

**WEST NILE VIRUS MOSQUITO SURVEILLANCE PROGRAM  
CLARK, FLOYD AND HARRISON COUNTIES, INDIANA 2004**

**and**

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

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

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

Miss Mollie McDonough, Floyd County, paid intern, supervised trapping, sorting and identified mosquitoes, editor of report

Mr. Steven Moberly, Clark and Harrison Counties paid intern, supervised trapping, sorting and identified mosquitoes.

Claude D. Baker, PhD, Professor of Biology, Indiana University SE

Mr. Douglas Bentfield, Clark County Health Department  
Mr. Daniel Ellnor, Floyd County Health Department, Editor  
Mr. Danny Schroeder, Harrison County Health Department

2004 Internship in Professional Practice: Steven Paul Moberly

Paid Laboratory Assistants: Holly Moberly and Aaron Fields  
Service Volunteer: Meghan McDonough

**2004 Ecology:**

Tim Deatrick, Steve Moberly, Nick Ellis, Dominique McCowan, Elizabeth Eilers, Julie Johnson, Ryan Miller, Rachel Brown, Trisha Campbell, Sherry Cox, Nathaniel Humes, Janice Judd, Nick Land, Jessica Oliver, Keith Pierce, Kevin Pierce, Sarah Ponsford, William Swartz, Brent Veach, Anita Norrington

**2004 Individual Study:**

Reuben Borrego, Tom Harris, Walter Phillips, Brian Schoen, Hunter Ferguson, Wm. Burden, Alicia Burden, Dominique McCowan, Debra Dean, Mary Catherine Walls, Craig Lalor, Stacey Rectenwald, Antwain Banks, Rachel Brown, Josh

Burris, Chris Ellis, Bryan Hargett, Patrick Harmon, James Holland, Brent Houin, Laundrea McDonald, Matt Phillips, Annamarie Prescott, Chris Saffran, Melissa Spear, Nick Spense, Wm. Swartz, Jamie Trowell, Jason Uhl, Jamie Vissing, Chris Washington, James Vest, Brittany Rieger, Anthony Schnell, Benjamin Hunter

#### 2004 Environment and People

Shallon Hill, Gabrielle Benfield, David Bradow, Kircie Brinkman, Wm. Burden, Karen Clive, Sarah Cospers, Ashley Embry, Jamey Evans, Hunter Ferguson, David Fletcher, Sherri Hamilton, Chris Hogue, Katharine Hurt, Jennifer Jamison, Janice Judd, Heather Roberts, Brian Schoen, Mary Catherine Walls, Marla Wilson, Deborah Woolsey

## QUICK SUMMARY

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 is currently an Eli Lilly III intern with the Clark County Health Department. 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 last year. 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.

IUS students also 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.

## **INTRODUCTION**

West Nile virus (WNV) was first detected in 1999 in New York City. The virus 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). Since its introduction, migratory birds have spread the virus to all states except Washington. WNV was detected in dead birds in Clark and Floyd counties in Indiana in 2001. This finding 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 reported positive mosquito pools in all three counties in 2003. The purpose of this report is to present a summary of our findings for 2004.

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 are available to the public on the Internet.

During the 2002, 2003 and 2004 surveillance program, 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 (McDonough, Moberly or Baker). The ability to test locally has greatly shortened the response time 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 last year, 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.

In May 2004, Dr. Claude Baker, Mollie McDonough and Steven Moberly were trained and certified by Indiana University, Bloomington in the use of the VecTest to quickly provide the local health departments with positive mosquito pool information (See Appendix I for the VecTest Biohazard II protocol).

For the 2004 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*. Dry weather conditions with drought amplification played an integral role in the spread of the virus during this current survey. September was the fifth driest on record for the region. These issues are discussed in greater detail in this report.

## 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 Holly Moberly and Aaron Fields. We also thank the following Indiana University faculty and staff, concerned residents, and local fire stations for providing secure sites for this study: Chancellor Sandra Patterson-Randles, Dean Ben Nassim, Dr. Gretchen Kirchner, Dr. John Click, Dr. Claude Baker, Ms. Diana Holman, 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 almost 80 students who made this investigation possible.

## VIROLOGY

West Nile virus belongs to a taxonomic group of viruses known as the Flaviviridae (4). The genus *Flavivirus* is part of the Japanese Encephalitis Antigenic Complex that encompasses about 60 viruses including the closely related St. Louis encephalitis virus (SLE). Cross-reactivity with SLE resulted in some confusion with WNV in the initial New York outbreak in 1999. Subsequent genetic sequencing, however, allowed the virus to be identified as WNV and not as SLE (5). WNV can be genetically divided into two lineages, only one of which has been directly linked to the human disease (6).

WNV is spherical in shape and is approximately 50 nm in diameter. It is a single-stranded RNA virus surrounded by an envelope (7). For an illustration of this virus, see Figure 1 (8). Recently, researchers have obtained their first 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).

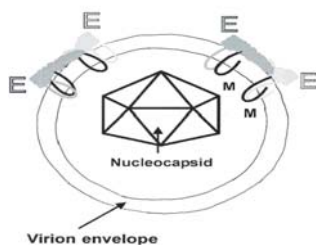


Figure 1. Flavivirus

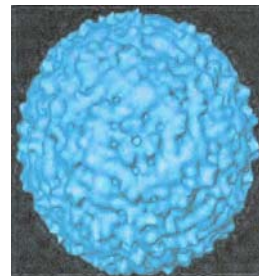


Figure 2. High Resolution Image of virus.

## ***EPIDEMIOLOGY***

Birds are the primary reservoir of WNV. The most common mode of transmission to humans is by mosquitoes that have bitten infected birds (10). Humans, horses, various mammals and some reptiles are known to contract the virus (10). Horses are particularly susceptible to the virus, but there is no evidence 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 immunosuppressed transfusion recipients (11). 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).

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.

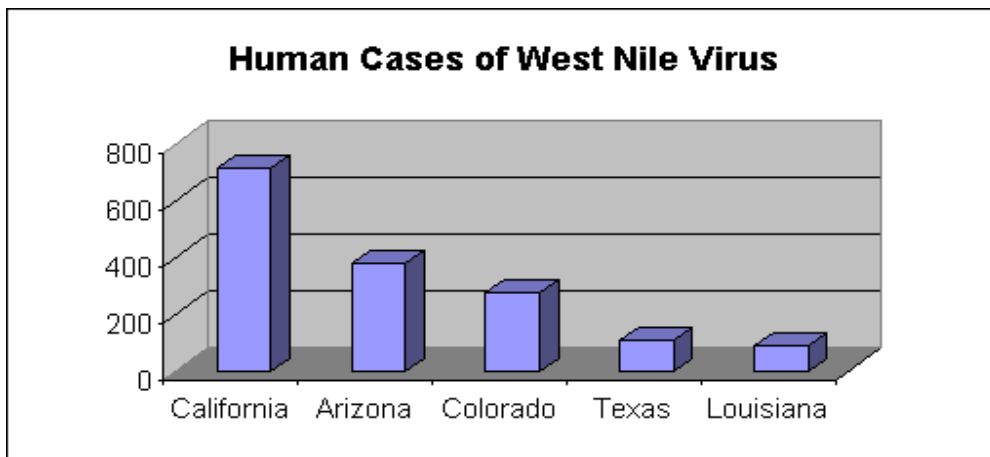
West Nile virus can presumably cross the placenta and infect the fetus (17). In 2002, a pregnant woman tested positive for WNV, and when her baby was born, the baby had signs of the disease, including decreased brain development in the temporal and occipital lobes (17).

## ***TRENDS IN HUMAN CASES***

Nationwide, the overall numbers of human cases reported to the CDC (2470 with 88 deaths) were not as high as 2003 (9862, 264 deaths) and 2002 (4156, 284 deaths), but the western states had a higher reported number of cases than in the eastern states. The spread of the disease 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 slowing the progression of the disease. WNV is cyclical and may resurge when the older immune birds perish (18).

The top five states with highest number of human cases for 2004 are all west or southwestern states (Figure 3). Indiana had 12 cases with one death.

**Figure 3. Human Cases of West Nile Virus.  
Top Five States (2004) as of January 11, 2005**



Source: <http://www.cdc.gov/ncidod/dubid/westnile/surv&control04Maps.htm>  
CDC data: California 771, 23 deaths, Arizona 391, 14 deaths, Colorado 276, 3 deaths, Texas 158, 8 deaths Louisiana 102, 7 deaths, Indiana 12, 1 death.

For those unfamiliar with the disease, the following summary is provided. WNV, since its initial American outbreak in New York City in 1999, marched westward across the North American continent affecting California in 2004 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.

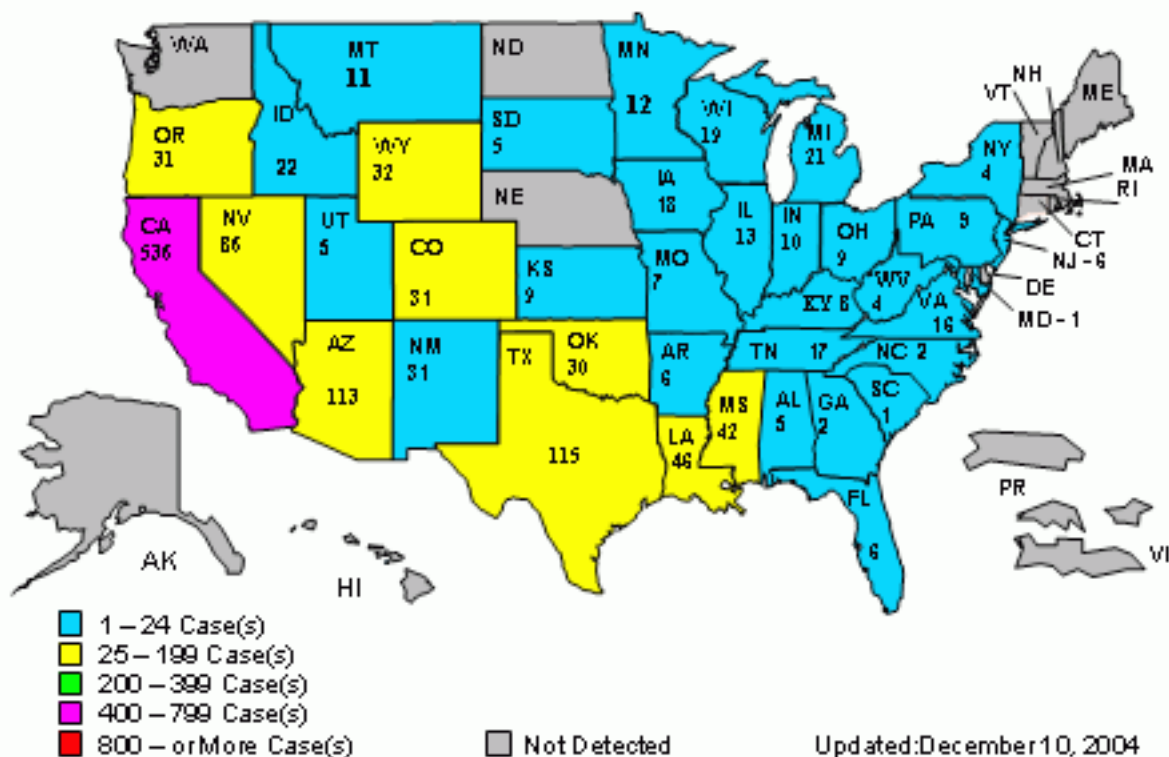




# West Nile Virus in 2004

States with an Equine Case(s)

Total Cases 1,341



<http://www.aphis.usda.gov/vs/nahps/equine/wnv/map2004.html>

Figure 6. 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 and 2004 to determine if the patterns were significantly different (Figs. 7, 8 and 9). In the first graph (2002), the mosquito season begins in March and April with significant rainfall. This is 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 amounts rainfall in July and the beginning of August were followed by the fifth driest September in history for this area.

Based on these data, it is tempting to conclude that the additional number of WNV pools was due to the drought at the end of August and the entire month of September.

Figure 7. Departure from Normal for Temperature and Precipitation for 2002

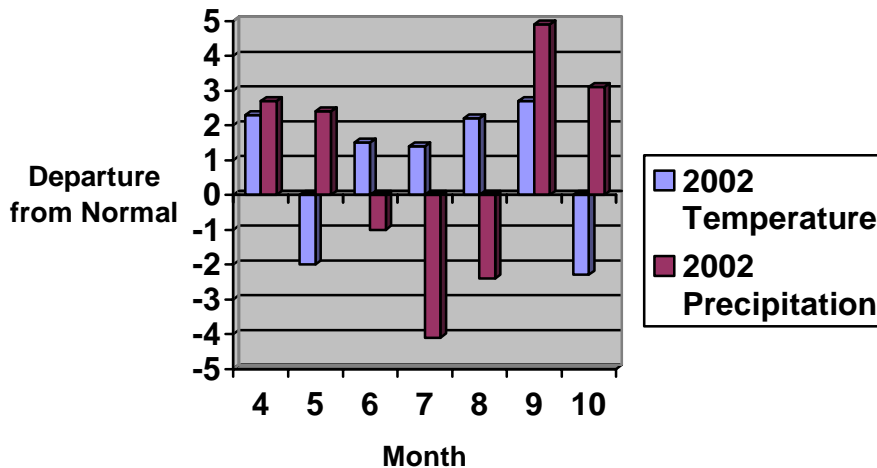


Figure 8. Departure from Normal for Temperature and Precipitation for 2003

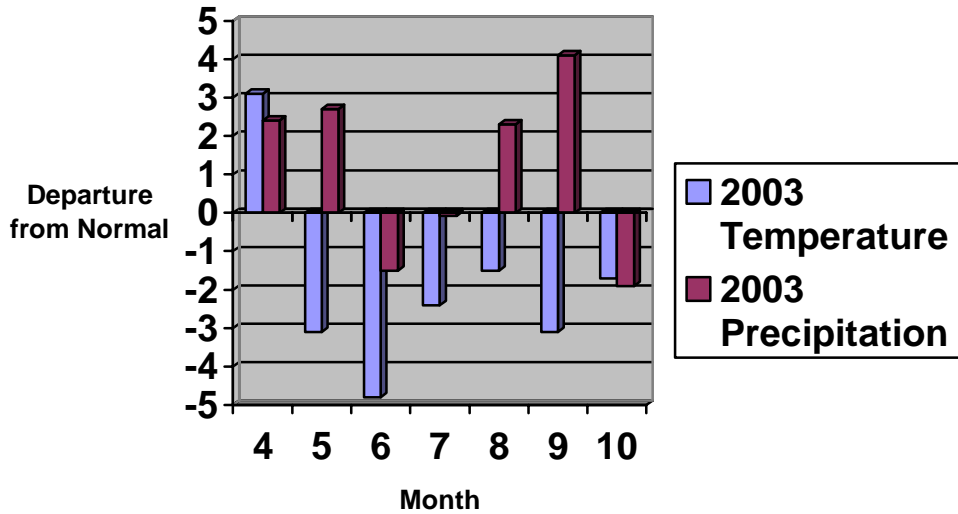
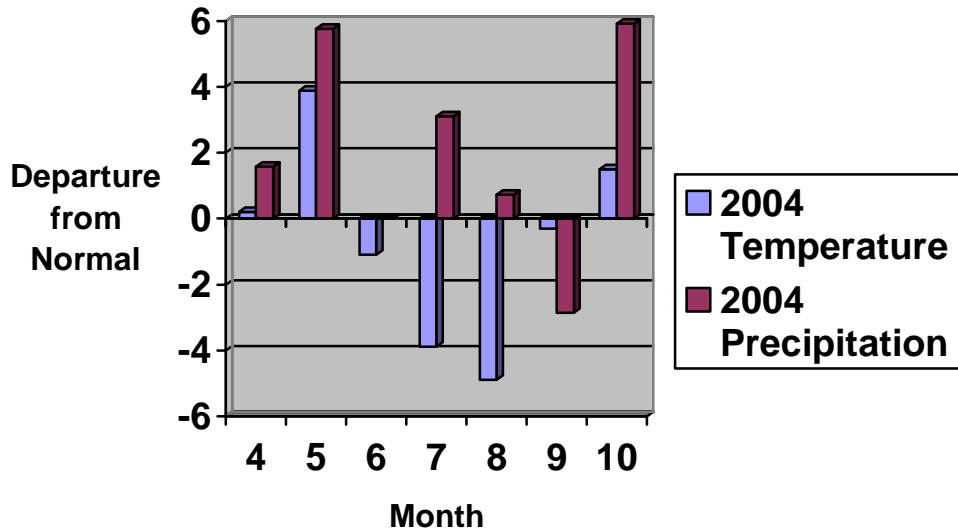


Figure 9. Departure from Normal for Temperature and Precipitation for 2004



## ***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<sub>2</sub> 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 10 and 11. Left: CDC Miniature Light Traps. Right: CDC Gravid Trap. Source: ([http://home.acceleration.net/jwhock/pd\\_512.htm](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 10 and 11 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). Miss 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 Miss Mollie McDonough and Mr. Steven Moberly performed the VecTest to detect the presence of WNV.

## **RESULTS AND DISCUSSION**

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. The positive pools were only found in three groups of mosquitoes *Culex pipiens/restuans*, *Aedes albopictus* and *Psorophora columbiae* (Tables 1, 2, 3).

**Table 1. Locations of Positive Mosquito Pools for Clark County Indiana, 2004**

<b>Location of Site (Township)</b>	<b>GPS Coordinates</b>	<b>Vector Species</b>
319 East Street (Borden)	N38° 24.929 W085° 46.834	<i>Culex pipiens/restuans</i>
2115 Sterling Oaks (Silver Creek)	N38° 27.043 W085° 54.660	<i>Culex pipiens/restuans</i>
Utica-Sellersburg Road (Utica)	N38° 20.898 W085° 41.938	<i>Culex pipiens/restuans</i>
Clark State Forest-Fire Tower (Monroe)	N/A	<i>Culex pipiens/restuans</i>
Clark State Forest- Horse trail (Monroe)	N/A	<i>Culex pipiens/restuans</i>
219 East Park Place (Jeffersonville)	N/A	<i>Culex pipiens/restuans</i>

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 were *Culex pipiens/restuans*. East Street and E. Park Place are 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.

**Table 2. Locations of Positive Mosquito Pools in Floyd County, Indiana 2004**

Location of Site (Township)	GPS Coordinates	Vector Species
743 Hausfeldt Lane (New Albany)	N38° 20.218 W085° 49.187	<i>Culex pipiens/restuans</i>
4135 Pauls Lane (Lafayette)	N38° 20.088 W085° 52.359	<i>Culex pipiens/restuans</i>
707 Pillsbury Lane (New Albany)	N38° 19.696 W085° 49.239	<i>Culex pipiens/restuans</i>
1216 Beechwood (New Albany)	N38° 18.171 W085° 48.605	<i>Culex pipiens/restuans</i>
1731 Depauw Ave (New Albany)	N 38° 18.046 W085° 48.747	<i>Culex pipiens/restuans</i>
2007 Player Place (New Albany)	N38° 18.183 W085° 49.633	<i>Culex pipiens/restuans</i>
2647 Alice Ave (New Albany)	N38° 18.972 W085° 48.120	<i>Aedes albopictus</i>
4811 Grantline Road (New Albany)	N38° 21.562 W085° 44. 016	<i>Culex pipiens/restuans</i>
4201 Grantline Road (New Albany)	N38° 20.661 W085° 49.306	<i>Culex pipiens/restuans</i>

We collected, sorted, identified and tested 10,023 mosquitoes in Floyd County. Floyd County was second in the state behind Marion County with 12 positive pools. Actually only nine locations tested positive; 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 *Aedes albopictus*, the Asian tiger mosquito. Since these are active avid daytime human biters, a concentrated spraying and mosquito reduction effort was undertaken at this location.

Eighty percent of the mosquitoes identified and tested in Floyd County were *Culex pipiens/restuans*. Forty-two percent of the mosquitoes collected 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.

The sites located on 4201 and 4811 Grantline Rd, Pillsbury Lane, and Hausfeldt Lane are located near the IUS campus near an active railway system and the interstate. One juvenile dead robin found at the Hausfeldt Lane station tested positive for WNV. The 1905 Grantline Rd station was considered a “hotspot” in 2003 did not produce positive results this year; however, the positive sites, Beechwood, Depauw Ave and Player Place, are less than one mile from last years “hot spot”. There are two main correlating factors on all of these sites. Both Falling Run Creek and CSX Railway pass through these areas. 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)	GPS Coordinates	Vector Species
12270 New Cut Rd (Morgan)	N 38° 22.711 W 086° 02.740	<i>Psorophora columbiae</i>
6990 Oak Park Rd (Jackson)	N 38° 18.116 W 085° 05.265	<i>Culex pipiens/restuans</i>
4525 Hwy 64 (Jackson)	N 38° 18.050 W 086° 02.515	<i>Culex pipiens/restuans</i>
4760 Crandall-Lanesville Rd (Franklin)	N 38° 16.161 W 085° 59.993	<i>Culex pipiens/restuans</i>

All 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. Crandall-Lanesville Rd site contained a pond and a small freshwater stream.

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 after all Jeff Boat, the Largest Inland Barge Builder in the US is 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. Refer to the Appendix III for our paper on this mosquito.

**Table 4. Mosquito Pools Containing a New Species of Mosquito: *Ochlerotatus japonicus*, the Japanese Rock Pool Mosquito 2004<sup>1</sup>**

<b>Clark Location and Twsp</b>	<b>Floyd Location and Twsp</b>	<b>Harrison Location and Twsp</b>
<b>934 East Chestnut Street Jeffersonville</b>	<b>Hwy111 mile 32 Franklin</b>	<b>5428 Critchlow Rd Boone</b>
<b>201 Cattail Rd Charlestown</b>	<b>4002 Scottsville Rd Lafayette</b>	<b>4525 State Rd 64 Jackson</b>
<b>319 East Street, Borden Wood</b>	<b>Grantline Methodist Cemetery New Albany</b>	<b>6990 Oak Park Jackson</b>
<b>Hwy60/Hwy111 Carr</b>	<b>Grant Line Firehouse New Albany</b>	<b>Wyandotte Ave Harrison</b>
<b>1903 Mt. Sterling Dr Charlestown</b>	<b>615 Park East Christian Center New Albany</b>	<b>Total = 8</b>
<b>4506 Brandon Circle Silver Creek</b>	<b>4201 Grantline Rd IUS Pond New Albany</b>	
<b>Otisco Twsp, State Health Lab</b>	<b>751 Hausfeldt Lane New Albany</b>	
<b>Total = 15</b>	<b>26262 St. Joe Rd New Albany Total = 18</b>	

(1) The State Health Lab identified an unknown mosquito from Otisco Twsp as *Oc. japonicus*.

## **REFERENCES**

1. Rappole, J.R., S.R. Derrickson and Z. Hubalek. 2000. Migratory Birds and Spread of West Nile Virus in the Western Hemisphere. CDC. Emerging Infectious Diseases. Vol. 6 (4): 11 pp.  
<http://www.cdc.gov/ncidod/eid/vol6no4/rappole.htm>. Via the Internet
2. State Department of Health's Surveillance of the West Nile virus. 2004. Available from: <http://www.in.gov/isdh/healthif/westnile/general.htm>
3. CDC, Division of Vector-Borne Infectious Diseases. West Nile Virus Available from: <http://www.cdc.gov/ncidod/dvbid/westnile/> Via the Internet
4. Division of Vector-Borne Infectious Diseases. West Nile Virus. Virology: Classification of West Nile Virus.  
<http://www.cdc.gov/ncidod/dvbid/westnile/virus.htm>. Via the Internet

5. Day, Jonathan F. and W.J. Tabachnick. 1999. Florida Medical Entomology On-line Publications PH1004. University of Florida, IFAS. <http://eis.ifas.ufl.edu/WNile/WNilemain3.htm>. Via the Internet
6. Petersen, Lyle R. and Anthony A. Martin. 2002. West Nile Virus: A Primer for the Clinician. *Annals of Internal Medicine*, 6 August 2002, Vol. 137(3): 173-177.
7. Leighton, F. A. *West Nile Virus*. April 2003. <http://wildlife.usask.ca/bookhtml/arbovirus/arbown.htm>
8. Lyle R. Petersen and John T. Roehrig. Picture of Flavivirus. 2001. (CDC web site): <http://www.cdc.gov/ncidod/eid/vol7no4/images/petersen2b.gif>.
9. Chang, K. Researchers Get Their First Close-Up Look at West Nile Virus. *New York Times*. Oct. 14, 2003. (Web Site): <http://www.nytimes.com/2003/10/14/health/14VIRU.html?ex=1066190400&en=df10ae0a51402dbf&ei=5059&partner=AOL>
10. Henley, Eric. *What FPs need to know about West Nile virus disease*. *Journal of Family Practice*. 52(9):711-713, September 2003.
11. Pealer, Lisa N., Marfin, Anthony A., Petersen, Lyle R., Lanciotti, Robert S., Page, Peter L., Stramer, Susan L., Stobierski, Mary Grace, Signs, Kimberly, Newman, Bruce. Kapoor, Hema. Goodman, Jesse L., Chamberland, Mary E. Transmission of West Nile Virus through Blood Transfusion in the United States in 2002. *New England Journal of Medicine*. 349(13):1236-1245, September 25, 2003.
12. Macedo de Oliveira, A., B.D. Beecham, S.P. Montgomery, R.S. Lanciotti, J.M. Linnen, C Giachetti, L.A. Pietrelli, S.L. Stamer and T.J. Safranek. West Nile Virus Blood Transfusion-Related Infection Despite Nucleic Acid Testing. *Transfusion*. 44: 1695-1699. December, 2004.
13. Sampathkumar, Priya. West Nile Virus: Epidemiology, Clinical Presentation, Diagnosis, and Prevention. *Mayo Clinic Proceedings*. 78(9):1137-1144, September 2003
14. West Nile Virus: What Ornithologist and Bird Banders Should Know. BIRDNET. 15 Apr 2003. Available via the Internet: <http://www.nmnh.si.edu/BIRDNET/WNV.html>
15. Update: Investigations of West Nile Virus Infections in Recipients of Organ Transplantation and Blood Transfusion. *MMWR*. 51(37): 833-836. September 20, 2002. Available from: <http://www.cdc.gov>

16. West Nile Virus (WNV) Infection and Breastfeeding: Information for Clinicians. Available from: <http://www.cdc.gov>. Last modified 16 July 2003
17. Clinical Guidance. Interim Guidelines for the Evaluation of Infants Born to Mothers Infected with West Nile Virus During Pregnancy. MMWR. 53(7); 27 Feb. 2004
18. 2004 West Nile Virus Activity in the United States (as of January 11, 2005) with 2003 information. Available from: <http://www.cdc.gov>.
19. West Nile Virus and Weather. Harvard Medical School, Center for Health and the Global Environment. Retrieved on January 20, 2004 at: <http://www.med.harvard.edu/chge/westnile.html>
20. Shaman, Jeffrey et al. 2002. Drought Induced Amplification of St. Louis encephalitis virus, Florida. CDC Emerging Infectious Diseases. Vol. 8, No. 6. 10 pp. Retrieved 01/20/04 at <http://www.cdc.gov/ncidod/eid/vol8no6/01-0417.htm>
21. J.W. Hock Company. 2002. The CDC Gravid Trap Model 1712. [http://home.acceleration.net/jwhock/pd\\_1712.htm](http://home.acceleration.net/jwhock/pd_1712.htm). Via the Internet.
22. Silverly, Russell E. 1972. Mosquitoes of Indiana. Indiana State Board of Health. 126 pp.

#### ADDITIONAL REFERENCES

Bledsoe, K.D. and R. L. Holladay. 1976. A Survey of the Mosquito Populations of New Albany Township, Floyd County Indiana to Discover Arbovectors of Saint Louis Encephalitis. Report to Floyd County Health Department. Prepared by B.J. Forsyth. Indiana University SE. 9 pp. Obtained from the Floyd County Health Department.

Andreadis, T.G., J.F. Anderson and C.R. Vossbrinck. 2001. Mosquito Surveillance for West Nile Virus in Connecticut, 2000: Isolation from *Culex pipiens*, *Cx. Restuans*, *Cx. Salinarius*, and *Culiseta melanura*. Emerging Infectious Diseases. Vol. 7 (4): 670-674. [http://www.medscape.com/viewarticle/414473\\_4](http://www.medscape.com/viewarticle/414473_4). Via the Internet

U.S. Fish and Wildlife Service. Division of Environmental Quality. Pesticide Issues. Fighting the West Nile Virus: Prevention Works Best. Internet Resource Available at: <http://contaminants.fws.gov/Issues/westnile.cfm>). Via the Internet.

O'Malley, Claudia. *Aedes vexans* (Meigen): An old foe. Proc. N.J. Mosquito Control Assoc. pp. 90-95.

NPIC's West Nile Virus Resource Guide. West Nile Mosquito Information. Available from: <http://npic.orst.edu/wnv/mosquito.htm> via the INTERNET.

MSNBC. West Nile Takes Toll on U.S. Birds. Available from: <http://www.msnbc.com/local/pencilnews/429322.asp> via the INTERNET. 2002.

State Department of Health's Surveillance of the West Nile Virus. 2002. Available from: <http://www.state.in.us/isdh/healthinfo/westnile/general.htm> via the INTERNET.

USDA. Update on the Current Status of West Nile Virus. Equine Cases of West Nile Virus Illness in 2002. Available from: <http://www.aphis.usda.gov/oa/wnv/wnvstats.html> via the INTERNET.

IN State Department of Health. Media Update on West Nile Virus. Available from: <http://www.state.in.us/isdh/healthinfo/westnile/news.htm> via the INTERNET. October 24, 2002.

USGS. West Nile Virus Maps- 2002. 2002 Surveillance Activity. Available from: [http://cindi.usgs.gov/hazard/event/west\\_nile\\_/west\\_nile.html](http://cindi.usgs.gov/hazard/event/west_nile_/west_nile.html) via the INTERNET. October 30, 2002.

USGS. West Nile Virus- Indiana Cumulative Veterinary Map. Available from: [http://cindi.usgs.gov/hazard/event/west\\_nile/indiana/in\\_vet\\_oct\\_16.html](http://cindi.usgs.gov/hazard/event/west_nile/indiana/in_vet_oct_16.html) via the INTERNET. October 17, 2002.

CNN.COM/Health. Blood Banks May Have West Nile Test by Summer. Available from: <http://www.cnn.com/2002/HEALTH/conditions/11/06/westnile.test.reut/> via the INTERNET. November 6, 2002.

University of Florida, Institute of Food and Agricultural Sciences. Florida Medical Entomology Laboratory. Available from: <http://fmel.ifas.ufl.edu/> via the INTERNET.

Centers for Disease and Prevention. Prevention: Avoid Mosquito Bites to Avoid Infection. Available from: <http://www.cdc.gov/ncidod/dvbid/westnile/> via the INTERNET. November 6, 2002.

CDC. Vertebrate Ecology: The Following 138 Bird Species Have Been Reported to CDC's West Nile Virus Avian Mortality Database from 1999- Present. Available from: <http://www.cdc.gov/ncidod/dvbid/westnile/birdspecies.htm> via the INTERNET. October 17, 2002.

CDC. MMWR. Possible West Nile Virus Transmission to Infant Through Breast Feeding- Michigan, 2002. Available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5139a1.htm> via the INTERNET. October 4, 2002.

CDC. West Nile Virus, Questions and Answers. Available from: <http://www.cdc.gov/ncidod/dvbid/westnile/ga/transfusion.htm> via the INTERNET. September 9, 2002.

CBSNEWS.COM. Dogs, Squirrels Falling Prey to West Nile. Available from: <http://www.cbsnews.com/stories/2002/09/24/health/main523121.shtml> via the INTERNET. Springfield, Ill. September 18, 2002.

United States Environmental Protection Agency. Office of Pesticide Programs. Mosquitoes: How to Control Them. Available from: <http://www.epa.gov/pesticides/citizens/mosquito.htm#fight> via the INTERNET. April 28, 1998.

Floyd County Health Department. Quick facts...About West Nile Virus. Indiana, 2002.

Center for Disease Control. Background: The Virus History and Distribution, DVVID. (serial online) October 7, 2002. Available from [www.cdc.gov](http://www.cdc.gov) via the INTERNET.

Center for Disease Control. Vertebrate Ecology, West Nile Virus. (serial online) April 19, 2002. Available from: [www.cdc.gov/ncidod/dvbid/westnile.htm](http://www.cdc.gov/ncidod/dvbid/westnile.htm) via the INTERNET.

Indiana Department of Health. West Nile Virus press release. (serial online) October 8, 2002. Available from: [www.in.gov/isdh/healthinfo/westnile/general.htm](http://www.in.gov/isdh/healthinfo/westnile/general.htm) via the INTERNET.

Rutgers University Microbiology Department. Species found Positive for West Nile Virus as of October 5, 2002. (serial online) October, 2002. Available from: [www.rci.rutgers.edu/~insects/poswn.htm](http://www.rci.rutgers.edu/~insects/poswn.htm) via the INTERNET.

Center for Disease Control, DVVID. West Nile Virus Questions and Answers "West Nile and Horses". (serial online) August 29, 2002. Available from: [www.cdc.gov/ncidod/dvbid/.html](http://www.cdc.gov/ncidod/dvbid/.html) via the INTERNET.

Center for disease Control. Questions and Answers: Prevention of West Nile Virus. (serial online) August 29, 2002. Available from: [www.cdc.gov/ncidod/dvbid/westnile/ga/prevention.htm](http://www.cdc.gov/ncidod/dvbid/westnile/ga/prevention.htm) via the INTERNET.

Stenson, Jacqueline. Reuters Health. West Nile Virus Vaccine Shows Promise. (Serial online) September 27, 2002.

Wilson, Gregory. Indiana State Health Commissioner Correspondence. November 20, 2002.

VecTest West Nile Virus Antigen Assay, Medical Analysis Systems, Incorporated Pamphlet. January 1, 2003.

## **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	Quantity
<i>Culex pipiens/restuans</i>	4449
<i>Aedes vexans</i>	710
<i>Aedes albopictus</i>	579
<i>Aedes triseriatus</i>	283
<i>Aedes trivitattus</i>	101
<i>Anopheles punctipennis</i>	46
<i>Orthopodomyia signifera</i>	19
<i>Anopheles quadrimaculatus</i>	13
<b><i>Ochlerotatus japonicus</i>*</b>	14
<i>Psorophora columbiae</i>	12
<i>Psorophora ciliata</i>	7
<i>Psorophora confinnis</i>	5
<i>Psorophora ferox</i>	4
<i>Psorophora howardii</i>	3
<i>Aedes dorsalis</i>	1
<i>Aedes sollicitans</i>	1

<i>Anopheles crucians</i>	1
<i>Culex quinquefasciatus</i>	1
<i>Culex territans</i>	1
<i>Culiseta melanura</i>	1
<i>Culiseta inornata</i>	1
<i>Psorophora cyanescens</i>	1
<i>Uranotaenia sapphirina</i>	1
Unknown	14
<b>Total Numbers</b>	6268

\*New species for Indiana 2004 and new Clark County record

### Species List for Floyd County 2004

Species	Quantity
<i>Culex pipiens/restuans</i>	8682
<i>Aedes (pool)*</i>	600
<i>Aedes albopictus</i>	286
<i>Aedes trivittatus</i>	178
<i>Aedes vexans</i>	114
<i>Aedes triseriatus</i>	72
<i>Anopheles punctipennis</i>	30
<b><i>Ochlerotatus japonicus</i>*</b>	18
<i>Culiseta inornata</i>	10
<i>Orthopodomyia signifera</i>	7
<i>Psorophora ferox</i>	4
<i>Psorophora howardii</i>	3
<i>Aedes sollicitans</i>	2
<i>Uranotaenia sapphirina</i>	2
<i>Anopheles quadrimaculatus</i>	2
<i>Aedes cinereus</i>	1
<i>Coquillettidia perturbans</i>	1
<i>Psorophora ciliata</i>	1
<i>Toxorhynchites rutilus septentrionalis</i>	1
Unknown	9
<b>Total Numbers</b>	10,023

\* This pool consisted of *albopictus*, *trivittatus*, *triseriatus*, and *vexans*.

\*New species for Indiana and new Floyd County record

## Species List for Harrison County 2004

Species	Quantity
<i>Culex pipiens/restuans</i>	2669
<i>Aedes trivittatus</i>	282
<i>Aedes triseriatus</i>	207
<i>Aedes albopictus</i>	196
<i>Aedes vexans</i>	194
<i>Anopheles punctipennis</i>	21
<i>Psorophora ciliata</i>	14
<i>Psorophora ferox</i>	9
<i>Orthopodomyia signifera</i>	8
<b><i>Ochlerotatus japonicus</i>*</b>	8
<i>Psorophora conifinnis</i>	7
<i>Psorophora howardii</i>	5
<i>Psorophora columbiae</i>	3
<i>Aedes dorsalis</i>	1
<i>Anopholes quadrimaculatus</i>	1
<i>Psorophora punctipennis</i>	1
<i>Uranotaenia sapphirina</i>	1
<i>Psorophora (unknown)</i>	1
Unknown	19
<b>Total Numbers</b>	3647

New species for Indiana and new Harrison County record

### III. *Ochlerotatus* paper

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

STEVEN P. MOBERLY, CRAIG LALOR AND MOLLIE  
MCDONOUGH

School of Natural Sciences  
Indiana University Southeast  
New Albany, IN 47150

BRAD FOSTER AND ADAM ESTES  
Indiana State Department of Health  
635 N. Barnhill Dr.  
Indianapolis, IN 46202

AND

DOUGLAS J. BENTFIELD

Clark County Health Department  
1320 Duncan Ave.  
Jeffersonville, IN 47130

Address to whom correspondence should be addressed:

Mr. Steven Paul Moberly c/o Dr. C.D. Baker  
Indiana University Southeast  
4201 Grantline Rd  
New Albany, IN 47150  
812-941-2251 Office  
502-445-7318 Cell  
812-941-2637 Fax  
[spmoberl@ius.edu](mailto:spmoberl@ius.edu) email address

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 *Ochlerotatus (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 & Turell 2001, Sardelis et al. 2002, Sardelis et al. 2003, Takashima & Rosen 1989, Turell 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.

The Clark, Floyd and Harrison County Indiana Health Departments and Indiana University SE provided funding for this project. Dr. Claude Baker, Professor of Biology at Indiana University SE served as project director and mentor. His help is gratefully acknowledged. We also acknowledge Dr. Dina M. Fonseca of the Smithsonian Institution and the Academy of Natural Sciences who verified the specimens using DNA sequencing techniques.

### **LITERATURE CITED**

Andreadis, T.G., J.F. Anderson, L.E. Munstermann, R.J. Wolfe & D.A. Florin. 2001. Discovery, distribution and abundance of the newly introduced mosquito *Ochlerotatus japonicus* (Diptera: Culicidae) in Connecticut, USA. *Journal of Medical Entomology* 38 (6):774-779.

CDC. 2001. Embargo on importation of *Dracaena* shipped in standing water. Via the Internet. <http://www.cdc.gov/od/oc/media/pressrel/r010702.htm>

Fonseca, D.M., S. Campbell, W.J. Crans, M. Mogi, I. Miyagi, T. Toma, M. Bullians, T.G. Andreadis, R. L. Berry, B. Pagac, M.R. Sardelis & R. C. Wilkerson. 2001. *Aedes (Finlaya) japonicus* (Diptera: Culicidae), a newly recognized mosquito in the United States: Analyses of genetic variation in the United States and putative source populations. *Journal of Medical Entomology* 38(2):135-146.

Linticum, K.J., V.L. Kramer, M.B. Madon, K. Fujioka and Surveillance-Control Team. 2003. Introduction and potential establishment of *Aedes albopictus* in California in 2001. *Journal of the American Mosquito Control Association*. 19 (4): 301-308.

Moore, C.G. & C.J. Mitchell. 1997. *Aedes albopictus* in the United States: Ten-year presence

and public health implications. *Emerging Infectious Diseases* 2 (3):329-334.

Peyton, E.L., S.R. Campbell, T.M. Candeletti, M. Romanowski, & W.J. Crans. 1999. *Aedes (Finlaya) japonicus japonicus* (Theobald), a new introduction to the United States. *Journal of the American Mosquito Control Association* 15 (2):238-241.

Sardelis, M.R & M.J. Turell. 2001. *Ochlerotatus j. japonicus* in Frederick County, Maryland: discovery, distribution, and vector competence for West Nile virus. *Journal of the American Mosquito Control Association*. 17 (2): 137-141.

Sardelis, M.R., D.J. Dohm, B. Pagae, B. Andre & M.J. Turell. 2002. Experimental transmission of eastern equine encephalitis virus by *Ochlerotatus j. japonicus* (Diptera: Culicidae). *Journal of Medical Entomology*. 39 (3): 480-484.

Sardelis, M.R., M.J. Turell & R.G. Andre. 2003. Experimental transmission of St. Louis encephalitis virus by *Ochlerotatus j. japonicus*. *Journal of the American Mosquito Control Association*. 19 (2): 159-162.

Takashima, I. & L. Rosen. 1989. Horizontal and vertical transmission of Japanese encephalitis virus by *Aedes japonicus* (Diptera: Culicidae). *Journal of Medical Entomology*. 26 (5): 454-458.

Turell, M.J., M.L. O'Guinn, D.J. Dohm & J.W. Jones. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *Journal of Medical Entomology*. 38 (2): 130-134.

Wilson, M.E. 1995. Travel and the emergence of infectious diseases. *Emerging Infectious Diseases*. 1 (2): 39-46.