrhynchos*) and domestic ducks (*Anas platyrhynchos* var. *domesticus*). Avian Dis. 2009;53(2):239-44.

Sirbu A, Ceianu CS, Panculescu-Gatej RI, Vazquez A, Tenorio A, Rebreanu R, Niedrig M, Nicolescu G, Pistol A. Outbreak of West Nile virus infection in humans, Romania, July to October 2010. Euro Surveill. 2011;16(2). pii: 19762.

Sonnleitner ST, Simeoni J, Schmutzhard E, Niedrig M, Ploner F, Schennach H, Dierich MP, Walder G. Absence of indigenous specific West Nile virus antibodies in Tyrolean blood donors. Eur J Clin Microbiol Infect Dis. 2012;31(1):77-81.

Sotelo E, Fernandez-Pinero J, Llorente F, Agüero M, Hoefle U, Blanco JM, Jiménez-Clavero MA. Characterization of West Nile virus isolates from Spain: new insights into the distinct West Nile virus eco-epidemiology in the Western Mediterranean. Virology. 2009;395(2):289-97.

Sotelo E, Gutierrez-Guzmán AV, Del Amo J, Llorente F, El-Harrak M, Pérez-Ramírez E, Blanco JM, Höfle U, Jiménez-Clavero MA. 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(1):11.

Sovada MA, Pietz PJ, Hofmeister EK, Bartos AJ. West Nile virus in American white pelican chicks: transmission, immunity, and survival. Am J Trop Med Hyg. 2013;88(6):1152-8.

Steinman A, Banet-Noach C, Simanov L, Grinfeld N, Aizenberg Z, Levi O, Lahav D, Malkinson M, Perk S, Shpigel NY. Experimental infection of common garter snakes (*Thamnophis sirtalis*) with West Nile virus. Vector Borne Zoonotic Dis. 2006;6:361-8.

Steinman A, Banet-Noach C, Tal S, Levi O, Simanov L, Perk S, Malkinson M, Shpigel N. West Nile virus infection in crocodiles. Emerg Infect Dis. 2003; 9(7): 887-9.

Swayne DE, Beck JR, Smith CS, Shieh W-J, Zaki SR. Fatal encephalitis and myocarditis in young domestic geese (*Anser anser domesticus*) caused by West Nile virus. Emerg Infect Dis. 2001;7:751-3.

Teehee ML, Bunning ML, Stevens S, Bowen RA. Experimental infection of pigs with West Nile virus. Arch Virol. 2005;150:1249-56.

Thompson NN, Auguste AJ, Coombs D, Blitvich BJ, Carrington CV, da Rosa AP, Wang E, Chadee DD, Drebot MA, Tesh RB, Weaver SC, Adesiyun AA. Serological evidence of flaviviruses and alphaviruses in livestock and wildlife in Trinidad. Vector Borne Zoonotic Dis. 2012;12(11):969-78.

Tiawsirisup S, Platt KB, Tucker BJ, Rowley WA. Eastern cottontail rabbits (*Sylvilagus floridanus*) develop West Nile virus viremias sufficient for infecting select mosquito species. Vector Borne Zoonotic Dis. 2005;5:342-50.

Timmermann U, Becker N. Mosquito-borne West Nile virus (WNV) surveillance in the Upper Rhine Valley, Germany. J Vector Ecol. 2010;35(1):140-3.

Travis D, McNamara T, Glaser A, Campbell R. A national surveillance system for WNV in zoological institutions. Available at: http://www.cdc.gov/ncidod/dvbid/westnile/conf/ppt/1a-travis.ppt.*

Trevejo RT, Eidson M. Zoonosis update: West Nile virus. J Am Vet Med Assoc. 2008;232:1302-9.

Trock SC, Meade BJ, Glaser AL, Ostlund EN, Lanciotti RS, Cropp BC, Kulasekera V, Kramer LD, Komar N. West Nile virus outbreak among horses in New York State, 1999 and 2000. Emerg Infec. Dis. 2001;7:745-7.

Tyler JW, Turnquist SE, David AT, Kleiboeker SB, Middleton JR. West Nile virus encephalomyelitis in a sheep. J Vet Intern Med. 2003;17:242-4.

United States Department of Agriculture. Animal and Plant Health Inspection Service, Veterinary Services [USDA APHIS, VS]. West Nile virus [online]. USDA APHIS; 2002 Dec. Available at: http://www.aphis.usda.gov/lpa/pubs/fsheet_faq_notice/fs_ahw nv.html.* Accessed 5 Dec 2002.

Valiakos G, Touloudi A, Iacovakis C, Athanasiou L, Birtsas P, Spyrou V, Billinis C. Molecular detection and phylogenetic analysis of West Nile virus lineage 2 in sedentary wild birds (Eurasian magpie), Greece, 2010. Euro Surveill. 2011;16(18). pii: 19862.

Van der Meulen KM, Pensaert MB, Nauwynck HJ. West Nile virus in the vertebrate world. Arch Virol. 2005;150:637-57.

Vasil'ev AV, Shchelkanov MIu, Dzharkenov AF, Aristova VA, Galkina IV, L'vov DN, Morozova TN, Kovtunov AI, Grenkova EP, Zhernovoĭ AV, Shatilova VP, Slavskiĭ AA, Petrenko MS, Chirkizov PF, Dybal' VD, Leont'ev EA, Gabbasov FB, Odolevskiĭ EA, Ibragimov RM, Idrisova RZ, Sokolova NN, Artiukh NP, Andreeva NI, Bondarev AD, Deriabin PG, Gromashevskiĭ VL, Nepoklonov EA, Aliper TI, L'vov DK. West Nile virus infection of agricultural animals in the Astrakhan region, as evidenced by the 2001-2004 serological surveys [abstract]. Vopr Virusol. 2005;50(6):36-41.

Venter M, Human S, van Niekerk S, Williams J, van Eeden C, Freeman F. Fatal neurologic disease and abortion in mare infected with lineage 1 West Nile virus, South Africa. Emerg Infect Dis. 2011;17(8):1534-6.

Venter M, Human S, Zaayman D, Gerdes GH, Williams J, Steyl J, Leman PA, Paweska JT, Setzkorn H, Rous G, Murray S, Parker R, Donnellan C, Swanepoel R. Lineage 2 West Nile virus as cause of fatal neurologic disease in horses, South Africa. Emerg Infect Dis. 2009;15(6):877-84.

Venter M, Steyl J, Human S, Weyer J, Zaayman D, Blumberg L, Leman PA, Paweska J, Swanepoel R. Transmission of West Nile virus during horse autopsy. Emerg Infect Dis. 2010;16(3):573-5.

Walker BL, Naugle DE, Doherty KE, Cornish TE. West Nile virus and greater sage-grouse: estimating infection rate in a wild bird population. Avian Dis. 2007;51:691-6.

Wang W, Sarkodie F, Danso K, Addo-Yobo E, Owusu-Ofori S, Allain JP, Li C. Seroprevalence of West Nile virus in Ghana. Viral Immunol. 2009;22(1):17-22.

Ward MP, Beveroth TA, Lampman R, Raim A, Enstrom D, Novak R. Field-based estimates of avian mortality from West Nile virus infection. Vector Borne Zoonotic Dis. 2010;10(9):909-13.

Wheeler S, Barker C, Fang Y, Armijos M, Carroll B, Husted S, Johnson W, Reisen W. Differential impact of West Nile virus on California birds. The condor. 2009;111:1–20.

Wheeler SS, Vineyard MP, Woods LW, Reisen WK. Dynamics of West Nile virus persistence in house sparrows (*Passer domesticus*). PLoS Negl Trop Dis. 2012;6(10):e1860.

Wodak E, Richter S, Bagó Z, Revilla-Fernández S, Weissenböck H, Nowotny N, Winter P. Detection and molecular analysis of West Nile virus infections in birds of prey in the eastern part of Austria in 2008 and 2009. Vet Microbiol. 2011;149(3-4):358-66.

World Organization for Animal Health [OIE]. Manual of diagnostic tests and vaccines for terrestrial animals [online]. Paris; OIE; 2012. West Nile fever. Available at: http://www.oie.int/fileadmin/Home/eng/Health_standards/tah m/2.01.20_WEST_NILE.pdf. Accessed 28 Feb 2013.

Wünschmann A, Shivers J, Carroll L, Bender J. Pathological and immunohistochemical findings in American crows (*Corvus brachyrhynchos*) naturally infected with West Nile virus. J Vet Diagn Invest. 2004;16:329-33.

Wünschmann A, Ziegler A.West Nile virus-associated mortality events in domestic Chukar partridges (*Alectoris chuka*r) and domestic Impeyan pheasants (*Lophophorus impeyanus)*. Avian Dis. 2006;50:456-9.

Yaeger M, Yoon KJ, Schwartz K, Berkland L. West Nile virus meningoencephalitis in a Suri alpaca and Suffolk ewe. J Vet Diagn Invest. 2004;16:64-6.

Zehender G, Ebranati E, Bernini F, Lo Presti A, Rezza G, Delogu M, Galli M, Ciccozzi M. Phylogeography and epidemiological history of West Nile virus genotype 1a in Europe and the Mediterranean basin. Infect Genet Evol. 2011; 11: 646–653.

Ziegler U, Angenvoort J, Fischer D, Fast C, Eiden M, Rodriguez AV, Revilla-Fernández S, Nowotny N, de la Fuente JG, Lierz M, Groschup MH. Pathogenesis of West Nile virus lineage 1 and 2 in experimentally infected large falcons. Vet Microbiol. 2013;161:263–73.

Ziegler U, Seidowski D, Angenvoort J, Eiden M, Müller K, Nowotny N, Groschup MH. Monitoring of West Nile virus infections in Germany. Zoonoses Public Health. 2012;59 Suppl 2:95-101.

Zhang Z, Wilson F, Read R, Pace L, Zhang S. Detection and characterization of naturally acquired West Nile virus infection in a female wild turkey. J Vet Diagn Invest. 2006;18:204-8.

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