CHAPTER 3 CASE

The Donor's Dilemma

The Donor's Dilemma

Usually, Russell found an excuse not to participate in company-sponsored blood drives, but for the first time he decided to donate blood. After filling out the donor eligibility form and passing the blood pressure, pulse, temperature, and blood-clotting tests, Russell sat down for his interview.

Russell interrupted the long list of "Have you ever?" questions with a question of his own. "What if I have West Nile virus?"

"West Nile virus is uncommon," the interviewer said. "Besides, all donated blood is tested for West Nile virus, even here in California where it's extremely rare." She glanced over his paperwork. "Let's see. You said you haven't had any fevers or headaches in the last week. Is there a reason that you think you might have it?"

"No, but I've heard that sometimes people don't have any symptoms," Russell responded. "I just got back from a hiking trip in Boulder, Colorado, over the Fourth of July weekend. There were news reports about a lot of cases of the virus there, and I'm still covered with mosquito bites."

"Well, if you have West Nile virus, we will find out. Lab tests on your blood will identify the presence of genetic material from the virus," the interviewer said reassuringly. "WNV can only be transmitted through blood transfusions if there are virus particles in the donated blood. In the United States, only a tiny fraction of blood donations last year tested positive for West Nile virus."

"So if I have West Nile virus, could you tell if I got it in Colorado?" Russell asked.

"Well, they can't tell from this blood screening, but other tests can identify the strain of WNV," she replied. "When West Nile virus first appeared in New York in 1999, all the samples were alike. But now mutations are showing up in the virus as it migrates to different areas. We're seeing different strains of the virus in different regions of the country."

"So did West Nile virus originate in New York?" Russell wondered.

"No," she said with a smile, "it's called *West Nile* for a reason."

Figure 3.1 A lab technician performs a PCR, which is used to make multiple copies of DNA. Blood donations are tested for WNV using a form of PCR.

CASE ANALYSIS

1. **Recognize potential issues and major topics in the case.** What is this case about? Underline terms or phrases that seem to be important to understanding this case. Then list **three to four** biology-related topics or issues in the case.

2. **What specific questions do you have about these topics?** By yourself, or better yet, in a group, list what you already know about this case in the "What Do I Know?" column. List questions you would like to learn more about in the "What Do I Need to Know?" column.

What Do I Know?	What Do I Need to Know?

3. Put a check mark by **one to three** questions or issues in the "What Do I Need to Know?" list that you think are most important to explore.
4. **What kinds of references or resources would help you answer or explore these questions?** Identify two different resources and explain what information each resource is likely to give that will help you answer the question(s). Choose specific resources.

Core Investigations

I. Transmission of West Nile Virus (WNV)

West Nile virus is an arbovirus (**ar**thropod **bo**rne) that infects birds, humans, and other animals. Although the virus was first detected in Uganda in 1937, the first bird and human cases of West Nile virus in the United States were reported in New York City in 1999. Since then it has spread throughout much of North America. Mosquitoes are the vectors for the virus, transmitting it to the animals that they feed upon. Although mosquitoes feed on many types of vertebrates, birds are the most likely source of the virus. The virus multiplies at a very fast rate in the blood of many bird species, producing a high viral titer (concentration of virus particles in blood). Many bird species are known as *reservoir hosts* for the virus because they can "store" a high concentration of virus particles in their blood. When a mosquito feeds on the blood of a reservoir host, it will likely take in enough virus particles to transmit the virus to another potential host (Figure 3.2).

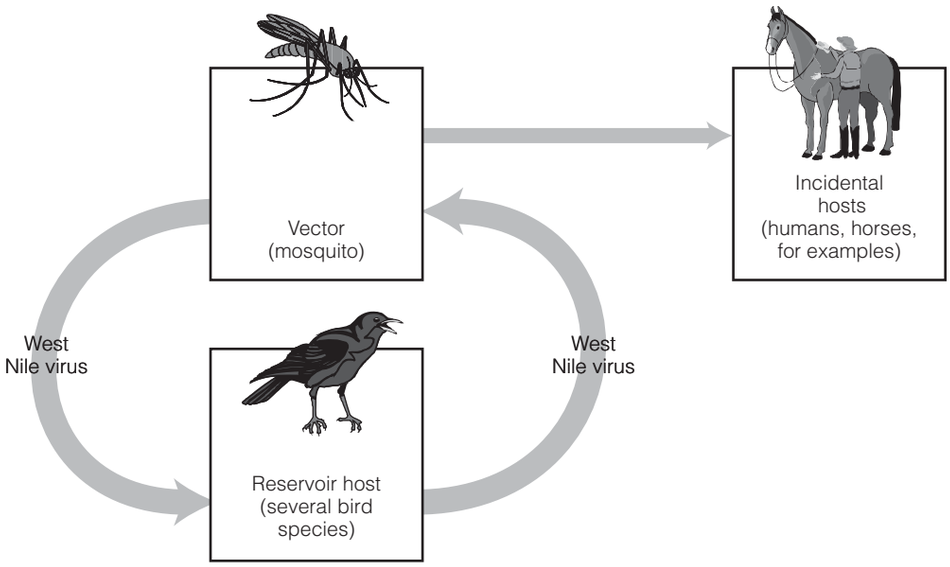

Figure 3.2 West Nile virus transmission cycle.

The interactions between infected birds and mosquitoes can quickly increase the incidence of WNV in a particular location, resulting in a cycle of viral amplification. The more mosquitoes there are, the more the virus is spread. The more birds that are present to be infected, the greater the number of virus particles that will be available to more mosquitoes.

Other animals, including humans, often serve as *incidental hosts*. Within incidental hosts, WNV is less efficient at multiplying; therefore, the concentration of the virus in the blood of these animals during infection is too low for mosquito vectors to pick up and transmit WNV to another host. Typically, these animals do not contribute to the cycle of amplification. West Nile virus can also be transmitted when an organism eats an infected organism. For example, crows that feed on the decaying flesh of other birds may contract West Nile virus through bird-to-bird transmission.

Eighty percent of humans who are infected with WNV show no symptoms. Twenty percent of those infected may experience fever, headache, fatigue, and body aches. Of the 20% with symptoms, only about 1 person in 150 develops encephalitis, a serious swelling of the brain that can

cause death. In cases of WNV, viremia—presence of virus in the blood—lasts approximately 6 days or less. The amount of genetic material produced from the virus (a measure of viral titer) averages less than 5,000 copies of the virus per milliliter of blood. By comparison, other forms of viral encephalitis can result in a titer of 25,000,000 copies per milliliter of blood.

Human-to-human transmission of WNV through blood and organ donation, as well as during pregnancy or nursing, has been reported. Screening tests, including nucleic acid amplification and antibody detection, have been developed for WNV. (You will learn more about nucleic acid amplification in Investigation II.) Antibody detection tests are not used for screening blood because by the time the immune system produces antibodies in detectable amounts, the majority of the virus particles have been destroyed.

1. Several alligator farms in the southeastern United States reported an unusually high number of alligator deaths between 2001 and 2003. WNV was determined to be responsible for many of these deaths. Blood samples from infected alligators revealed high titers (some of which were higher than the titers in reservoir host bird species) for WNV. Considering that an adult alligator's hide is too thick for mosquitoes to penetrate (except for a few areas of soft tissue, such as inside the mouth and around the eyes), what are some other ways in which the alligators might have acquired WNV?

2. How would you add alligators to the transmission cycle shown in Figure 3.2?

3. Although humans produce low titers of WNV particles in their blood and don't serve as reservoirs for this vector-disseminated disease, human-to-human transmission of WNV is possible. Explain how a transfusion of infected blood can result in the dissemination of WNV.

II. Critical Reading

Before delving further into this investigative case, you first should read Concepts 17.1, 17.2, and 17.4, "Types of Point Mutations" in Concept 17.5, and Concepts 19.1 and 19.3. You might also want to do two Chapter 17 Activities on the Campbell website (http://www.masteringbio.com) or CD-ROM—*Overview of Protein Synthesis* and *Translation*.

In "The Donor's Dilemma," Russell wondered if it would be possible to tell where someone contracted West Nile virus. This is indeed possible. West Nile virus is an RNA virus. Like other RNA viruses, it has a high mutation rate; therefore, the nucleic acid sequence of a virus strain in New York could be quite different from a virus strain found in Egypt, for example. Many strains of WNV have been identified, and information about their nucleic acid sequences are stored in publicly available databases such as GenBank.

The sequences found in these databases are actually DNA sequences. In a laboratory, it is possible to create a DNA version of an RNA genome by using enzymes called reverse transcriptases. The newly

constructed DNA sequence can be compared quickly to the sequences stored in databases by using powerful software to perform the comparisons. In the following activity, you will manually compare a short sequence of DNA (50 nucleotides out of 11,000) from six samples of WNV collected in Africa and Europe (Table 3.2). This particular sequence is part of the gene that codes for a portion of the virus's envelope protein (*E* gene).

1. Before you begin your analysis of the nucleotide sequences, use the data in Table 3.1 to make a prediction about the sequence that you would expect to be most similar to the one from Egypt. Make a second prediction about the one you would expect to be most dissimilar. Include number, country, and year.

Table 3.1 Identification of DNA Samples for a Portion of the Envelope (*E*) Gene of WNV (Berthet et al., 1997)

No.	Country	Year
1	Egypt	1951
2	France	1965
3	Senegal	1979
4	Senegal	1990
5	Uganda	?*
6	Madagascar	1986

*The specific year in which this sample was gathered in Uganda is unknown; however, it was after 1951.

Most similar:

Reason:

Most dissimilar:

Reason:

2. To analyze the sequences in Table 3.2 (see the next page), you will use manual methods that were used by geneticists until the development of computer-based methods. However, to make your comparison easier, a software program has been used to align the sequences in the table. The basic technique for comparing sequences has three steps:

Examining the sequences for noticeable differences in length

Comparing the sequences nucleotide by nucleotide

Translating the sequences from codon to amino acid

a. Consider Sequence 1, the oldest sequence from the West Nile region of Egypt, to be the standard for comparison. Examine the sequences shown in Table 3.2 for noticeable differences in length. Gaps in sequences are sometimes inserted by the computer as it

aligns the rest of the sequence. These gaps are not present in the actual nucleic acid; however, they show up in the computer's output and often indicate certain kinds of mutations. Which of the sequences has either a deletion (gaps leading to a shorter length) or an insertion (leading to longer length)? Which type of mutation is it? Indicate, by column number, the affected nucleotides.

Table 3.2 Alignment of Six Sequences of Part of a WNV Gene for Envelope Protein*

	1 10 20 30 40 50
1	CCAACCACTGTGGAGTCGCATGGAAACTACTCCACACAGATTGGGGCCAC
2	CCAACCACTGTGGAGTCGCATGGAAACTACTCCACACAGATTGGGGCCAC
3	CCGACGACCGTTGAATCTCATGGCA AGATAGGGGCCAC
4	CCAACCACTGTGGAGTCGCATGGAAACTACCCCACACAGATTGGGGCCAC
5	CCAACGACCGTTGAATCTCATGGCAGTTATTCAGCACAGATAGGGGCCAC
6	CCGACGACTGTTGAATCTCATGGCAATTATTCAACACAGGTTGGGGCCAC

*(Note that published DNA sequences, such as those shown here, are always the nontemplate strand of DNA; thus, it is directly comparable to mRNA. By replacing the *T*s with *U*s, these sequences can be directly translated using Figure 17.5 in your text. These are only fragments of the *E* gene sequence shown with the 5′ end to the left. The WNV genome is an open reading frame that starts before these first 50 nucleotides of the *E* gene.)

b. Next, analyze the differences in the columns of nucleotides to identify point mutations. Use a straightedge to keep your place, a highlighter, and a pen. Examine each vertical column in Table 3.2 starting at the left to look for variations from Sequence 1. If the nucleotides in a column match those of the standard sequence, highlight them. If there are deviations from the standard, circle them with the pen. For example, the first column contains all *C*s, so the whole column should be highlighted. The third column has two *G*s that vary from the A in Sequence 1. The two *G*s would be circled in pen and all the *A*s highlighted. How many total point mutations did you identify?

c. Determine the percentage of point mutations in sequences 2 through 6 (number of point mutations/number of nucleotides in sample × 100%). Sequence 2 is done for you as an example. (*Note:* For Sequence 3, count only the nucleotides present in the sequence.)

Sequence 2 = (0/50) × 100% = 0%

Sequence 3 =

Sequence 4 =

Sequence 5 =

Sequence 6 =

Which sample shows the greatest difference in nucleotides from Sequence 1? Explain. (*Note:* The 12 missing nucleotides in Sequence 3 should be considered as one deletion mutation rather than 12 point mutations because this deletion most likely occurred as one event.)

d. In the third and final step in comparing sequences, translate each of the six sequences from codon to amino acid, using Figure 17.5 in your textbook. Then you will be able to observe the consequences of the different mutations on the resulting polypeptides. Normally, you would expect to see a start codon (AUG), but assume instead that the reading frame begins with the first nucleotide at the 5′ end. Write in the appropriate amino acids under the DNA sequences in Table 3.2.

e. Examine each sequence. How many amino acids differed from the standard in sequences 2 through 6? Which amino acids changed?

Sequence 2:

Sequence 3:

Sequence 4:

Sequence 5:

Sequence 6:

What does this information reveal about the effects of the mutations on the *E* gene and the protein it codes for?

f. How many point mutations were involved in the amino acid differences you found? In Table 3.2, draw an asterisk by those nucleotides that made these differences.

g. How many of the point mutations were nonsense mutations? How many were silent mutations?

h. Compare your answers in 2c to those in 2f. Is the percentage of point mutations related to how many amino acids are changed? Explain your response.

i. Is it likely that the deletion mutation is also a frameshift mutation? Explain.

j. Now that you have identified, categorized, and determined the consequences of the various mutations in these sequences of WNV, how do these results compare to your predictions in question 1?

III. West Nile Virus: Viral Structure and Life Cycle

West Nile virus is a relatively small, spherical virus whose genome is single-stranded RNA (ssRNA), which also serves as the messenger RNA (mRNA) coding for viral proteins (Figure 3.3). This genetic material is contained within an inner protein coat called a capsid. Like many other animal viruses, WNV also has a membranous envelope derived from the host cell. This membrane surrounds the capsid and has numerous glycoproteins (the E protein) encoded by the viral genome. These glycoproteins are located on the outer surface of the envelope and function in the recognition of potential host cells. You analyzed the sequence of a portion of this viral envelope glycoprotein gene in Investigation II.

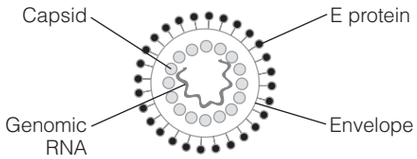

Figure 3.3 West Nile virus structure.

1. Animal viruses are classified by the type of nucleic acid found within the capsid. Using Table 19.1 in your textbook and the clues provided in the passage above, identify the classes for WNV and HIV. Provide an example of another virus from the same class for each.

WNV Class _____	HIV Class _____
Example:	Example:

2. Compare the structure of WNV to that of HIV (see Figure 19.8 in your text).

3. How do the RNA molecules of these two viruses differ in number and function? In your response, consider the role of both in the formation of mRNA.

4. Compare and contrast the reproductive life cycle of WNV (Figure 3.4) to that of HIV (see Figure 19.8 in your text).

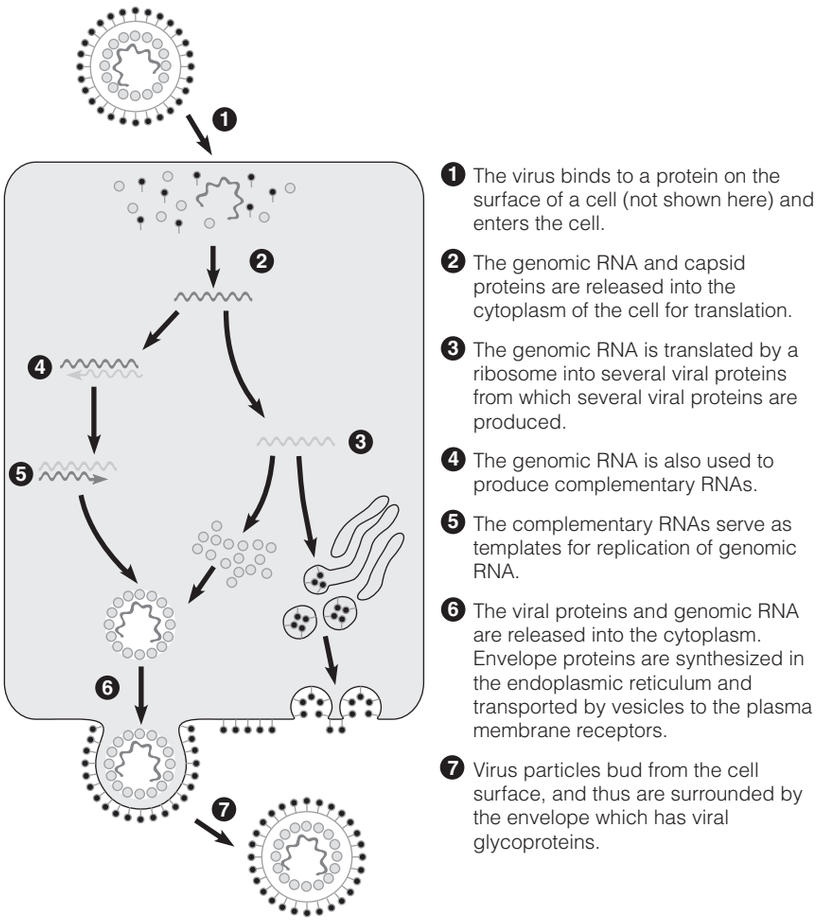

Figure 3.4 Simplified reproductive cycle of the West Nile virus.

5. Many viruses, including WNV, cold viruses, and flu viruses, reproduce in the host for a short time before being destroyed by the host's immune system. This production of new virus particles occurs during a period in which virus particles are present in the blood (viremia). If Russell, the blood donor in the case, had been infected with WNV, he could safely make future donations once the viremia had passed and his blood no longer contained virus particles. In contrast, a person infected with HIV can never give blood. Examine the life cycle of HIV and suggest a reason for this. (*Note:* An immune system response is usually initiated by recognition of "nonself" molecules on the surface of infected cells.)

IV. Testing Blood Donations for WNV

To prevent human blood-to-blood transmission of WNV, all blood donations since June 2003 have been tested for the presence of WNV particles. The test used is called reverse transcription–polymerase chain reaction (RT-PCR).

A PCR cannot be run without DNA. Since WNV does not contain DNA, its RNA must be isolated and reverse transcribed (RT) to form complementary DNA (cDNA). (See Figure 20.8 for more information on PCR and Concept 20.4 for more information on RT-PCR.) When donor blood is tested for WNV, RNA is extracted from the blood sample. Individuals who are in the viremic phase of WNV will have West Nile virus RNA present in their blood, as well as other types of RNA, including their own. The mixed sample of "unknown" RNAs is reverse transcribed to create a mixed sample of cDNAs.

PCR utilizes polymerase enzymes and specific DNA "primers" to amplify (make many copies of) a targeted DNA sequence. Primers are short, single-stranded DNA molecules that match up to the two ends of the targeted DNA sequence and are necessary for the initiation of DNA synthesis. The DNA that matches up to the primers is then repeatedly duplicated in cycles of PCR, until it reaches detectable levels.

Primers specific for WNV cDNA are used in the PCR test referred to in this case. If WNV is present in the blood sample, then the cDNA will be amplified successfully. The primers ensure that a fragment will be amplified from this cDNA only. (For more information, see Khanna et al., cited in the references at the end of this investigative case.)

1. Why are primers needed for initiation of DNA synthesis using PCR? How do PCR primers differ from the primers in cells? (Hint: See Figure 16.16.)

2. The following cDNA sequences (A–D) were obtained by reverse transcription of RNA samples from donated blood. One of the WNV primers used in RT-PCR has the following sequence.

3′ GGCTGCTGGCAACTT 5′

Circle the cDNA sequence below that would be targeted by this WNV primer.

a. 5′ GGCTGCTGGCAACTT 3′

b. 5′ CCGACGACCGTTGAA 3′

c. 5′ TATAACCGTCCAAGTT 3′

d. 5′ CCGGCCTAGCATAGAA 3′

3. Explain how primers control which cDNA is being amplified.

4. The day after Russell's blood sample was tested for WNV, he was told that the results were positive. What organisms were likely involved in Russell's infection with WNV? Is it likely he will pass on the disease?

Additional Investigations

V. Tracking West Nile Virus

A. Origin of the West Nile Virus in the United States. WNV was first isolated in Uganda in 1937 and has since spread throughout Africa and other parts of the world. As an emerging disease, WNV continues to generate both public and scientific interest. Researchers are exploring questions about its origin, evolution, transmission by multiple vectors and host tissues, replication in multiple hosts, detection, and vaccine potential. Central to these investigations are the use of molecular data, including nucleic acid sequences, and the use of bioinformatics (the application of computer science and mathematics to genetic and other biological information).

When WNV was first detected in New York City in 1999, researchers wanted to know where it came from and how it arrived. To propose an answer to these questions, using methods similar to those used in the analysis in Investigation II, you can look at similarities between a New York strain (NY99) of WNV isolated from a Bronx Zoo flamingo in 1999 and strains of WNV isolated from different parts of the world. (*Note:* In Table 3.3, only a portion of the genomes were compared—specifically, a portion of the envelope protein gene. A software program called CLUSTALW was used to align the nucleic acid sequences found in these strains, and then a second program called BOXSHADE was used to display the sequences from the most similar to the least similar compared to the NY99 strain. The Case Book website provides links to instructions for using these programs.)

Table 3.3 BOXSHADE Plot of Aligned WNV *E* Gene Sequences from Various Strains

The BOXSHADE program automatically generates several colors to indicate properties of nucleic acids. To learn more, go to the Biology WorkBench website (see References).

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          NY99 CCAACTACTGTGGAGTCGCACGGAAACTACTCCACACAGGTTGGAGCCACTCAGGCAGGGAGATT
      ISRAEL98 CCAACTACTGTGGAGTCGCACGGAAACTACTCCACACAGGTTGGAGCCACTCAGGCAGGGAGATT
   MOROCCO 96 CCAACCACTGTTGAGTCTCATGGTAACTACTCCACACAGATTGGGGCCACTCAGGCAGGGAGATT
      ITALY98 CCAACCACTGTGGAGTCGCATGGAAACTACTCCACACAGATTGGGGCCACTCAGGCAGGGAGATT
    SAFRICA99 CCAACCACTGTGGATTCGCATGGTAACTACCCCACACAGATTGGGGCCACTCAGGCAGGGAGATT
   ROMANIA96 CCAACCACTGTGGAGTCGCATGGAAACTACTTCACACAGATTGGGGCCACTCAGGCAGGGAGATT
  TAJIKISTAN99 CCAACCACTGTGGAGTCGCATGGAAACTATTTCACACAGATTGGGGCCACTCAGGCAGGGAGATT
MADAGASCAR88 CCGACGACTGTTGAATCTCATGGCAATTATTCAACACAGGTTGGGGCCACCCAGGCTGGAAGATT
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1. Scientists at the Centers for Disease Control and Prevention (CDC) concluded that NY99 most likely was transported to New York from Israel. Does the information in Table 3.3 support this conclusion? How many differences in sequence are there between the two samples? What other conclusion could you draw from comparing the NY99 and ISRAEL98 strains?

2. Which strain is the most dissimilar to NY99? How many differences did you find between this strain and NY99? Do you find this result surprising? Explain.

3. How do you think WNV arrived in New York City? Consider what you've learned previously about transmission of this disease.

B. Spread of WNV in the United States. Since 1999, WNV has been carefully monitored. The CDC maintains resources including regional data and maps to track the spread of WNV in the United States. For example, the map in Figure 3.5 reflects both vector (mosquito) and host (birds, horses, humans, and so on) data collected by the CDC. Human cases reported in any state from 1999 through 2002 are distinguished by cross-hatching.

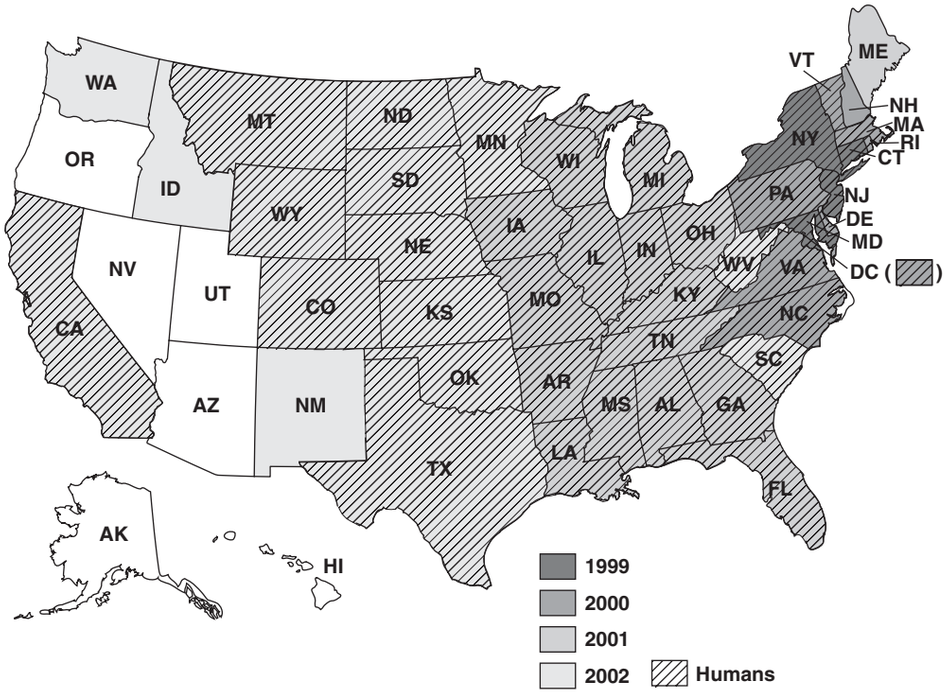

Figure 3.5 West Nile virus in the United States (1999–2002). (Source: CDC)

1. Construct a line graph that shows the number of states reporting the presence of WNV from 1999 through 2002.

2. Is proximity to known outbreaks of WNV a factor in its spread? Looking at the map in Figure 3.5, describe geographic factors that seem to influence the spread of WNV. Explain.

3. Examine the map in Figure 3.6 and compare it to that shown in Figure 3.5. In three to four sentences, describe the extent of spread in 2006.

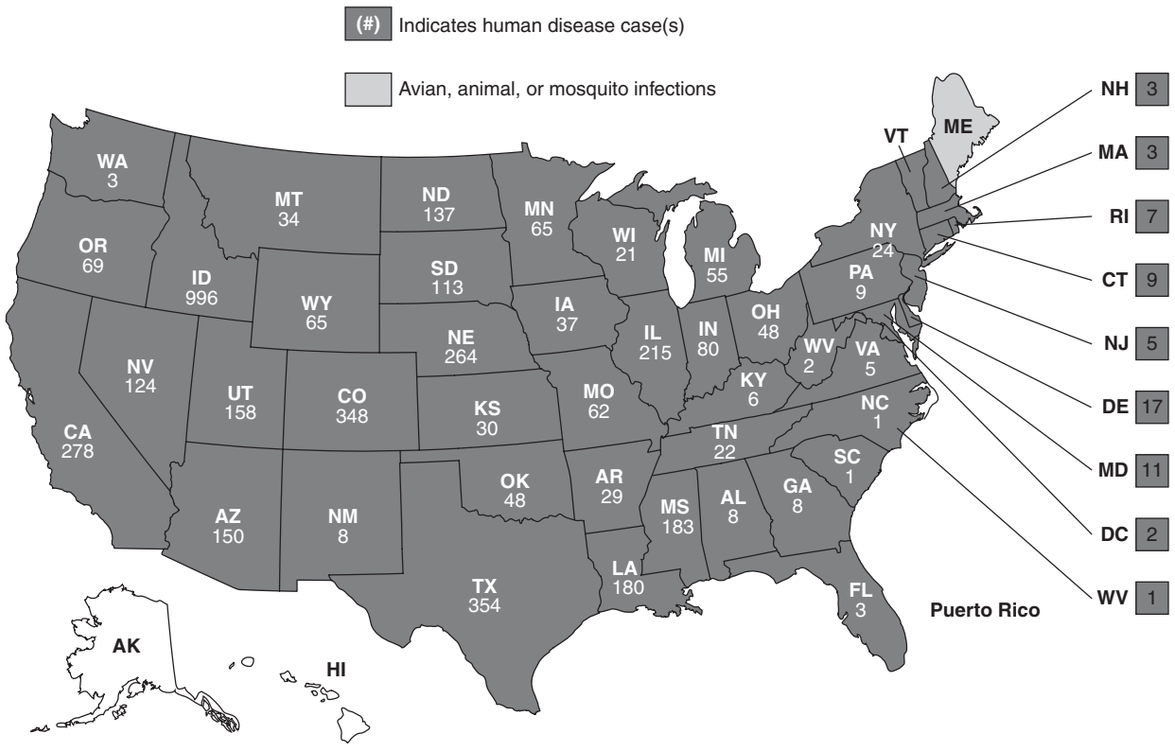

Figure 3.6 West Nile virus activity in the United States (2006), CDC: http://www.cdc.gov/ncidod/dvbid/westnile/Mapsactivity/surv&control06Maps.htm

VI. Open-Ended Investigation

You may wish to visit the West Nile Virus Problem Space to use tools, methods, and data to explore the global spread and evolution of WNV.

The West Nile Virus Workbench Lab (Kiser 2004) provides instruction on using the data and bioinformatics tools.

Additional Potential Investigations are listed in the WNV Problem Space at http://bioquest.org/bedrock/problem_spaces/wnv/curr_resources.php.

References

Berthet, F.-X., H. G. Zeller, M.-T. Drouet, J. Rauzier, J.-P. Digoutte, and V. Deubel. Extensive nucleotide changes and deletions within the envelope glycoprotein gene of Euro-African West Nile viruses. *Journal of General Virology,* 78(9):2293–297, 1997.

Note: Table 3.2 presents an alignment of published DNA sequences of WNV, edited for length. We obtained these sequences from GenBank using identifiers provided by Berthet et al. (see reference above). The sequence identifiers are: EGY-HEg101/51, FRA-PaH651/65, SEN-AnD27875/79, SEN-ArD78016/90, UGA-MP22/?, and MAD-ArMg956/86. We then used the nucleic acid alignment tool CLUSTALW on these sequences. The Biology Workbench was the interface that provided the nucleic acid tools and access to the San Diego Supercomputer. It may be freely accessed at http://workbench.sdsc.edu.

Khanna, M., K. J. Henrickson, K. Harrington, C. R. Waters, J. Meece, K. Reed, and S. Shukla. "Multiplex PCR–EHA Compared to 'Real Time' Taqman for the Surveillance and Diagnosis of West Nile Virus." Prodesse Inc., Waukesha, WI, Medical College of Wisconsin, Milwaukee, and Marshfield Clinic Research Foundation, Marshfield, Wis. Presented at the 11th International Conference on Infectious Diseases, March 2004, in Cancun, Mexico. http://www.prodesse.com/resources/ICID_2004_WNV.pdf

Kiser, Stacey. West Nile Virus Workbench Lab. 2004. http://bioquest.org/bedrock/problem_spaces/wnv/curr_resources.php (accessed July 2, 2007).