April 26, 2007

To Whom It May Concern,

Although we are still in the process of testing some of our samples from 2006, I wanted to give you an update on our findings so far, as well as tell you about the results of our work on WNV in mammals in 2005, that wasn't available when I wrote last year's report. I will send an additional report when our testing and analysis on all the 2006 specimens is complete.

We were able to perform this work through your helpful cooperation, and for this we are very grateful. If you have any questions about this summary or have additional interest in any other aspect of the study, please don't hesitate in contacting me.

In addition to this report, we recently published a paper on some of the findings from our research in a journal called the Proceedings of the Royal Society which can be found on the following website (the 8th paper down the list):
http://www.conservationmedicine.org/marm_kilpatrick.htm
Kilpatrick, A.M., Daszak, P., Jones, M.J. , Marra, P.P., Kramer, L.D. 2006. Host heterogeneity dominates West Nile virus transmission. Proceedings of the Royal Society of London B: Biological Sciences 273 (1599) 2327-2333. PDF
(click on the "PDF")

We are continuing to analyze data from all the years of our project and will keep you updated on our findings! In addition, we were fortunate to gain additional funding from the National Science Foundation to expand the study and have added several new sites this year. We hope that you will allow us to continue to use your property for our study.

Thanks you again for your help in making our research possible.

Sincerely,

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West Nile virus and Lyme disease transmission

along a forest-to-urban gradient in Maryland and Washington DC

From May through October 2006 we visited 9 sites in Maryland and Washington DC region as a part of our project to understand West Nile virus transmission; for example, why do some areas seem to have more infected birds, mosquitoes or people than other areas?

At each of our sites we:

1) performed a census or survey of the bird community,
2) captured, banded, and took blood samples from birds
3) trapped mosquitoes using dry ice (CO_2) baited CDC light traps, and CDC gravid traps (for egg-laying mosquitoes) baited with organically rich water
4) collected mosquitoes using a backpack mounted aspirator that still had blood in their stomachs
5) trapped mammals and took blood samples
6) NEW: estimated tick densities using a cloth dragging method

Over the past six months the NY State Dept. of Health has been testing our samples for:

1) the mosquitoes we trapped were tested for West Nile virus
2) the birds and mammals are being tested for antibodies to West Nile virus
3) we sequenced the DNA in the blood of mosquitoes that we caught in traps and with the backpack mounted aspirator to determine what animal they fed on
4) NEW: we tested the ticks for several diseases including Lyme, tularemia, and ehrlichiosis

In total, between May and October, 2006, we trapped 4,092 resident birds, 490 mammals (228 in 2005), 41,072 mosquitoes (487 with blood in their stomachs), counted 6,232 birds on our censuses, and collected 4760 ticks. These are again substantially higher numbers of birds and mosquitoes trapped than in 2004, partly as a result of working with additional residents who were kind enough to let us work on their property. Below I describe the data from each of these components and some of the preliminary conclusions that we can draw from these data.

Mosquito Abundance and West Nile virus Prevalence

Figure 1, below, shows the abundance of three different types (genera) of mosquitoes and the fraction that were infected with West Nile virus from each of our sites in 2005. *Culex* mosquitoes bite mostly birds, but sometimes feed on mammals (including humans). In contrast, *Aedes*, *Ochlerotatus*, and *Anopheles* mosquitoes all feed primarily on mammals (including humans). The fraction of mosquitoes that are infected with West Nile virus is labeled "Cx. pipiens/restuans MIR" which denotes the *Culex pipiens* & *Culex restuans* Minimum Infection Rate. These two species of mosquitoes are the primary mosquitoes for transmitting West Nile virus. The Minimum Infection Rate (MIR) is a measure that mosquito control people and scientists use to describe the fraction of mosquitoes that are infected with West Nile virus. It is equal to 1000 times the fraction of mosquitoes that are infected with West Nile virus. People use this measure because the fraction of mosquitoes infected with West Nile virus (and other viruses) is usually very low (1 in a 1000), so instead of having to write 0.001 or 0.002, they just write 1 or 2. One last thing that is important when looking at Figure 1 is that the y-axes (the vertical dimension) are drawn on a logarithmic scale, so that each little tick mark is a large jump. On the y-axis, $10^2 = 100$, whereas $10^1 = 10$, and $10^0 = 1$. So, the graphs show a span of 1000-fold.

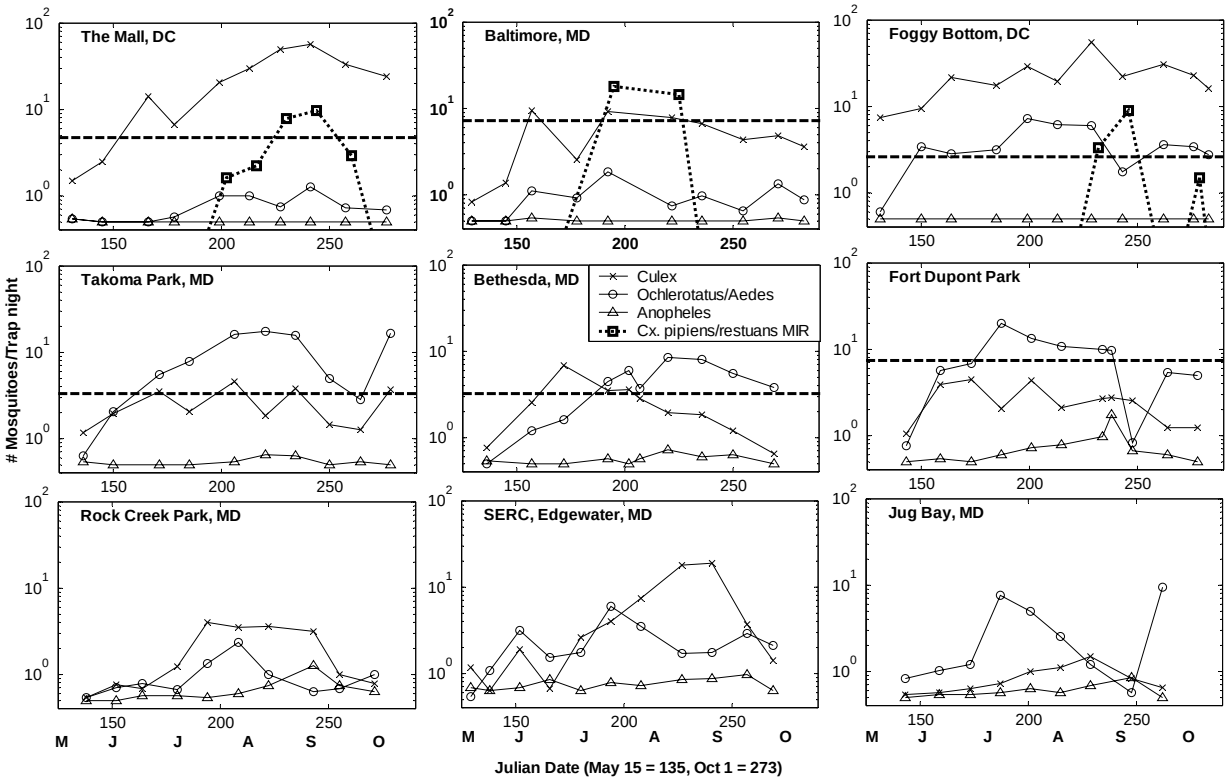

Figure 1. 2006 *Abundance of Culex, Aedes/Ochlerotatus and Anopheles mosquitoes and WNV prevalence (MIR) on a log scale. Horizontal dashed lines are season average MIRs and squares are MIRs for a two day period. No WNV-infected mosquitoes were trapped in our CDC light traps at the bottom 3 sites.*

Here's what these data tell us:

1) Several patterns in mosquito abundance and WNV prevalence first observed in 2004 appear to be repeated each year (2004, 2005, 2006). This is very useful information, because it means that hotspots for disease are consistent year after year, and suggests that if we can understand why these are hotspots, we can make generalizations that will likely be true in future years. Some of the repeated patterns are:
    a. There are many more *Culex* mosquitoes at the urban sites (The Mall, Foggy Bottom and Baltimore) but very few *Aedes and Ochlerotatus* (mostly mammal biting) mosquitoes at two of the urban sites (The Mall and Baltimore) and more at the urban site that has some forest habitat (Foggy Bottom).
    b. There are especially large numbers of *Aedes* mosquitoes at the two residential sites (Takoma Park and Bethesda). The Asian tiger mosquito (*Aedes albopictus*) that some of you may be familiar with (it is black with white stripes on its legs and body) is in this group.
    c. There is strong evidence of West Nile virus transmission at the 6 most urban sites, and no evidence of transmission at the two most forested sites (SERC, and Jug Bay). This means that the risk of being infected with West Nile virus while in intact forested areas is essentially zero.
    d. Infected mosquitoes are first around in late July at the Mall and in Baltimore, but a full month later (late August) in Foggy Bottom.
2) There are also some important differences between the three years (2004, 2005, 2006).

a. The abundance of *Culex* mosquitoes (the key players in West Nile virus transmission) was highest in 2004, then 2005, then 2006. However, West Nile virus prevalence in mosquitoes was much higher in 2006 than 2005 and about the same as 2004, suggesting that factors other than mosquito abundance are also quite important.
b. We found our first infected mosquito at Rock Creek Park in 2006, caught in a gravid trap. We had no evidence of transmission to birds at this site in 2005, but 6% of the young of the year cardinals were exposed to West Nile virus at Rock Creek in 2004. In 2006 almost 25% of cardinals at this site had been exposed to West Nile virus (see Figure 4 below). This suggests that transmission of West Nile virus at this site in 2006 was the most intense of the three years so far.

West Nile virus antibody prevalence in Birds

Figures 4 and 5 show the 2005 prevalence or fraction of the young of the year (or Hatch year birds) and adult birds that had West Nile virus antibodies from May-September for a few species at each site, using the 4- letter abbreviations (see legend). Birds that have antibodies to West Nile virus have been bitten by a WNV infected mosquito and have survived the infection. The exposure of birds is indicated by the "steepness" of the lines which indicates the increase in the prevalence or fraction of hatch-year birds that have West Nile virus antibodies over time.

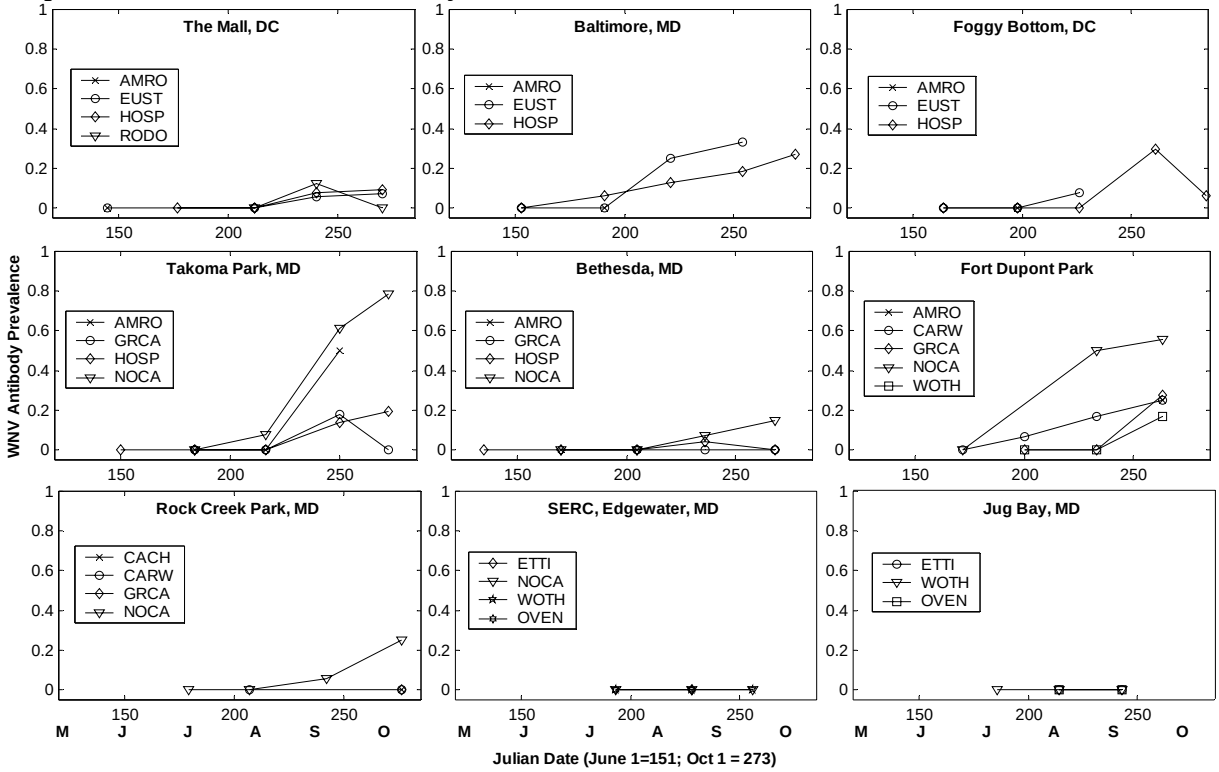

Figure 4. *2006 West Nile virus antibody prevalence (fraction of birds with antibodies) for young of the year birds at our 9 sites. Each point represents an average of 10.9 (range 4-32) birds. AMRO-American Robin, CARW – Carolina Wren, CACH – Carolina Chickadee, ETTI – Tufted Titmouse, EUST – European Starling, GRCA – Grey Catbird, HOSP – House Sparrow, NOCA – Northern Cardinal, OVEN – Ovenbird, RODO – Rock Dove, WOTH – Wood Thrush.*

Here's what these data tell us:

1) The exposure of several native birds to West Nile virus in 2006 was highest at Takoma Park and Fort Dupont Park (Northern Cardinals, NOCA 60-80% by October, Carolina Wrens, CARW 30%). The exposure of house sparrows (HOSP) and European starlings (EUST), two birds introduced from Europe, are higher at Takoma Park, Baltimore, and Foggy Bottom, and lower at The Mall.
2) At Rock Creek Park approximately 30% of young of the year cardinals were exposed to West Nile virus, whereas there was no evidence of transmission at this site in 2005.
3) The fraction of young of the year birds that had antibodies to West Nile virus was zero at the two forested sites (SERC and Jug Bay)
4) The fraction of young birds that had antibodies to West Nile virus was different for different species, and partly reflects mosquitoes' feeding preferences for each species, as well as their susceptibility to dying from West Nile virus. As in previous years, Northern Cardinals showed the highest exposure, whereas the exposure of House Sparrows and pigeons appeared to be lower.

For the adults (Figure 5) the West Nile virus antibody patterns are quite different.

5) As in 2004 and 2005, the prevalences for many species are essentially flat over the season, with a little bit of fluctuation, suggesting that adult birds of most species are not exposed to West Nile virus.
6) However, adult Northern Cardinals showed strong evidence of exposure at Takoma Park, and some evidence of exposure at Bethesda.
7) Taken with our data from 2004 and 2005, we believe that adults of some species are bitten by mosquitoes (N Cardinals), whereas for most other species, once they get past their first summer, they are bitten much less often.

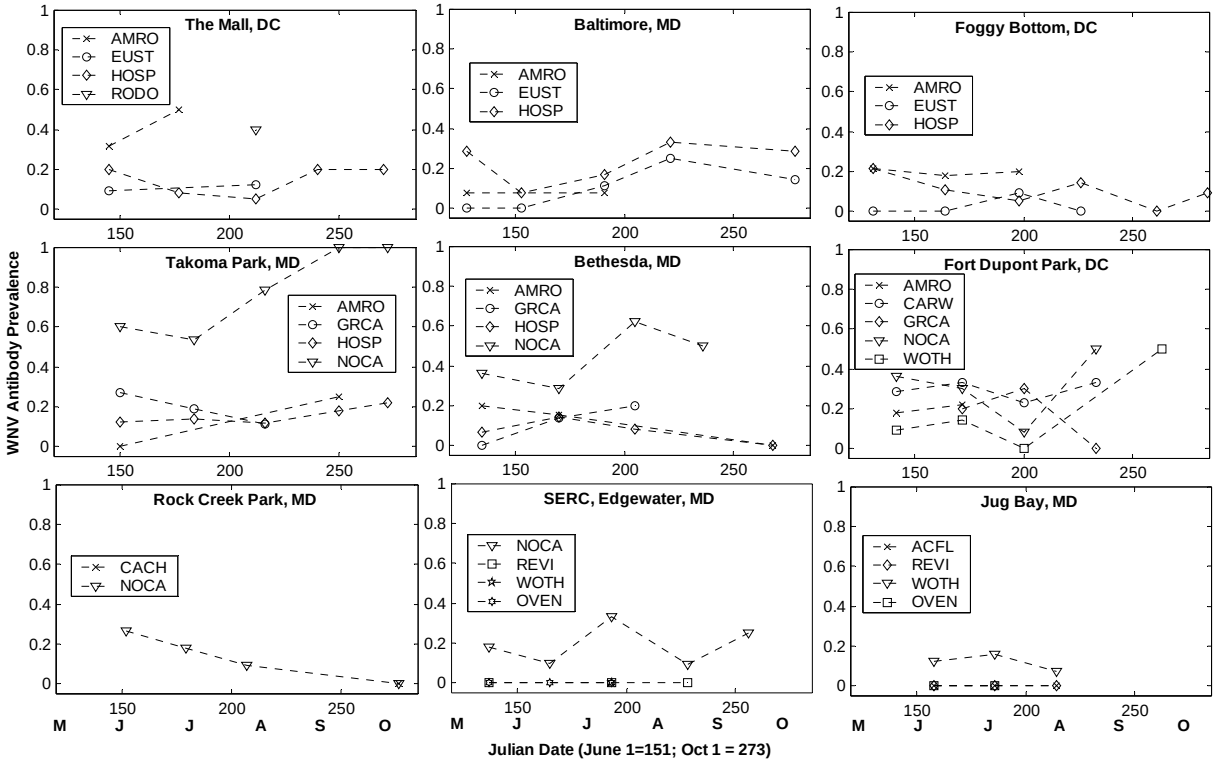

Figure 5. *2006 West Nile virus antibody prevalence (fraction of birds with antibodies) for adult birds at our 9 sites. Each point represents an average of 7.4 (range 4-37) birds. ACFL -*

Acadian Flycatcher, AMRO American Robin, CAWR – Carolina Wren, CACH – Carolina Chickadee, ETTI – Tufted Titmouse, EUST – European Starling, GRCA – Grey Catbird, HOSP – House Sparrow, NOCA – Northern Cardinal, OVEN – Ovenbird, REVI – Red-eyed vireo, RODO – Rock Dove, WOTH – Wood Thrush.

West Nile virus antibody prevalence in Mammals, 2005

Table 1 shows the antibody prevalence in wild mammals caught at 7 of our 9 sites in 2005 (2006 samples are still being tested). These data show that:

1. WNV antibody prevalence varied significantly among species: eastern gray squirrels were 5.5 times more likely to have WNV antibodies than eastern chipmunks (*Tamias striatus*) and 4.5 times more than white footed mice (*Peromyscus leucopus*).
2. Prevalence increased with capture date for juveniles, but not for adults, suggesting that as with birds, adults are bitten by mosquitoes and exposed to WNV less than juveniles.
3. West Nile virus antibody prevalence increased with the urbanization index of the site, so squirrels living in urban areas were more likely to be exposed to WNV.

Table 1. Percent West Nile virus antibody prevalence (sample size in parentheses) in adult (A) and juvenile (J) wild mammals at 7 sites in Washington DC and Maryland, caught between June 14, 2005 and September 17th, 2005 except where noted.

Site	UI*	Age	*E. Chip-munk*	*E. Gray Squirrel*	*Opossum*	*White footed mice*	*Raccoon*	*Norway Rat*
Baltimore	91.2	J		0 (3)				
		A		64 (14)				50 (2)
Foggy	75.5	J		20 (10)		50 (2)		
Bottom†		J**		43 (7)				
		A		52 (23)		50 (2)		50 (6)
		A**		100(6)				
Fort Dupont	38.8	J		100 (2)	20 (5)			
Park		A		75 (8)	60 (5)		50 (2)	
Takoma	50.4	J		0 (2)	71 (7)			
Park‡		J**		50 (6)				
		A		65 (20)	50 (6)		100 (2)	
		A**		100 (5)				
Bethesda§	41.5	J	0 (4)			100 (1)		
		A	22 (11)	67 (15)				
Rock Creek	27.8	J		0 (5)		0 (1)		
Park¶		A	16 (6)	30 (20)		0 (3)	100 (3)	
SERC#	16.2	J			50 (4)	0 (10)	0 (1)	
		A		100 (1)	25 (4)	0 (6)	0 (1)	

*UI - Urbanization Index; *Additional mammals sampled:* †House mouse, *Mus musculus* (1 WNV+ adult, 1 WNV- juvenile); ‡Big brown bat, *Eptesicus fuscus* (1 WNV- adult), Little brown bat, *Myotis lucifugus* (1 WNV+ adult); §Little brown bat, *Myotis lucifugus* (1 WNV+ adult) ¶Groundhog, *Marmota monax* (1 WNV- adult); #Domestic cat (1 WNV- juvenile), Groundhog, *Marmota monax* (1 WNV- adult, 1 WNV+ adult), E. cottontail rabbit, *Sylvilagus floridanus* (1WNV- adult); ** Samples from April, 2006

Tick Abundance and prevalence, 2006

We performed 28,460m (~28.5km or ~18 miles) of tick drags, and in doing so collected 4760 ticks of three species, deer ticks or black-legged ticks (*Ixodes scapularis*), lone star ticks (*Amblyomma americanum*), and dog ticks (*Dermacentor variabilis*). We tested nymphal and adult deer ticks for Lyme disease (*Borrelia burgdorferi*), nymphal and adult lone star ticks for Tularemia (*Francisella tularensis*), and Ehrlichiosis (*Ehrlichia chaffeensis*), and nymphal and adult dog ticks for Ehrlichiosis and Rocky mountain spotted fever (*Rickettsia rickettsii*).

Testing of these ticks showed that:

1. Of the 13 adult and 153 nymphal deer ticks, 6 adults and 22 nymphs were infected with Lyme disease. The prevalence varied from 0% to 60% across the sites.
2. The 4 adult and 5 nymphal dog ticks were all negative for both pathogens.
3. Of 8 adult and 140 nymphal lone star ticks, only 1 was positive for Ehrlichiosis (collected at Patuxent on 8/30/06), and all were negative for Tularemia.

The abundance of ticks also varied substantially across the sites. The density of infected deer ticks (per square meter of dragging), which is an indicator of the risk of being bitten by an infected tick, is shown in Figure 6. The risk of encountering an infected tick was zero at the residential and urban sites and variable at the park and forested sites.

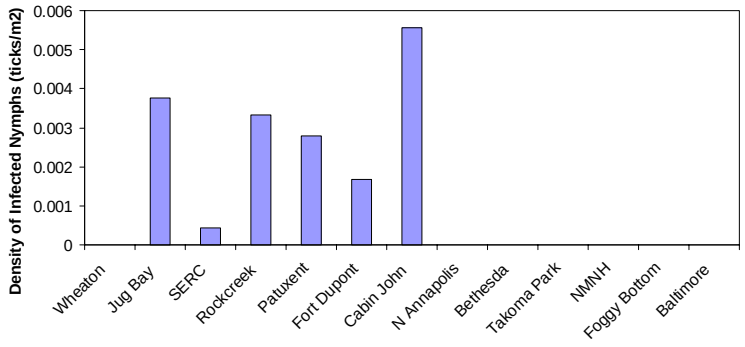

Figure 6. Density of Lyme disease-infected deer tick nymphs across 13 sites, with forested and park sites to the left (Wheaton to Cabin John) and residential and urban sites to the right.