WEST NILE VIRUS MOSQUITO SURVEILLANCE PROGRAM CLARK, FLOYD AND HARRISON COUNTIES, INDIANA
2002-2005

INDIANA UNIVERSITY SOUTHEAST
BIOLOGY DEPARTMENT
4201 GRANTLINE RD
NEW ALBANY, IN 47150

A CAPSTONE STUDY AND SERVICE PROJECT WITH THE CLARK COUNTY, FLOYD COUNTY AND HARRISON COUNTY, INDIANA HEALTH DEPARTMENTS

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2005

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Field and Laboratory Assistants: Mollie McDonough and D. McCowan, Floyd County

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2004 Environment and People

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Unpaid Interns: Megan Gettelfinger and DeLynn Rutherford

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Eastern High School, Pekin, IN: Dawn Dietrich; Boy Scout Service: Patrick Click

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EXECUTIVE SUMMARY

West Nile virus was first detected in southern Indiana in 2001 in dead crows and bluejays (Family Corvidae). A significant die off of corvids suggested that the virus was using birds as a reservoir and that mosquitoes obtaining virus from birds would begin to affect the human population in subsequent years. This fear prompted the health departments to initiate mosquito-monitoring programs.

Following a service request from the local southern Indiana Health Departments, mosquito collections began in Fall 2002 with an Indiana University SE Ecology

class collecting 20 sites in Clark County and 12 in Floyd County. Ms. Shea Leffler developed a mosquito identification PowerPoint, and Dr. Claude Baker developed a book of mosquito identification photographs. Mosquitoes were bagged and shipped to the Indiana Department of Health where they were identified and tested for West Nile virus. In Clark County, 3 of the sites tested positive for West Nile virus while in Floyd County 5 of the 12 sites were positive for WNV. These results suggested that WNV was well established in our area and was being amplified during drought conditions in late summer and fall. This timing coincides with a population surge in the house mosquito, *Culex pipiens*. In theory, during drought conditions, normal water supplies dwindle, and birds rely more on stagnant water areas where the *Culex* are abundant.

In 2003, Harrison County requested assistance with their mosquito problems. During this year, almost 70 students collected and identified nearly 5000 mosquitoes finding 6 positive WNV pools; 2 in Clark County, 3 in Floyd County and 1 in Harrison County. One finding during 2003 was that high mosquito populations were associated with failing septic systems in both Clark and Floyd Counties. By locating the mosquito hot spots, we significantly increased our collection numbers in subsequent years.

In 2004, almost 80 students received individual study credit or service hours for working on the West Nile virus project at Indiana University SE. Two students were summer interns with the health departments, and one was an Eli Lilly III intern with the Clark County Health Department. The 21st Century Scholars from Austin schools also participated in the study. These students collected, sorted and identified almost 20,000 mosquitoes, representing 30 species, taken in the field in Clark, Floyd and Harrison Counties, Indiana. Of these mosquitoes, 23 mosquito pools tested positive for West Nile virus compared to six in 2003. Seven were found in Clark County; 12 were from 9 locations in Floyd County, and 4 were from Harrison County. Forty-two percent of the mosquitoes in Floyd County were *Culex pipiens/restuans* taken from an area with failing septic systems. Thirty-three of the 35 positive pools were a single species group, *Culex pipiens/restuans*. The health departments aggressively treated positive locations in all counties.

In 2004, IUS students discovered a new exotic Asian mosquito in Clark County, the Japanese rock pool mosquito. *Ochlerotatus japonicus.* Small numbers of the new species for Indiana were identified at 18 locations in Clark, Floyd and Harrison Counties, mostly near existing active railroad tracks. State Health Lab officials positively identified the species. Molecular genetic testing revealed that our population is most closely related to a population of these mosquitoes found only in Hokkaido, Japan. We believe that our mosquitoes arrived in southern Indiana via international commerce. The Asian rock pool mosquito is medically important from a public health perspective. It can carry the viruses responsible for West Nile virus, St. Louis encephalitis, eastern equine encephalitis and

Japanese encephalitis. The new mosquito record was published in the *Proceedings of the Indiana Academy of Science*.

In 2005, about 50 Indiana University students collected, sorted and identified almost 25,000 mosquitoes, representing 21 species, taken in the field in Clark, Floyd and Harrison Counties, Indiana. Of these mosquitoes, 49 mosquito pools tested positive for West Nile virus compared to 23 in 2004 and six in 2003. Nearly all the positive pools were from Floyd County. Only one positive pool was found in Clark County. No positive pools were reported from Harrison County.

One reason for the low number of positive pools in Clark County was an aggressive treatment strategy. Steven Moberly obtained certification in mosquito pesticide application as a registered technician with the Clark County Health Department. With this certification, he was able to treat many mosquito "problem sites" discovered in previous years. Additionally, a team of IUS students actively sought out additional "problem sites". If a trap recovered approximately 50 mosquitoes in one night, that location was investigated for a treatment strategy. Public mosquito complaints were also investigated and where appropriate, larvicides were applied.

With this added tool for controlling West Nile virus, the number of mosquitoes collected after treatments began was, for the most part, sustained at numbers well below 50 mosquitoes per night. Furthermore, the number of sites testing positive for West Nile virus in Clark County dropped from twelve in 2004 to only one in 2005. This trend was not observed in adjacent Floyd County.

In addition to the larvicide treatment strategy, we were successful in rearing substantial numbers of mosquitofish in an isolated pond located on Ms. Rica Kanzinger's property in Floyd's Knobs. We were able to transfer these fish into locales lacking an extant fish population. Additional work will be done in 2006 to determine the effectiveness of mosquitofish in lowering the larval mosquito population.

Forty-eight positive pools were taken from 8 locations in Floyd County. A very high percentage of the mosquitoes were *Culex pipiens/restuans* taken from an area with failing septic systems. The health departments aggressively treated positive locations and other area hot spots in all counties.

In 2005, we determined that the Japanese rock pool mosquito, *Ochlerotatus japonicus,* discovered in 2004 carries West Nile virus in Clark County, Indiana. Although this species has been found to harbor West Nile virus in other states, this is the first confirmation that it carries the virus in Indiana.

This program has been beneficial to both the agencies and Indiana University. Based on the numbers of students who have worked on the project, the overall value to the health departments has been nearly $500,000. In addition, IUS

developed a web page for Clark County. More information on this webpage is given below. Students also hand delivered mosquito prevention brochures to neighborhoods with vector problems.

From this pool of students, one was employed full-time by the Clark County Health Department. The Clark, Floyd and Harrison County Health Departments employed five students part-time. Two of the students employed by the Health Departments were Eli Lilly III interns with the Clark County Health Department. These 15 week internships paid about $5000- to each student. Another was awarded a Summer Fellowship at IUS and another was a research fellow in Fall 2005.

The university was the recipient of some $20,000 in tuition from students taking Individual Study. More important, however, was the positive press coverage. All local media outlets carried stories about the student-directed WNV program. One administrator conjectured that the value to the university was in the $500, 000 range. One of the news stories was picked up by the Associated Press and reached national outlets. Students were featured several times during IU basketball halftimes. Dr. Baker and students were on National Public Radio, and they presented their work to the IU Board of Trustees and to potential donors at the Academy at Cedar Farm. IUS students were featured speakers at two Indiana Environmental Health Association conferences. Students presented papers in the Indiana Academy of Science and all the student research conferences in Indiana. They received awards at the IUS undergraduate research conferences and at the IT Making IT Happen presentation. The new mosquito find was published in the senior section of the Indiana Academy of Science. A summary of the work will be presented to the National Undergraduate Research Conference in April 2006.

Other students, not directly involved in the monitoring, assisted by handing out brochures in residential neighborhoods with reported mosquito problems. One student penned a new brochure for our area. Paul Ranney, as part of his Lilly III internship, developed a website for the Clark County Health Department. The URL is listed below:
http://www.clarkhealth.net/info.htm

RECOMMENDATIONS

1. Our evidence and published information suggest that human outbreaks of West Nile fever are related to high temperatures and drought. Several days with temperatures above 90^0 F are necessary for the viral load to reach high levels in mosquitoes.

2. Normally, the above conditions are met in July through September and not in every year.

3. We recommend, therefore, that serious monitoring should be done only in those months where human infection is likely; July-September.

4. Special attention should be given to the above set of conditions—several consecutive abnormally hot days above 90^0 F and drier than normal conditions that force birds into less desirable water supplies where *Culex* mosquitoes are present.

5. We recommend that "hot spot" investigation with larvicide treatment should continue. In Clark County, we have shown that this protocol, when initiated early, may be effective in reducing West Nile viral load.

6. We recommend the continued use of mosquitofish in areas where they are unlikely to enter the natural stream ecosystem.

7. We recommend that the Health Departments should aggressively work to eliminate areas with failing septic tanks. We have shown that these areas are susceptible to developing high viral loads in *Culex* mosquitoes. Also, source reduction of abandoned tires, abandoned swimming pools, birdbaths, and small water filled containers should continue.

8. Because the dollar value of the IUS service effort has been in the $120k to $150k range per year, we believe the time is ripe for each department to consider the addition of a full-time vector control and monitoring person.

Submitted:

January 20, 2006

INTRODUCTION

In 2002, Indiana University Southeast students, staff and faculty initiated a West Nile virus-monitoring program with the local southern Indiana county health departments. The fundamental premise behind this effort was that college students would gain field and laboratory experience while providing a much needed service for our local communities. A reasonable estimate is that IUS has provided about 12,000 service hours over a four-year period. The purpose of this report is to present a four-year summary of our effort.

BACKGROUND INFORMATION

West Nile virus (WNV), first detected in 1999 in New York City, possibly was carried into the US by an infected bird, one of over 2700 commercial birds entering in 1999 through John F. Kennedy airport (1). Following introduction, migratory birds have spread the virus to all states. The finding of dead WNV infected birds in 2001 prompted the initiation of a surveillance program conducted by students at Indiana University Southeast (IUS), and funded by the Clark and Floyd County Health Departments. The successful program was expanded into Harrison County in June 2003. IUS students, in addition to monitoring, found the first Indiana specimens of the Asian rock pool mosquitoes, *Ochlerotatus japonicus*, in 2004, and in 2005, this mosquito species was found to carry West Nile virus.

Our monitoring program is part of a nationwide surveillance effort (2,3). ArboNet is the national electronic surveillance system established by the Centers for Disease Control (CDC) to assist states in tracking WNV and other mosquito-borne diseases. The positive mosquito pool information for southern Indiana was transferred electronically by appropriate agencies onto state and CDC maps that is available to the public on the Internet.

During the 2002-2005 surveillance programs, mosquitoes were collected at selected stations and “hot spot” locations where mosquito problems had been reported. Once collected, all were sorted to the species level and sent to the Indiana State Department of Health for analysis, or were tested at IUS by three certified technicians (Nelson, McDonough, Moberly or Baker). The ability to test locally greatly shortened the response time required to treat affected areas.

The VecTest, a WNV and Saint Louis encephalitis antigen panel assay, is a rapid detection dipstick test for both viruses. This test, introduced in 2003, provides positive or negative results in 15 minutes. The only negative aspect is that considerable virus must be present, whereas the State Health Laboratory can detect the virus in a single mosquito.

For the 2004 and 2005 surveillance program, we collected significantly more mosquitoes than in previous years, found significantly more positive mosquito pools and discovered a new exotic species for Indiana, *Ochlerotatus japonicus.* This does not necessarily mean that that West Nile virus is increasing in the area. Actually we found only one positive location in Clark County and none in Harrison County in 2005. We were able to positively identify and treat several hot spots in Clark and Harrison Counties before problems developed. In Floyd County, we identified and continually monitored areas where WNV was prevalent.

Dry weather conditions with drought amplification played an integral role in the spread of the virus during this study, but it now appears that areas of failing septic systems have the highest incidence of positive mosquito pools.

ACKNOWLEDGMENTS

We acknowledge the assistance of the following agencies and individuals: Douglas Bentfield, Daniel Ellnor and Danny Schroeder of the Clark, Floyd and Harrison County Health Departments. These agencies provided funding and equipment for the project. IUS provided funding for Steven P. Moberly, Dawn Dietrich, Holly Moberly and Aaron Fields. Steven P. Moberly and Paul Ranney were Eli Lilly III interns. We also thank the following Indiana University faculty and staff, concerned residents, and local fire stations for providing secure sites for this study: Dr. Sandra Patterson-Randles, Chancellor of IUS, Dr. John Moody, Mr. Eugene Seals, Dr. Glenn Mason, Essroc Corporation in Speed, IN, Neil's Salvage Yard in Palmyra, Mr. Earl Copple, Mr. S. Wright of Clarksville, Mr. Kenny Adams of Sellersburg, Kenny's Auto Parts of Clarksville, Dr. John Click, DDS, Mr. Robert and Ms. Rica Kanzinger of Floyds Knobs, Mickey Freiberger of Mike's Tavern in Floyd's Knobs, Dr. Joe Hollingsworth, Mr. Matt Lacy, student at IUS, Mr. Craig Lalor, student at IUS, Mr. Tom Stutesman of Bethlehem, IN, Ms. Kelly Jenkins of Jeffersonville, Tunnel Mill Boy Scout Camp, Mr. Ben Coons, Dean Ben Nassim, Dr. Claude Baker, Ms. Barbara Cocke, Ms. Diana Holman, Mr. Aaron Holman, Mr. David Kaiser, Dr. Gretchen Kirchner, Mrs. Donna Lopp, and New Albany Fire Stations along Grantline Road, Scottsville Road Fire Station, and Carr Township Fire Station in Clark County. We also thank Brad Foster and Adam Estes at the Indiana State Department of Health for their identification of the new mosquito species. Dr. Dina Fonseca of the Smithsonian verified the identification using mitochondrial DNA sequencing techniques. Finally, we would like to thank the 200 or more students who made this investigation possible.

VIROLOGY

West Nile virus (Family Flaviviridae, Genus *Flavivirus*) is part of the Japanese Encephalitis Antigenic Complex that encompasses about 60 viruses including the closely related St. Louis encephalitis (SLE) virus (4). Cross-reactivity with SLE resulted in some confusion with WNV in the initial New York outbreak in 1999.

Subsequent genetic sequencing, however, indicated the virus was WNV (5). WNV can be genetically divided into two lineages, only one of which has been directly linked to the human disease (6).

The spherical WNV virion is approximately 50 nm in diameter. Inside an envelope derived from the host cell is a single-stranded RNA virus surrounded by an icosahedral nucleocapsid (7). For an illustration of this virus, see Figure 1 (8). Researchers at Purdue University have obtained a high-resolution look at the West Nile virus; this image is presented in Figure 2 (9). The virus can cause a disease that ranges in symptoms from asymptomatic to fatal encephalitis (10). The percentages of symptoms are as follows: about 80% are asymptomatic, about 20% will develop a mild illness, and a very small percentage will develop severe, potentially fatal, encephalitis (7). Thus, for every symptomatic patient, there are hundreds of individuals who show little or no signs for WNV.

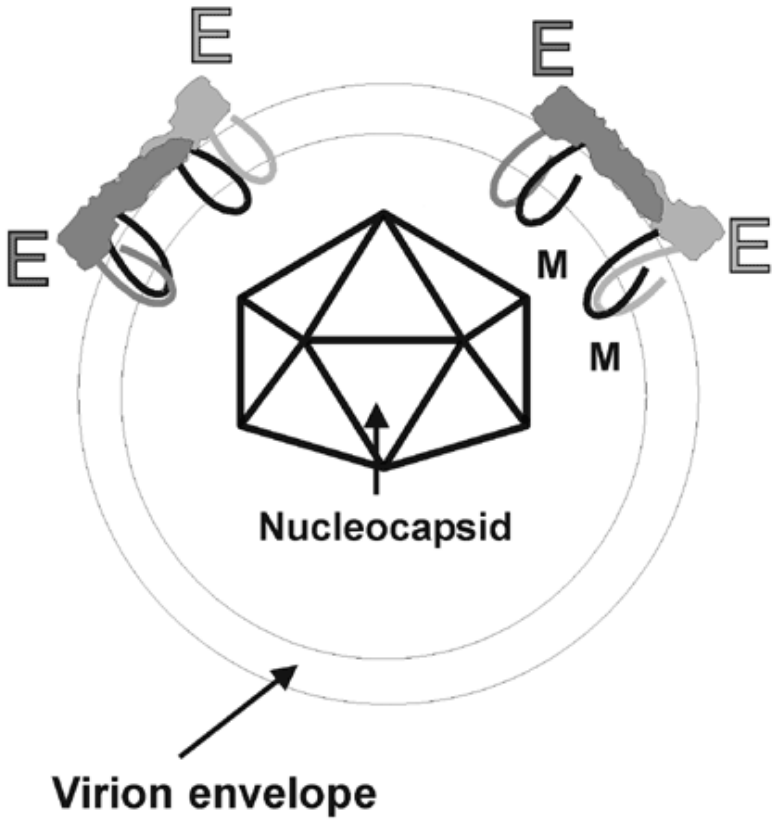


Figure 1. Flavivirus. An icosahedral nucleocapsid encloses the RNA. Emerging Infectious Diseases. Vol. 7, No. 4, 2001.

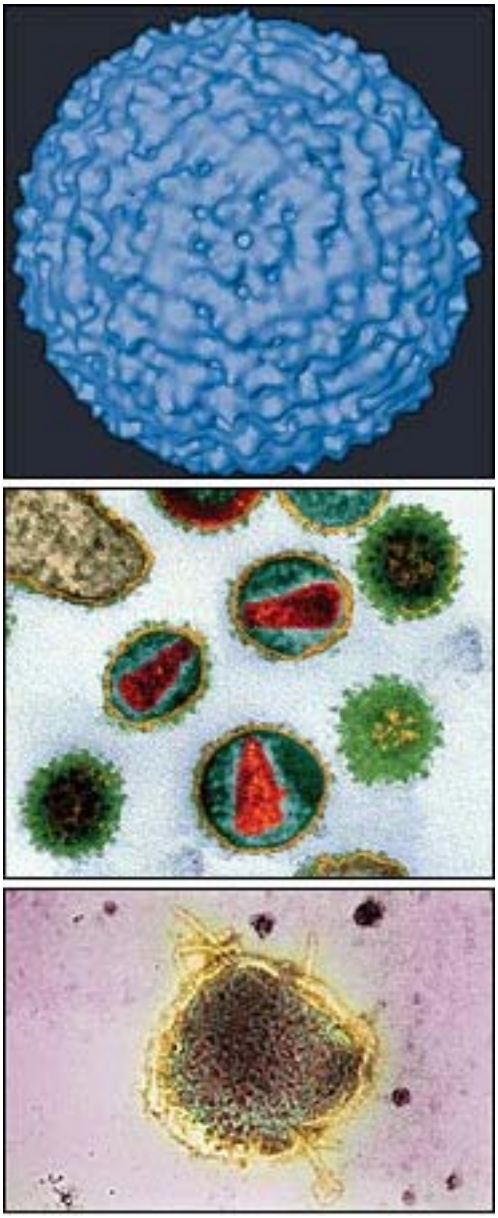


Figure 2. High Resolution Image of Virus. Surface proteins snap together to form a smooth sphere. Source: New York Times. Published October 14, 2003

EPIDEMIOLOGY

Birds are the primary reservoir of WNV, but a novel feature of this virus is the astounding number of different organisms it has infected. For example, it was reported in 2006 that a 12-year old harbor seal developed West Nile polioencephalomyelitis (See Vet Path 2006 Jan;43(1):58-61). The most common mode of transmission to humans is by mosquitoes that have bitten infected birds (10). Humans, horses, various mammals and some reptiles like baby alligators are known to contract the virus (10). Horses are particularly susceptible to the

virus, but no evidence suggests that WNV can be transmitted from equines to humans or other animals.

A study published in the *New England Journal of Medicine* discussed blood transfusions as a means of viral transmission (11). The study showed that 16 donors might have been responsible for the transmission to 23 mostly elderly or immunosupressed transfusion recipients (11). Since 2003, the blood supply has been screened thereby greatly reducing the chance of transfusion transmission. In 2003, a Nebraska man developed WNV encephalitis even though the transfusions he received had been screened (12).

Other modes of transmission reported may include needle sticks, organ transplants, breast milk and transmission across the placenta to the fetus (13). On August 19, 2004, the CDC reported the possibility of WNV infection via a dialysis clinic in Georgia. Two ill patients treated on the same machine tested positive for WNV; one died.

In 2002, two laboratory workers involved in West Nile work were stuck with needles while performing an autopsy on an infected animal (14). Both workers contracted the disease, and it was determined that the source was the needle sticks because the workers had no other risk factors for the disease (14).

In 2002, four organ recipients contracted the virus, and all received their transplanted organs from a single donor (15). The donor presumably received the virus in a blood transfusion (15). In 2005, WNV infections were confirmed in organ recipients in New York and Pennsylvania (MMWR Dispatch, October 5, 2005/54(Dispatch); 1-3). Unlike blood donation screening of all organ donors is not currently required or routinely performed.

A new mother, in 2002, possibly received WNV from a blood transfusion (16). Her breast milk also tested positive for the virus, and since she had been breastfeeding her newborn baby, the baby was tested for the virus (16). The infant tested positive, and the mode of transmission was presumed to be through the breast milk, as the baby had no other contact with any known causes of the disease (16). The benefits of breastfeeding still outweigh the risk of spreading West Nile to the infant. The CDC recommends standard precautions for avoiding mosquito bites for mothers who are breastfeeding.

West Nile virus can presumably cross the placenta and infect the fetus (17). In 2002, a pregnant woman tested positive for WNV, and when her baby was born, the baby had signs of the disease, including decreased brain development in the temporal and occipital lobes (17). Additional studies have been inconclusive with little evidence that the fetuses were infected (CDC, West Nile Virus Topics, Pregnancy and Breast-feeding).

TRENDS IN HUMAN CASES

WNV, since its initial American outbreak in New York City in 1999, has followed a somewhat predictable pattern. The high recorded numbers of mosquito pools, human cases and equine cases occur in the second year before the avian and human population has developed sufficient immunity to the virus. As time progresses, birds and humans develop immunity to the virus, thus reducing the number of West Nile fever and encephalitis cases.

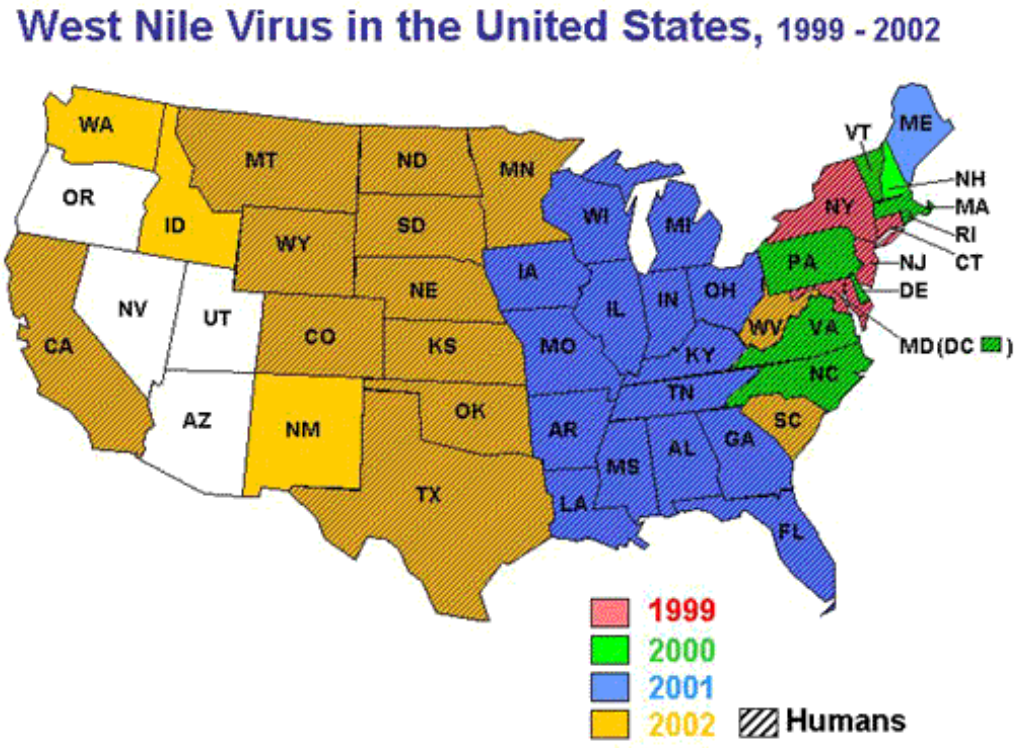


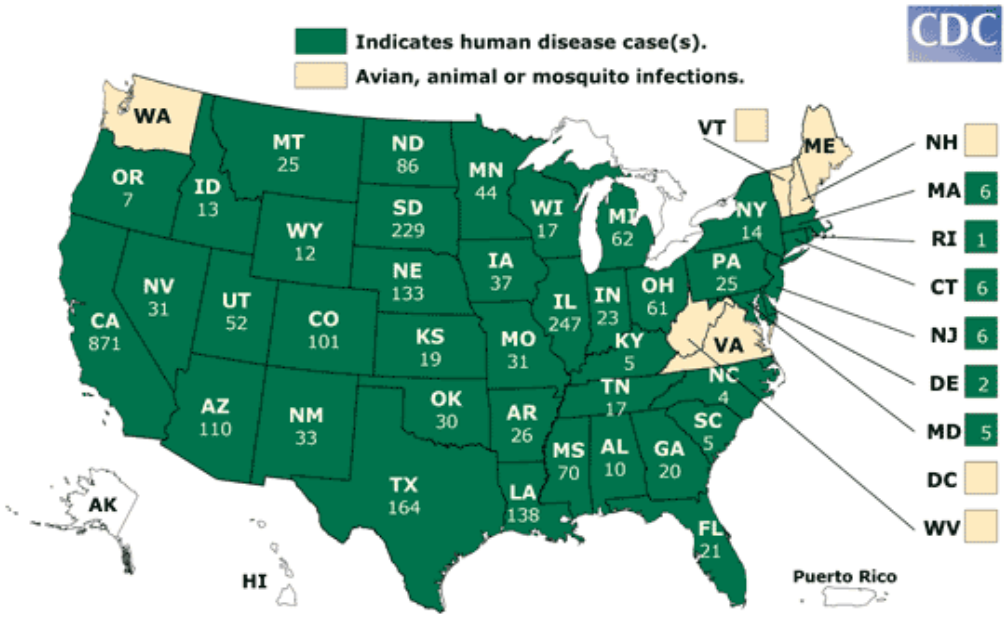
Fig. 3. Map Illustrating Westward Expansion of West Nile Virus. Source: CDC

The disease has now marched westward across the North American continent (Fig. 3) affecting California in 2004 and 2005 in much the same way Illinois was affected in 2002 (884 cases, 64 deaths) and Colorado in 2003 where 2974 cases with 64 deaths were reported (13). Looking back at 2002, Colorado only reported 14 human cases with no deaths. Thus, 2005 is a pivotal year because the virus has been found in all states, and the number of cases should be declining because birds and humans are developing immunity to the virus. WNV is cyclical and may resurge when the older immune birds perish and when certain specific weather patterns occur; hot and dry for more than two or three weeks (18).

Nationwide, in 2005, some 2819 WNV cases have been reported to the CDC with 105 deaths (CDC, Statistics, Surveillance, and Control, Jan. 10, 2005). This

is slightly higher than the overall numbers of human cases reported to the CDC (2470 with 88 deaths) in 2004, but not even close to the record high in 2003 (9862, 264 deaths) and 2002 (4156, 284 deaths). California had the highest number of cases (871).

Figure 4. Human Cases of West Nile Virus as of January 2006



Source: http://www.cdc.gov/ncidod/dubid/westnile/surv&control04Maps.htm

Interestingly, the number of cases in Illinois rebounded from 60 and 54 in 2004 and 2003 to 247 in 2005. Most of these cases were recorded near Lake Michigan where drought conditions were prevalent (Fig. 5). This appears to be a case of drought amplification of the virus.

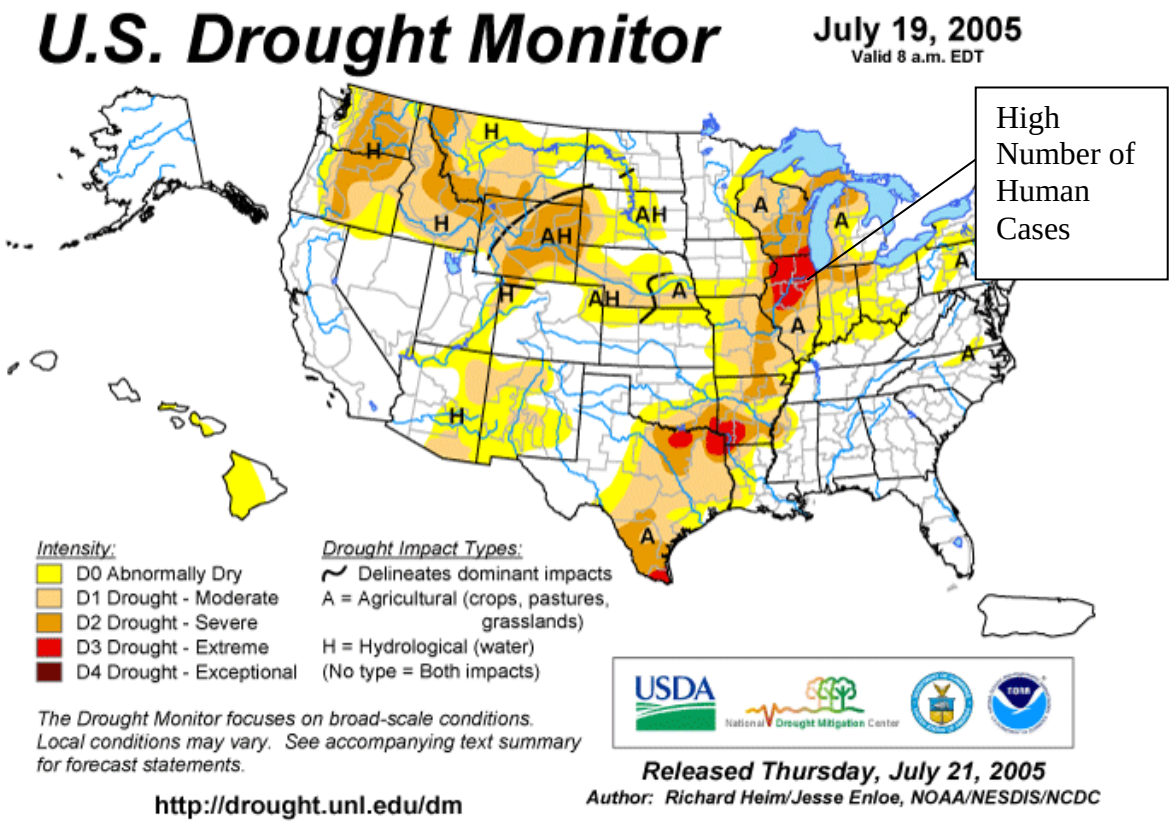
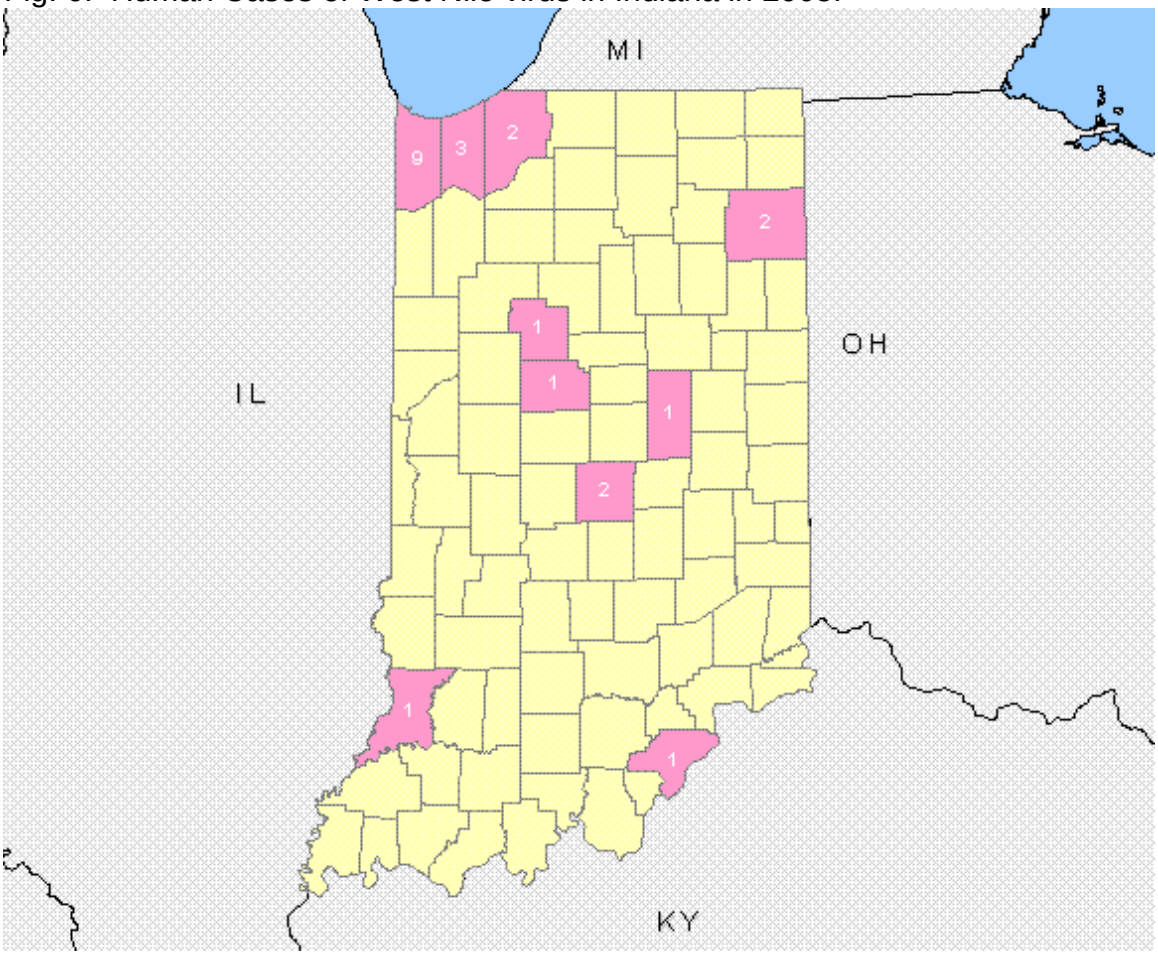


Fig. 5. Drought Conditions in Illinois in 2005

Human Cases of WNV for 2005 (Indiana)

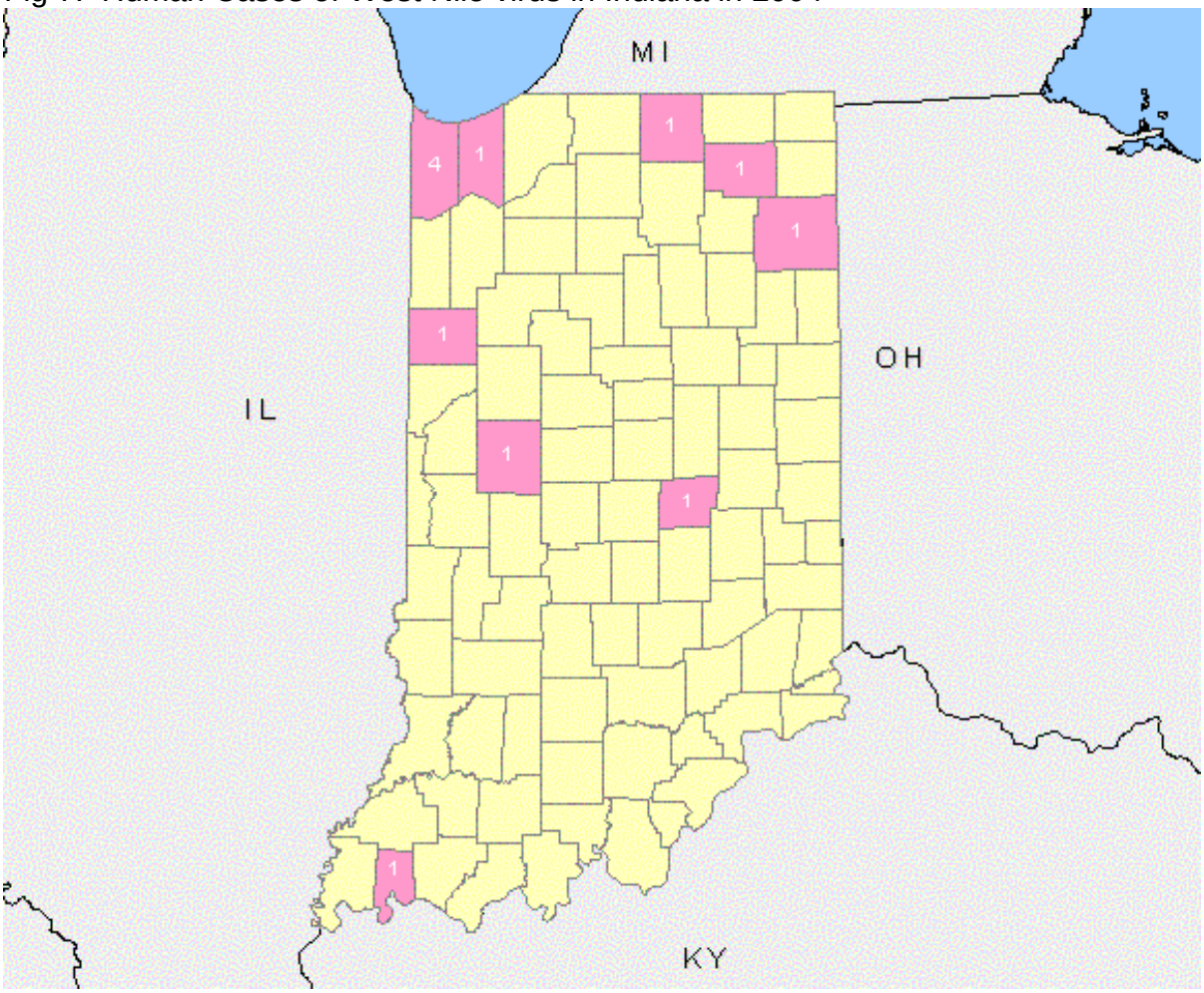
Reported human cases and deaths resulting from WNV infection have been very low in Indiana in the past two years. This year there were 23 cases with one death while some twelve Indiana citizens contracted WNV in 2004 with one death (Fig. 4). One human case was reported for our area in Clark County in 2005 with no human cases in 2004. Despite much effort, we were unable to locate positive mosquito pools near the person's residence. Some 47 cases with 4 deaths were reported in 2003 (Fig. 5). In the highest year (2002), Indiana had 293 cases with 11 deaths.

Fig. 6. Human Cases of West Nile virus in Indiana in 2005.



Source: http://westnilemaps.usgs.govin_human.html

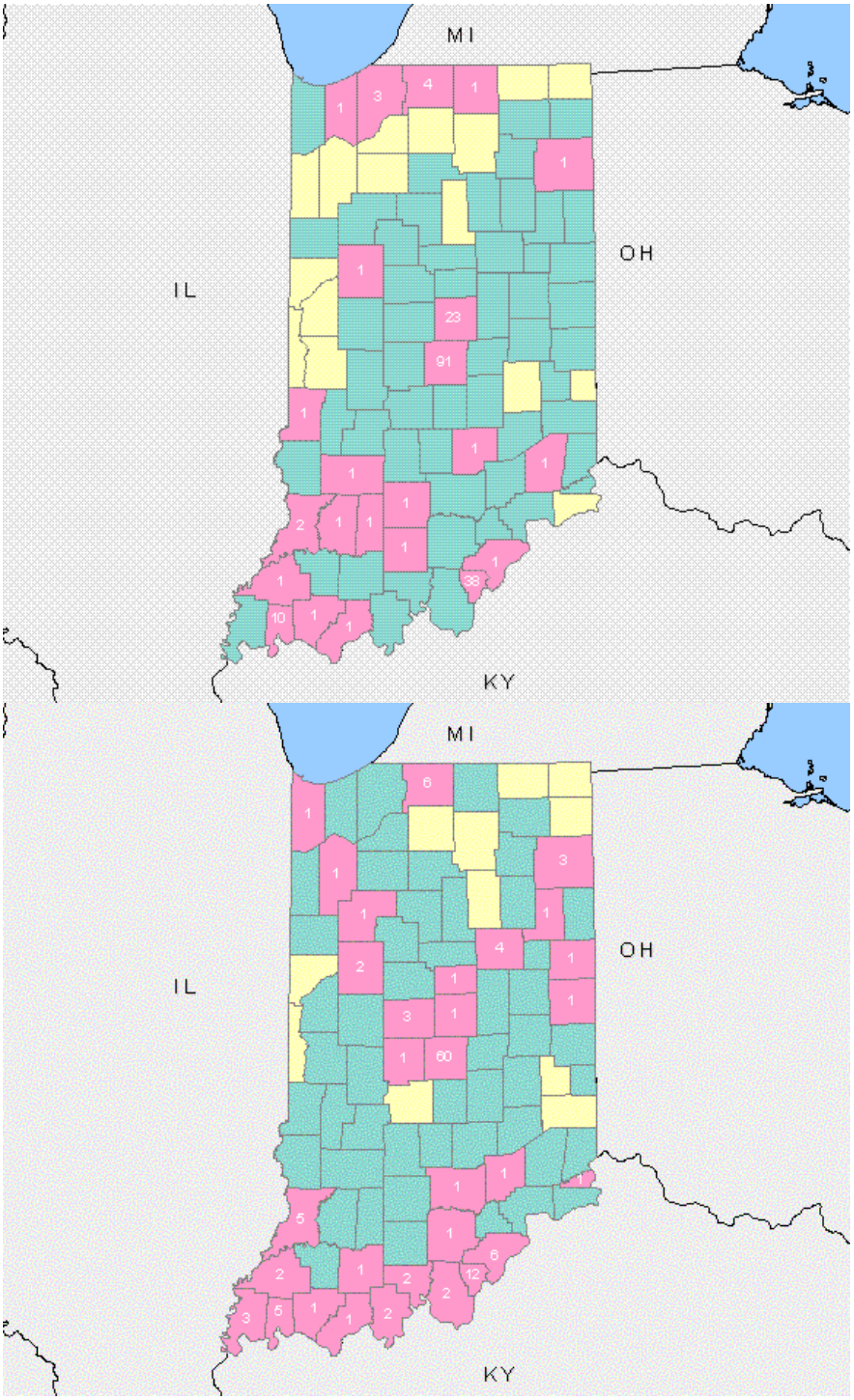
Fig 7. Human Cases of West Nile virus in Indiana in 2004



Source: http://westnilemaps.usgs.gov/us_human.html

Positive mosquito pools of WNV for 2005 (Indiana)

Some 187 positive mosquito pools were reported in 2005 compared with 153 positive mosquito pools were reported for 2004. The highest number was 91 in Marion County compared with 60 in 2004. Our 38 positive pools in 2005 and 12 positive pools in Floyd County in 2004 were the second highest number in both years. Clark County tied for third with 6 pools in 2004, but dropped to 1 pool in 2005.



Figs. 8 & 9. Positive Mosquito Pools of WNV for 2005 and 2004 respectively in Indiana
Source: http://westnilemaps.usgs.gov/us_mosquito.html

TRENDS IN EQUINE POPULATIONS

Over 1100 cases were noted in 2005 and 1300 equine in 2004, but cases of WNV have declined significantly in eastern states because a vaccination is now available, and most horses are now immune to the disease (Figs 10 & 11). Most equine cases have been recorded in California (456 in 2005 and 536 in 2004). Only 6 cases were reported in Indiana compared to 10 cases reported for Indiana last year.

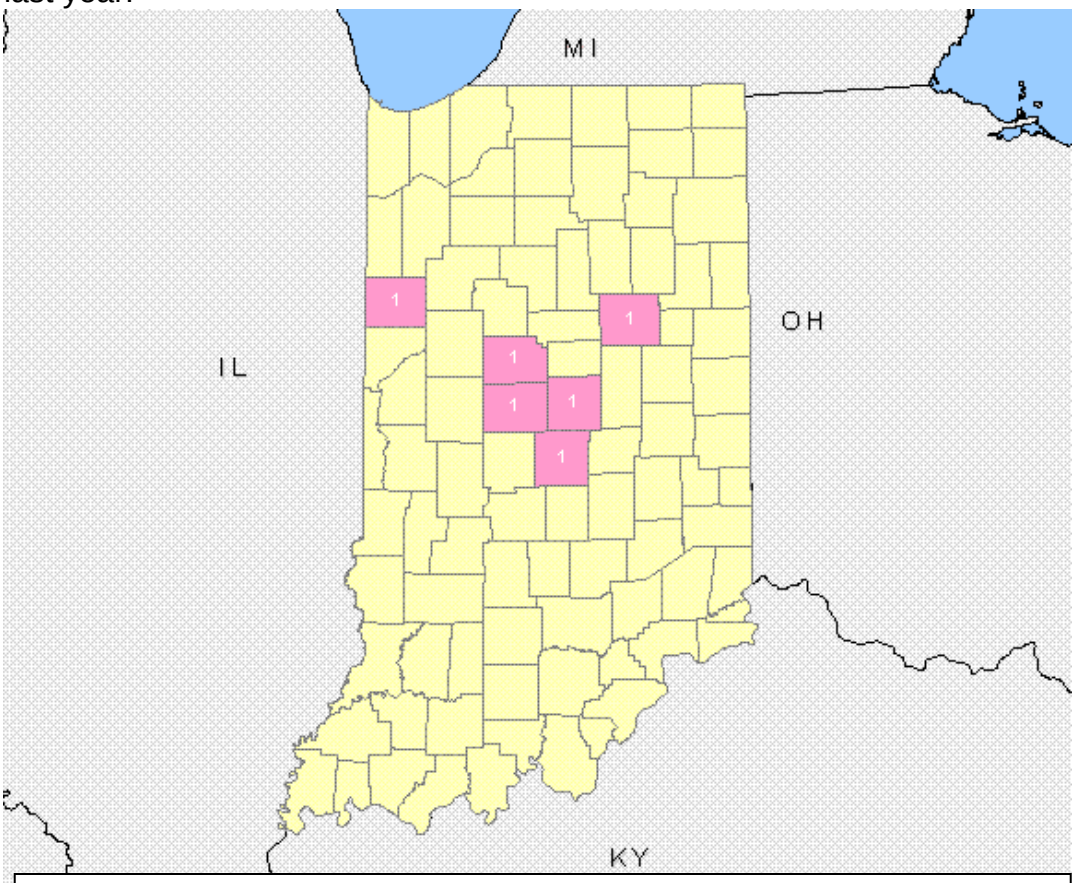


Figure 10. Equine Cases of West Nile virus in Indiana in 2005.

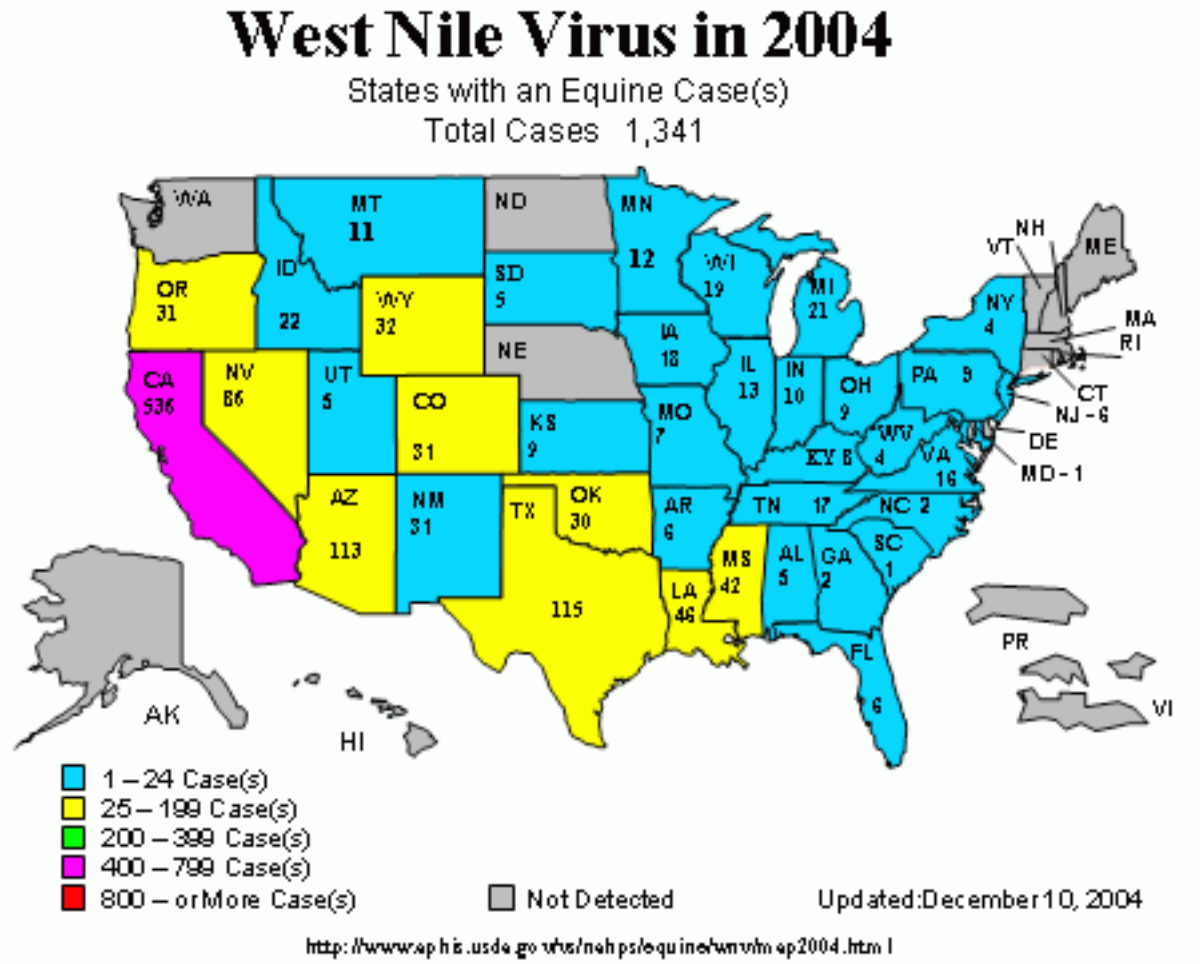


Figure 11. Equine cases of WNV in 2004.
Source: http://www.aphis.usda.gov/vs/nahps/equine/wnv/map2004.html

FUTURE PATTERNS

Once immunity has been established in humans, birds and other animals, WNV may follow a pattern similar to that found in Africa where most humans have developed immunity and contain antibodies to the disease. Periodic outbreaks may occur when bird immunity has declined and young birds without immunity comprise a majority of the population. As with the similar St. Louis encephalitis, these outbreaks will most often be associated with wet spring weather patterns followed by drought during summer and early fall.

WEATHER AND WEST NILE VIRUS

Scientists are studying the relationship between weather and the severity of outbreaks of WNV and St. Louis encephalitis. Large outbreaks of WNV and St. Louis encephalitis occurred in Midwestern states after prolonged periods of

drought (19). Some 10 of 12 outbreaks of St. Louis encephalitis were associated with two or more months of consecutive drought. The increase in mosquito borne illness during lack of rainfall may be a result of ***drought-induced amplification*** (20). When a summer-fall drought occurs, water sources for birds are reduced significantly. Birds are eventually drawn to water supplies where mosquitoes are abundant. Many of these are organically enriched sites where *Culex pipiens* are present—drains, catch basins, areas of failing septic tanks, and stagnant ponds (19).

We compared the departures from average temperature and precipitation for 2002, 2003, 2004 and 2005 to determine if the patterns were significantly different (Figs. 12, 13, 14 & 15). In the first graph (2002), the mosquito season begins in March and April with significant rainfall. This was followed by a period of above average temperatures and very little rain in the summer months. This is the classic pattern for drought-induced amplification. In 2003, above average rainfall occurred in the spring, but the summer was much cooler with average to above average rainfall. In 2004, significant rainfall amounts in July and the beginning of August were followed by the fifth driest September in history for this area. In 2005, the mosquito season began with dry but cool weather followed by a burst of rainfall in late August and drier conditions in September.

Based on these data, it is tempting to conclude that the higher number of WNV pools in 2004 and 2005 was due to the dry and hot conditions in late August and September.

Figure 12. Departure from Normal for Temperature and Precipitation for 2002

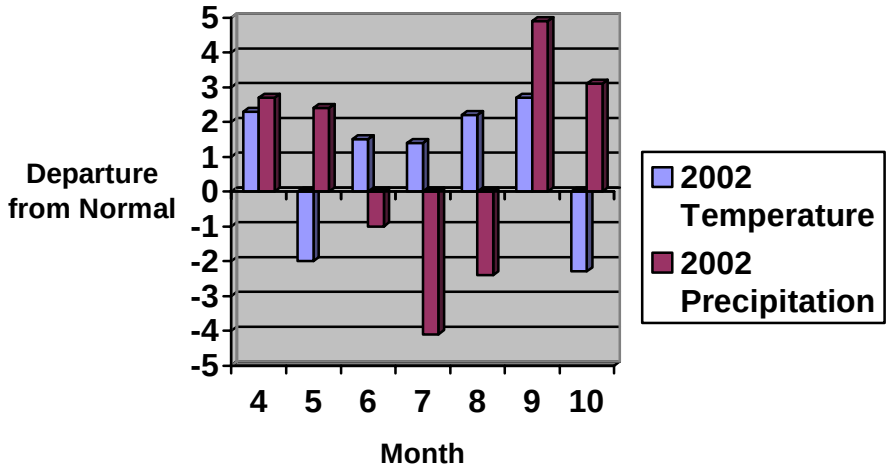


Figure 13. Departure from Normal for Temperature and Precipitation for 2003

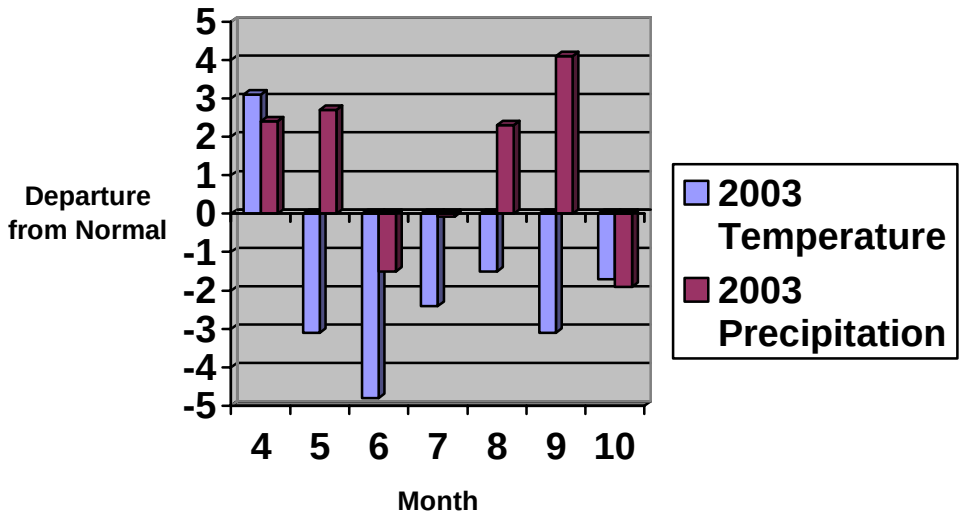


Figure 14. Departure from Normal for Temperature and Precipitation for 2004

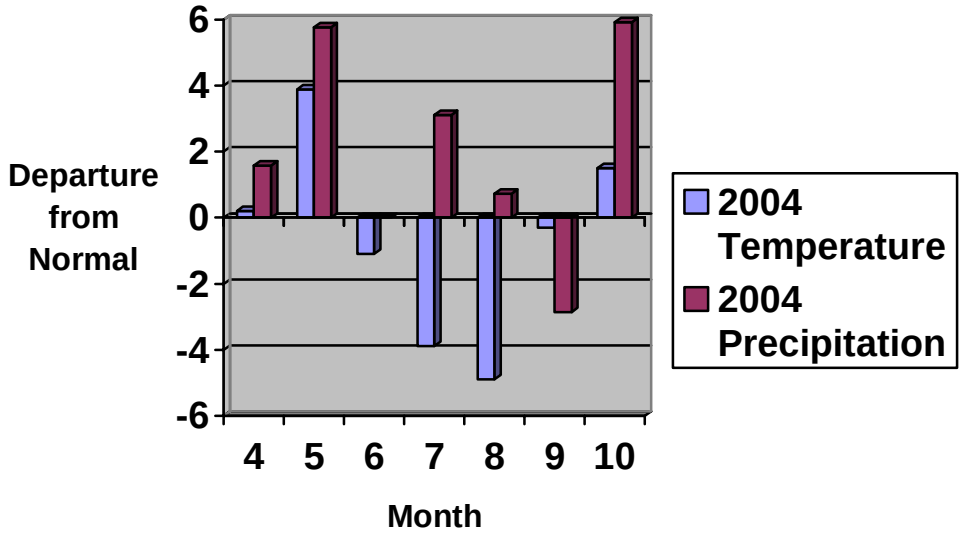
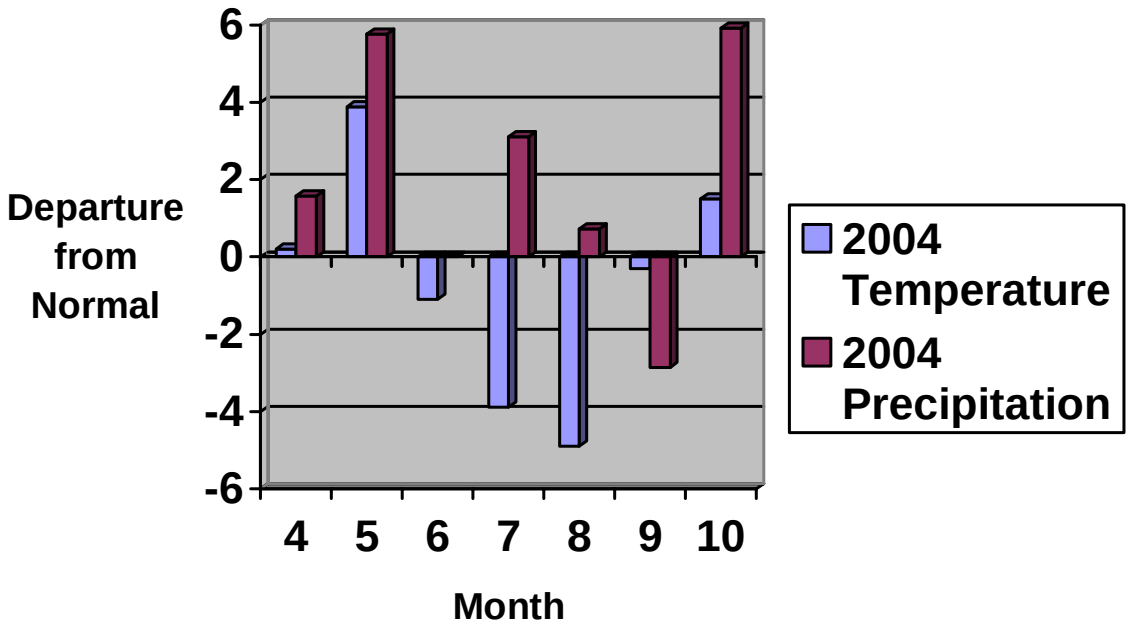


Figure 15. Departure from Normal Temperature and Precipitation in 2005



MATERIALS AND METHODS

The CDC Miniature Light Traps used in this study are portable sampling devices for the collection of mosquitoes and sand flies. Some traps came equipped with photocells and operated only after sundown; others operated continuously and had to be manually turned on and off. Both styles required the use of dry ice for producing a CO_2 mosquito attractant. The dry ice was made at IUS or purchased locally and placed in perforated Styrofoam containers or in perforated plastic thermos jugs.

The CDC Gravid Trap was specifically designed for the selective capture of gravid *Culex* mosquitoes. An oviposition attractant was prepared using 1 oz. of dried brewer's yeast and lactalbumin powder, 1 lb. of hay and 30 gallons of tap water. The solution was placed into a fifty-gallon closed container and allowed to infuse and incubate for five days. A one-half gallon portion of oviposition media was poured into the pan placed below the trap. Females were attracted to the media's odor, landed on it, and were then swept upward into the net (21).


Figures 16 and 17. Left: CDC Miniature Light Traps. Right: CDC Gravid Trap. Source: (http://home.acceleration.net/jwhock/pd_512.htm).

Floyd, Clark and Harrison County Health Departments provided CDC Miniature Light Traps and CDC Gravid Traps, manufactured by the John W. Hock Company. Figures 16 and 17 are photographs of these traps.

Indiana University SE supplied laboratory space, dry ice, microscopes, supplies and keys for identification of the mosquitoes. IUS biologists also worked to organize a much needed color reference key to the species. This time-consuming task greatly increased our accuracy and ability in identifying each specimen. Finally, each student was trained to use the equipment.

The CDC Miniature Light Traps and Gravid traps were used in Clark, Floyd and Harrison Counties. Protocols for proper use and assembly of these traps were provided with the traps. Briefly, the CDC Miniature Light Trap was assembled according to procedure and then was suspended from a tree limb or other stationary object. The CDC Gravid Trap was placed on the ground near vegetation or standing water in accordance with the manufacturer's recommendations.

Traps were dispersed at southern Indiana locations chosen by the Floyd, Clark and Harrison County Health Departments and/or students. In some cases, Dr. Baker and students surveyed the area selecting locations for the traps and obtained the property owner's permission to place a trap at that location. Other IUS professors, staff employees and students provided locations for mosquito trapping.

Traps were assembled near a suitable collecting site at dusk and allowed to operate continuously. The traps remained in place for a total of four trap nights. The following morning nets were first tied off and then removed from trap assembly. The samples were then transported to Indiana University Southeast where they were placed in the freezer in Life Sciences Building Room #170. The

frozen samples were removed from the net and placed in plastic bags or vials for identification.

The frozen mosquito samples were identified to species level using dissecting microscopes, available keys and equipment (22). Ms. Mollie McDonough or Mr. Steven Moberly, along with other students, performed the identifications. The identified frozen mosquitoes were placed in labeled bags and were shipped frozen to Indianapolis where the Indiana State Department of Health used PCR Amplification to determine presence of WNV. Dr. Michael Sinsko, Head Entomologist for the ISDH, reported back only positive WNV results. A large number of the collected mosquitoes were reserved at the IUS campus where Ms. Mollie McDonough and Mr. Steven Moberly performed the VecTest to detect the presence of WNV.

RESULTS AND DISCUSSION

In Tables 1, 2, and 3 the positive pools for the study period from 2002-2005 are presented. In 2005, we expected to find positive pools in each county, but we did not expect the number to increase this year. A very dry September contributed to the increase by concentrating birds with the WNV carrying *Culex* mosquito particularly near areas with failed septic tanks (Tables 1, 2, 3).

Table 1. Locations of Positive Mosquito Pools for Clark County Indiana, 2002-2005

Location in Clark County	Species Testing Positive
2002	
Essroc Corporation Quarry, Speed, IN	*Culex pipiens/restuans*
24815 Mahan Rd., Marysville, IN	*Culex pipiens/restuans*
Weidner Lane off Allentown Road, Union Township.	*Culex erraticus*
2003	
1900 Winburn Drive	*Cx. pipiens/restuans*
17130 SR 60 Hoosier Hills Golf Course	*Anoph. punctipennis*
2004	
319 East Street (Borden)	*Culex pipiens/restuans*
2115 Sterling Oaks (Silver Creek)	*Culex pipiens/restuans*
Utica-Sellersburg Road (Utica)	*Culex pipiens/restuans*
Clark State Forest-Fire	*Culex*

Tower (Monroe)	*pipiens/restuans*
Clark State Forest-Horse trail (Monroe)	*Culex pipiens/restuans*
219 East Park Place (Jeffersonville)	*Culex pipiens/restuans*
2005	
Carr Township Hwy 111/60	*Ochlerotatus japonicus*

Some 6,268 mosquitoes were collected, sorted and identified in Clark County during the 2004 study. In Clark County, there were six positive WNV mosquito pools found during 2004. All mosquitoes testing positive for WNV in 2004 were *Culex pipiens/restuans*. East Street and E. Park Place are located in downtown Borden and Jeffersonville. E. Street contained several open containers with active mosquito larvae. E. Park Place in Jeffersonville is located a block from Jeffboat (Largest Inland Barge Builder). Utica-Sellersburg Rd was a mosquito-breeding site involving failing septic systems. The Clark State Forest was an arboreal setting with available tree holes, ponds and other breeding areas. Only one positive pool was found in Clark County in 2005. This was, however, an important finding since this is the first time that the new mosquito*, Ochlerotatus japonicus*, was found carrying West Nile virus.

Table 2. Locations of Positive Mosquito Pools in Floyd County, Indiana 2002-2005

Location of Site (Township)	**Vector Species**
2002	
5020 Kres Lane, Floyds Knobs, IN	*Culex erraticus*
	Stegomyia albopicta (Asian tiger mosquito)
4201 Grantline Road, New Albany, IN; IUS near stream by Activities Building	*Culex erraticus*
1919 Grantline Rd behind VFW	*Stegomyia albopicta*
1408 Vance Ave, New Albany, IN	*Culex erraticus*
1410 Vance Avenue, New Albany, IN	*Anopheles quadrimaculatus, Common malaria mosquito*

	Uranotaenia sapphrina, Sapphire-lined mosquito
2003	
1905 Grantline Rd.	*Cx. pipiens/restuans*
4002 Scottsville Rd near Jenny Lane	*Cx. pipiens/restuans*
6636 Riley Ridge Rd.	*Cx. pipiens/restuans*
2004	
743 Hausfeldt Lane (New Albany)	*Culex pipiens/restuans*
4135 Pauls Lane (Lafayette)	*Culex pipiens/restuans*
707 Pillsbury Lane (New Albany)	*Culex pipiens/restuans*
1216 Beechwood (New Albany)	*Culex pipiens/restuans*
1731 Depauw Ave (New Albany)	*Culex pipiens/restuans*
2007 Player Place (New Albany)	*Culex pipiens/restuans*
2647 Alice Ave (New Albany)	*Stegomyia albopicta*
4811 Grantline Road (New Albany)	*Culex pipiens/restuans*
4201 Grantline Road (New Albany)	*Culex pipiens/restuans*
2005	
4002 Scottsville Rd	*Culex pipiens/restuans*
4002 Scottsville Rd	*Culex pipiens/restuans*
4002 Scottsville Rd	*Culex pipiens/restuans*
4126 Paul's Lane	*Culex pipiens/restuans*
4126 Paul's Lane	*Culex pipiens/restuans*
4126 Paul's Lane (2 pools)	*Culex pipiens/restuans*
816 Market Street (3 pools)	*Culex pipiens/restuans*
4002 Scottsville Rd (3 pools)	*Culex pipiens/restuans*
4126 Paul's Lane (2 pools)	*Culex pipiens/restuans*
816 Market Street	*Culex pipiens/restuans*
4126 Paul's Lane	*Culex pipiens/restuans*
4126 Paul's Lane (2 pools)	*Culex pipiens/restuans*
4002 Scottsville Rd	*Culex pipiens/restuans*

1240 Vance Avenue	*Culex pipiens/restuans*
Scottsville/Deerwood Plant	*Culex pipiens/restuans*
4126 Paul's Lane (3 pools)	*Culex pipiens/restuans*
4126 Paul's Lane (2 pools)	*Culex pipiens/restuans*
4126 Paul's Lane	*Culex pipiens/restuans*
4002 Scottsville Rd (2 pools)	*Culex pipiens/restuans*
Beechwood Avenue Alley (2 pools)	*Culex pipiens/restuans*
4002 Scottsville Rd	*Culex pipiens/restuans*
Highlander Village Highway 150	*Culex pipiens/restuans*
4126 Paul's Lane	*Culex pipiens/restuans*

We collected, sorted, identified and tested 10,023 mosquitoes in Floyd County in 2004 and 9185 with an additional 1838 near sewage package plants in the Knobs in 2005. Floyd County was second in the state behind Marion County with 12 positive WNV pools in 2004 and 38 in 2005. Actually only nine locations tested positive in 2004; the 12 positive pools were due to duplicate positive sites on different dates. Floyd County had one site, Alice Avenue, with a positive result with *Stegomyia albopicta*, the Asian tiger mosquito. Since these are active avid daytime human biters, a concentrated spraying and mosquito reduction effort was undertaken at this location. In 2005, 38 positive pools were found at nine sites. Most of these pools (24) were found in two adjacent sites with failing septic tanks. Despite continuing treatments, positive pools were found during all summer months (15 in August and 11 in September).

Eighty percent of the mosquitoes identified and tested in Floyd County in 2004 and 95.6% in 2005 were *Culex pipiens/restuans.* Most of the mosquitoes collected in 2004 and 2005 came from Pauls Lane and 4002 Scottsville Road sites, where failing septic systems are prevalent. Pauls Lane tested positive for WNV in 2003; so it was not surprising that this area tested positive in 2004 and 2005.

The other sites testing positive in Floyd County were located either within the city or in outlying portions of the county near package sewage treatment plants studied by Dawn Dietrich. Most of the city locations were near branches of Falling Run Creek or near the CSX railroad tracks.

There are three main correlating factors on all of these sites: Failing septic systems, package sewage treatment plants and urban creeks and transportation systems. Both Falling Run Creek and CSX Railway pass through Floyd County. Falling Run Creek is a significant breeding area for mosquitoes, because, as an

urban creek, it has multiple non-point sources of pollution emptying into it, old tires, bottles and discarded containers whereas CSX railway is an active transportation system with numerous small pools and water filled ditches along the tracks.

Table 3. Locations of Positive Mosquito Pools in Harrison County, Indiana 2004

Location of Site (Township)	**Vector Species**
2003	
Harrison Springs Road near White Cloud	*Ae. vexans*
2004	
12270 New Cut Rd (Morgan)	*Psorophora columbiae*
6990 Oak Park Rd (Jackson)	*Culex pipiens/restuans*
4525 Hwy 64 (Jackson)	*Culex pipiens/restuans*
4760 Crandall-Lanesville Rd (Franklin)	*Culex pipiens/restuans*
2005 No Positive Pools	

Harrison County had no positive pools in 2005. All previous Harrison County stations were less than one mile from a local highway or interstate. The New Cut Rd and the Oak Park Rd locations both had septic or sewer odors present during the study. The Oak Park location contained large numbers of tires that had active mosquito larvae at the time of the site surveys. Hwy 64 had temporary standing water that provided a breeding ground. The Crandall-Lanesville Rd site contained a pond and a small freshwater stream.

Ochlerotatus japonicus sites

The first official Indiana site of *Ochlerotatus japonicus* was 934 East Chestnut Street in Jeffersonville. Our first hypothesis on its origin was that the species was brought downriver on a barge or boat to Jeff Boat. The largest inland barge builder in the US is only one block from the site. The DNA of the specimen was tested and indicated that the DNA of that particular mosquito is most similar to a population found in Hokkaido, Japan; whereas the eastern species analyzed came from elsewhere in Japan. Afterwards, continued collection revealed that the mosquito was spreading and was found in all three counties and nine townships. There were 41 Japanese Rock Pool mosquitoes found and identified in 2004 and 720 in 2005. Dawn Dietrich found most of the 2005 specimens near package sewage treatment plants in Floyd Knobs, Indiana. Refer to an Appendix for our paper on this mosquito.

Table 4. Mosquito Pools Containing a New Species of Mosquito: *Ochlerotatus japonicus,* the Japanese Rock Pool Mosquito 2004-2005

Clark Location and Township	Floyd Location and Township	Harrison Location and Township
2004		
934 East Chestnut Street Jeffersonville	Hwy111 mile 32 Franklin	5428 Critchlow Rd Boone
201 Cattail Rd Charlestown	4002 Scottsville Rd Lafayette	4525 State Rd 64 Jackson
319 East Street, Borden Wood	United Methodist Cemetery New Albany	6990 Oak Park Jackson
Hwy60/Hwy111 Carr	Grant Line Firehouse New Albany	Wyandotte Ave Harrison
1903 Mt. Sterling Dr Charlestown	615 Park East Christian Center New Albany	Total Number of Specimens = 8
4506 Brandon Circle Silver Creek	4201 Grantline Rd IUS Pond New Albany	
Otisco Twsp, State Health Lab	751 Hausfeldt Lane New Albany	
Total Number of Specimens = 15	26262 St. Joe Rd New Albany Total Number of Specimens = 18	
2005		
Hwy60/Hwy111 Carr	United Methodist Cemetery New Albany	2085 Highway 150 Salvage Yard Morgan
8614 Marion Martin Rd Oregon	4201 Grantline Rd New Albany	9127 Old Dam 43 Taylor
1801 Shirley Ave Jeffersonville	1905 Grantline Rd New Albany	9570 Baptist Church Rd Posey
5010 Hwy 62 Utica	Tomahawk Dr New Albany	N Rehoboth Rd and Hwy 111 Boone
10110 Hwy 3 Oregon	Main St RR Tracks New Albany	4385 Elizabeth New Middletown Rd Webster
1616 Allentown Rd Silver Creek	9033 Richland Dr Georgetown	Crandall Br/Angel Run Franklin
2515 Lincoln Dr Jeffersonville	6119 Hwy 111 New Albany	5287 Bloomington Trail Monroe

11218 Hwy 60 Silver Creek	**112 Butler New Albany**	**1185 Banner Ave Harrison**
Bethel Christian Church, Bethlehem	**3622 Gray Fox New Albany**	**3025 Skyline Dr Blue River**
319 E Street Wood	**4002 Scottsville Rd Lafayette**	**4855 Hwy 337 Spencer**
4618 New Washington Rd, Bethlehem	**1240 Vance Ave New Albany**	**7800 Oak Park Rd Jackson**
1903 Mt Sterling Dr Silver Creek	**133 Grantline Center New Albany**	**4760 Lanesville Rd Boone**
Total Number of Specimens=76	**Wymberly Woods Sewage**	**2931 Harrison Heth Rd Heth**
	Chimneywood Sewage	**80111 Granny's Way Heth**
	Highlander Point Sewage	**1235 N Hwy 11 SE Posey**
	Deerwood Sewage	**1100 Green Rd Posey**
	Total Number of Specimens=48 Total Sewage Plant Plant Specimens=507	**12650 Old State Rd 135 Morgan**
		2085 Hwy 150 Morgan
		6715 Pioneer Trail Jackson
		Oak Park Rd Jackson
		6280 St Louis Rd Spencer
		4760 Lanesville Rd NE Franklin
		1999 Ave of the Emporer Posey
		1445 Harrison Ave Harrison
		4915 Corydon Ramsey Rd Jackson
		810 Keeling Ave Morgan
		1295 Fairview Church Rd Harrison
		3370 Hwy 64 NW Blue River
		Rocky Hollow & Lickford Bridge Rd

		Washington
		Total Number of Specimens=89

We predicted last year that *Ochlerotatus japonicus* would begin to occupy habitats similar to those in its native habitat in Japan. In our 2005 monitoring, we found that the new mosquito had invaded all areas of the counties and is now found in wooded arboreal habitats. A good example is Rocky Hollow and Lickford Bridge Road in Harrison County. This is a remote area with ample forested habitat. A strange twist to this study is that Dawn Dietrich found *Ochlerotatus japonicus* near sewage package plants in Floyds Knobs. It seems plausible that the mosquitoes were attracted to the smell and gases released by the plants. Most of these plants are near small streams and are in forested locations.

CONCLUSION

Our four years of mosquito monitoring have given us ample time to reflect upon the occurrence of the virus in our particular area. A common theme is nearly all West Nile virus outbreaks, regardless of location, is heat and drought (See, for example, http://www.medicalecology.org/diseases/westnile.westnile.htm). Emergence in the human population is associated with prolonged periods of hot, dry weather followed by a significant rain event. It is under these conditions that the virus infections seems to shift over to humans. Apparently, the hotter the temperature the higher the viral load in mosquitoes. Birds, during drought conditions, will often select water supplies that harbor *Culex* larvae and have large numbers of *Culex* mosquitoes in the area. When these mosquitoes hatch from water tainted with faulty septic water, birdbaths, abandoned swimming pools, tires in alleys and small containers, they become infected by biting birds and the virus is amplified by the hot weather. The next blood meal might come from a human, and this is when humans are most susceptible to the disease. It is known that 2002 was a drought year for most of the eastern United States with hot, dry conditions in the Midwest. In 2005, northern Illinois had severe drought and high temperatures for most of the summer, and human cases rebounded in this region.

With these conditions in mind, we believe we are prepared to provide solid recommendations regarding monitoring and treatment to the county health departments.

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Appendix I. VECTEST

In 2004, Dr. Claude Baker, Ms. Mollie McDonough and Steven Moberly were certified to use the VecTest on the IUS campus for the detection of WNV in mosquito populations collected in Clark, Floyd and Harrison counties. The following describes the VecTest and the protocol used on campus.

The VecTest is a WNV (WNV) Antigen Assay produced by Medical Analysis Systems, Incorporated. The VecTest WNV Antigen Assay is a rapid immunochromatographic assay intended for the qualitative determination of WNV antigen in infected mosquitoes. Results from this assay can enable public health teams to:

- Continuously monitor mosquito vectors
- Focus vector control and eradication efforts
- Deliver cost-effective prevention of the disease

Assays that detect the disease-causing agents and pathogens in field populations of arthropods, such as mosquitoes, make it possible to monitor the spread of disease, to identify areas where there is risk of humans and other domestic animals contracting disease, and to more efficiently target arthropod control measures. The emergence of WNV in the Western Hemisphere has prompted the development of several assays for the detection of West Nile antigen in vector species. Growth of the virus in cell culture and PCR-based

molecular methods remain the standard for virus identification. These methods require many reagents and multiple steps in a lengthy procedure that must be performed using specialized instruments within the laboratory.

The VecTest WNV Antigen Assay uses monoclonal antibodies against WNV and the *Flavivirus* group to identify the presence or absence of viral antigen specific to WNV in mosquitoes. The VecTest WNV Antigen Assay is a rapid, one step wicking assay providing rapid results, ambient storage and requiring no specialized equipment.

Lack of a vaccine and the many unknowns concerning the transmission rate and involved vectors necessitate mosquito control measures as the only means of controlling the spread of the disease in human populations. The VecTest WNV Antigen Assay provides a time-efficient and cost-effective method of controlling the spread of WNV.

VecTest Protocol

Procedure Outline (to be performed in a Class II biohazard hood):

1. Place up to 50 female mosquitoes into a plastic culture tube provided in the VecTest kit.
2. Dispense 2.5 mL of Grinding Solution onto the mosquitoes and add four copper-coated ball bearings provided by the VecTest kit.
3. Vortex the capped tube for 1 minute at high speed until the mosquito pool is homogenized into a slurry. (A centrifugation step may be performed to remove excess mosquito debris before running the test)
4. Dispense 250 uL of mosquito homogenate into a conical tube provided, place the tube into the tube stand provided, and insert a test strip from the canister with the arrows pointing down. (Replace the desiccant cap on the canister to protect the remaining strips from moisture). **WAIT 15 MINUTES FOR THE TEST TO BE COMPLETED**
5. Determine the test results by removing the test strip and comparing it to the pictorial sample provided on the back of the VecTest insert.
6. Autoclave hazardous waste and discard appropriately.

II. Species List

Species List for Clark County 2004

Species	2004	2005
Culex pipiens/restuans	4449	4414
Aedes vexans	710	185
Stegomyia albopicta	579	260
Aedes triseriatus	283	151
Aedes trivitattus	101	4

Anopheles punctipennis	46	21
Orthopodomyia signifera	19	15
Anopheles quadrimaculatus	13	0
Ochlerotatus japonicus*	14	76
Psorophora columbiae	12	1
Psorophora ciliata	7	0
Psorophora confinnis	0	0
Psorophora ferox	4	1
Psorophora howardii	3	0
Aedes dorsalis	1	0
Aedes sollicitans	1	0
Aedes hendersoni	0	3
Anopheles crucians	1	0
Culex quinquefasciatus	1	0
Culex territans	1	1
Culiseta melanura	1	1
Culiseta inornata	1	0
Culiseta moristans dyari	0	3
Psorophora cyanescens	1	0
Uranotaenia sapphirina	1	2
Unknown	14	0
Total Numbers	6268	5172

*New species for Indiana 2004 and new Clark County record

Species List for Floyd County 2004

Species	**2004**	**2005**	**Sewage Study**
Culex pipiens/restuans	8682	8782	1302
*Aedes (pool)**	600	0	0
Stegomyia albopicta	286	286	4
Aedes trivittatus	178	0	0
Aedes vexans	114	1	4
Aedes triseriatus	72	24	0
Aedes hendersoni	0	0	1
Aedes atlanticus	0	0	1
Anopheles punctipennis	30	11	2
Ochlerotatus japonicus*	18	48	524
Culiseta inornata	10	0	0
Orthopodomyia signifera	7	0	0
Psorophora ferox	4	0	0
Psorophora howardii	3	0	0
Aedes sollicitans	2	0	0
Uranotaenia sapphirina	2	0	0
Anopheles quardrimaculatus	2	0	0

Aedes cinereus	1	0	0
Coquillettidia perturbans	1	0	0
Psorophora ciliata	1	0	0
Toxorhynchites rutilus septentrionalis	1	0	0
Unknown	9	33	
Total Numbers	10,023	9,185	1,838
***** This pool consisted of *albopictus, trivtittus, triseriatus, and vexans.*			

***New species for Indiana and new Floyd County record**

Species List for Harrison County 2004

Species	**2004**	**2005**
Culex pipiens/restuans	2669	6251
Aedes trivittatus	282	2894
Aedes triseriatus	207	79
Stegomyia albopicta	196	181
Aedes vexans	194	165
Aedes cinereus	0	1
Anopheles punctipennis	21	4
Anopheles barberi	0	4
Culiseta melanura	0	1
Psorophora ciliata	14	1
Psorophora ferox	9	203
Orthopodomyia signifera	8	7
Ochlerotatus japonicus*	8	89
Psorophora confinnis	7	0
Psorophora howardii	5	1
Psorophora columbiae	3	1
Aedes dorsalis	1	0
Anopholes quadrimaculatus	1	1
Uranotaenia sapphirina	1	6
Psorophora (unknown)	1	0
Unknown	19	0
Total Numbers	3647	9889

New species for Indiana and new Harrison County record
*New Middletown Rd, Aaron Holman, Collector

III. *Ochelrotatus* paper

DISCOVERY OF AN EXOTIC ASIAN MOSQUITO (*OCHLEROTATUS JAPONICUS,* DIPTERA: CULICIDAE) IN SOUTHERN INDIANA, USA

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ABSTRACT. Indiana University Southeast discovered the first specimens of *Ochlerotatus japonicus,* the Asian rock pool mosquito, in Indiana in July 2004. The Indiana State Health Laboratory and the Smithsonian Institution

verified the specimens. This invading non-indigenous species is medically important because it is a known vector of several arboviruses including West Nile virus and St. Louis encephalitis.

Key Words: Discovery, *Ochlerotatus japonicus*, Indiana

INTRODUCTION

We report the discovery and first Indiana appearance of *Ochlerototus* (*Oc.) japonicus,* the Asian rock pool mosquito, in Clark County, southern Indiana, USA, 2004. In July 2004, the first specimens of *Oc. japonicus* were discovered in mosquito nets set by Indiana University Southeast. Mr. Brad Foster and Mr. Adam Estes at the Indiana State Department of Health identified the specimens. In August 2004, Dr. Dina Fonseca of the Smithsonian Institution verified the identification using mitochondrial DNA sequencing techniques.

SITE DESCRIPTION

The species was first trapped in urban and rural areas characterized by the presence of water-filled artificial containers such as old tires and other receptacles, an existing active railway, small drainage systems with pooled water and older trees with tree holes. This corresponds well with published data from New Jersey, Connecticut and New York where *Oc. japonicus* were found in similar habitats (Andreadis et al. 2001). A minimal number of specimens (1-3) were taken at each site. For the entire 2004 sampling period (May-November), we found Oc. japonicus at 13 of 81 sampling locations. All except three sites were near the southern Indiana railway system. Of the remaining locations, two were in Harrison County near a tire dump and used car parts dealer and an area of natural sinkholes. A Floyd County site was near an area of failing septic systems.

BACKGROUND INFORMATION

***Ochlerotatus japonicus* probably was introduced in shipments of used tires entering New York and New Jersey from Asia (Peyton et al. 1999). Since the initial discovery in 1998, the mosquito has been moving westward (Fonseca et al. 2001). Since *Oc. japonicus* is a non-migratory species with a flight range of less than 1 mile, its distribution is facilitated by continental trade and transit (Fonseca et al. 2001). Studies have shown that another Asian mosquito, *Aedes (Ae.) albopictus*, may have arrived in Texas in a shipment of used tires and then spread across the country along the interstate highway system (Moore & Mitchell 1997). *Oc. japonicus* could be**

migrating in a similar fashion hitching a ride in tires and other containers found on barges, trains and trucks.

DISCUSSION

Travel and the rapid transcontinental movement of products are known to be potent forces in the emergence of disease (Wilson 1995). Invading non-indigenous species can cause public health problems, and introductions are expected to rise (Fonseca 2001). In addition to used tires, *Ae. albopictus* and *Oc. japonicus* recently have been found in a variety of shipments including Lucky Bamboo an ornamental lily in the genus *Dracaena*, imported machinery, containers and in several airplanes (Linthicum et al. 2003).

***Oc. japonicus* is medically important from a public health perspective because it is capable of spreading the viruses responsible for West Nile Virus, St. Louis encephalitis, eastern equine encephalitis, and Japanese encephalitis (Sardelis & Turrell 2001, Sardelis et al. 2002, Sardelis et al. 2003, Takashima & Rosen 1989, Turrell et al. 2001). Although studies are needed to determine host preference in the United States, in Japan, the mosquito readily bites humans who venture into forested habitats (Peyton et al. 1999). Unlike many other mosquitoes, *Oc. japonicus* readily adapts to colder conditions and in Japan is capable of surviving snowy winters (Fonseca et al. 2001). Since *Oc. japonicus* mosquito pools testing positive for West Nile virus have been found elsewhere, it is possible that the recorded incidence of the virus will be extended into late fall and early winter.**

The discovery of *Oc. japonicus* in southern Indiana verifies the importance of existing mosquito control and monitoring programs. The selection of natural predators and competitors, elimination of mosquito habitat, and regulation of insects on trade vessels are a few of the many actions being taken to slow the introduction of foreign species. On a local level, public awareness and education are key factors; however, research into the spread of these vectors is of equal importance. Today's unprecedented swift transit of goods has set the stage for the spread and emergence of additional infectious diseases. It is essential, therefore, to research how these mosquitoes disperse and take action to protect our local environments.

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