Spring 2012

Investigation of West Nile Virus Infection Rates in Cx. tarsalis at Medicine Lake Wildlife Refuge and Ninepipe Wildlife Refuge 2011

Joseph Maricelli
Carroll College, Helena, MT

Investigation of West Nile Virus Infection Rates in *Cx. tarsalis* at Medicine Lake Wildlife Refuge and Ninepipe Wildlife Refuge 2011

Honors Thesis
Carroll College
Department of Natural Sciences
Helena, Montana

Joseph W. Maricelli
4/1/2012

This thesis for honors recognition has been approved for the Department of Natural Sciences by:

Dr. Samuel Alvey (Thesis Advisor)	Date
Dr. Grant Hokit (Reader)	Date
Dr. Jamie Dolan (Reader)	Date

Acknowledgements

I would like to thank my thesis advisor Dr. Samuel Alvey as well as the other advisors in the Carroll College West Nile Virus Project, Dr. Grant Hokit and Dr. Jennifer Geiger for their guidance and opportunity to conduct this research. Thanks to all past and present West Nile Virus Project interns for aiding me in the completion of this thesis. I would especially like to thank Kellie Kalbfleisch, Paige Esposito, and Max Bernt who played vital roles assisting me with protocols and trapping. Brian Murphy was vital to mosquito trapping, as well as, provided a figure for this thesis. I acknowledge Montana INBRE (grant P20 RR16455-09 to Dr. Grant Hokit) for funding my research. Special consideration must be given to the state of Montana as well for additional funding and collaboration on viral detection. I wish to thank Dr. Gerald Shields whose mentoring and continued support made this paper possible. Lastly I would like to thank all my family and friends who have loved and supported me throughout this entire process.

Table of Contents

Acknowledgements……..iii

Abstract……v

List of Tables……vi

List of Figures……..vii

Introduction……1

Methods and Materials……5

Results……..10

Discussion……14

Literature Cited……17

Abstract

This study focused on the infection rate of West Nile virus in two *Culex tarsalis* mosquito populations in Montana. Medicine Lake Wildlife Refuge (MLWR) and Ninepipe Wildlife Refuge (NWR) *Cx. tarsalis* populations were chosen due to the variance in past viral incidence. Viral incidence at MLWR was thought to be higher due to higher infection rate in the *Cx. tarsalis* population possibly from favorable environmental factors. This study suggests, however, that high viral incidence may be a result of high *Cx. tarsalis* population as a proportion of the total mosquito population. Samples were homogenized and purified for RNA using a series of centrifugations through a specialized filter. A RT-PCR and Taqman assay then determined viral presence. No positive samples were collected from either collection site; a pooled infection rate program utilizing a 95% confidence interval determined there was no statistical difference in infection rates. Future studies can build upon this research by increasing sample number, recording differing temperatures and precipitation levels at both sites, and incorporating a temporal study in addition to the spatial analysis.

List of Tables

Table 1. Ninepipe Collection Dates……..…..7

Table 2. Mosquito Totals at Medicine Lake and Ninepipe…….……10

Table 3. Number of Extractions by Date and Site at Medicine Lake……..11

Table 4. Comparison of Infection Rates Percent…….12

Table 5. Comparison Infection Rate at MLWR and NWR 2009-2011……13

List of Figures

Figure 1 Map of Collection Sites (Courtesy of Brian Murphy)……...……6

Introduction

West Nile Virus (WNV) was first introduced to the United States in 1999, and by 2004 had spread to the entire continental U.S. (Brault et al. 2009). The speed at which the virus spread leads to concern for the health risks to humans, as well as equine and avian populations, which are most adversely affected by the disease (Brault et al. 2009). In an attempt to elucidate methods of transmission and risk assessment, the Centers for Disease Control and Prevention (CDC) has set up a nationwide WNV activity report which can be examined by state and county to help with preventative measures (Gubler et al. 2003). My study's aim is to help expand and strengthen current risk assessment models for the transmission of WNV by better understanding mosquito infection rates throughout the state of Montana.

WNV is an arbovirus from the family *Flavivirdae* which is part of the Japanese encephalitis complex (Marra et al. 2004). While most human WNV infections are asymptomatic, infection can cause a range of symptoms from a mild fever lasting a few days to extreme cases of encephalitis, meningitis, or myelitis (Brault et al. 2009).

The most competent and common arthropod vector of the virus in the western United States is the mosquito, *Culex tarsalis* (Brault et al. 2009, Goddard et al. 2002, Turrell et al. 2005). The preferred blood feeding hosts of *Cx. tarsalis* are birds, especially those most commonly found in the order Passeriformes (Kent et al. 2009). Over 280 species are susceptible to WNV infection in the United States with corvids being particularly predisposed to development of disease (Kramer et al. 2007). Many passerines infected with WNV are able to support sufficient viremia in their blood-stream to be efficient amplifying vectors for the virus to pass on to more mosquitoes in the future

(Molaei et al. 2006). *Cx. tarsalis* are particularly effective at transmitting the virus to humans due to its opportunistic feeding habits that allow it to transition from an amplifying host to a bridging vector (Sugumaran et al. 2009). *Cx. tarsalis* maintain a feeding preference for avian blood meals throughout the spring and early summer, but during the later summer, when the number of avian hosts decrease as mosquito densities increase, *Cx. tarsalis* are forced to switch to mammalian hosts (Kent et al 2009).

Using GIS tools to spatially map incidence of WNV transmission over the course of 2002-2008, Sugumaran (et al. 2009) determined that, although the spread of WNV became endemic, there were common WNV "hot spots", especially in the Great Plains region, extending into Eastern Montana. Precipitation levels were suggested to be the most significant factor in determining risk assessment of WNV transmission with a maximum of 200 mm of moisture from May-July being the ideal conditions for high rates of human infection (Sugmaran et al. 2009). Dohm (et al. 2002) determined that increased environmental temperature correlated positively with mosquito transmission and dissemination of WNV. These combined factors help explain why certain areas are more susceptible to WNV transmission.

My goal was to determine if the infection rate of WNV in *Cx. tarsalis* was actually higher in a designated WNV "Hot Spot" as observed by Johnson (et al. 2010) at Medicine Lake Wildlife Refuge (MLWR) in Eastern Montana during the late summer, a time at which viral transmission may be highest in *Cx. tarsalis* due to precipitation levels (Sugmaran et al. 2009) and high temperatures (Dohm et al. 2002). I then compared the MLWR results to an area of putative lesser WNV transmission at Ninepipe Wildlife Refuge (NWR) in Western Montana at the same time. Both sites have average

precipitation levels of less than 200 mm between May and July as suggested by Sugmaran (et al. 2009): 173.99 mm for MLWR and 146.05 mm for NWR. The average maximal temperatures for the collection months of July and August are 28.6º C in MLWR and 27.5º C in NWR (U.S. Climate Data 2012). In a laboratory setting at temperatures of 30º C and greater, 90% of *Cx. tarsalis* disseminated a viral infection from imbibing an infected host within four days, with intermediate to no dissemination at 26º C (Dohm et al. 2002). Vertical transmission of the virus in mosquitoes is possible and the rate of gestation for mosquitoes eggs decreases as temperature increases allowing viral infection to grow exponentially in warmer temperatures among the mosquitoes (Almiron et al. 2010). MLWR has over a degree Celsius higher average maximum temperature than NWR which could account for the higher incidence of WNV infection at MLWR (CDC 2012). If the two sites have significantly different infection rates, one possible conclusion could be that environmental conditions, particularly temperature, may play a role in WNV infection between the sites. A possible explanation if the results are not significantly different would be that larger *Cx. tarsalis* populations leads to the increased incidence of WNV transmission while the overall infection rate in *Cx. tarsalis* remains constant. Other explanations that could account for varying levels include the amplifying bird hosts, but an analysis of the relationship between bird hosts and *Cx. tarsalis* infection rate is beyond the scope of this paper.

The null hypothesis of this study is that the infection rate of WNV will be the same in all *Cx. tarsalis* populations tested across the state. If the infection rate is the same, that may suggest the best prevention techniques to avoid further human and equine transmission would be to better control and reduce *Cx. tarsalis* population size to

minimize transmission. Alternatively, if the infection rate is higher at MLWR than in the NWR sites then it could suggest that there are one or more environmental factor(s) that are increasing the transmission of WNV to the mosquitoes and further studies will be needed to determine them.

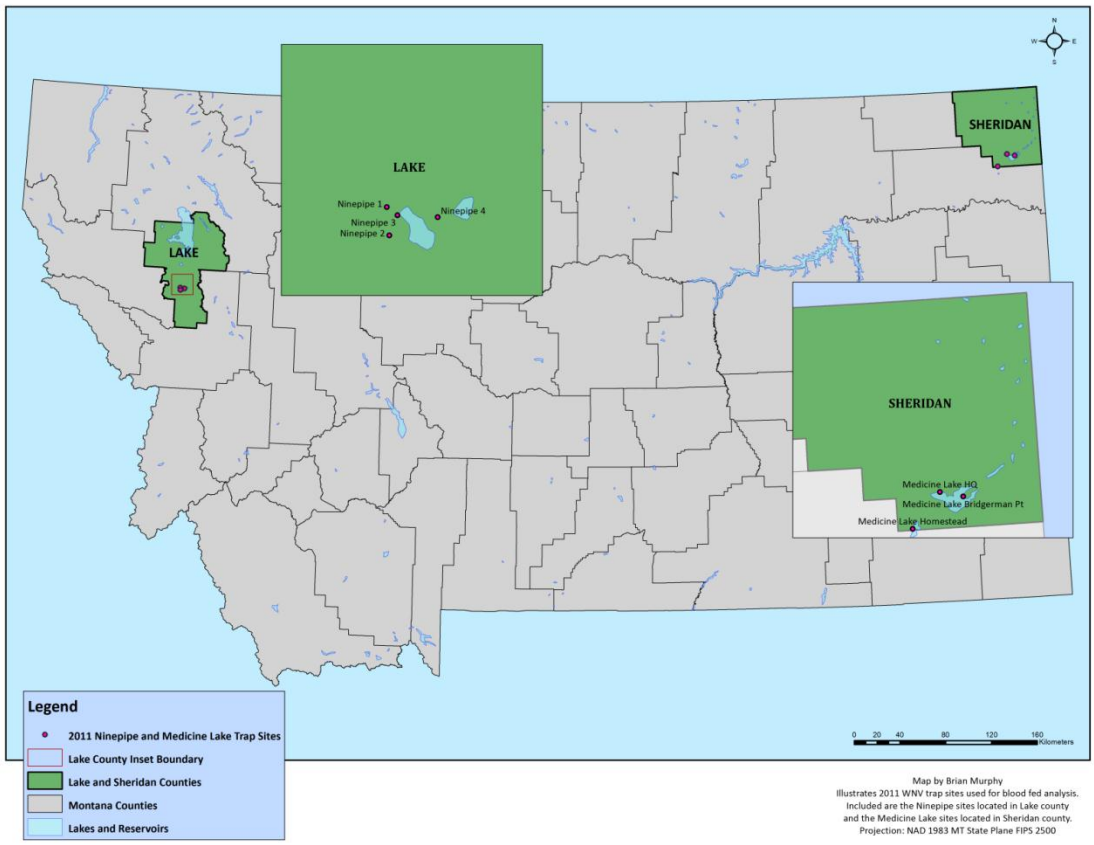
Methods and Materials

Study Area:

The study area for mosquito collection was the Medicine Lake Wildlife Refuge (MLWR) in Sheridan County, Montana near the towns of Medicine Lake and Homestead. Three separate trap sites were chosen: the waterfront by the MLWR headquarters (GPS UTM Coordinates 13 E 0540623 N 5370152), the waterfront by a pelican colony (E 0546225 N 5368540), and the waterfront by a marshy area near Homestead (E 0531582 N 5360086). All three had easy access to water, with little to no tree cover. The vegetation was thick tall grasses, with cattails growing sparsely at the Homestead and headquarters sites.

The second study area was a series of trapping sites west of the Continental Divide around the Flathead Lake area near Ronan at the Ninepipe Wildlife Refuge (NWR). Here, there were four collection sites that where sampled throughout the summer as part of the Carroll College WNV Project. Ninepipe 1 was located in an open area around sedge and a single tree cover next to a pond by agricultural land (11 E 714997 N 5259180). Ninepipe 2 was also located in an open marshland next to water with higher sedge and cattails (E 715480 N 5256933). Ninepipe 3 was located in a bushy outcropping with surrounding trees and large canopy cover (E 715916 N 5258453). Ninepipe 4 was located in extremely thick bush and tree canopy cover with close access to water (E 718835 N 5258574). The trapping sites in context of the state and in relation to each other can be seen in figure 1.

Fig. 1 Map of Collection Sites (Courtesy of Brian Murphy)



Mosquito Collection:

Mosquito trapping at MLWR took place from 7/29/11 to 8/6/11 with no trapping occurring on 7/30-7/31 due to faulty equipment. Traps were set in the early evening between 7-9 PM at the three sights and collected the next morning between 7-9 AM. The CDC light traps used CO_2 tanks as mosquito attractants throughout the week; dry ice was also used from 8/1/11 to 8/5/11 in addition to the tanks to make up for lost data from the two missed nights of trapping. Trapped mosquitoes were placed in a -20º C freezer in order to better preserve the RNA of the samples.

The Western Montana trap sites were collected in the same manner but on a weekly or biweekly schedule depending on the site (Table 1).

Table 1 Ninepipe Collection Dates

Ninepipe Collection Dates
6/27/2011
7/5/2011
7/11/2011
7/18/2011
7/25/2011
8/1/2011
8/8/2011
8/16/2011
8/26/2011

Mosquito Sorting:

Using Swift M28 Zoom Stereo dissecting microscopes, the group of students assigned to the Carroll College WNV Project individually sorted and counted each sample for female *Cx. tarsalis* by date and location. Male and non *Cx. tarsalis* mosquitoes were included in the total so that a total percent *Cx. tarsalis* in the sample could be determined. Samples were kept on ice at all times during the procedure so degradation of any viral RNA was assumed to be minimal.

Homogenization:

The *Cx. tarsalis* samples were divided into groups of 50 and placed into MP Lysing Matrix A tubes that contained a ceramic bead. Under a sterile hood in BSL-II conditions, 1000µL of QIAGEN RNA Later and 500µL of BA-1 Diluent were added. The BA-1 Diluent was made up of 100 mL of M199-H, 1 mL 1M Tris at pH 7.6, 33.3 mL 30% BSA, and 1.35 mL 100x anti-anti (Johnson et al 2010). After the solutions had been added to the tubes they were placed into a Fast Prep FP120 bead beater at a speed setting of 5.5 for 30 seconds to homogenize. The mosquito homogenate was then kept in a -80º C freezer until RNA extraction.

RNA Extraction:

The extraction was performed using a Qiagen QIAcube using the RNeasy® Fibrous Tissue Mini Kit protocol (RNeasy® Fibrous Tissue Handbook 2006). The protocol consisted of a series of elution centrifugations using differing pH solutions through a specialized filter to bind the nucleic acids from the homogenate. A DNase digest was then preformed on the filter to isolate RNA in the sample. Ten samples at a time were prepared under a sterile hood in a BSL-II lab and then placed into the QIAcube. The extracted RNA was then stored in a -80º C freezer.

RT-PCR and Taqman Assay:

The RNA was analyzed for WNV by a RT-PCR and Taqman Assay using the same primers, solution concentrations, and run time protocol as Lancioti (et al. 2000). The RT-PCR was prepared in a different sterile hood than the extraction to prevent contamination. During the plating process all samples were run in duplicate to prevent false positives and each sample was run using both primer and probe sets. If only one of the duplicates was positive that sample was rerun. The RT-PCR was run on a BioRad iQ5™.

Determining Infection Rate:

The data from the results of the RT-PCR were placed into an excel program created by Biggerstaff (2009) to determine infection rate in the mosquito pools. Using a two T pool test at a 95% confidence interval the program was able to compare for statistical difference between infection rates at two different sites. The program determines infection rate through minimum infection rate (MIR) calculations in context

with a maximal likelihood estimation (MLE) calculation. The program also incorporates skewness-correction and can be run for unequal pool size.

Previous Years Result:

The WNV project at Carroll College has two years of prior data from samples run using the same techniques as presented above. A study of the two sites individually had not been done up to this point, but previous data at the sites were run through the Biggerstaff (2009) program and compared to the results of this year's experiment.

Results

Collection Results:

The two sites yielded vastly different mosquito totals and percentages of *Cx. tarsalis* among the total collections (Table 2). Both the MLWR and NWR trap sites produced high yields of mosquitoes with each study area totaling over 31,000 mosquitoes. These results are somewhat skewed when considering that there were fewer trap sites and days spent trapping at MLWR; however the temporal aspect, coinciding dates of trapping at each study area, was not taken into consideration for this study. The infection rate program could also compensate for the skewed results in determining statistical difference. In addition, the percentage of *Cx. tarsalis* out of total mosquitoes collected was over 90% at all MLWR sites, while the highest percent total in the west was 12.9% at Ninepipe 1, with all other sites below 10%. It should also be noted that Homestead and Ninepipe 4 account for over half of the total mosquitoes collected at both study areas.

Table 2 Mosquito Totals at Medicine Lake and Ninepipe

Medicine Lake			
Site	Total Mosquitoes	Total *Cx. tarsalis*	% *Cx. tarsalis*
Headquarters	5002	4661	**93.2**
Pelican Point	11720	11069	**94.4**
Homestead	19427	17970	**92.5**
TOTALS	**36149**	**33700**	**93.2**
Ninepipes			
Site	Total Mosquitoes	Total *Cx. tarsalis*	% *Cx. tarsalis*
Ninepipes 1	3991	516	**12.9**
Ninepipes 2	4875	273	**5.6**
Ninepipes 3	3110	255	**8.2**
Ninepipes 4	19347	644	**3.3**
TOTALS	**31305**	**1688**	**5.4**

Cx. tarsalis Extraction and RT-PCR Results:

Since *Cx. tarsalis* numbers were significantly lower at NWR, all collected samples were able to be extracted as 38 pools of 50 moquitoes. However, the much larger 33,700 *Cx. tarsalis* sample from MLWR was not completely processed due to time restrictions and expenses needed to complete what would have been over 800 mosquito pools. So 100 *Cx. tarsalis* pools were extracted in the Carroll College Undergraduate lab from various sites and dates from the MLWR collections and 100 additional pools were sent to the state labs of Montana totaling 200 RNA extractions from MLWR (Table 3). Despite running 238 extractions, none of the samples were positive for WNV.

Table 3 Number of Extractions by Date and Site at Medicine Lake

Dates	Headquarters	Pelican Point	Homestead	**TOTALS**
7/29/2011	10	1	5	**16**
8/1/2011	5	10	20	**35**
8/2/2011	N/A	10	20	**30**
8/3/2011	10	10	15	**35**
8/4/2011	10	20	15	**45**
8/5/2011	10	15	10	**35**
8/6/2011	1	N/A	3	**4**
TOTALS	**46**	**66**	**88**	**200**

Infection Rate Results:

Since none of the mosquito samples were positive for WNV, the data suggest that the infection rates for MLWR and NWR were extremely low, at least in 2011. The confidence interval for MLWR was between 0% and 0.38% infection rate, which overlaps completely with the NWR interval of 0% and 2.76%. The smaller number of

Cx. tarsalis collected and tested from NWR compared to the MLWR collections explain why the interval is much more spread out to account for the greater chance of error. The two sample pool analysis program created by the Biggerstaff (2009) calculated the infection rates to have a difference of 0 showing that there is not a statistical difference in viral prevalence in the mosquito population between MLWR and NWR (Table 4).

These results were then pooled with the data from the WNV project at Carroll College from 2009 and 2010 as well. Although positive samples were detected in the previous years' collections at MLWR, there was no statistical difference from MLWR and NWR in either of the years (table 5)

Table 4 Comparison of Infection Rates Percent 2011

Medicine Lake					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.00	**0.00**	**0.38**	200	0	10000
Ninepipes					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.00	**0.00**	**2.76**	38	0	1316
Comparison of Both					
MLWR Inf Rate	**NLWR Inf Rate**	**Inf Rate Diff**	**Lower Limit**	**Upper Limit**	
0	**0**	**0**	0	2.76050238	

Table 5 Comparison Infection Rate in Easter and Western Montana 2009-2011

Ninepipes National Wildlife Refuge Infection Rates					
2009					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.00	0.00	**6.07**	11	0	541
2010					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.00	0.00	**3.92**	18	0	887
2011					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.00	0.00	**2.76**	38	0	1316
Medicine Lake Wildlife Refuge Infection Rates					
2009					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.68	0.12	**2.23**	60	2	2963
2010					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.53	0.03	**2.56**	38	1	1900
2011					
Infection Rate	**Lower Limit**	**Upper Limit**	**Num Pools**	**Num Pos Pools**	**Num Individuals**
0.00	0.00	**0.38**	200	0	10000

Discussion

The infection rates between the two separate *Cx. tarsalis* populations in MLWR and NWR were not statistically different, so the null hypothesis could not be rejected. A higher percentage of *Cx. tarsalis* were expected at MLWR than NWR. This was hypothesized due to previous studies in the area (table 5) and the higher average maximum temperature at MLWR, and was supported by the collection results (Table 2) The data suggest that since the infection rates were not statistically different that different precipitation and temperature levels are not the underlying reason for the high incidence of avian, human, and equine cases of WNV in Eastern Montana, especially Medicine Lake. However, previous studies have shown that temperature and precipitation levels do affect transmission of the virus (Dohm et al. 2002, Sugumaran et al. 2009). The lack of data supporting these prior studies may be due to MLWR and NWR precipitation and temperature levels being too similar to see varying infection rates, with their average summer maximal temperatures only one degree Celsius apart. One alternative explanation may be due to the increased population size at MLWR and not an increased infection rate. In this example, environmental factors could play a role in viral transmission by affecting reproduction rates and growth conditions of the *Cx. tarsalis* populations instead of inherently effecting infection rates.

This finding is further supported by previous years' studies where positive samples of the virus were detected. Despite the detection of positive samples in Eastern Montana there was still not a statistical difference in infection rates when run through the Biggerstaff program (Table 5). The lack of positive samples also corroborates with the CDC WNV surveillance ArboNET which reported only one incidence of WNV in

Montana during 2011 for humans, birds, and equines (CDC 2012). These data give a possible explanation to the low and statistically equal infection rate as well as the absence of virus detection.

The results from this study could highlight key areas to improve upon disease prevention by putting more focus on controlling *Cx. tarsalis* populations rather than concern about the reservoir host amplification process. The reasoning is that by decreasing the *Cx. tarsalis* population the chance of infection goes down even if there is high viremia in avian host blood, because the infection rate is so minuscule. However, these results appear contrary to similar studies by Dohm (et al. 2002) and Sugumaran (et al. 2009) regarding transmission of WNV and the importance of environmental factors. Despite having less than 200 mm of precipitation May-July and having temperatures over 27º C neither site contained a positive sample.

Further and more specific research needs to be completed before the data can be supported. The study does not incorporate the full viral cycle by not including avian amplification. The results from this experiment cannot rule out the possibility of an avian driven transmission cycle instead of mosquito driven. This study could be strengthened through increasing the sample size, decreasing experimental variables so individual environmental factors can be accounted correctly, and continuing to perform the experiment for multiple years. More *Cx. tarsalis* samples need to be collected from both sites, but particularly the NWR site to create a more precise and accurate infection rate interval. In addition, both collection areas need to be collected on the same dates in order to calculate a temporal analysis to collaborate with the spatial analysis to eliminate confounding factors that make distinguishing between the two difficult and provide a

stronger comparison between the sites. A more complete assessment of environmental differences at the sites by recording temperature and precipitation levels will allow for a quantifiable comparison in connection with the infection rate data. If this study's results coincide with future studies they may provide answers to better prevention techniques as well as explain high prevalence of WNV in specific regions.

Literature Cited

Almiron, W.R., G.P. Batallan, M.S. Contigiani, L.A. Diaz, and F.S. Flores. 2010. Vertical transmission of St. Louis encephalitis virus in Culex quinquefasciatus (Diptera: Culicidae) in Cordoba, Argentina. Vector Borne Zoonotic Dis. 10:999.

Biggerstaff, B.A. 2009. Pooled Infection Rate, Version 4th Ed. CDC, Fort Collins, CO.

Brault, A.C. 2009. Changing Pattern of West Nile Virus Transmission: altered vector competence and host susceptibility. Vet. Res. 40:43.

Centers for Disease Control and Prevention (CDC). West Nile virus. Updated 20 February, 2012. Accessed 5 March 2012. <http://www.cdc.gov/ncidod/dvbid/westnile/Mapsincidence/surv&control09IncidMaps.htm>.

Dohm, D.J., M.L. O'Guinn, and M.J. Turell. 2002. Effect of Environmental Temperature on the Ability of Culex pipiens (Diptera:Culicidae) to Transmit West Nile Virus. J. Med. Entomol. 39 (1):221-225.

Goddard, L.B., A.E. Roth, W.K. Reisen, and T.W. Scott. 2002. Vector Competence of California Mosquitoes for West Nile Virus. Emerg. Infect. Dis. 8:12-17.

Gubler, D.J., L.R. Peterson, J.T. Roehrig, G.L. Campbell, N. Komar, and R.S. Nasci. 2003. Epidemic/Epizootic West Nile Virus in the United States: Guidelines for Surveillance, Prevention, and Control. http://www.cdc.gov/ncidod/dvbid/westnile/resources/wnv-guidelines-aug-2003.pdf

Kent, R., L. Juliusson, M. Weissman, S. Evans, and N. Komar. 2009. Seasonal Blood-Feeding Behavior of Culex tarsalis (Diptera:Culicidea) in Weld County, Colorado, 2007. J. Med. Entomol. 46 (2):380-390.

Kramer, L.D. L.M. Styer, and G.D. Ebel. 2008. A Global Perspective on the Epidemiology of West Nile Virus. Annu. Rev. Entomol. 53:61-81.

Johnson, G., N. Nemeth, K. Hale, N. Lindsey, N. Panella, and N. Komar. 2010. Surveillance for West Nile Virus in American White Pelicans, Montana, USA, 2006–2007. Emerg. Infect. Dis. 16:3.

Lanciotti, R.S., A.J. Kerst, R.S. Nasci, M.S. Godsey, C.L. Mitchell, H.M. Savage. 2000. Rapid Detection of West Nile Virus from Human Clinical Specimens, Field-Collected Mosquitoes, and Avian Samples by a TaqMan Reverse Transcriptase-PCR Assay. J Clin Microbiol. 38:4066–71.

Marra, P.P., S. Griffing, C. Caffrey, A. M. Kilpatrick, R. McLean, C. Brand, E. Saito, A.P. Dupuis, L. Kramer, and R. Novak. 2004. West Nile Virus and Wildlife. BioScience 54:393-402.

Molaei, G., T.G. Andreadis, P.M. Armstrong, J.F. Anderson, and C.R. Vossbrinck. 2006. Host Feeding Patterns of Culex Mosquitoes and West Nile Virus Transmission, Northeastern United States. Emerg. Infect. Dis. 12:3

Sugumaran, R., S.R. Larson, and J.P. DeGroote. 2009. Spatio-temporal Cluster Analysis of County Based Human West Nile Virus Incidence in the Continental United States. Internat. J. Health Geograph. 8:43.

Turell, M.J, D.J. Dohm, M.R. Sardelis, M.L. O'Guinn, T.G. Andreadis, and J.A. Blow. 2005. An Update of the Potential of North American Mosquitoes (Diptera;Culicidae) to Transmit West Nile Virus. J. Med. Entomol. 42 (1):57-62.

US Climate Data. 2011. http://www.usclimatedata.com/