Examining the global status of West Nile virus with focus on current challenges in disease prevention in humans and animals

by

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Abstract

West Nile virus (WNV) continues to be a worldwide threat to the health of humans and animals. The virus has been known to cause disease in humans since 1937, and its range continues to expand. While infection is typically asymptomatic, this virus can cause serious illness and death in the host. There are also significant expenditures of resources to control and prevent its morbidity in humans, domestic animals, and wildlife. It is a mosquito-borne disease with many challenges for surveillance and control that include some underlying issues with climate change. Currently, there are no licensed vaccines for humans or treatments for infection beyond supportive care, but there are several licensed vaccines for use in animals. The purpose of this report is to examine the global status of WNV and the current challenges of controlling this disease threat in humans and animals. This report will also review new strategies in vaccine development and other preventative measures.

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Chapter 1 - Review of West Nile Virus

West Nile virus (WNV) is an important flavivirus that originated in the West Nile region of Africa, and it has since spread to far reaches of the globe, primarily causing disease in humans, horses, and birds. This chapter will provide a review of WNV, discussing disease outbreaks, transmission routes, human and animal disease presentation, diagnostics, and treatment strategies.

WNV and other medically important flaviviruses

Other than WNV, additional flaviviruses of medical importance include yellow fever virus (YFV), dengue virus (DENV), Japanese encephalitis virus (JEV), St. Louis encephalitis virus (SLEV), and Zika virus (ZIKV). These viruses are primarily transmitted by *Culex* or *Aedes* mosquitoes, but WNV and ZIKV can occasionally be transmitted by infected biological material, like blood or organ transplants. Only Japanese encephalitis has an effective vaccine for humans. Dengue fever also has a vaccine, but its use is not widespread due to the risk of adverse reactions. With the exception of DENV, flaviviruses do not cause significant disease in most people. However, if they do cause disease, it can be severe. Interestingly, the ranges of these viruses can overlap without one displacing the other. For example, both SLEV and WNV occur in similar regions within the United States (Lillibridge et al., 2004). Several flaviviruses are compared in **Table 1** and **Table 2** based on transmission routes, vectors, symptoms, and availability of vaccines.

Table 1: Flaviviruses (JEV, SLE, Zika)

Virus	**Japanese Encephalitis**	**Saint Louis Encephalitis**	**Zika**
Typical Transmission	Mosquito	Mosquito	Mosquito
Atypical Transmission	n/a	n/a	Transplacental, Sexual, Blood Transfusion, Organ Donation
Primary Vector	*Culex*	*Culex*	*Aedes*
Typical Symptoms	None, Fever and Headache, Gastrointestinal Pain and Vomiting	None, Fever and Headache	None, Fever, Rash, Malaise, Conjunctivitis, Muscle and Joint Pain
Rare Complications	High Fever, Headache, Neck Stiffness, Disorientation, Coma, Seizures, Spastic Paralysis, Death	High Fever, Headache, Neck Stiffness, Disorientation, Coma, Seizures, Spastic Paralysis, Death	Fetal Abnormalities: Microcephaly, Abortion, Stillbirth, Premature Birth
Treatment	Supportive	Supportive	Supportive
Primary Range	Asia	North America	Africa, North and South America, Asia
Human Vaccine	Yes	No	No

Adapted from: The World Health Organization (WHO) and CDC websites, 2019.

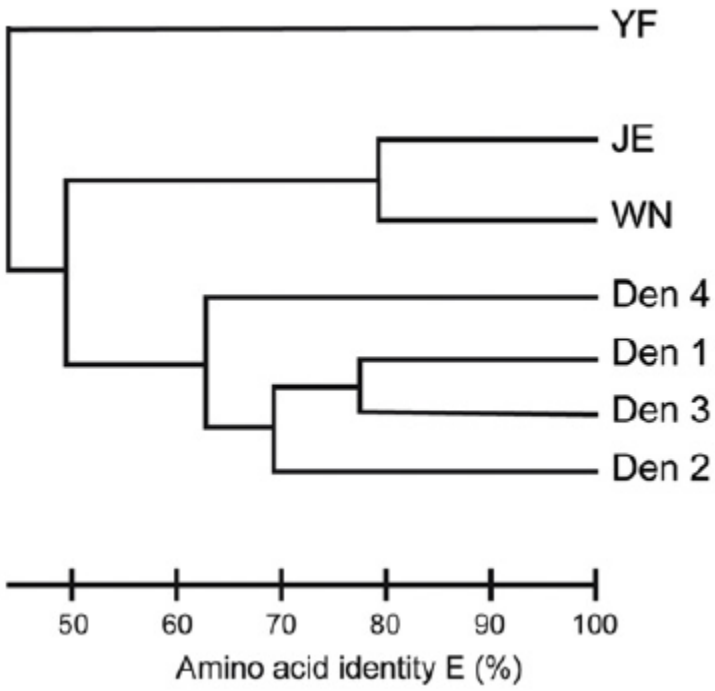
Table 2: Flaviviruses (WNV, YFV, DENV)

Virus	**West Nile**	**Yellow Fever**	**Dengue Fever**
Typical Transmission	Mosquito	Mosquito	Mosquito
Atypical Transmission	Contact with infected biological material	n/a	n/a
Primary Vector	*Culex*	*Aedes*	*Aedes*
Typical Symptoms	None, Fever, Head and Body Aches, Nausea, Vomiting, Rash	None, Fever, Muscle Pain, Nausea or Vomiting	High Fever, Severe Headache, Eye Pain, Joint/Muscle pain, Nausea, Vomiting, Swollen Glands or Rash
Rare Complications	Neuroinvasivene Disease: Encephalitis and/or Myelitis, Death	Jaundice: High Fever, Liver and Kidney Malfunction, Bleeding from Mouth, Nose, Eyes, and Stomach, Death	Plasma Leaking: Respiratory Distress, Severe Bleeding, Fluid Accumulation, Organ Impairment, Death
Treatment	Supportive	Supportive	Supportive
Primary Range	Worldwide	Africa and South America	Worldwide Tropics
Humans Vaccine	No	Yes	Yes-Conditional

Adapted from: The World Health Organization (WHO) websites, 2019.

Figure 1 shows the genetic relationships among some of the important flaviviruses based on the genomic sequence of the envelope protein. JEV and WNV are the most closely related flaviviruses with YFV being the most distant virus (Heinz and Stiasny, 2012). DENV has four serotypes, and while infection with one serotype of DENV confers protection from that specific serotype, it does not confer protection from the others. Therefore, a human can potentially become infected with DENV four times depending on serotype. Each successive infection increases the chance for severe dengue symptoms (WHO, 2019).

Figure 1: Phylogenetic Relationships Among Flaviviruses

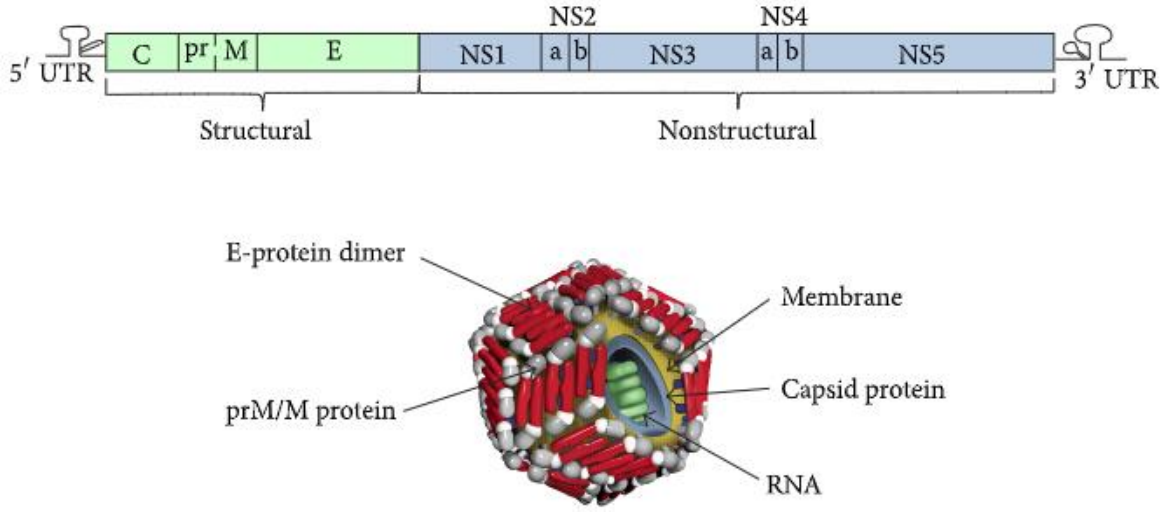


Adapted from: Heinz, F.X., Stiasny, K. (2012). Flaviviruses and their antigenic structure. J Clin Virol, 55(4):289–95.

Viral description and properties

WNV belongs to the genus *Flavivirus* in the family *Flaviviridae*. As mentioned above, it is an arthropod-borne virus (or arbovirus) with a positive sense single-stranded RNA genome (Martin-Acebes and Saiz, 2012). The virus is enveloped and icosahedral in structure, measuring about 50 nm in diameter. As depicted in **Figure 2**, the viral genome is about 11,000 nucleotides in length, encoding for three structural (capsid, envelope, and premembrane) and five nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins (Martin-Acebes and Saiz, 2012; Heinz and Stiasny, 2012). The main target of neutralizing antibodies is the envelope (E) protein (Heinz and Stiasny, 2012). Therefore, most vaccines use E proteins and/or premembrane (prM) proteins as antigen targets.

Figure 2: West Nile Virus Genomic and Virion Structure



Adapted from: Chancey, C., Grinev, A., Volkova, E., and Rios, M. (2015). The global ecology and epidemiology of West Nile virus. Biomed Res Int, 376230.

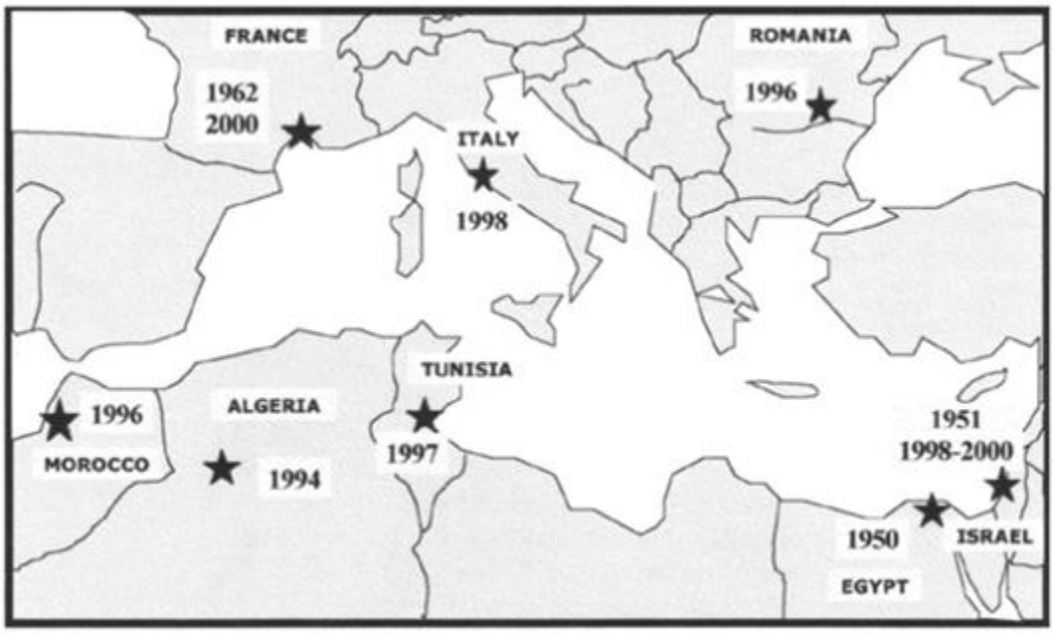
History of Old World and New World outbreaks

Old World outbreaks

WNV was first isolated in the West Nile region of Uganda in 1937. It was initially discovered during an expedition conducted to study YFV (Smithburn, 1940). The exact numbers of cases during these early outbreaks within the Old World countries are difficult to determine, because not every case was confirmed by laboratory testing. The first known outbreak was reported in Israel in 1951 within a small settlement of about 300 people. While there were no fatalities, 41% of the human population became ill, and the virus was also isolated from chickens and cattle in the area (Bernkopf, 1953). Later, studies from this same region found antibodies in eight species of mammals and nine species of birds. This suggested that the virus was endemic to the area at the time (Hurlbut, 1956).

The virus continued to spread out and into the Mediterranean region. **Figure 3** shows the geographical locations of some of the early outbreaks of WNV in the Mediterranean region. There were reports of WNV in Egypt, France, Algeria, Morocco, Tunisia, Israel, and then, France once again (Murgue et al., 2001). The illnesses associated with these outbreaks are shown in **Table 3**. These outbreaks caused illness in both humans and horses (Murgue et al., 2001). The first outbreak in Europe was in Bucharest in 1996 with 835 patients, resulting in 17 fatalities (Murgue et al., 2001).

Figure 3: West Nile Outbreaks in the Mediterranean, 1950 to 2000



Adapted from: Murgue, B., Murri, S., Triki, H. (2001). West Nile in the Mediterranean basin: 1950–2000. Ann N Y Acad Sci, 951:117-26.

Table 3: Summary West Nile Cases in the Mediterranean, 1951-2000

Year	Location	Human Illness	Human Deaths	Horse Illness	Horse Deaths
1951-1952	Egypt	123	0	0	0
1962-1965	France	13	1	80	20
1994	Algeria	50	8	0	0
1996	Morocco	1	0	94	42
1997	Tunisia	173	8	0	0
1998-2000	Israel	400	18	18	0
2000	France	0	0	76	21

Adapted from: Murgue, B., Murri, S., Triki, H. (2001). West Nile in the Mediterranean basin: 1950–2000. Ann N Y Acad Sci, 951:117-26.

New World outbreaks

WNV was observed in North America for the first time in New York in 1999. This outbreak resulted in 50 human cases with 5 human deaths. In addition to the human cases, there were many bird deaths, primarily of crows (CDC MMWR, 1999). Since 1999, there have been two major outbreaks in North America. The first was during the summer of 2002. This included 4,156 human cases that resulted in 2,354 patients with meningoencephalitis and 284 deaths (Sejvar, 2003). The second outbreak occurred in 2012. There were 5,674 cases in all of the lower 48 states with 286 deaths. Fifty-one percent of these cases were neuroinvasive (CDC, 2019). WNV continues to be a concern for humans with an incidence rate of about 0.4 cases per 100,000 people since the 2012 outbreak (CDC, 2019).

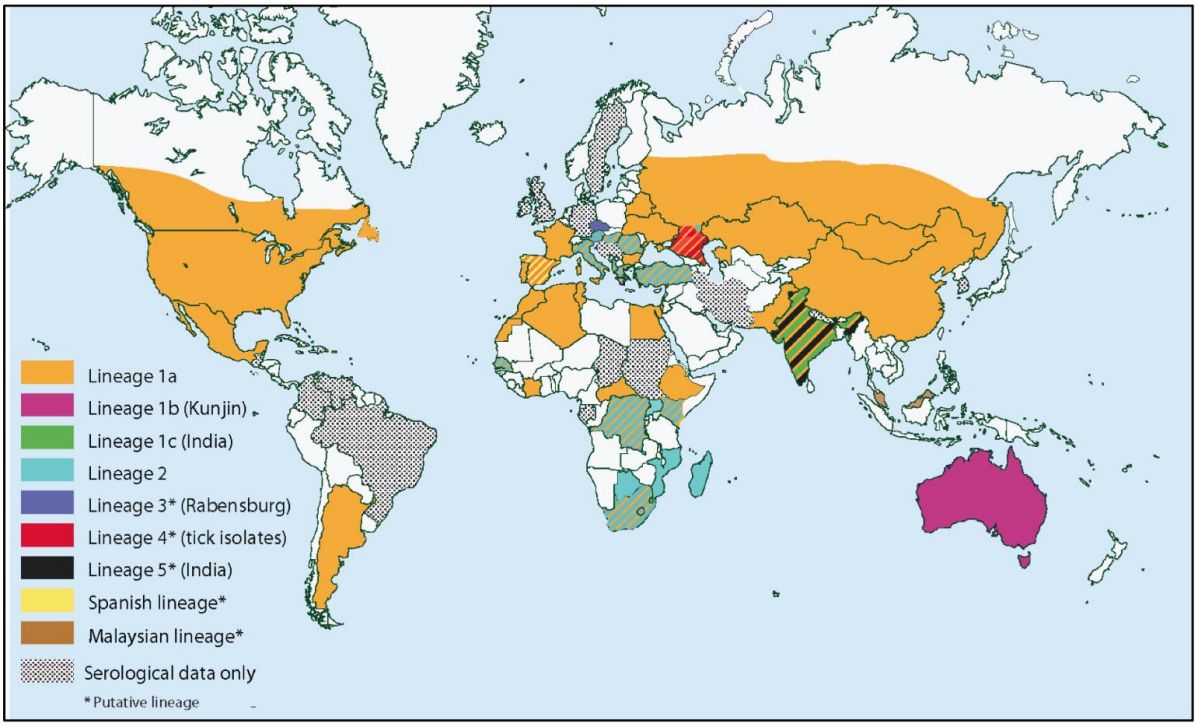
It is still not clear how WNV was introduced to North America. It could have been transported within infected mosquitoes or birds on ships, or possibly in migratory birds that crossed the ocean (Rappole, J.H., et al. 2000). The strain of WNV that initiated the epidemic in North America in 1999 was most similar to a strain isolated from a dead goose in Israel in 1998 (Lanciotti et al., 1999).

WNV has also been found in humans, horses, and birds in South America. The first record of WNV in South America was from a Columbian horse in 2005 (Castro-Jorge et al., 2019). Brazil has seen WNV in both horses and birds (Vieira et al., 2015). While South America does have cases of WNV, there have not been any major outbreaks (Elizondo-Quiroga and Elizondo-Quiroga, 2013).

Virus strains

There are different strains of WNV, and depending on the strain, there are variances in mortality and neuroinvasiveness (Beasley et al., 2005). Current evidence suggests that there are at least 5 lineages of WNV (Bondre et al., 2007). Lineages 1 and 2 appear to be the main source of human disease (Pérez-Ramírez et al., 2017). Research on vaccines for WNV has also demonstrated cross-protection between lineages 1 and 2 (Iyer and Kousoulas, 2013). **Figure 4** shows the distribution of the WNV lineages across the world.

Figure 4: Distribution of West Nile Virus Lineages



Adapted from: Ciota, A.T., Kramer, L.D. (2013). Vector-virus interactions and transmission dynamics of West Nile virus. Viruses, 5(12):3021–47.

Reservoir hosts

Birds are the primary reservoir of WNV, and the primary vector for transmission is *Culex* mosquitoes. Secondarily, it can also be transmitted by *Aedes* and *Ochlerotatus* mosquito species.

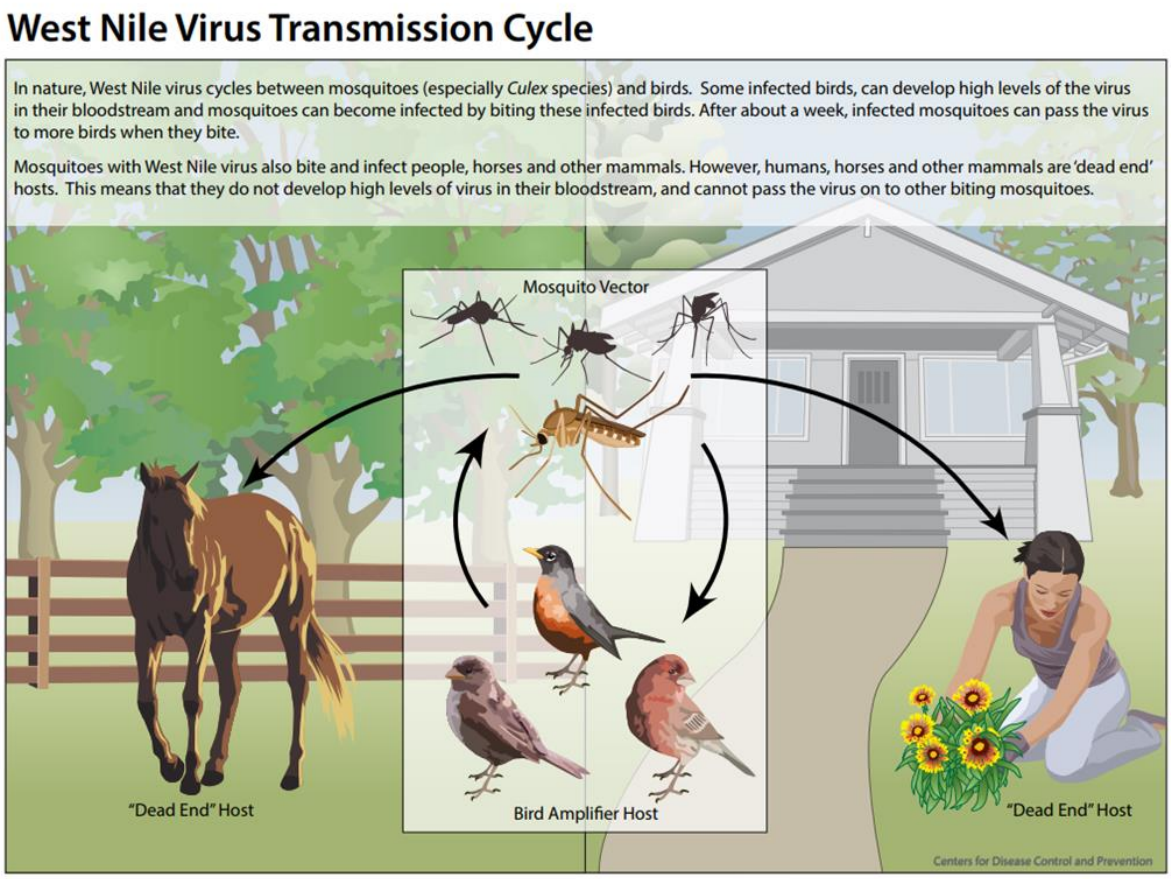
American robins (*Turdus migratorius*) are a major amplifying host for WNV. Because robins are distributed over most of North America, they may be the most important reservoir on the continent (Kilpatrick et al., 2006).

While birds are the primary reservoir, other animals can be infected as well. However, most are considered dead end hosts, because they do not develop a high enough viremia to transmit the virus to a mosquito vector. Humans and horses are susceptible to infection and can show signs of disease. Other animals, like dogs and monkeys, can be infected, but typically show no signs of disease (Martin-Acebes and Saiz, 2012).

Transmission routes

WNV is typically spread through the bite of an infected mosquito. The typical life cycle is shown in **Figure 5**. WNV is normally transmitted between mosquito and bird, but it can be transmitted to other hosts. It can also be transmitted transplacentally, through organ donation, dialysis, breastfeeding, and needle-stick injury among humans (CDC, 2019). There may be other routes, such as fecal-oral and predation in animals (Klenk et al., 2004).

Figure 5: West Nile Virus Transmission Cycle



Adapted from: Centers for Disease Control and Prevention (CDC) website.

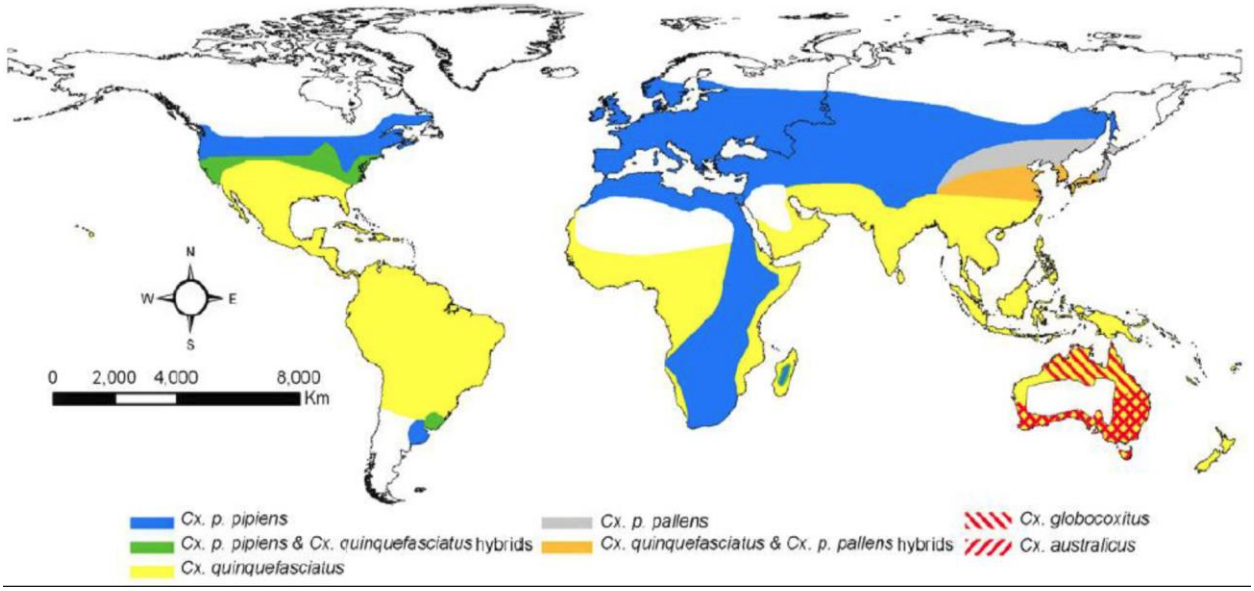
One study looked at several other arthropods for possible transmission of the virus. Fleas, two species of flies, lice, mites, and nine species of ticks were investigated. While WNV was isolated from all of these insects, the virus was not transmitted from any of these arthropods, with the exception of one tick species, *Ornithodoros savignyi*. Transmission occurred once under experimental conditions, but it could not be replicated without first injecting the virus directly into the tick (Hurlbut, 1956).

Virus and vector range

WNV is circulating on all continents except Antarctica (Ciota and Kramer, 2013). Interestingly, seroprevalence rates among people living along the Nile River, the most likely origin of the virus, are still moderately high, approaching 60% (Sejvar, 2003).

Culex spp. mosquitoes are the main WNV vectors. Secondarily, it can be transmitted by *Aedes* and *Ochlerotatus* species of mosquitoes (Work et al., 1955). As discussed above, another arthropod that may be infected with WNV is one species of tick (*Ornithodoros savigny*). However, the importance of this arthropod as a transmission vector appears to be minimal (Hurlbut et al., 1956). **Figure 6** demonstrates the ranges of some *Culex* mosquito species.

Figure 6: WNV Vector Range



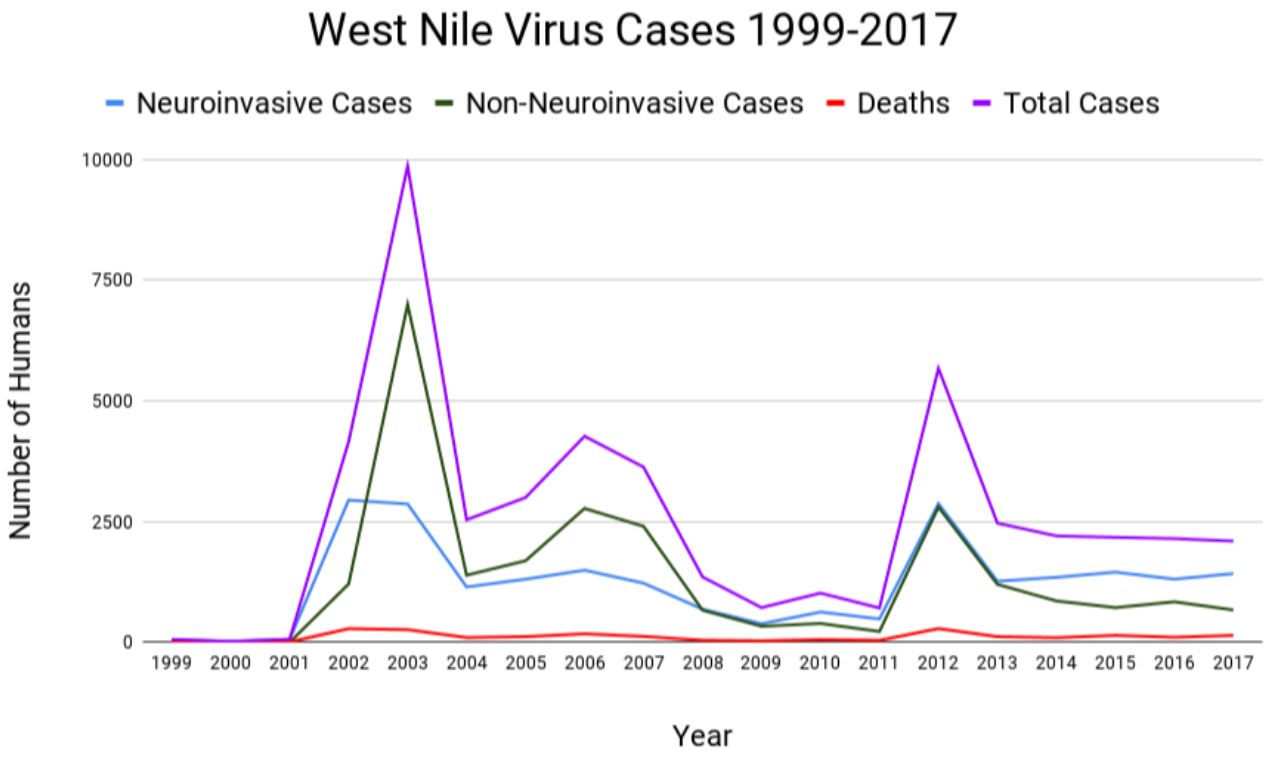
Adapted from: Ciota, A.T., Kramer, L.D. (2013). Vector-virus interactions and transmission dynamics of West Nile virus. Viruses, 5(12):3021–47.

Interestingly, mosquito saliva has components that assist in obtaining a blood meal, including immunomodulatory compounds that promote viral transmission like WNV (Schneider and Higgs, 2008).

Human disease

WNV can cause disease in humans and is the main cause of mosquito-borne disease in the United States. WNV outbreaks are more common in the summer months, when mosquitoes are more active. As a reportable disease to the CDC, the total numbers of human cases in the United States are represented in **Figure 7**. Since its introduction to the U.S. in 1999, there have been a total of 50,830 cases with 24,656 patients developing neuroinvasive disease and 2,330 numbers of deaths (CDC, 2019).

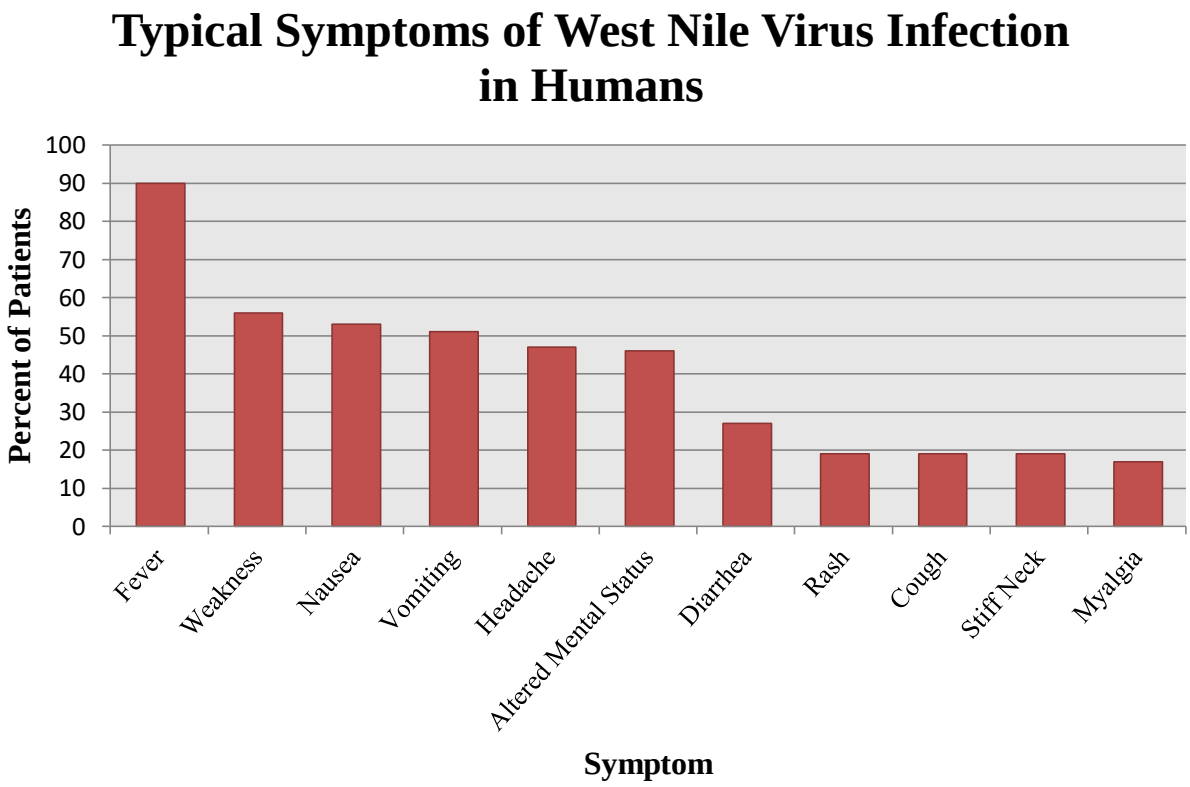
Figure 7: Types of Cases of WNV in the United States



Adapted from: Centers for Disease Control and Prevention (CDC) website.

Most humans infected with West Nile are asymptomatic, and only 1 in 5 will develop illness (CDC, 2019). Symptoms typically develop 2 to 15 days post-infection and last 2 to 5 days, but can last several weeks (Martin-Acebes and Saiz, 2012). Of those that are symptomatic, the most common symptom is febrile illness. This can also include aches, joint pain, vomiting, diarrhea, and/or rash. Fatigue and weakness can last for weeks or months. About 1 in 150 people will develop serious disease, typically encephalitis or meningitis (CDC, 2019). Individuals over 75 years old or having diabetes is associated with an increased risk of death (Nash et al., 2001). WNV will kill about 1 in 10 people who show encephalomyelitis symptoms (CDC, 2019). Typical WNV symptoms are shown in **Figure 8**.

Figure 8: West Nile Virus Symptoms in Humans



Adapted from: Nash, D., Mostashari, F., Fine, A., et al. (2001). The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med, 344,:1807–14.

West Nile displays an “iceberg” pattern of disease. This means that most individuals will be infected and asymptomatic, while a moderate number will be infected and have mild symptoms. Finally, a few individuals develop severe symptoms of disease (Martin-Acebes and Saiz, 2012). This can lead to underreporting of actual disease prevalence. The CDC estimates that only 20% of WNV infections result in disease.

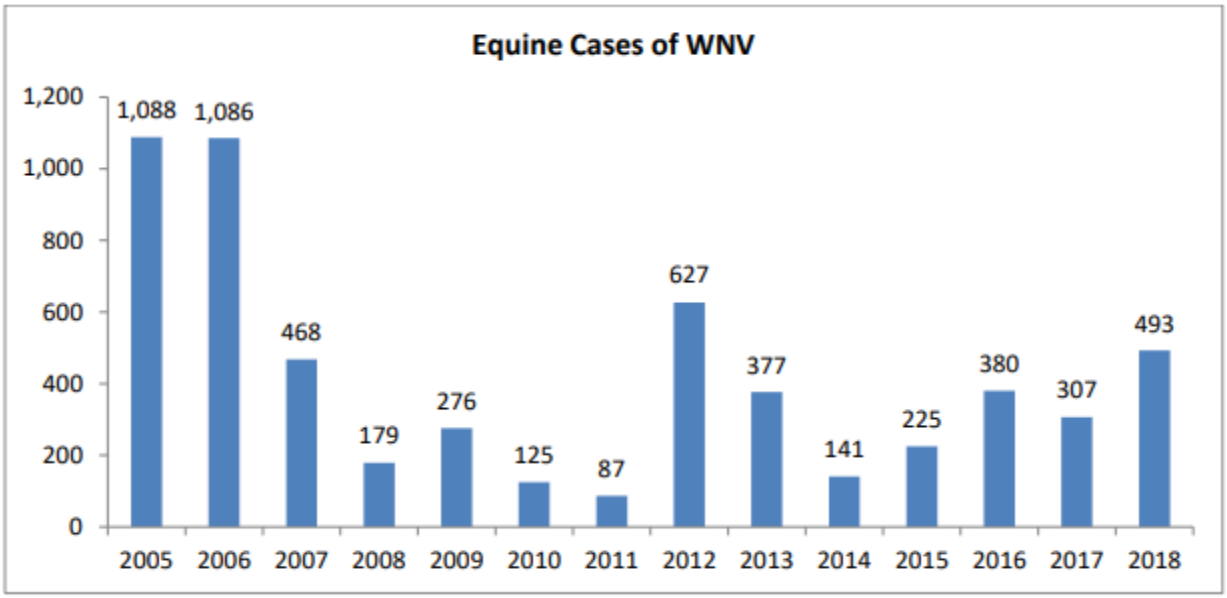
There is no specific antiviral protocol for treatment, and most cases are managed with supportive care (CDC, 2019). With regard to prevention, there are no licensed vaccines for use in humans.

Animal disease

Equine

WNV affects horses and cases are reported to the USDA. Since 2005, there have been 5,858 reported equine cases in the United States (USDA, 2019). **Figure 9** shows the yearly distribution of these cases.

Figure 9: Equine Cases of WNV 2005-2018



Adapted from: USDA Equine West Nile Virus Case Reporting and Surveillance Information. (2019).

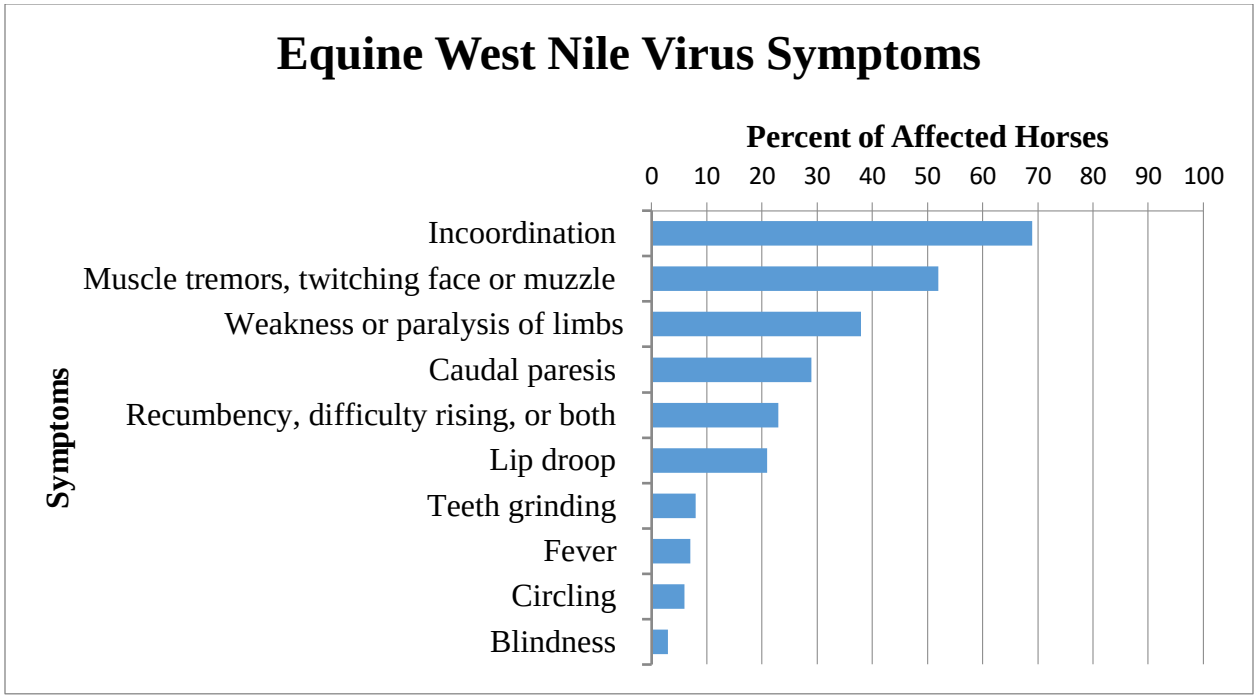
Disease transmission in horses is from a bite of an infected mosquito (EDCC, 2017). Following the bite, the incubation period is about 7-10 days. While most cases of equine WNV are asymptomatic, if symptoms do occur, they typically last about 3 weeks (Martin-Acebes and Saiz, 2012).

WNV infection can be difficult to detect. This is because viremia in horses is short lived and clinical signs, if any, often appear as the viremic stage is ending (Castillo-Olivares and Wood, 2004). After viremia, horses can be tested for WNV IgM antibodies. While a positive test is usually indicative of infection, some horses may not produce enough antibodies to test positive while still being infected (Long et al., 2006).

About 10% of horses infected with WNV develop neurological disease (Castillo-Olivares and Wood, 2004), and the most common symptoms of affected horses are shown in **Figure 10**. The typical symptoms are neurological and are similar to those of a spinal cord injury (Castillo-

Olivares and Wood, 2004). These include incoordination, muscle tremors, weakness or paralysis of limbs and caudal paresis (Ndiva et al., 2008). Once neurological symptoms appear, about 40% of these horses will either die or be euthanized (Castillo-Olivares and Wood, 2004). Treatment is supportive only (EDCC 2017), and outbreaks can result in 25-45% mortality. Fortunately, this number has been greatly reduced by the introduction of vaccination (Martin-Acebes and Saiz, 2012).

Figure 10: Equine WNV Symptoms



Adapted from: Schuler L.A., Khaitsa M.L., Dyer N.W., Stoltenow C.L. (2004). Evaluation of an outbreak of West Nile virus infection in horses: 569 cases (2002). J Am Vet Med Assoc, 225(7):1084–89.

The economic impact of WNV in horses can be significant. There have been no nation-wide studies in the United States on the economic impact of WNV in horses, but there have been a few studies conducted by different states. In North Dakota, the cost of the 2002 WNV outbreak was estimated to be about $1.5 million dollars; in Colorado and Nebraska, the cost was

estimated to be about $2.75 million dollars; and in Texas, it was estimated to be about $1.2 million dollars (Ndiva et al., 2008). These costs are associated with vaccination, treatment, death of the animal, and vector control.

There are currently available vaccines available for horses. These vaccines and future vaccine strategies will be discussed in more detail in Chapter 3.

Avian

WNV has caused significant decline in some bird populations, sometimes by more than 50%. Species affected include corvids, chickadees, wrens, and thrushes (Kilpatrick, 2011). Hooded crows (*Corvus corone sardonicus*) have a high mortality rate from WNV. House sparrows (*Passer domesticusare*) are also important hosts (Work, 1955). Some birds, like turkeys, seem to be minimally affected by WNV (Swayne et al., 2000).

Clinical signs in WNV infected birds are often nonspecific, and birds are often simply found dead. If birds do become symptomatic, they can show signs of neurologic abnormalities, general depression and weakness. Most birds showing clinical signs die within a week (Ludwig et al., 2002).

The economic cost of WNV to birds is difficult to calculate. Most of the avian species affected are not considered species of major economic importance. There may be two exceptions to this, game birds and zoo birds.

Hunting provides states with revenue, and the decline of game birds has an economic impact. Ruffled grouse (*Bonasa umbellus*) are an example of this. Their numbers are in decline due to the dual pressures of habitat loss and WNV infection (Stauffer et al., 2018). This has led the state to shorten hunting seasons and increased expenditures of state resources to study species decline. At least $1,250,000 was spent on forest management in 2019 in an attempt to control

the population decline (Williams, 2019). The decline of these game birds could also lead to a reduction of state funds if hunters choose to hunt and spend money in other states.

Birds kept in zoos or conservation facilities have a value based on both their ability to bring people to the zoo and in their conservation status. Great effort is expended to maintain and breed rare birds. The exact value of these efforts is difficult to calculate and have not been published. Even though zoos and aquariums may participate in surveillance of WNV, they also worry they will see a decline in ticket sales if WNV is discovered on their grounds (Nolen, 2001).

There is no commercially available vaccine for birds. Some zoos and wildlife centers use the horse vaccine on their birds despite the lack of studies demonstrating its efficacy or safety in birds (USGS, 2019). Of the few studies on birds, there have been limited results. It appears California condors can produce neutralizing antibodies from an experimental plasmid DNA vaccine, but not to the licensed formalin-inactivated horse vaccine (Chang et al., 2007). A study vaccinating falcons with three different horse vaccines showed a reduction of clinical signs and only partial protection from disease (Angenvoort, 2014).

Alligator

Alligators (*Alligator mississippiensis*) are also susceptible to WNV. They typically acquire the virus from infected mosquitoes, but can also become infected by eating infected animals (Klenk et al., 2004). One possible source of this is when they are fed horsemeat as part of the diet (Sejvar, 2003). There is also the possibility of fecal-oral transmission of the virus in co-housed alligators (Klenk et al., 2004). Alligators also produce a high enough viremia to infect mosquitoes, suggesting they are active vectors and not just dead end hosts (Klenk et al., 2004).

Clinical signs in alligators include anorexia, lethargy, and abnormal swimming patterns, such as swimming in circles and being unable to submerge (Jacobson et al., 2005). Infection with WNV can also be a factor that compromises the overall health of the animal and allows for secondary infection. Hatchlings tend to die from WNV infections, whereas juveniles tend to die from secondary infection brought on by infection with WNV (Sejvar, 2003).

WNV infection is particularly problematic for alligator farmers. One alligator farm in Florida reported a 3.3% loss in 2002 (Jacobson et al., 2005). The total value of the Florida alligator harvest in 2002 was $3,941,416 (FFWC, 2019). This means the commercial loss in 2002 could have been $130,066. The average value of a Florida alligator in 2002 was $143.47, but has risen to $332.96 in 2017 (FFWC, 2019). This suggests the potential economics loss from WNV could have been $283,615 in 2017.

There is one commercially available vaccine for WNV in alligators.

Animal models of disease

Different types of animal models can be used to study different effects of disease in humans as well as to assess vaccine efficacy. There are several things to consider when choosing an animal model to study. These include: the system or effect to be studied, acquiring the animals, and the facility for testing. WNV is classified as biosafety level (BSL-3) agent and requires specialized containment and training of personnel. The use of smaller animals, like rodents, can be advantageous. This is because they are easier to handle and less expensive than larger animals. Below, different animal models of West Nile infection will be discussed, including strengths and weaknesses of each model. **Table 4** summarizes some of the common animal models of WNV depending on study type, infection route, and human application.

Rodents

Mice are a frequently used to study WNV. Mice are easy to acquire and study, but they have significant drawbacks as human models. Observations in mice following infection are not always transferrable to humans. To study the disease, mice are typically inoculated with WNV by different routes. With the exception of dermal inoculation, inoculation by any other route is not the natural route of infection by the virus. This difference can affect study outcomes in ways that may or may not be relevant. Even dermal inoculation can be less than ideal, because it removes the variables present with the mosquito, such as the saliva components and viral load (McGruder et al., 2016). Since there are multiple strains of WNV, mouse models are useful for looking at variances in morbidity across these different strains (Beasley et al., 2005).

Nonhuman primates

Primates are frequently used to study WNV. For example, a study of macaques was performed to examine the duration of viremia and the humoral response. This is difficult to study in humans, because most cases present for medical examination only after the patient is symptomatic. This likely occurs 2-3 weeks after infection, so there is not always virus left in the blood to detect (Ratterree et al., 2004).

Nonhuman primates are also used for vaccine development to determine the level of protection following WNV challenge. For example, a study of squirrel monkeys was undertaken to explore the possibility of a recombinant vaccine for WNV. These animals were chosen because they are easier to use than larger nonhuman primates due to their size of about 1 kilogram each (Brandler et al., 2012).

When considering nonhuman primate models for West Nile disease, there are often non-target disease considerations to take into account. These include the pathogens that primates can

carry that may also pose a disease risk to humans who are handling these animals. Therefore, baboons may be a useful model in some situations because they are readily available, do not carry hepatitis B virus, and can be infected with WNV (Wolf et al., 2006).

Primates are more closely related to humans than mice, but they are not always ideal models for study. One limitation of nonhuman primate models is that they do not typically display symptomatic infection. Another limitation is that older nonhuman primates, like macaques, display less susceptibility to WNV than mice and humans (Wertheimer et al., 2010).

Table 4: Examples of Animal Models

Animal Model	Study Type	Infection Route	Human Application
Mouse	Systemic	Intraperitoneal or Subcutaneous	Some overlap with human symptoms
Mouse	Geriatric	Intraperitoneal	Risks associated with aging
Mouse	Persistent Infection	Intraperitoneal or Subcutaneous	Long-term morbidity
Mouse	Central Nervous System	Intracranial	Neurovirulence
Mouse	Cutaneous Infection Route	Intradermal	Natural mosquito infection
			(McGruder et al., 2016)
Primate/Macaque	Viremia	Intradermal	Duration of viremia
Primate/Macaque	Humoral Response	Intradermal	Antibody response kinetics
			(Ratterree et al., 2004)
Mouse	Different Strains	Intraperitoneal/ Intracerebral/ Intranasal	Possible differences in virulence/morbidity
Hamster	Different Strains	Intraperitoneal	Compare to mouse studies for consistency
			(Beasley et al., 2005)
Primate/Squirrel Monkey	Vaccine Development	Intravenous	Producing a recombinant vaccine
			(Brandler et al., 2012)
Primate /Baboon	Proof of Susceptibility	Intradermal	Vaccine development
			(Wolf et al., 2006)
Hamster	Proof of Susceptibility	Intraperitoneal	Additional model for study
			(Xiao et al., 2001)
Primate/Macaque	Geriatric	Intravenous or Subcutaneous	Risks associated with aging
Primate/Macaque	Immunocompromised	Subcutaneous	Risks associated with immune deficiency
Primate/Macaque	Mosquito Saliva	Intravenous or Subcutaneous	Risks associated with exposure to mosquito saliva
			(Wertheimer et al., 2010)

Adapted from studies by: McGruder et al., 2016, Ratterree et al., 2004, Beasley et al., 2005, Brandler et al., 2012, Wolf et al., 2006, Xiao et al., 2001, and Wertheimer et al., 2010.

Diagnostic assays

There are three basic diagnostic techniques for WNV. One is identification of the virus based on genomic sequencing. Another is the detection of antibodies to the virus. A third technique is attempting to isolate the virus in culture. Selection of the appropriate diagnostic assay depends on the stage of infection.

Virus isolation

Early in the course of the disease and during the viremic stage of infection, virus isolation and confirmatory identification using immunostaining would provide a definitive diagnosis. Unfortunately, WNV can be challenging to isolate (Sambri et al., 2013), particularly from dead end hosts with low viremia. This method has the additional challenge of requiring a BSL-3 facility. As the disease progresses and viremia levels decrease, the detection of antibodies is a more appropriate strategy.

Antibody testing

Antibody testing is used to determine the later stages of infection or past infection. The immunoglobulin type can be useful in determining the stage of infection and severity of neuroinvasive disease. Detecting IgM against WNV in serum can indicate early infection, while detecting IgG against WNV in serum can indicate later stages of disease. Detecting IgM in cerebrospinal fluid is a positive test for central nervous system neuroinvasive disease, because IgM does not cross the blood-brain barrier.

One of the main challenges with antibody testing is cross-reactivity with other flaviviruses (Martin-Acebes and Saiz, 2012). This is important because the geographical ranges of the various flaviviruses can overlap (Lillibridge et al., 2004), and some of the flaviviruses

have similar symptoms. Also, previous vaccination for WNV in animals and/or other flaviviruses can interfere with test results (Shirafuji et al., 2009).

There are several different types of antibody assays available for the diagnosis of WNV infection: plaque reduction neutralization test (PRNT), hemagglutination inhibition test (HIT), immunofluorescence assay (IFA), and the enzyme-linked immunosorbent assay (ELISA). See **Table 4** and **Table 5** that summarizes the principles of each assay, antibody targets, advantages, disadvantages, and application.

The PRNT is frequently used to test for WNV. It measures neutralizing antibodies by comparing plaque lysis with a control. This test is mainly used to detect past WNV infection. The main drawbacks with this test are that it is time consuming, must be performed in a BSL-3 facility, and does not differentiate between IgG and IgM (Beck et al., 2013).

The HIT is used primarily as a screening tool. It is useful for screening large numbers of samples so that presumptive positives can be sent on to confirmatory testing by more accurate tests. Some advantages of this test are that it is inexpensive and does not require a BSL-3 facility. Some disadvantages are that it is not species specific and can react to other flaviviruses (Beck et al., 2013).

The IFA is typically used in the investigation of clinical cases. It has the advantage in that it can differentiate between IgM and IgG and associated stage of infection. The assays are commercially available, inexpensive, and fast, but they do have some cross reactivity problems between different flaviviruses (Beck et al., 2013).

The ELISA is a common, inexpensive, screening tool with commercial availability. This assay has a high level of sensitively, but a low level of specificity. It requires confirmatory testing and will cross-react with other flaviviruses (Beck et al., 2013).

Polymerase chain reaction (PCR)

PCR testing allows for precise identification of the infecting agent. Flaviviruses can have overlapping ranges, and WNV can have symptoms similar to other flavivirus (Martin-Acebes and Saiz, 2012) and encephalomyopathy-causing diseases. WNV typically also has a low viremia in dead end hosts, like humans, so the amplification from PCR is necessary to obtain enough of the RNA for identification. Many other tests have problems with cross-reactivity. Using PCR eliminates this problem.

Table 5: Antibody Tests (PRNT, HIT) for WNV

Test	**Plaque Reduction Neutralization Test (PRNT)**	**Hemagglutination Inhibition Test (HIT)**
Principle	Neutralization of virus attachment to cells or post-binding steps	Inhibition of virus-induced erythrocyte aggregation
Antibody Targets	Neutralizing epitopes in the E protein	E protein
Advantages	-Not species specific	-Inexpensive -Not species specific -Can be performed outside BSL-3 facility
Disadvantages	-Less sensitive than ELISA -Takes 1 week to complete -Requires BSL-3 facility -No differentiation between IgM and IgG	-Less Sensitive than ELISA -Less Specific than PRNT -Cross-Reaction with other flaviviruses -No differentiation between IgM and IgG
Applications	Confirms past WNV infection	Screening method: requires additional confirmatory testing

Adapted from**:** Beck, C., Jimenez-Clavero, M. A., Leblond, A., Durand, B., Nowotny, N., Leparc-Goffart, I., … Lecollinet, S. (2013). Flaviviruses in Europe: Complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. Int J Environ Res Public Health, 10(11):6049–83.

Table 6: Antibody Tests (ELISA, IFA) for WNV

Test	**Enzyme-Linked Immunosorbent Assay (ELISA)**	**Immunofluorescence Assay (IFA)**
Principle	Antibody binding on well plates coated with antigen and revealed by enzyme-conjugated antibodies with a photometric reaction	Antibody binding to virus-infected cells coated on microscope slides are revealed by fluorophore-conjugated antibodies
Antibody Targets	Whole virus or recombinant proteins	Whole Virus
Advantages	-Rapid, a few hours -Inexpensive -Commercially available -Sensitive -Can detect both IgM and IgG -Can be performed outside BSL-3 facility	-Fast: 1-2 days -Available Commercially -Differentiation between IgM and IgG -More specific than ELISA -Can be performed outside BSL-3 facility
Disadvantages	-Less specific than VNT -Cross-Reaction with other flaviviruses	-Less specific than PRNT -Cross-Reaction with other flaviviruses
Applications	Screening method: requires additional confirmatory testing	Investigation of clinical cases

Adapted from: Beck, C., Jimenez-Clavero, M. A., Leblond, A., Durand, B., Nowotny, N., Leparc-Goffart, I., … Lecollinet, S. (2013). Flaviviruses in Europe: Complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. Int J Environ Res Public Health, 10(11):6049–83.

Treatment for WNV infection

Treatment of WNV disease is supportive care. This can include supervision in the intensive care unit and use of artificial ventilation (Petersen and Marfin, 2002). Other treatments have been attempted, but with little success. One of the challenges to finding a more specific protocol is the limited number of human cases available for trials. WNV cases, particularly the neuroinvasive cases, are sporadic. This makes enrolling an adequate number of patients challenging. There have been some published results for the following treatments, but they

typically involve only a few people (CDC). Also, any therapy against neuroinvasive disease would have to pass the blood-brain barrier (Diamond, 2005).

There have been some attempts with passive antibody prophylaxis (Iyer and Kousoulas, 2013); however, treatments with intravenous immunoglobulin have produced mixed results (Beasley, 2011). Monoclonal antibody treatments have been attempted; however, differences among the WNV lineages make this approach less effective outside the lineage the antibodies were developed from (Beasley, 2011). A recombinant monoclonal antibody study of an experimental drug called MGAWN1 was conducted in 2009. The results from this study are available at ClinicalTrials.gov. Unfortunately, this study had to be terminated early due to a lack of participants. Another study was conducted in 2003 on a treatment called Omr-IgG-am. There appeared to be no differences in outcomes between the drug and placebo, and this drug is no longer available in the United States (ClinicalTrials.gov).

Treatment with interferon-alpha (IFN-α) has been shown to increase survivability in mice (Morrey et al., 2004). However, using IFN-α in humans has not been well studied. For example, one study of two people demonstrated improvement (Andre et al., 2005). However, a study of two people that showed improvement from disease following treatment when most people recover from infection with supportive care is probably not all that meaningful. The effectiveness of IFN-α is mainly against early viral replication. This is problematic with WNV, because viremia typically decreases as symptoms appear (Diamond, 2005).

Ribavirin has been attempted as a treatment for WNV infections. Ribavirin is an antiviral agent used in the treatment of viral infections. It is effective against WNV in cell culture; however, in infected animals and humans, it is not very effective (Diamond, 2005).

Mycophenolic acid is typically used as an anti-rejection drug in organ transplantations. It has been effective against WNV in culture, but not in human and animal studies. While it does show some effect against WNV, it has immunosuppressive properties that may actually increase mortality (Diamond, 2005).

RNA interference (RNAi) has also been attempted. RNAi is a natural component of cells that appears to regulate gene expression by breaking down RNA, and it may be a natural defense against viruses. It has been shown to be helpful in reducing mouse mortality if given before viral challenge (Bai, et al., 2005).

Unfortunately, in some treatments, like IFN-α, ribavirin, and RNAi, the most successful treatment attempts were given to mice in studies before viral challenge. While this may affect survivability, it is not a practical treatment regimen for an individual who is already infected with WNV. Studies of ribavirin and IFN-α in humans have not been helpful in the treatment of the virus (Petersen and Marfin, 2002).

Prevention strategies

Other than vaccination strategies that will be discussed in Chapter 3, prevention of WNV infection is primarily through vector control. Three vector control methods include environmental control, chemical control, and biological control (WHO, 2019).

Environmental control involves mechanical changes to the environment. These changes seek to remove mosquito breeding and feeding sites by installing window screens and mosquito nets, removing standing water, and individuals wearing long-sleeved clothing (CDC, 2019).

Chemical control includes using pesticides. This includes indoor and targeted outdoor spraying as well as the use of pesticides on clothing and skin when outdoors (CDC, 2019).

Pesticides can target any or all of the mosquito life stages. Some of the challenges with insecticides include preventing harm to non-target species and mosquitoes developing resistance (Benelli et al., 2016).

Biological control often involves using natural predators or bioengineered mosquitoes. These include altering adult mosquitoes to make them non-fertile so when they breed, they do not produce viable offspring. Natural predators can be utilized to reduce larval populations. These predators include fish, amphibian larva, and copepods. Additionally, fungi and bacteria can be used. These naturally produce toxins that are toxic to mosquitoes. The main challenge with biological control is preventing the release of an organism that will harm the local environment (Benelli et al., 2016).

Overall, WNV has had a major impact on human and animal health over a long period of time, and there has been improved understanding of disease outbreaks, transmission routes, disease presentation, diagnostics, and treatment strategies. The information presented in this chapter provides a link to the next two chapters that discusses some of the significant challenges of controlling this disease in the future and the importance of developing effective vaccines for disease prevention.

Chapter 2 - Challenges of Controlling Disease Outbreaks

The spread of WNV is a One Health challenge. Controlling disease outbreaks of WNV around the globe is challenging for many interconnected reasons when among humans, animals, and the environment. This chapter will focus on some of the important interrelated issues associated with the attempt to control this vector-borne disease with focus on the role of climate change. One Health recognizes that humans are part of the wider ecosystem and how individual health, population heath, and ecosystem health are all connected (Lerner and Berg, 2015). Multiple disciplines contribute to the concept of One Heath, including environmental heath, ecology, veterinary medicine, public health, human medicine, molecular and microbiology, and the economics of health (Lerner and Berg, 2015). WNV is important to approach from a One Health standpoint because it touches so many areas: it is a human and animal disease, it is controlled primarily through environmental manipulation, and the dynamics of the ecosystem determine its prevalence.

WNV as a One Health challenge

Related to One Health, globalization of the world economy and the associated movement of people and animals have led to the distribution of pathogens outside their native range. Public health entities can be unprepared when a non-native pathogen arrives in an unexpected location. WNV provides a case study into ways a pathogen can invade new territory, and why surveillance and vigilance to the unexpected is important. Possible ways for delivery of a virus out of its native location include inadvertent transportation of mosquito vectors, trade in or migration of bird hosts, and human travel of infected humans.

One of the primary hosts of WNV in North America is the American robin (*Turdus migratorius*). In one study, it was proposed that the increase in robin population due to human urbanization may have contributed inadvertently to the spread of WNV in North America (Kilpatrick, 2011). The possibility of migratory birds spreading WNV is supported by another study in North Dakota that found horse infections had a similar distribution to the concentration of migratory birds (Schuler et al., 2002).

Surveillance

Sentinel animals are often used to determine if WNV is circulating in geographical regions. Good sentinel species have three necessary characteristics: 1) they must be susceptible to infection, 2) they need to produce antibodies, and 3) they must not be killed by the infectious agent (Langevin et al., 2001).

Several sentinel animals have been tested for WNV antibodies to determine exposure. Both horses and dogs develop neutralizing antibodies to WNV, but cats do not (Schmidt and El Mansoury, 1963). Horses meet the criteria listed above, but they are poor sentinel animals because their infections do not typically precede human infections (Martin-Acebes and Saiz, 2012). Dogs are a possible sentinel animal for WNV, because they can show a higher seroprevalence rate than humans (Rocheleau et al., 2017). Chickens meet all three criteria and are used for sentinel animals in some locations (Langevin et al., 2001). Regarding other birds, the observation of a large number of dead birds in a region is considered a risk factor for human WNV infection (Mostashari et al., 2001). In particular, previous studies have shown that high levels of dead crows correlate with human WNV cases (Eidson et al., 2001).

Mosquitoes can be sampled directly for evidence of arbovirus circulation. Local public health agencies will often sample mosquito populations to screen for arboviruses. Their ability to sample mosquitoes in the region typically depends on the availability of financial resources for this program. This limitation makes the statistically preferred method of random sampling difficult, so they may be forced to focus on specific areas instead based on a limited budget. The best areas to focus on mosquito surveillance should be places where vector and host contact is expected to be high (Gu et al., 2008). An example of a comprehensive surveillance strategy is provided by Harris County, Texas. This county uses several approaches to survey for WNV. They include trapping and testing of mosquitoes, testing of both live and dead birds collected from the region, identification of human WNV cases, and serosurveillance of shelter dogs (Lillibridge et al., 2004).

Climate change

Climate change is impacting the environment and changing the global landscape. These changes include rising sea level, decreases in snow and ice, increasing rain in some areas and increasing drought in others, and intensifying cyclone activity (IPCC, 2007). Increasing environmental temperatures have also been shown to correlate with more human WNV cases (Soverow et al., 2009). This may be due to the influence of rising temperatures on WNV transmission vectors.

Increasing temperatures due to climate change have complex effects on *Culex* mosquitoes. Up to a point, higher environmental temperatures increase *Culex* development. Slight increases improve development rates, but over a threshold, they lower overall survival rates (Ciota et al., 2014). This suggests increasing global temperatures may see an expansion of

Culex mosquito range, or it may simply shift the range to locations with more favorable temperatures. This would likely move WNV out of some locations and into others.

According to Paz (2015), climate change could have the several effects as described below. Rising temperatures could increase mosquito growth rates, viral transmission efficacy, and growth rates of vector populations. Decreasing temperatures typically increase the interval between blood meals and the incubation time of the virus in the vector. A complex variable is changes in precipitation. Some mosquito vectors increase with more water and some with less water. While more water could lead to more breeding areas for mosquito vectors, less water would bring bird hosts in closer contact to vectors as both are drawn to remaining water locations in times of drought.

WNV can be transmitted vertically (mother to offspring) and can overwinter in hibernating mosquitoes (Anderson and Main, 2006). This contributes to the continued circulation of the virus. Warmer temperatures mean shorter hibernation times and more opportunities for transmission.

WNV is a challenging disease to control. Between the mosquitoes and the birds, it can travel great distances. Multi-faceted surveillance and control are necessary to understand where it is and where it may be next. Climate change continues to alter vector and host range in ways that are difficult to predict.

Chapter 3 - Vaccines and Vaccine Development for West Nile Virus

Vaccines and other preventive measures for WNV exist and have been licensed for animals, but not humans. This chapter will review currently licensed veterinary vaccines for various species and discuss new approaches for vaccine development, including some human vaccines that are either in development or currently in clinical trials. Vaccines for veterinary use and currently in use for human clinical trials will be discussed based on the type of vaccine.

Vaccines by type

In general, there are several approaches to vaccine development. Among these are inactivated virus vaccines, live attenuated virus vaccines, DNA vaccines, and recombinant viral vaccines, and chimeric vaccines (Iyer and Kousoulas, 2013). More details about each WNV vaccine will be provided below based on relevance to animals and humans. A general overview of vaccine types is further summarized in **Table 6**. Inactivated vaccines are the entire virus particle. This particle is inactivated and cannot cause disease. Because the virus does not replicate, it often does not provoke an adequate immune response by itself and requires an adjuvant, and often multiple doses. Live attenuated vaccines are a weakened version of the virus. These typically produce a strong immune response that can last many years. Rare complications with this type of vaccine include a reversion to virulence and being unsuitable for immunocompromised individuals. DNA vaccines use the body's own cells to produce the part of the virus that is targeted by antibodies. Unfortunately, these vaccines typically produce a weak immune response and often require some form of external stimulation to for uptake into the cell. Subunit vaccines are simply the immunogenic particles of the virus. These have similar concerns to inactivated vaccines. Chimeric vaccines combine virus parts into a new virus.

Sometimes the types can overlap. For example, there is a human vaccine in development that is both live attenuated and chimeric. The act of combining the two viruses attenuates the final viral particle (Dayan et al., 2013).

Table 7: Overview of Vaccine Types

Vaccine Type	Composition	Pros	Cons
Inactivated	Whole killed virus	-Easy to make -Cannot cause disease -More stable	-Short duration of immunity -Often require several doses -Typically require adjuvant
Live Attenuated	Weakened virus	-Stimulate strong immune response -Require few doses -Long duration of immunity	-Can possibly revert to pathogenic form -Not typically useable in immunocompromised individuals -Need cold chain
DNA	Only the portion of viral genome that makes the antigen	-Produces antigen with body's own cells	-Needs external stimulation to be taken up by host cells -Weak antibody response
Subunit	Immunogenic particles: often surface proteins	-Lack significant part of the virus genome and cannot replicate in the host	-Typically require multiple doses
			(Milligan and Barrett, 2015)
Chimeric	Parts of different viruses are combined	-The use of an established vaccine can be a shortcut to a new vaccine	-Can cause unexpected issues from the host virus
			(Iyer and Kousoulas, 2013)

Adapted from: Milligan, G., and Barrett, A. (2015). Vaccinology: An Essential Guide. Wiley Blackwell; Saadatian-Elahi, M., Aaby, P., Shann, F., Netea, M.G., Levy, O., Louis, J., et al. (2016). Heterologous vaccine effects. Vaccine, 34:3923–30; Iyer, A.V., Kousoulas, K.G. (2013). A review of vaccine approaches for West Nile virus. Int J Environ Res Public Health 10(9):4200–23.

Heterologous vaccine effects

While not an actual vaccine, heterologous vaccine effects are important to consider. Heterologous vaccine effects are the "off-target" effects of a vaccine (Saadatian-Elahi et al., 2016). The important effect in this instance is how a flavivirus vaccine in the same family may

modulate the response to natural WNV infection or immunity by allowing the body to recognize the disease based on a similar antigenic stimulation by another disease it has already been exposed to. Because the viruses are related, attempts have been made to determine if there is a mechanism of cross protection for WNV from the use of DENV and JEV vaccines. Overall, these attempts have been unsuccessful (Iyer and Kousoulas, 2013).

Unfortunately, there can be negative heterologous effects as well, and this is particularly evident in DENV and antibody-dependent enhancement of disease. Antibody-dependent enhancement of disease is when existing antibodies enhance the uptake of viral particles by the host cell and cause severe disease. While this process is not well understood, a subset of people will have more severe symptoms after their initial infection because of this (Rothman, 2011).

Veterinary vaccines

Alligator

Boehringer Ingelheim Vetmedica, Inc. also produces a vaccine for alligators, which the USDA apparently classifies as "fish" for the purposes of regulation (USDA, 2019). This vaccine is formalin-inactivated whole virus. It requires two doses to be effective (USDA, 2019). This vaccine does not appear to be commercially marketed and has no trade name.

Equine

This section will focus mainly on equine vaccines, as this is the largest market and most studied. The horse vaccines are available singly or in a multivalent product. A list of equine

vaccines is available in **Table 8** that provides information on the manufacturers and the different vaccine types they produce.

Inactivated vaccines

Inactivated vaccines have the advantage of being uncomplicated to produce, but they typically require revaccination yearly, or more, due to their short duration of protection (Milligan and Barrett, 2015). Most available WNV vaccines are inactivated.

DNA vaccines

DNA vaccines have been developed for crows and horses. There was a DNA vaccine available for horses for a short time (Iyer and Kousoulas, 2013), but it was discontinued for undisclosed reasons. A similar version has been developed for potential human use (Amanna and Slifka, 2014).

Chimeric viral vaccines

The PreveNile vaccine developed by Intervet was briefly recalled. This was due to an allergic reaction to a stabilizer. It was later re-released without the allergenic ingredient (Dayan et al., 2013), but appears to be currently unavailable.

Table 8: Equine WNV Vaccines

Commercial vaccine name	Manufacturer	Vaccine type	Antigen	Doses	Immune duration
West Nile Innovator	Ft. Dodge Zoetis Inc./Pfizer	Whole inactivated	WNV	2	Annual or more often
West Nile-Innovator DNA (discontinued)	Ft. Dodge Zoetis Inc./Pfizer	Plasmid DNA	Premembrane protein and envelope protein	2	Annual or more often
Vetera	Boehringer Ingelheim	Whole inactivated	WNV	2	At least a year
Recombiteck (discontinued after buy-out?)	Merial (Boehringer Ingelheim)	Canarypox expressing PrM/E	Premembrane protein and envelope protein	2	Annual
PreveNile (recalled and re-released)	Intervet (Merck)	Live attenuated YF17D backbone expressing WNV PrM/E	Premembrane protein and envelope protein	1	Annual
EquiNile	Intervet (Merck)	Formalin inactivated YF17D backbone expressing WNV PrM/E	Premembrane protein and envelope protein	2	Annual or more often

Adapted from: Brandler, S., and Tangy, F. (2013). Vaccines in development against West Nile virus. Viruses, 5(10):2384–2409; Dayan, G. H., Pugachev, K., Bevilacqua, J., Lang, J., and Monath, T. P. (2013). Preclinical and clinical development of a YFV 17 D-based chimeric vaccine against West Nile virus. Viruses, 5(12);3048–70.

Human vaccines

Human vaccines in development

While no WNV vaccines are available for humans, there are several currently in development. Additionally, there have been other vaccines developed for other flaviviruses. YFV and JEV have vaccines that work well. The DENV vaccine has been problematic. In unexposed individuals, it actually increases the risk of severe illness. However, in individuals

already exposed to one type, it does protect against the other three types without increased risk of adverse effects (WHO, 2019).

One of the largest barriers to developing a WNV vaccine in humans is the cost-benefit analysis. Mathematical modeling was performed to estimate the costs associated with vaccination. In general, it was more cost-effective to vaccinate older people than younger ones. Targeted vaccination of older people was found to be the most cost-effective option (Shankar, 2017).

Human vaccines by type

Inactivated vaccines

There are two inactivated human vaccines in development. One is inactivated with hydrogen peroxide, and one is inactivated with formaldehyde. Both vaccines require multiple doses, with the hydrogen peroxide vaccine requiring two doses to achieve neutralizing antibodies in 50% of participants (Ulbert, 2019).

Recombinant viral vaccines

A study was done on the possibility of using a recombinant measles vaccine for a human WNV vaccine. Vaccine efficacy in a primate model was demonstrated, but there were some concerns about using measles as a carrier. These concerns mainly involved the possibility of previous measles vaccination interfering with the effectiveness of this recombinant vaccine (Brandler et al., 2012).

Human vaccines in clinical trials

The development of a WNV vaccine for humans has not been prioritized. This is likely because disease outbreak is sporadic and neuroinvasive disease is rare. However, WNV vaccines have been developed and are currently in clinical trials. While most humans do not exhibit symptoms for WNV infection, it would be beneficial to have a vaccine for the elderly and immunocompromised individuals.

There are a few WNV vaccines in clinical trials. Clinical trials have 4 phases that lead to the approval and continued oversight of new biologics. An overview of clinical trial phases is described in **Table 9.** Phase 1 trials are used to determine the safety of a potential new drug on humans. This phase typically enrolls 20-80 people. Phase 2 trials are used to determine the dosing, method of delivery, and effectiveness in a target group of humans. These trials typically have 80-300 people. Phase 3 trials are used confirm efficacy and safety in a large group of patients. A sample of 300-10,000 patients is more likely to show rare events that would not be noticeable in a smaller sample. Phase 4 trials are used to continue to evaluate efficacy and safety after a biologic has been licensed (Milligan and Barrett, 2015).

Table 9: Clinical Trial Phases

Clinical Trials				
Phase	**1**	**2**	**3**	**4**
Purpose	-Assess safety of new drug -First time drug is used on humans -Determine type and extent of immune response	-Examine safety and effectiveness in a targeted disease group -Determine: -Safety profile -Immunogenicity -Dose regimen -Method of delivery	-Confirm efficacy and safety in a large patient group	-Post licensure efficacy -Extended safety -Look for rare side effects -Duration of protection -Effects on herd immunity
Area of Study	-How the drug moves in the body -How the drug works in the body	-Results of various doses	-Looking for events that may not be evident in a smaller sample size	-Continued evaluation of safety and efficacy
Number of Individuals	20-80	80-300	300-10,000	10,000+
Locations	One	Typically One	Several	Several

Adapted from: Milligan, G. and Barrett, A. (2015). *Vaccinology: An Essential Guide*. Hoboken, New Jersey. Wiley Blackwell.

Human vaccine candidates are summarized in **Table 10** and described below. rWN/DEN4Δ30 is a chimeric attenuated WNV vaccine that uses an attenuated strain of DENV with WNV proteins. This vaccine was tested in 28 volunteers who were 50-65 year old (Pierce et al., 2017). While the sample size is small, all participants were in the at-risk age group. There appeared to be no adverse effects related to the vaccine and the seroconversion rate was 95% after a single dose (Pierce et al., 2017). Also of interest, this chimeric vaccine has shown the ability, although limited, to reproduce in birds and mosquitoes (Pierce et al., 2017). This raises some ethical questions about the possibility of introducing a new virus into the world.

HydroVax-001 is a hydrogen peroxide inactivated vaccine. It was in a Phase 1 trial with 96 subjects receiving either the vaccine or a placebo. There were no serious adverse events, but

the seroconversion rate was not high. Two dosing volumes were tested, 1mcg and 4mcg. The 1mcg dose had a seroconversion rate of either 0% ($PRNT_{50}$) or 41% (ELISA) after the second dose, and the 4mcg dose had a seroconversion rate of either 31% ($PRNT_{50}$) or 75% (ELISA) after the second dose (Woods et al., 2019).

VRC-WNVDNA020-00-VP is a DNA vaccine for WNV that was tested in a Phase 1 trial. It is a plasmid that produces the E membrane protein and premembrane protein and was produced in bacterial culture. The study included 30 adults with a mean age of 44 years old. There were no vaccine-related adverse effects. A neutralizing antibody response was detected in 96.6% of the subjects after 2 doses (Ledgerwood et al., 2011).

ChimeriVax-WN02 is a chimeric vaccine for WNV in Phase 2 clinical trials. This vaccine is very similar to both the equine version of the vaccine and the YFV vaccine because it uses the same YFV vector (Dayan et al., 2013). This vaccine was given to 498 participants across the United States. The age groups were nearly evenly split between 18-65 and 65+ with an average age of 63. Participants were given either a low, medium, high, or placebo dose once. The most common adverse effects were headache and fatigue. Seroconversion was greater than 90% by day 28 in the vaccine group (Clinicaltraials.gov, 2019).

Table 10: Human WNV Vaccine Candidates

Human WNV Vaccine Candidates				
Vaccine Candidate	**Antigen**	**Vaccine Type**	**Clinical Trial Phase**	**Notes**
ChimeriVax -WN02	Membrane Protein E and premembrane protein	Live attenuated chimeric -yellow fever virus	One Phase 1 Two Phase 2	Neutralizing antibodies in >90% of individuals after one dose
rWN/DEN4 Δ30	Membrane Protein E and premembrane protein	Live attenuated chimeric -Dengue type 4	1	Neutralizing antibodies in 89% of individuals after two doses
VRC-WNVDNA020-00-VP	Plasmid expressing the membrane Protein E and premembrane protein	Plasmid DNA	1	Neutralizing antibodies in >90% of individuals after two doses
HydroVax-001	Whole WNV virion	Inactivated with hydrogen peroxide	1	Neutralizing antibodies in 50% of individuals after two doses

Adapted from: Brandler, S., and Tangy, F. (2013). Vaccines in development against West Nile virus. Viruses, 5(10):2384–2409; Kaaijk, P., and Luytjes, W., (2018). Are we prepared for emerging flaviviruses in Europe? Challenges for vaccination. Hum Vaccin Immunother, 14(2):337–344; Ulbert, S. (2019). West Nile virus vaccines – current situation and future directions, Hum Vaccin Immunother, Jul 10:1-6.

Vaccines come in a variety of types, but most WNV vaccines are killed. There are several animal vaccines, but none for humans. There are several potential candidate vaccines for human WNV vaccines, but none have passed clinical trials. While there is not economic incentive for a general human WNV vaccine, there is a need for one for older adults.

Chapter 4 - Conclusions

WNV is a continuing threat to human and animal health. While there are many important flaviviruses, WNV has the broadest impact worldwide among both animals and humans. In less than 100 years, it has gone from unknown to a worldwide disease.

WNV has been circulating globally for at least 150 years, and it continues to be a problem for both humans and animals. With no specific treatment and limited vaccine options, there is a huge deficiency in management of WNV illness. Vector control is the best way to control WNV, but it still has major human health impacts. Vaccine development for both humans and animals is ongoing. This is important because WNV will likely continue to cause illness in the foreseeable future.

WNV does not typically cause disease in humans, but when it does it can be severe. Older humans are particularly at risk. There has been some progress to producing a vaccine, but it has been slow. Vaccinating young people is probably not necessary, but a WNV vaccine could reduce morbidity and mortality in older people. Another point to consider is the different WNV lineages because some appear to be more virulent than others. If a more virulent strain moves into a populated area, it could significantly increase mortality.

WNV vaccination is a multi-faceted issue. Vaccination for animals is both available and economical, but WNV vaccination for humans is tricky. Because WNV rarely causes disease in humans, it is not a priority for vaccination development. Also, flavivirus vaccines can be challenging. While JEV and YFV have effective vaccines, DENV has a complicated one. DENV does have 4 serogroups, so it may be exceptional in this regard, but the fact that WNV has different disease-causing lineages seems to be a problem. It is unknown if WNV would have disease enhancing problems with antibodies from another lineage. WNV has a greater impact on

animals than humans. It causes significant mortality in birds, horses and alligators. The economic impact of wild bird deaths is difficult to measure. WNV may be a primary reason for death and decline in some species, or just another point of pressure to push a species into severe decline. While the economic impact of the loss of game bird hunting revenue can probably be measured, the impact on biodiversity has unknown repercussions. Horses are particularly sensitive to WNV, with 40% or more horses dying after they show symptoms of disease. This has a significant economic impact both cost of treatment and prevention. Vector control and vaccination continue to be a significant expenditure for horse owner. WNV is a significant problem for alligators. It has the potential to directly or indirectly kill large numbers of young animals, which causes significant economic loss to farmers. Additionally, alligators may not be a dead-end host and may actively spread the disease. Addressing this possibility appears to be an underappreciated risk when attempting to control the disease.

It is impossible to predict what climate change will do to the range of WNV, but some educated guesses can be made. As the planet warms, it provides greater opportunity for the expansion of this disease. Warmer temperatures generally increase vector fecundity and transmission, but only to a point. Because WNV can infect a few different mosquitoes, it will probably have the opportunity to increase its range.

WNV can be challenging to study. It is impractical to have infected mosquitoes biting experimental animals in a laboratory setting. This necessitates the inoculation of animals manually. The manual inoculation removes what may be critical components in the mosquito's saliva that may affect infection. The BSL-3 status of WNV also requires a specialized biosecurity environment. This imposes some practical limits on the types of animals that can be studied. For example, getting a horse or an alligator into a BSL-3 lab is considerably more

difficult than using rodents or small monkeys. Another challenge to studying WNV is the sporadic nature of serious disease. It is difficult to obtain a large enough group of affected individuals to investigate new treatments.

WNV can be difficult to detect, but there are good options for this. There are commercially available assays for screening that require confirmation with PCR. The most challenging aspect of WNV detection is that some tests require utilization of a BSL-3 lab.

WNV prevention is only through vector control. Mosquitoes carry a variety of diseases; so many municipalities have mosquito control measures in place. Often all that is needed is to add WNV specific surveillance to these efforts.

Because WNV affects so many animals, it is a One Health issue. It is necessary for animal and human health agencies to work together. This has been difficult in the past, but fortunately, this is changing.

WNV poses many challenges, but they are slowly being addressed. There is a need for a vaccination in older humans, but severe disease is fortunately rare. While the horse vaccination is effective, it only provides protection for a year. Hopefully some of the newer vaccinations will provide a longer duration of immunity that can bring down the economic costs. The alligator vaccine appears to work well, but it likely only lasts a year. Vaccinating alligators is challenging, but they are typically harvested at less than 4 years, and they seem to be less likely to die as they get older. The inactivated vaccine for alligators may be adequate as it is. There is a real need for a bird vaccine. Some bird owners have been vaccinating with the horse vaccine in an attempt to protect their birds. The effects of this are unknown.

It is important to continue surveillance, especially with the continuing warming of the planet. It will also be important to continue to try to understand the differences in the lineages and how they may be more or less dangerous.

In our global world, it is important to recognize how everything is connected and how all health agencies need to work together to recognize and control disease. If we fail to communicate quickly and efficiently across agencies, we could miss a more serious disease threat in time to stop it from doing significant harm. In closing, the following quote from author R.S. Desowitz (2002) illustrates the importance of this:

> "In comparison, we are fortunate that it was the relatively benign West Nile virus that was America's trial run for out readiness to diagnose and contain the unexpected pathogen. How then should we grade our medical watchdogs? For the report card, let me synoptically run the New York West Nile story by you…and you be the judge. There is retrospective evidence that the first bird death occurred on or about May 21 and the first clinically significant human case about eight weeks later. About two weeks later, an alert hospital physician and veterinary pathologist independently called attention to what were considered separate outbreaks in birds and humans. Conclusive identification of the West Nile virus was made during the third week of September, *four months from the death of the first bird*, and one and a half months from the first professional observation of what was happening to birds and people…is this any way to run a business that deals with protecting the public's health?"

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