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 an avian virus that can cause fatal disease in some species of mammals, reptiles and birds. Most clinical cases occur in humans and horses. Approximately 80% of infected humans remain asymptomatic; 20% have flu-like symptoms. Less than 1% develop meningitis, encephalitis or acute paralysis, but some of these cases are fatal or result in permanent impairment. Neurological signs are also seen in some infected equids, and occasionally in other mammals. Outbreaks have been reported in alligators. Some newer isolates of West Nile virus seem to be particularly virulent. Before 1994, disease occurred only sporadically in humans and horses, or as relatively small epidemics in rural areas, and severe neurological signs were uncommon in most outbreaks. Until 1999, West Nile virus was also confined to the Eastern Hemisphere. However, severe outbreaks were reported in Algeria, Romania, Morocco, Tunisia, Italy, Russia and Israel between 1994 and 1999, and West Nile virus spread to North America in 1999. An increased incidence of neurological disease and a higher case fatality rate have been associated with these viruses. Some recent viral isolates also cause clinical signs in birds. Consequently, West Nile fever has emerged as a significant human and veterinary health concern in the Americas, Europe, the Mediterranean basin and other areas.

The effects of West Nile virus have been particularly dramatic in North America, where widespread epidemics have occurred in horses and humans. Although vaccination can control the disease in equids, no human vaccine is available, and thousands of people become ill in the U.S. and Canada each year. Many North American species of birds have also been affected by this virus. It has killed large numbers of crows, blue jays and other corvids, as well as American robins, house wrens, eastern bluebirds, tufted titmice, chickadees and sage grouse. Outbreaks have been reported in domesticated geese, pheasants and partridges, and occasional cases occur in captive psittacine birds. Many species of zoo birds have been affected. The effects of this virus on threatened or endangered species could be significant. California condors and greater sage grouse, which are both susceptible to this virus, are of particular concern. The West Nile virus found in the U.S. has become established in Canada, parts of Central and South America and the Caribbean, and it is continuing to spread. If this virus were introduced into Hawaii, it could be disastrous for some native birds.

Etiology

West Nile virus is an arbovirus in the *Flavivirus* genus of the family Flaviviridae. This virus belongs to the Japanese encephalitis virus complex or serogroup. There are at least two genetic lineages of West Nile virus. Lineage 1 viruses, which have caused most recent outbreaks, can be divided into three clades (1a, 1b and 1c). Lineage 1 contains both virulent and attenuated viruses. Many of the virulent viruses in recent outbreaks belong 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 virulent strains. A variant of this virus, called WN02, has recently become the predominant strain in the U.S. and Canada, and NY99 seems to have disappeared. A few attenuated strains of WNV have been isolated in the Americas since 2003. Clade 1b consists of Kunjin viruses, a subtype of WNV found in Australia, and clade 1c contains viruses found in India. Lineage 2 viruses, which occur mainly in Africa, often cause asymptomatic infections or mild disease. Virulent lineage 2 viruses also exist. One lineage 2 virus has caused encephalitis in raptors in Central Europe.

New isolates from the Czech Republic, Russia and Malaysia might represent new lineages of WNV. Some recent studies suggest that this virus can be classified into as many as five lineages.

Geographic Distribution

West Nile viruses are found throughout much of the world including Africa, parts of Asia and Europe, the Mediterranean region, the Middle East, Australia and the Americas.

West Nile virus first occurred in the Western Hemisphere when a lineage 1a virus was introduced into New York in 1999. Since that time, WNV has spread north into Canada, and south into Central and South America and the Caribbean. As of January 2009, evidence for this virus has been found as far south as Colombia, Argentina and Venezuela. It has not yet been introduced into Hawaii or some other islands. Lineage 1a viruses also occur in northern and central Africa, the Middle East and parts of continental Europe. Although seroconversion to WNV has been reported in sentinel chickens in the U.K., recent surveillance has found no evidence for circulating viruses in wild birds, horses or humans. Lineage 1b (Kunjin virus) occurs in Australia, and lineage 1c viruses are found in India.

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 isolated from sick raptors in Central Europe (Hungary). Other lineage 2 strains may also occur outside Africa; one apparently avirulent strain, isolated from a migrating bird on Cyprus in 1968, was recently shown to belong to this lineage.

Transmission

West Nile virus is primarily transmitted by mosquitoes. In North America alone, there is evidence of infection in at least 59 species. *Culex. pipiens, Cx. quinquefasciatus, Cx. restuans, Cx. salinarius* and *Cx. tarsalis* are particularly efficient vectors. Other species including *Culex nigripalpus*, *Aedes albopictus*, *Aedes vexans* and *Ochlerotatus triseriatus* may also be important in transmission. Transovarial transmission has been demonstrated in some species of mosquitoes. Dormant mosquitoes that survive the winter may also harbor WNV. Other arthropods may 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. Hippoboscid 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. The level and duration of viremia varies with the species. In endemic regions, this 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 spread the virus to humans, horses and other incidental hosts. Migratory birds may carry West Nile virus into new areas. In some birds, viremia can persist for more than three months, possibly contributing to the overwintering of the virus. Some species of crows, jays, magpies, gulls and other birds can also shed WNV in oral and cloacal secretions, and can transmit the virus directly. Evidence for horizontal transmission was reported during an outbreak in domesticated geese. Experimentally infected turkeys and chickens can excrete WNV in feces for a few days. The infectivity of virus in avian feces decreases dramatically after 24 hours. This virus 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 may eat infected mosquitoes.

Most mammals become infected via mosquito bites. Carnivorous mammals and reptiles (e.g., cats and alligators) can also be infected by ingesting tissues that contain this virus. WNV-contaminated horsemeat was implicated in one outbreak in alligators. Most non-avian species, including humans and horses, are dead-end hosts and do not transmit WNV to mosquitoes. However, this may not be the case for some species of squirrels, chipmunks, rabbits, cats and alligators, which have higher levels of viremia. In Russia, at least one species of frog (*Rana ridibunda*) develops high viremia and is a potential reservoir host. Direct transmission has been reported between alligators during close contact, possibly via fecal shedding of virus. Chipmunks and squirrels can excrete WNV in feces, oral secretions and/or urine, and might also be able to transmit the virus horizontally. It also occurs in the urine of experimentally infected hamsters. Experimentally infected American bullfrogs and green iguanas have low viremia, but can shed very small amounts of virus in oral and/or cloacal fluids. Transplacental transmission has been reported in experimentally infected sheep and mice. The epidemiological significance of mammalian, reptilian and amphibian hosts in the maintenance of WNV remains to be established.

Humans usually become infected via mosquito bites, but some cases have occurred in people who handled infected birds or tissues from infected alligators. One 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 shed WNV in secretions or excretions, but the virus can be transmitted by blood transfusions and in organ transplants. Rare cases of transplacental transmission and probable transmission in breast milk have also been reported.

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 iodophores. It is also inactivated by UV light and gamma irradiation, as well as heat [30 min. at 56°C (133°F)].

Infections in Humans

Incubation Period

The incubation period is two to 14 days.

Clinical Signs

Human illness has been classified into two forms: West Nile fever, which is relatively mild and flu-like, 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 also be seen. 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.

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 are common in patients with neuroinvasive disease, and 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; syndromes that have been reported include chorioretinitis, uveitis, vitritis and optic neuritis. Myocarditis, pancreatitis, orchitis and fulminant hepatitis have been seen in some outbreaks.

A life-threatening hemorrhagic syndrome has been seen in a few West Nile cases in Africa, and was recently reported in a patient in the U.S.

Communicability

Person to person transmission does not occur during casual contact; however, West Nile virus 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.

Diagnostic Tests

In humans, West Nile virus infections are often diagnosed by serology. Diagnostic criteria include a rising titer or the presence of IgM in serum or cerebrospinal fluid (CSF). IgM in CSF indicates a recent infection; however, anti-WNV IgM can persist in the serum of some individuals for more than a year. For this reason, IgM in serum is suggestive but not definitive. Enzyme-linked immunosorbent assays (ELISAs) are the most commonly used serological tests. Other tests include the plaque reduction neutralization (PRN) test, indirect immunofluorescence (IFA) and hemagglutination inhibition. Two rapid tests, an optical fiber immunoassay and a microsphere-based fluorescence immunoassay, have recently been developed. In some serological tests, cross-reactions can occur with closely related flaviviruses including yellow fever, Japanese encephalitis, St. Louis encephalitis or dengue viruses. For this reason, positive reactions in ELISAs or other tests may be confirmed with the PRN test.

West Nile virus, viral antigens or nucleic acids can sometimes be detected in tissues, CSF, blood and other body fluids. WNV can usually be found in the blood of patients with West Nile fever, during the first few days after the onset of illness. Reverse-transcription polymerase chain reaction (RT-PCR) assays are often used to screen blood supplies for transfusion. However, viremia usually disappears before the onset of neurological signs, and viral RNA is generally absent from the serum of patients with neuroinvasive disease. CSF can be tested with RT-PCR, although this is rarely done in clinical practice. Immunohistochemistry to detect viral antigens is mainly used postmortem in cases of fatal neurological disease. Virus isolation requires level 3 biosafety containment, and is rarely performed. Virus isolation from CSF and brain tissue is often unsuccessful.

Treatment

No specific treatment, other than supportive care, is available. Intensive care and mechanical ventilation may be required in some cases. Various therapies including interferon, antisense nucleotides and intravenous immunoglobulin are being tested in clinical trials. 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 the 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.

Veterinarians, wildlife rehabilitators, wildlife biologists and others should practice good biosecurity and hygiene when handling 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. In some circumstances, respiratory protection may be needed during close contact. Protective clothing and gloves should be used when performing necropsies, especially on birds, squirrels and alligators.

Human vaccines are not yet available, but some vaccines have entered clinical trials.

Morbidity and Mortality

West Nile fever and neuroinvasive disease usually occur in humans during warm weather, when mosquitoes are active. In temperate regions of the U.S., the number of cases peaks between July and October, but infections have been reported from April to December. An outbreak among crows during the winter, when mosquitoes were not active, raises the possibility that zoonotic cases could occur by direct contact year-round.

Most human infections 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. Patients with encephalitis are often older than those who develop meningitis or flaccid paralysis. Recipients of organ transplants are particularly susceptible to neuroinvasive disease; if infected, they are estimated to have a 40% chance of developing this form. Underlying diseases such as diabetes and autoimmune syndromes are also associated with more severe clinical signs. West Nile neuroinvasive disease is rare in healthy patients under the age of 30 years, although it can occur.

Case fatality rates reported during outbreaks vary from 4% to 15%. The overall case fatality rate for West Nile neuroinvasive disease 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.

The impact of WNV has been much greater in North America than in the Eastern Hemisphere. More than 2500 cases of West Nile fever or neuroinvasive disease are reported each year in the U.S., and many mild cases are probably not reported. A new North America variant, WN02, can replicate more rapidly in mosquitoes than the original virus; this may have contributed to a dramatic rise in the number of West Nile fever cases since the 1999-2001 seasons. In the U.S., relatively few people have antibodies to WNV, and epidemics are expected to occur each summer. Surprisingly, far fewer clinical cases or deaths have been reported in Central and South America. The reason for this pattern of disease 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.

Infections in Animals

Species Affected

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 Charadriiformes (shorebirds), Falconiformes (hawks, eagles, vultures and related species) and Strigiformes (owls) can also transmit the virus to mosquitoes. Many birds, particularly species found in the Eastern Hemisphere, carry the virus asymptomatically. Other species may become ill. In North America, commonly affected wild birds include corvids (crows, ravens, magpies and jays), American robins (*Turdus migratorius*), eastern bluebirds (*Sialia sialis*), chickadees (*Poecile* sp.), tufted titmice *(Baeolophus bicolor)* and

house wrens (*Troglodytes aedon*). Owls, hawks, falcons, kestrels, eagles and vultures have also been killed by WNV. Outbreaks have been reported in domesticated geese. Infections in gallinaceous birds (order Galliformes) vary with the species. Chickens and turkeys seroconvert but remain asymptomatic. However, greater sage grouse (*Centrocercus urophasianus)* are highly susceptible, and outbreaks have occurred in chukar partridges (*Alectoris chukar*) and Impeyan pheasants (*Lophophorus impeyanus*). One case was reported in a wild turkey (*Meleagris gallopavo* ssp). Psittacine birds are relatively resistant to disease, but cases have occasionally been seen in cockatoos, macaws, parrotlets, rosellas, lories, sun conures (*Aratinga solstitialis*), budgerigars (*Melopsittacus undulatus*), cockatiels (*Nymphicus hollandicus*) and various species of parrots. Emus, penguins, pigeons, flamingos, American white pelicans (*Pelecanus erythrorhyncos*) cormorants, gulls, bronze-winged ducks (*Anas specularis*), sandhill cranes (*Grus canadensis*) and other species have also been affected. Overall, WNV-infections have been reported in nearly 300 species of North American birds since 1999.

Among mammals, disease occurs mainly in equids (horses, donkeys and mules). Clinical cases have also been reported in alpacas, sheep and reindeer (*Rangifer tarandus*), as well as in wild squirrels, harbor seals (*Phoca vitulina*), Indian rhinoceroses (*Rhinoceros unicornis*), a wolf, a Barbary macaque (*Macaca sylvanus*) and a white-tailed deer (*Odocoileus virginianus*). Dogs and cats appear to be readily infected, but rarely become ill. Antibodies to WNV have been found in many species including cattle, goats, pigs, deer, lemurs, bats, skunks, bears, foxes, raccoons, opossums, rabbits, non-human primates, small rodents and insectivores. Experimental infections have been established in a variety of mammals: mice, hamsters, cats and rhesus monkeys developed mild to severe clinical signs, but rabbits, pigs, guinea pigs, dogs and hedgehogs (*Erinaceus europaeus)* remained asymptomatic. Among reptiles, outbreaks have been reported only in alligators, but some experimentally infected garter snakes (*Thamnophis sirtalis*) died, and antibodies have been found in turtles. Experimental infections have also been reported in green iguanas (*Iguana iguana*). Some amphibians including lake frogs (*Rana ridibunda*) and North American bullfrogs (*Rana catesbeiana*) can be infected with WNV. Some species of mammals, reptiles and amphibians including squirrels (*Sciurus* sp.), eastern chipmunks (*Tamias striatus*), eastern cottontail rabbits (*Sylvilagus floridanus*), alligators (*Alligator mississippiensis*) and lake frogs (*Rana ridibunda*) may be capable of transmitting WNV to mosquitoes, although their importance as reservoir hosts is still uncertain.

Incubation Period

The incubation period in horses is three to 15 days. The incubation period in most species is unknown.

Clinical Signs

Birds

Some species of birds carry WNV asymptomatically, while others develop clinical signs. Birds in the Eastern Hemisphere are generally unaffected by most viruses circulating there. Exceptions include a lineage 1a virus that causes severe disease in domesticated geese in Israel, and a lineage 2 virus that has killed some raptors in Hungary. In North America, some species of birds have subclinical infections, but others may become ill.

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 or 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, circling and seizures have been reported. Myocarditis is sometimes seen at necropsy. Sudden death also occurs. However, 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. Most clinically affected birds have died or been euthanized due to their deteriorating condition.

Affected wild birds are usually found dead, and the clinical signs in many species have not been well described. 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. In these grouse, clinical signs progressed to the end stage of disease within hours.

Mammals

Most horses are infected asymptomatically. 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. 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% may have residual defects such as weakness in one or more limbs, decreased exercise tolerance, muscle atrophy or behavioral changes.

A few clinical cases have been reported in ruminants. Frequently, only a single animal has been affected on a farm. Occasionally, a few other animals became ill around the same time. Most sheep, alpacas, reindeer and white-tailed deer have had neurological signs that resembled the syndrome in horses. In many cases, these were 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 to 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 to 2 days, particularly in reindeer, but some animals have been 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 wolves. 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 include decreased conscious proprioception, 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 mild recurrent myopathy has been 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 has also been recovered from the brain of a cat with neurological signs. Experimentally infected cats were transiently lethargic and had fluctuating fevers, but neurological signs were not seen.

Neurological signs have also been reported in other species of mammals. 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. Progressive neurological signs including tremors, involuntary spasms, muscle stiffness, swallowing difficulties and hind flipper weakness were reported in a harbor seal that died. This seal was also inappetent and weak, with intermitted diarrhea and vomiting, and labored breathing. Similar tremors and twitching were seen in another captive seal for four days, but this animal recovered. West Nile virus was suspected as the cause of depression, lethargy, partial anorexia and a drooping lip in two Indian rhinoceroses during an outbreak at a zoo. Both animals recovered. Neurological signs were reported in a Barbary macaque in a zoo. Fatal encephalitis has also been seen in experimentally infected mice, hamsters and rhesus monkeys. Experimentally infected pigs remained asymptomatic.

Reptiles

In alligators, the clinical signs included anorexia, lethargy, weakness and neurological signs such as 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.

Fatal infections have been reported in experimentally infected garter snakes. Some snakes died without previous clinical signs. 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 occurs in some avian species. Birds known to shed West Nile virus in oral secretions and/or feces include domesticated geese,

corvids, ring-billed gulls (*Larus delawarensis*) and some raptors. Experimentally infected turkeys and chickens can excrete this virus in feces for a few days, and one outbreak was reported in people who worked at an infected turkey farm. Other avian species may also be capable of shedding the virus.

Some chipmunks and squirrels also excrete WNV in oral secretions, feces and/or urine, and may be capable of horizontal transmission. This virus has been found in the urine of experimentally infected hamsters. Alligators can transmit West Nile virus during close contact, possibly via fecal shedding. Very small amounts of virus have been transiently found in the oral and cloacal fluids of experimentally infected green iguanas, and in the cloacal fluids of experimentally infected American bullfrogs.

Post-Mortem Lesions

Birds

A wide variety of gross and microscopic lesions have been reported in birds. Some birds may be thin or emaciated, but others are in good body condition. Gross hemorrhages of the brain, splenomegaly, meningoencephalitis, myocarditis, pancreatitis and chronic inflammation of the adrenal gland are relatively common. Less often, there may be hemorrhages in the lungs or intestines, lymphoplasmacytic enteritis, renal congestion or mottling, hepatic or splenic necrosis, ovarian necrosis or evidence of disseminated intravascular coagulation. Gross lesions are minimal or absent in some infected birds, including some psittacines. No lesions appear to be pathognomonic for WNV, and the lesions are not consistent between species.

During outbreaks among geese in the Middle East, some birds were dehydrated and in poor condition. Lesions included subcutaneous hemorrhages around the joints, pale lungs, pale beaks, and petechial hemorrhages in the splenic capsule. An enlarged gall bladder, severe thymic and cloacal bursa atrophy, and excess cerebrospinal fluid were also seen. Consistent gross lesions that could be attributed to WNV infection have not been reported in crows.

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. 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 have also been seen in some dogs. Mild to moderate lymphocytic myocarditis, myocardial necrosis and mild focal hepatic necrosis were reported 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

In horses, 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.

Isolation of West Nile virus is definitive in all species, but this test is time-consuming and requires level 3 biosafety containment. It is not performed in many laboratories. 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 immunofluorescence or RT-PCR. WNV is difficult to recover from live horses because the viremia is usually low-level and short-lived. At necropsy, this virus can sometimes be found in the brain and spinal cord of horses with neurological signs. Recovery of WNV is usually easier in birds, which tend to have higher viral titers. Nevertheless, viremia may be low in some avian species, even in clinical cases. At necropsy, WNV can often be isolated from avian heart, brain and/or liver, and sometimes from other tissues. Viremia has not been studied extensively in ruminants; in most cases, WNV seems to be undetectable or present at low levels.

RT-PCR can be used to detect viral RNA in equine 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 birds, West Nile virus RNA can be found in samples of the brain, spinal cord, and parenchymal tissues. In live birds, it may be detected in oral and cloacal swabs and/or serum samples. RT-PCR has been valuable as a postmortem test in various mammals including dogs and a wolf cub. Some RT-PCR tests may not detect lineage 2 viruses.

Viral antigens can be detected with immunohistochemistry, antigen capture ELISAs and rapid immunochromatographic "dipstick" assays. Immunohistochemistry is used as a postmortem test. It may detect WNV antigens in equine brain and spinal cord, and avian brain, heart, kidney, spleen, liver, intestine and lung. Because the CNS does not contain large quantities of virus, some infected horses are not detected by this test. Immunohistochemistry has also been used to diagnose WNV infections in other species including dogs and a wolf. The antigen-capture ELISA can detect antigens in avian tissues. This test is not used in horses, because the viremia is too low. The 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.

In live horses, clinical cases are usually diagnosed by serology. 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. Serological tests used in horses include IgM capture ELISA (MAC-ELISA), IgG ELISA, hemagglutination inhibition (HI) and plaque reduction neutralization (PRN) assays. The recombinant fluorescent microsphere immunoassay can also be used to test equine sera. Cross-reactions can occur with closely related flaviviruses in ELISAs, HI and some other tests. The PRN test can distinguish antibodies to these viruses by testing for more than one virus in parallel. PRN is used to confirm positive or equivocal ELISAs.

The HI and PRN tests are also used in birds. Some ELISAs are species-specific, and can only be used in the species for which they have been standardized. In addition to horses, this includes some but not all species of birds. Epitope blocking ELISAs, which are not species-specific, have been developed. Virus neutralization tests have been used for serological diagnosis in ruminants including camelids.

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

Several commercial vaccines are available for horses in the U.S. and other countries, and one vaccine has been licensed for geese in Israel. Vaccines are sometimes used "off label" to protect sensitive birds or other species. For instance, in an effort to minimize the impact of West Nile virus on endangered California condors, captive condors have been vaccinated since 2003, and attempts have been made to vaccinate wild chicks in the nest.

Susceptible species should be protected from mosquitoes as much as possible. West Nile encephalitis occasionally occurs in vaccinated horses, and mosquito control measures should not be neglected. Topical repellents should be used on horses and other susceptible animals 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 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 mosquitoes 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 should be weighed against the risk of infection in each species. For example, there is a significant risk of disease in horses and some species of birds during an outbreak, 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 in an area.

Quarantines may be helpful in species suspected or known to transmit the virus horizontally. Carnivores should not be fed meat that might be contaminated with WNV. One outbreak occurred in alligators that had been fed WNV-infected horsemeat. Preventing dogs and cats from hunting or eating birds and rodents may reduce the risk of exposure in these species.

Morbidity and Mortality

West Nile virus is usually a seasonal disease. Most cases in birds occur 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 in crows during the winter. Similarly to WNV in humans, widespread morbidity and mortality has been reported in horses and some species of wild birds in North America, but this has not been the case in Central and South America.

Birds

In areas where WNV has been endemic for decades, the prevalence of infection in wild birds ranges from 10% to greater than 50%. Birds found in the Eastern Hemisphere are rarely affected by WNV. In contrast, some North American populations exposed to this virus for the first time have suffered high mortality rates. Highly susceptible species include American crows (*Corvus brachyrhynchos*), black-billed magpies (*Pica hudsonia*), ring-billed gulls, house finches (*Carpodacus mexicanus*) and greater sage grouse; all of these species have mortality rates up to 100% when infected experimentally. Mortality rates of 75% in blue jays (*Cyanocitta cristata*), 53% in fish crows (*Corvus ossifragus*) and 16% in house sparrows (*Passer domesticus*) have also been reported. In wild populations, severe effects have been seen in corvids including crows, jays and magpies. Overall, the number of crows in the U.S. is estimated to have fallen by 30%, but in some localized areas, much greater declines have been seen. Populations of blue jays, American robins, eastern bluebirds, chickadees, tufted titmice and house wrens have also declined either after intense epidemics or over longer periods. In some cases, the number of birds has fallen across their entire range; in others, the decreases were regional. Some species such as blue jays and house wrens have apparently recovered after epidemics; other populations remained smaller than normal. Some local populations of greater sage grouse have been severely affected, with nearly all of the breeding birds dead. In the Powder River basin (PRB) 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%. One study estimated the annual mortality rate from WNV infections in raptors to be 7-15%. In some species of birds, WNV-resistant populations may be emerging. Some surveys also suggest that WNV may have reached its peak prevalence in the U.S., and the effects on bird species overall may be decreasing. The abundance of some birds including Baltimore orioles (*Icterus galbula*), chipping sparrows (*Spizella passerina*), eastern towhees (*Pipilo erythrophthalmus*), northern cardinals (*Cardinalis cardinalis*) and white-breasted nuthatches (*Sitta carolinensis*) does not seem to have been affected by WNV.

Among poultry, young geese seem to be particularly susceptible to West Nile virus. 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. During 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 young chickens and turkeys are infected asymptomatically.

In zoos and rehabilitation centers, West Nile virus 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 New-World species of birds (20%) than Old-World birds (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. 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.

Mammals

Among mammals, West Nile outbreaks occur mainly in equids. Many infections are asymptomatic, and high seroprevalence rates may be seen in some endemic areas. In 1959, 54% of the horses, donkeys and mules in Egypt were seropositive. During outbreaks, 10-43% of infected horses may develop neurological signs. In experimentally infected horses, the morbidity rate has varied with the methodology. In one study, only one of 12 horses experimentally infected by mosquito vectors developed encephalitis. The other 11 horses seroconverted but remained asymptomatic. Higher rates of encephalitis and fever were seen when foals and horses were infected subcutaneously or intravenously: 4 of 9 animals in two studies became ill. The reported case fatality rate in horses varies from 23% to 57%, depending on the outbreak; in the U.S., it is approximately 30-40%. Approximately 80-90% of horses that recover return to full function; the remainder have some residual neurological defects.

Clinical cases seem to be uncommon in most other species of mammals, but asymptomatic infections may be frequent. Although only rare clinical cases have been reported in dogs and cats, WNV antibodies have been found in 8-37% of dogs in South Africa, and 2-26% of dogs in localized areas of the U.S. In one U.S. study, the seroprevalence rate was 9% in cats. No clinical signs have been seen in experimentally infected dogs, and only mild, non-neurological signs were reported in experimentally infected cats. Approximately 26% of camels, 20% of sheep

and 18% of goats in Nigeria had antibodies to WNV, but outbreaks have not been reported in these animals. In the U.S., clinical cases have been seen in a few sheep, alpacas and reindeer; illness usually occurred in only one to a few animals in the herd. Symptomatic infections have not been reported in pigs; however, approximately 3-10% of domesticated pigs in India, and 22% of the feral pigs in Florida, Georgia, and Texas were seropositive. Antibodies have also been reported in many species of wild mammals; some species may be infected frequently. In the U.S., up to 63% of striped skunks, up to 46% of raccoons, and up to 49% of squirrels in some areas have antibodies to WNV. High seroprevalence rates have also been reported among wild lemurs in Madagascar. Morbidity rates in most wild mammals are unknown; however, the morbidity rate in experimentally infected fox squirrels was 10%. The case fatality rate seems to be high in mammals that develop neurological disease. Most clinically affected sheep, alpacas, reindeer, dogs, cats, wolves and deer 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 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.

Internet Resources

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

CDC. West Nile Avian Mortality Database
http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm

Public Health Agency of Canada. Material Safety Data Sheets
http://www.phac-aspc.gc.ca/msds-ftss/index.html

The Merck Manual
http://www.merck.com/pubs/mmanual/

The Merck Veterinary Manual
http://www.merckvetmanual.com/mvm/index.jsp

United States Animal Health Association.
Foreign Animal Diseases.
http://www.vet.uga.edu/vpp/gray_book02/fad/index.php

United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS). 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/eng/normes/mmanual/a_summry.htm

OIE Terrestrial Animal Health Code
http://www.oie.int/eng/normes/mcode/A_summry.htm

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*Link defunct as of 2009