West Nile virus Ecology*

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Overview of West Nile Virus biology

In 1999 a disease new to the Western Hemisphere was discovered in New York City with the death of hundreds of corvids and other birds as well as unexplained neurological disease in humans. The infectious agent was West Nile Virus (WNV), a pathogen first identified in Uganda in 1937 (Hayes et al., 2005; Kilpatrick, 2011). WNV is related to viruses causing diseases such as St. Louis encephalitis, Eastern Equine Encephalitis, Dengue fever, and yellow fever. The virus and the disease it causes spread to the West Coast in four years (Kilpatrick, 2011), and had been found in all contiguous US states by 2005 (DeBiasi and Tyler, 2006). Humans are in fact "dead end" hosts; the virus can not be transmitted from us. Instead, the virus cycles primarily between birds, which are often called amplification hosts or reservoir hosts since they are the "reservoir" of infections for humans, and mosquitoes (Hayes et al., 2005, Fig. 1). The mosquitoes, primarily *Culex* spp.

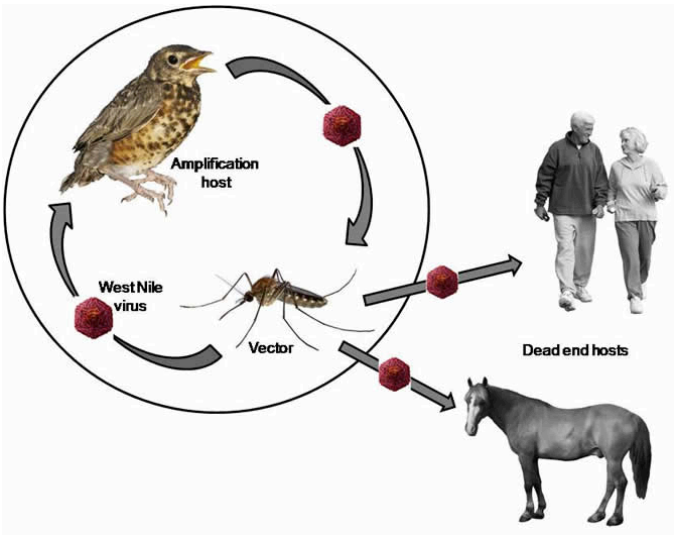

Figure 1: The general transmission cycle of West Nile Virus. From: `http://www.vetmed.wisc.edu/WNV/background.html`

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(Hamer et al., 2008), vector the virus from host-to-host, injecting a bit of the virus when they take their blood meal. The WNV rapidly replicates within the newly infected host, which makes it much more infectious to the next mosquito that happens to feed upon it. It is important to note that birds may not remain infectious for long; their immune systems quickly regulate and even clear the virus population. Also of importance: there is no evidence that WNV can be transmitted by direct contact between infected humans, birds, or other animals, living or dead (Hayes et al., 2005). (It can, however, be transmitted through the placenta from mother to unborn child and in blood transfusions, which necessitates testing of blood donors.)

It is important to note that most (about 80%) WNV infections are asymptomatic, and of those people showing symptoms, most have a mild, flu-like illness (Centers for Disease Control and Prevention, 2011). However, WNV, like other viruses of the Flaviviridae, can infect the central nervous system, causing meningitis or encephalitis, collectively called "neuroinvasive" disease, which can be fatal, especially among the elderly (Hayes et al., 2005). Neuroinvasive disease occurs in about 1 in 150 WNV infections, or less than 1% (DeBiasi and Tyler, 2006), and about 10% of people with these serious neurological symptoms will die.

The incidence[1] of WNV infection and neuroinvasive disease has not been evenly distributed across the US, either at the onset of the epidemic or as the pathogen spread. To some degree this is because WNV was a "virgin ground" epidemic, meaning that the entire population of North America was susceptible. When a pathogen invades an area where many hosts (e.g., humans, birds) are susceptible, there is usually a large peak in incidence. You can see the wave of WNV infection moving from New York in 2001 to the West Coast by 2004 in the series of maps in Fig. 2 from Hayes et al. (2005) (and look at the CDC for a longer, more complete set of maps). As the hosts recover and become immune, the epidemic fades.

There are many other explanations for the observed heterogeneity in WNV incidence as well including environmental conditions, host community ecology, and socioeconomic factors. Since mosquito bites are the source of infection and, as we have read, mosquito development

[1] **Incidence** is defined as the number or better yet the proportion[2]of *new* cases within a specified number of time. Incidence of neuroinvasive disease cases, for instance, is generally reported as the number of new cases per 100,00 in a given year. It measures the rate at which the disease is acquired. In contrast, the **prevalence** of a disease is the proportion of the population that has the disease *at a given time*, regardless of when the disease was acquired. It is measure of the disease burden. **Seroprevalence**, like prevalence, is not a rate, but the proportion of the population that has antibodies to the agent. It measures how much of the population has been *exposed* to the pathogen.

[2]In this way we can account for differences in population size. For instance Montana has very few people and thus few cases of neuroinvasive disease, while New York is large and has many cases. The *proportion* of people developing WNV-associated neuroinvasive disease, however, is much higher in Montana.

and population growth are controlled by temperature and moisture (among other factors[3]), it would seem important to identify environmental conditions that correlate to mosquito density and rates of infection.

We might also expect land use change, such as urbanization, and socioeconomics to directly or indirectly alter the availability of mosquito habitat, the make up of host communities, and even the responses of the human population to WNV risk (Kilpatrick, 2011; Harrigan et al., 2010). Indeed, the first directives to the public were to cover up when outdoors; use insect repellant; avoid outdoor activity at dawn and dusk, when mosquitoes are most abundant; and get rid of standing water, such as in clogged rain gutters or old tires, where mosquitoes can breed.

The influence of bird diversity in WNV dynamics has also received a great deal of attention. In the US, WNV has been found in at least 48 species of mosquitoes (but again, *Culex* spp. are the principle vectors), several dozen mammalian species (and exposure in 100 species!; Root, 2013), and an amazing 330 species of birds (Centers for Disease Control and Prevention, 2012)—hence the focus on birds! Several of these bird species are very susceptible to WNV infection; their populations declined drastically as WNV swept through an area (LaDeau et al., 2011). Other species are

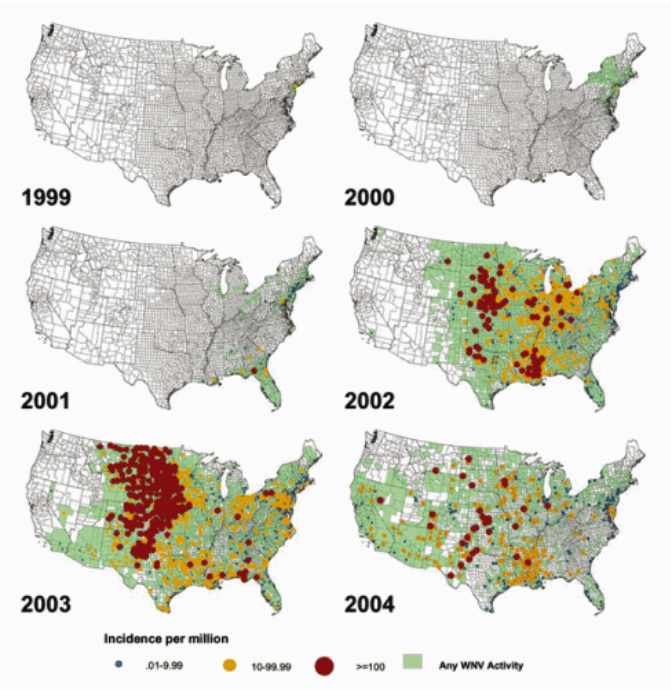

Figure 2: The spread of West Nile virus in the United States from 1999 to 2004. The dots are incidence by county (blue = 0.01 - 9.99, yellow = 10 - 99.99, red $\geq$ 100 per million new cases of neuroinvasive West Nile virus disease) and the green represents areas where there was any reported presence of WNV infection.

[3] In fact, the biting rate of mosquitoes is temperature-dependent, but not, perhaps, for the reasons you might initially expect. As you may know, only female mosquitoes blood-feed because they need the protein to produce eggs. They generally take a blood meal, then develop and lay several eggs, then repeat the cycle. The length of time between a blood feeding and oviposition, the "gonotrophic cycle," is much shorter at higher temperatures, thus the less time between mosquito bites (Paaijmans et al., 2013)!

good (competent) hosts, because they do not die from the infection and are highly infection to mosquitoes. (Note that mosquitos only bite living animals.) Because of these differences in the "reservoir competence" among bird species (Kilpatrick et al., 2006), several studies have suggested that the "dilution effect" may be at work in the WNV system because areas with greater diversity of birds tend to have less risk of WNV infection to humans (e.g., Ezenwa et al., 2006; Swaddle and Calos, 2008). Others have suggested that this relationship between bird diversity and WNV risk is inconsistent or even spurious (Loss et al., 2009; Hamer et al., 2011). Still, it is worth considering how biodiversity can affect human health (Swaddle and Calos, 2008).

Class exercise: finding a metric of risk to humans

Clearly, there are many ecological hypotheses that may help explain why some areas (or periods of time) present a greater risk of WNV infection than others (i.e., why the risk to humans is so heterogeneous). Your challenge in this lab is to articulate and then test one hypothesis for this heterogeneity in risk to humans. That is, what factor(s) do you think control or predict WNV infections in humans? But first, as a class, we will determine which of several types of infection data best predict the patterns of human infections.

While WNV-associated disease is a "reportable" illness, meaning that doctors must report cases of WNV infection to their local health officer, which then submits the information to the Centers for Disease Control and Prevention (CDC), actual infections in humans are fairly rare and can be difficult to identify quickly with confidence. Most state and federal agencies, as well as researchers, thus track the risk to humans using animal surveillance data, including incidence of infections in mosquito samples, birds, and other non-human vertebrates. Included in these data are "sentinels," which are animals, in this case chickens and/or horses, that are deployed in a given area and regularly checked for infection. We have two related questions to address:

1. Which type of WNV infection data (Bird, Mosquito, Sentinel or Veterinary) has the strongest relationship with the distribution of Human Disease Cases *in space*?

2. Which of these types of WNV infection data best predict the number of human cases *in time*?

Ideally our metric would perform well both in space and in time, but we may not be so lucky.

While it would be nice to concentrate just on the state of Washington, since we live here and it is thus relevant to us, there has been little WNV activity in Washington since the pathogen first arrived (e.g., in 2012 there were only four human cases and similarly few cases

of infection in other animals). We will thus concentrate our efforts on California, which is large, is well studied, and has had a great deal of WNV activity.

First, you will need to extract data from USGS WNV Maps for Bird, Human, Mosquito, Sentinel and Veterinary[4] infections. (For efficiency, break up into groups. Each group can then compare one kind of data with human cases over all of the years.) The maps and data for human infections can be found at these urls (and you can then click on the links on each page to get to the Bird, Mosquito, Sentinel, and Veterinary data):

2012: `http://diseasemaps.usgs.gov/2012/wnv_ca_human.html`

2011: `http://diseasemaps.usgs.gov/2011/wnv_ca_human.html`

2010: `http://diseasemaps.usgs.gov/2010/wnv_ca_human.html`

2009: `http://diseasemaps.usgs.gov/2009/wnv_ca_human.html`

2008: `http://diseasemaps.usgs.gov/2008/wnv_ca_human.html`

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Determining spatial association

Again, our first task is to determine the spatial association between each type of data and human incidence. To keep it simple[5] let's ask how often counties with *at least* one human case had *at least* one infected dead bird (or mosquito or sentinel or veterinary infection). In each year (for a given type of non-human data) you want to construct a 2×2 matrix of the number of counties with both human and bird cases, those with just human or just bird

[4] Almost all veterinary cases involve horses, which are *very* susceptible to WNV.

[5] We are making several simplifying assumptions. For instance, we are assuming that a county without any dead birds was actually *looking* for dead birds, just as hard as in other counties, and just did not find them. We are also assuming that population densities do not vary a great deal since we are using the number of cases rather than proportions of the population. Epidemiologists spend a great deal of time trying to account for actual differences in surveillance effort, etc., in order to provide more robust estimates of association, but for our purposes these assumptions are OK.

cases, and those with no cases in birds or humans. In 2003, for instance, this is what the data would look like:

		Infected birds?	
		No	Yes
Human cases?	Yes	1	2
	No	52	3

It may help to note that California has 58 counties; your table should thus add up to 58.

In the general case this will look like this:

		Infected birds/mosquitoes/sentinels?	
		No	Yes
Human cases?	Yes	a	b
	No	d	c

If all of the counties fall into cells b and d (i.e., either a county has both types of cases, or neither) then we have a perfect association between the types of data. We will rarely get that lucky. Instead we will use the phi-coefficient of association to measure the degree of association. It ranges from -1 if the two types of cases are perfectly, negatively associated to 0 if there is no association (i.e., random) to $+1$ if the cases are perfectly associated. The equation, using the letters from the previous table, is:

$$\phi = \frac{bd - ac}{\sqrt{(a+b)(c+d)(a+c)(b+d)}}. \tag{1}$$

You can do this calculation by hand, or you could use the online calculator at `http://www.vassarstats.net/tab2x2.html` (we formatted our table to match how data is entered in this table). In the case of birds and human data in 2003 we get a value of $\phi = 0.48$, which is moderate, positive association.

Assignment: Your group needs to repeat this process for the type(s) of data you chose for each of the 10 years. It would be useful to graph the values of *phi* against time, which will make it easier to see what is happening and to compare your metrics of risk with those used by other groups.

Determining temporal association

Our second task as a class is to determine which type of data best predicts human cases through time. Again, we will keep things simple in our analyses. We will just keep track of the *total* number of cases of each kind in the *whole* state of California in each year. The last two years of human and bird data, for instance, would look like this:

Year	Bird cases	Human cases
2012	1644	479
2011	692	158
⋮	⋮	⋮

A simple plot of the Human cases against the bird cases (or whatever metric you have) will be informative, so please create one. You might even fit a line through the points, but it is probably not correct to assume that, in the current example, infections in birds *lead to* or *cause* human cases (although there is a reasonable case for an *indirect* causal relation since birds amplify WNV infection in mosquitoes, which then infect humans). So instead of regressing human cases against bird cases we will look for correlations between them. We will use the Pearson product-moment correlation (a pretty standard type of correlation). You are welcome to calculate this by hand—just search online for the formula or look in a stats book—but I'm guessing you would rather have the calculations done for you. So navigate to an online calculator, like that at the same site we used above. You can enter the values in by hand at `http://www.vassarstats.net/corr_stats.html` or copy-and-paste values from a spreadsheet at `http://www.vassarstats.net/corr_big.html`. Look for the correlation coefficient, r. Again, this will range from -1 if the two types of cases are perfectly, negatively associated to 0 if there is no association (i.e., random) to $+1$ if the cases are perfectly associated.

Assignment: For this section, you should contribute to the class a graph of human cases against your metric of interest, as well as the correlation coefficient. Once everyone is finished we will compare the associations between each type of data and human cases to find the best metric(s) of risk to humans. Think about what a strong association implies about WNV biology[6].

Group exercise: test a new hypothesis explaining WNV risk

The last part of this lab, which you will do in your individual groups, is more open-ended, like most real-world ecology. We want you to 1) develop a **new, unique** hypothesis (**not the same one you just did as a class**) explaining the spatial and/or temporal variability in WNV risk to humans, 2) devise a way to test this hypothesis using publicly accessible data, 3) clearly state your expectations given this data *if* your hypothesis is correct, and 4) evaluate the evidence for or against your hypothesis.

[6] "Correlation doesn't imply causation, but it does waggle its eyebrows suggestively and gesture furtively while mouthing 'look over there.' " - Randal Monroe, `http://xkcd.com/552/`

Discuss with your group what you think drives WNV infections in humans. If you are having difficulty thinking of hypotheses, refer back to the introduction, talk with your TA, or look around at scientific sources online for inspiration. Drawing the causal links between different factors (e.g., X causes Y, which leads to Z) can help you be clear in your thinking. As much as possible, try to root your hypothesis in the ecological principles you have learned in this class.

Data sources: There are many sources of data online. You can use any of those listed below or, if you find a source you would like to use somewhere else online, just get the approval of your TA before proceeding. Also note that you can address the question at the local, state, or national level, whichever best matches your question.

- The CDC makes their WNV statistics and maps available online at `http://www.cdc.gov/westnile/statsMaps/`. You can also find a lot more information about WNV infections by looking around their site.
- The National Atlas of the United States has a lot of maps and mapping tools. You can create your own custom maps with physical, climatic, and even biological data using the MapMaker tools (`http://nationalatlas.gov/mapmaker`).
- The United States Historical Climatology Network (US HCN) (`http://cdiac.ornl.gov/epubs/ndp/ushcn/ushcn.html`) also includes a lot of historical data.
- The PRISM Climate Group (`http://prism.nacse.org/`) has 30-year "normals" for precipitation and the minimum, mean, and maximum temperature for the last several decades.
- The North American Breeding Bird Survey (BBS; `http://www.pwrc.usgs.gov/bbs/`) has a tremendous amount of information on bird diversity across the US through time.
- The USDA National Agriculture Statistics Service has a database and maps of croplands available here: `http://nassgeodata.gmu.edu/CropScape/`

Format of abstract and figure

You will need to turn in an abstract detailing your hypothesis and related logic, your tests of this hypothesis, your prediction, and your findings, as well as a figure showing your data analysis with a descriptive caption. In science—indeed, in powerful writing in general—brevity and clarity are essential. Thus, this assignment will be very similar to the 12-sentence paper you turned in for your first lab. Make every word count!

Focusing on one of the seven tests we conducted in lab, your abstract will consist of:

1. **Introduction** (1–2 sentences). Here you need lay out the context and logic that leads to your hypothesis and research. If we were writing about a test of the hypothesis that bears with larger fat reserves hibernate longer, we might start out by discussing the energetic costs of hibernation: "Although hibernating bears lower their heart rate and ventilation as well as their basal metabolism, they are still expending energy during this time. . . "

2. **Hypothesis** (1 sentence). A brief, clear statement of the hypothesis you set out to test. This is not the same thing as an expectation. If you are unclear on the different consult your TA. It might start out with, "We hypothesized that . . . "

3. **Methods & Predictions** (2–3 sentences). Describe the data and analyses you used to test your hypothesis. This will include the sources (i.e., where the data came from), the spatial and temporal scale (e.g., county, state, USA; time period), and a brief description of the data (e.g., duration of hibernation times as measured by the time between when a bear entered its den and when it emerged). You also need to state your prediction, which should be closely related to both your hypothesis and your data and analyses.

4. **Results** (1–2 sentences). Summarize in text format your results (i.e., "There was a strong, positive relationship between the percent fat of the bear's mass and the duration of hibernation."). The focus should be on relationships that are meaningful to your hypothesis.

5. **Conclusion** (1 sentence). Here you just need to make a conclusion about whether your hypothesis was strongly/weakly supported, or not. Keep it brief!

Figure and caption. Finally, you will generate a figure illustrating the relationship between your factor of interest and human risk of infection in space or time. That is, it should illustrate the test of your hypothesis. You need to label the axes clearly and make sure that it is clear what the points or lines or bars represent. Your caption should explain what the x- and y-axes represent, where the data came from and represent (e.g., 10 years of data on . . .), and how many observations they represent. You also need to describe the relationship (e.g., positive, negative, unrelated) between the variables or groups and the test statistic and P-value of any statistics you used to come to this conclusion.

Grading rubric for West Nile virus lab assignment

Criteria	Points	Possible
Introduction provides context and logic clear		1
Hypothesis is clear and coherent		1
Data are accurately, comprehensively described (sources, scale, etc.)		1
Method of testing hypothesis is clearly explained		1
Prediction is laid out in relation to hypothesis and test		1
Results and meaningful relationship(s) clearly describe		1
Conclusion is logical and flows from results		1
Figure illustrates test of hypothesis		1
Figure is correctly labeled, clear, etc.		1
Caption explains the results in a clear manner, includes statistics		1
Total		10

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