

How is West Nile Virus Effecting the United States?

By: Laura Koscomb

Submitted to:
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Program Director
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Abstract

West Nile Virus (WNV) is a sometimes-fatal flavivirus that first appeared in the US in 1999. When WNV appeared in our country not only was there a human outbreak but there was also an equine outbreak in the eastern half of the country. There have now been outbreaks of WNV in all states in humans, and the majority has had equine outbreaks as well. At this time there is no vaccine against WNV for humans, however there are two vaccines for horses. If an epidemic occurs there will be no vaccine to help prevent the virus and this could possibly become out of control. Research on this virus is undeniably important because of the threat that faces our country if there is an outbreak of this dangerous virus.

Introduction

West Nile Virus (WNV) is a new threat to our country, and it is not only a threat to humans but also horses and birds. West Nile virus was first isolated in 1937 in a woman from the West Nile region of Uganda (Background: Virus History and Distribution, 2004). In the late 1950's this virus became recognized as a severe cause of meningitis in Israel. Meningitis is the inflammation of the brain and spinal cord and is most often times fatal (Meningitis, 2006). There were not many outbreaks of West Nile until 1994 when one occurred in Algeria. Before 2003, there were six other outbreaks, including the United States from 1999-2003, which included both human and equine cases. The first reported cases of equine West Nile were recorded around 1960 in Egypt and France and has since led to an outbreak in Morocco in 1996, Italy in 1998, the United States in 1999, and France in 2000. The research on West Nile in horses really began to gain interest once the virus came to the United States and has recently made many advances. There have been two vaccines developed for horses and also advances in the treatment of horses once they acquire this virus. Since viruses are always changing it is difficult to keep up with the new strains, but the vaccine progress appears to be working. As of now West Nile has been positively identified in either a human, avian, or equine subject in all of the states except for Oregon, Alaska, and Hawaii (Background: Virus History and Distribution, 2004). Research on this virus is undeniably important because of the threat that faces our country if there is an outbreak of this dangerous virus.

WNV is from the Family Flaviviridae and Genus Flavivirus Japanese Encephalitis Antigenic Complex. A flavivirus is from the family flaviviridae but is also known as a group

b arbovirus, which contain several subgroups and species (Flavivirus, 2006). Ticks or mosquitoes transmit most flavivirus'. In this case West Nile is transmitted by mosquitoes and most commonly by *Culex pipiens*, which are also known as the northern house mosquito. These mosquitoes are known the best for transmitting St. Louis encephalitis (SLE), and are found mostly in suburban places especially where there is polluted or standing water (Crans, n.d.). They are also becoming the most common mosquito to transmit WNV to birds, which are the main carriers of WNV. However humans and horses are the dead end hosts or the accidental carriers.

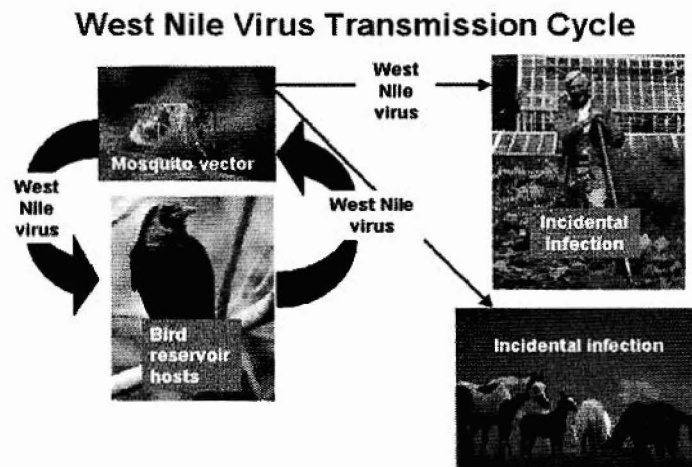


Figure 1. West Nile Virus Transmission Cycle. This is the cycle in which WNV is supposed to follow, but since mosquitoes also bite humans and horses they too are infected. (www.cdc.gov/ncidod/dvbid/westnile/cycle.htm)

West Nile is really only supposed to be going from birds to mosquitoes but unfortunately *Culex pipiens* also bites humans and horses which causes the virus to spread to them also (See Figure 1). This virus is not contagious from person to person and from horse to horse with the exception of a blood transfusion or some other form of blood-to-blood contact. However WNV is still thriving in the eastern part of the United States and

precautions must be taken to protect people from this. This includes not just getting rid of standing water in people's yards and wearing protective clothing along with not going out at dawn and dusk in the summer months. A vaccine needs to be developed for people especially since the results of this virus can be so devastating. This virus causes swelling of the brain and spinal cord so even if the patient does recover there is a chance that they will have paralysis or permanent neurological disorders. Even though only about one in every one hundred-fifty people will develop a severe illness from this, the United States was lucky that the strain was not horribly devastating this year and a vaccine should be developed not when a really bad outbreak happens (National Institute of Allergy and Infectious Diseases, 2000).

A lot of research has been done already on WNV, especially from 1999, because that is when WNV appeared on the east coast. There has been a lot of testing done on the mosquitoes that transmit this virus and also on the isolation of WNV. For example there was a study done from 1999-2003 about the epidemiology of WNV. In this study mosquitoes were trapped over the state over five years and the results were compared and contrasted (Andreadis, 2004). The results were very interesting because this virus spread across the state starting in Fairfield County at the New York border all the way into Windham County. After the mosquitoes were collected they went through a series of testing such as reverse transcription-polymerase chain reaction (RT-PCR) and gene amplification. The results helped scientists realize the genetic variations from the different sample and then they figured out what these modifications mean (Andreadis, 2004).

Another side of WNV research is the genetic approach to the virus. Scientists at the Connecticut Agricultural Experiment Station, in New Haven, are doing a large amount of work on this subject in Connecticut although research is also being done elsewhere. In this

experiment 82 isolates from Connecticut were identified from nine species of birds, five species of mosquitoes, and one striped skunk. The virus was then identified through RT-PCR analysis and then compared to WN-NY99, which is the strain of WNV from New York (Anderson, 2001). The nucleotide sequencing was compared and 30 genetic changes were identified between the WN-NY99 and the samples obtained from the birds, mosquitoes, and the skunk (See Figure 2).

Reverse transcriptase-PCR sequences of isolates that differ from WN-NY99

Isolate no.	Host	Sequence (passage number)	Sequence (passage number)
1	Crow	1032 T to C (P ₁)	1032 T to C (P ₃)
2	Crow	721 C to T, 905 G to A, 940 T to C (P ₁)	721 C to T, 905 G to A, 940 T to C (P ₃)
4	Crow	673 A to G (P ₁)	673 A to G (P ₃)
6	<i>Cx. Pipiens</i>	933 C to T, 1047 C to T (P ₀)	933 C to T, 1047 C to T (P ₂)
11	Crow	456 C to T, 516 T to C (P ₀)	456 C to T, 516 T to C (P ₂)
12	Cooper's hawk	867 T to C (P ₀)	867 T to C (P ₁)
15	Crow	381 C to T, 711 C to T (P ₀)	381 C to T, 711 C to T (P ₂)
18	Crow	528 T to C, 543 A to G (P ₀)	528 T to C, 543 A to G (P ₃)
33	Blue jay	None (P ₀)	None (P ₁)
46	<i>Cx. restuans</i>	None (P ₀)	None (P ₂)
57	Canada goose	858 C to T (P ₀)	858 C to T (P ₁)
64	Striped skunk	795 T to C, 812 A to G, 858 C to T (P ₀)	795 T to C, 812 A to G, 858 C to T (P ₁)
69	<i>Cs. melanura</i>	726 A to G, 858 C to T, 951 G to A (P ₀)	726 A to G, 858 C to T, 951 G to A (P ₂)

Figure 2. Reverse transcriptase-PCR sequences of isolates that differ from WN-NY99. Comparison of 921-nt sequence (genome positions 205-1125) of WN-NY99 to Connecticut West Nile virus isolates in different passages in Vero cell culture. (<http://www.pnas.org/cgi/content/full/98/23/12885/T2>)

The majority of the changes occurred in the 921-nt region of the viral region beginning at nucleotide position 205 and ending at 1125. Once these results were compared there was a sort of clustering of 26 isolates that was found to have originated in Stamford, Connecticut (See Figure 3).

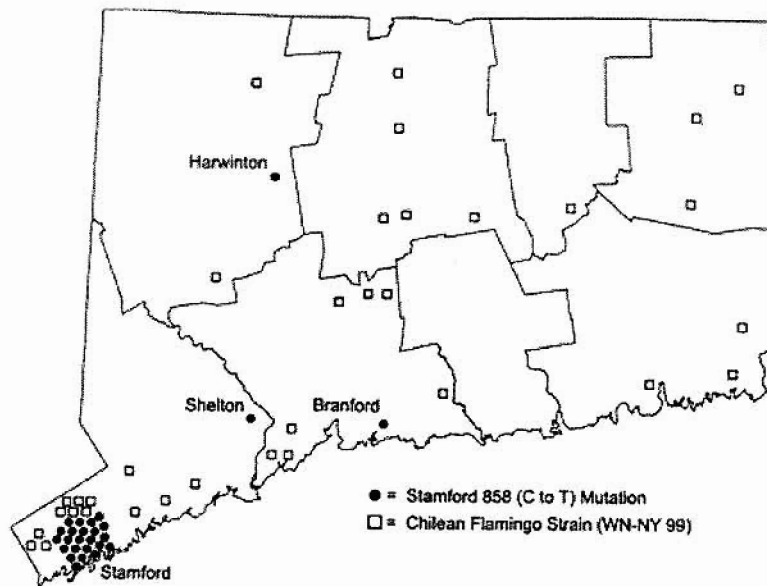


Figure 3. Map of Connecticut Identifying Towns with Positive West Nile Virus. Map of Connecticut showing county boundaries and the distribution of WN virus isolates with sequences identical to WN-NY99 (□) and isolates containing the C to T mutation at genome position 858 shown in the majority of the isolates from Stamford (●). (<http://www.pnas.org/cgi/content/full/98/23/12885/F2>)

Thus the results were strains were retested but the same results were concluded and it has been found that these results are totally valid and the mutations are not related to mistakes from culturing, RNA extraction, or PCR procedures. After the conclusion of this experiment it was found that WNV is adapting to North America and it will continue adapting which is making the virus harder to follow (Andreadis, 2001).

It is almost impossible to completely stop a virus from moving and mutating which makes the work of scientists much more difficult. In order for scientists to develop a vaccine against a virus they try to find out if the virus follows a certain pattern in its' mutations. If a pattern can be identified then the scientists will be able to create a vaccine that directly stops that strain of the virus. So far scientists have not been able to develop a vaccine for WNV that is ready to be used in humans; however two vaccines have been created for horses.

One of these vaccines is made by Fort Dodge and is called West Nile Innovator. This was the first vaccine to be approved for use in horses and is an attenuated version of WNV so it is almost impossible for the horse to contract WNV from this vaccine. This vaccine is administered intramuscularly where the first dose is given and then three to six weeks later the second dose is given. Not only does this vaccine protect against WNV but it can also be produced in combinations that protect against other viruses such as Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis (WEE), and Venezuelan equine Encephalomyelitis (VEE). It can also be produced to include Tetanus in the vaccine (West Nile Virus, 2006).

The other vaccine that has been created for horses is called Recombitek and it is produced by Merial. In this vaccine instead of having an attenuated version of WNV, the virus is being administered through a vector of Canary Pox. Neither the Canary Pox nor the WNV can replicate inside the horse because there is only a part of the WNV virus injected into the Canary Pox. This vaccine is also effective because it works very quickly and has also been proven effective in foals and adult horses.

Both vaccines have been proven effective and there are more that are still in clinical testing, which could make the choice of veterinarians and horse owners more difficult

because there are going to be so many vaccines now. Producing these vaccines is very difficult because West Nile Virus is considered to be a Bio Safety Level Three (BSL 3) type of virus, which means that a person must be specially certified along with other specific qualifications (See Figure 4).

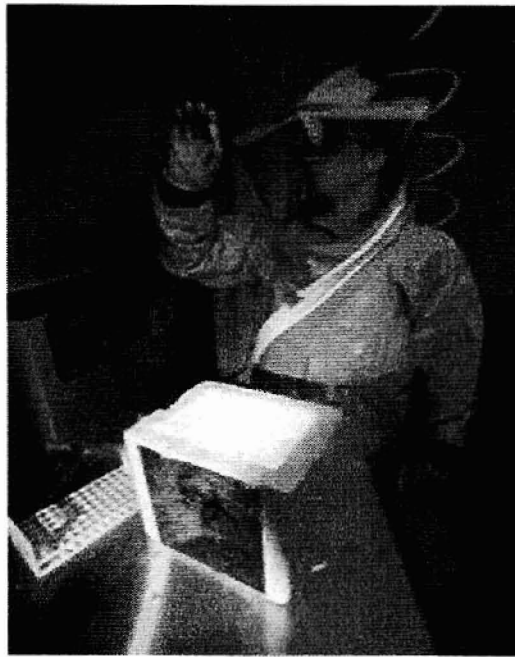


Figure 4. A scientist looking at WNV in a BSL 3 laboratory. In order to work with WNV a person must go through special training to be able to work with dangerous viruses, along with taking special precautions. (<http://www.cannabisculture.com/forums/uploads/822096-WestNile1.jpg>).

So far the more molecular side of WNV has been discussed, however there is another entire side of WNV, and that is called fieldwork. There would be no advances made in WNV without scientists that are working on collecting mosquitoes, birds, and other WNV positive animals. There are two different ways to trap mosquitoes and which method is used depends on which mosquitoes are desired. For example, in a study done by scientists, they were attempting to find *Culex pipiens* so they decided to use a different kind of trap that attracts mostly *Culex pipiens* (American Mosquito Control Association, 2005).

However this kind of collection is for the mosquitoes that are already fully-grown and flying in the air, there is also another kind of collection. This collection is for mosquitoes that do not usually carry infectious diseases and they are found in vernal pools. In Connecticut the definition of a vernal pool is, "Vernal Pools are small bodies of standing fresh water that are most obvious in the landscape during the spring of the year. They are usually temporary in nature. In order to meet the definition of a vernal pool, a wetland must have the following physical characteristics: (1) it contains water for approximately two months during the growing season (2) it occurs within a confined depression or basin that lacks a permanent outlet stream (3) it lacks any fish population (4) it dries out most years, usually by late summer."(What is a Vernal Pool?, n.d.) When doing mosquito collection from vernal pools traps are not needed because the mosquitoes have not yet developed wings so they submerged in the water of the vernal pool. So to collect these vernal pool dwelling mosquitoes a dipper is used, otherwise known as a long pole with a small cup on the end, and the person can either stand on the side of the pool or in the pool and collect samples. Once the dipper has been submerged under the water bring the cup back and inspect the cup for mosquitoes and other creatures that live in the vernal pool; larval mosquitoes can be identified by their distinct shape (See Figure 5).



Figure 5. Mosquito collection in a vernal pool. This shows how when the person dips the dipper into the vernal pool and then brings it back to see if it is a good sample. A good sample is considered to have a good amount of mosquitoes along with some other organisms that are common in vernal pools.

(http://www.sanctuary.org.nz/research/projects/images/profile0006/amy-snell-mosquitoes_clip_image002_0001.jpg)

Larval mosquitoes have a long body with a siphon that is shaped like a 'y' on the end and also a small black head that is at the bottom. It seems as if the mosquitoes are floating upside-down because their siphon is at the top near the water and their head is the lowest point in the water, however the siphon must be at the top of the water in order for the mosquitoes to receive oxygen. (See Figure 6).

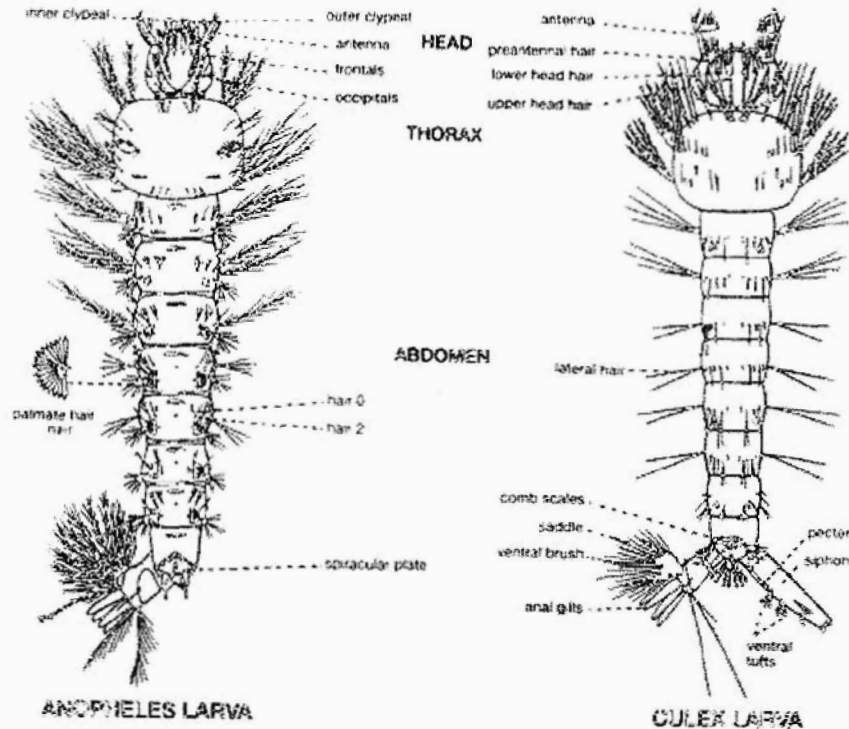


Figure 6. Diagram of mosquitoes in their larval stage. Pictures like these are often used for the identification of the mosquito larvae once they are collected. That is why these pictures are so detailed and specific.

(<http://www.tuscolacounty.org/mosquito/images/larvadig.gif>)

In this picture the siphons are facing down, however if these mosquitoes are found in vernal pools the picture is flipped over. Once the mosquitoes have been collected they are usually kept in the water they were collected in for a while until they can be separated from the other organisms from the vernal pool. Then they will most likely be put into a strong alcohol so preserve them and then they will be identified and the results will help scientists keep track of the mosquito population.

Since the arrival of WNV in the US in 1999 many advances have been made in tracking WNV along with the development of vaccines for WNV. In the future there will be

more advances made and from those advances there could possibly be a WNV vaccine for humans or even a way to keep this virus from spreading.

References

- American Mosquito Control Association. (2005). *Traps*. Retrieved June 8, 2006 from <http://www.mosquito.org/mosquito-information/traps.aspx>.
- Anderson, John F, et al. (2001). A phylogenetic approach to following West Nile virus in Connecticut. *PNAS*, 98, 12885-12889.
- Andreadis, Theodore G, et al. (2004). Epidemiology of West Nile Virus in Connecticut: A Five-Year Analysis of Mosquito Data 1999-2003. *Vector-Borne and Zoonotic Diseases*, 4, 360-378.
- Crans, Wayne J. (n.d.). *Culex Pipiens: The Northern House Mosquito*. Retrieved November 17, 2005 from <http://www.rci.rutgers.edu/~insects/cxpip.htm>.
- National Institute of Allergy and Infectious Diseases. (2000). *NIAID Research on West Nile Virus*. Retrieved December 2, 2005 from <http://www.niaid.nih.gov/factsheets/westnile.htm>.
- No author. (2004). *Background: Virus History and Distribution*. Retrieved December 6, 2005 from <http://www.cdc.gov/ncidod/dvbid/westnile/background.htm>.
- No author. (2006). *Flavivirus*. Retrieved December 10, 2005 from <http://en.wikipedia.org/wiki/Flavivirus>.
- No author. (2006). *Meningitis*. Retrieved December 10, 2005 from <http://en.wikipedia.org/wiki/Meningitis>.
- No author. (2005). *Prevention*. Retrieved November 12, 2005 from http://www.michigan.gov/emergingdiseases/0,1607,7-186-25805_25823-75771--,00.html.
- No author. (2004). *Recombitek*. Retrieved May 10, 2006 from <http://www.equinewnv.com/>.
- No author. (2006). *West Nile Virus*. Retrieved May 10, 2006 from <http://www.agdepartment.com/Programs/Livestock/BOAH/WestNile.htm>.
- No author. (n.d.). *West Nile Virus: Laboratory Evaluation*. Retrieved November 9, 2005 from <http://www1.umn.edu/eoh/hazards/hazardssite/westnilevirus/labeval.html>.
- No author. (n.d.). *What is a Vernal Pool?*. Retrieved June 8, 2006 from <http://www.uri.edu/cels/nrs/paton/whatisavp.html>.