

Mosquito Surveillance

Surveillance Protocol



Summary Paragraph about the Disease

Four mosquito-borne diseases (all arboviruses) are the focus of the West Virginia Mosquito Surveillance Program's surveillance efforts: La Crosse encephalitis (LAC), West Nile (WN) encephalitis, Zika virus (ZIKV) disease, and St. Louis encephalitis (SLE). Activities and responsibilities outlined include trapping, identification, and testing of mosquitoes across the state from May to October when mosquitoes are most active.

Local Health Responsibilities

1. Respond to citizen complaints concerning mosquito and mosquito-borne disease
 - a. Conduct mosquito surveillance at the complaint site based on local health capacity
 - b. Seek assistance from the Public Health Entomologist if needed.
2. Conduct environmental assessments of human arboviral cases.
 - a. Complete an "Environmental Assessment Form" (see Appendix) for potential human arboviral disease cases within your jurisdiction within one week of notification.
 - b. Provide prevention education about arboviruses to citizens during environmental assessments, including specific recommendations for controlling mosquitoes around the home and mosquito bite prevention to the patient and other residence of the home. If possible, mosquito abatement activities should include removal of standing water and water holding containers.
 - c. Become familiar with DIDE's Integrated Pest Management plan and employ these guidelines when possible.
3. Conduct adult mosquito surveillance (based on capacity) in a timely and accurate manner and ensure that mosquito samples are in good testing condition.
 - a. Set and collect mosquito traps at localities for which permission has been obtained.
 - b. Record mosquito collection information on the "Mosquito Surveillance Collection" Form in the Appendix. Collection information includes collecting locality, collection dates, and collecting method.
 - c. Local health departments with previous experience and expertise in mosquito taxonomy will submit mosquito samples prepared for immediate arboviral testing. Pool mosquitoes into sets of 10 to 50 adult mosquitoes of the same genus from the same location and same end collection date. (The Asian tiger mosquito, *Aedes albopictus*, will be maintained in their own testing pools separate from other *Aedes* species).
 - i. Keep adult mosquitoes frozen at -70 to -80°C until submitted to the state health department for arboviral testing.

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- ii. Complete the “West Virginia Office of Laboratory Services (OLS) Arbovirus Laboratory Animal Specimen Submission” Form for each mosquito pool sample: <http://www.wvdhhr.org/labservices/forms/index.cfm>. Handwrite trap information on the form and include the number of specimens of each species collected from each trap in the ‘Comments’ field.
 - iii. Submit mosquito samples to the OLS within **five days** of collection through the mail, physically delivered to OLS or arranging with the Public Health Entomologist to pick up the samples.
- d. Local health departments being trained in mosquito taxonomy or unable to complete mosquito identification will submit samples with the “Mosquito Surveillance Collection” Form in the Appendix. Mosquitoes can be submitted in the original mosquito trap collection bag, sealed plastic bags, or individual cryovials.
- i. Keep adult mosquitoes frozen at -70 to -80°C until submitted to the state health department for identification and arboviral testing.
 - ii. Submit mosquito samples to OLS within **five days** of collection through the mail, physically delivered to OLS or arranging with the Public Health Entomologist to pick up the samples.
- e. Review WN virus minimum infection rate (MIR) data and other arboviral infection data when sent by Division of Infectious Disease Epidemiology (DIDE).
- f. Implement recommended control measures based on WN virus MIR data. See ‘Recommendations for Responding to Increased Mosquito Viral Activity.’

State Health Responsibilities

1. Refer citizen complaints concerning mosquito and mosquito-borne disease to the local health department.
 - a. Record detailed information from mosquito complaint phone calls in yearly database. These calls can be taken by the “Epidemiologist On-Call” in the Division of Infectious Disease Epidemiology or the Public Health Entomologist.
 - b. Information will be provided to the local health department for follow-up. The Public Health Entomologist may assist the local health department as needed.
2. Assist with environmental assessments of human arboviral cases when needed.
 - a. Maintain an up-to-date arboviral case investigation form that can be used for the environmental assessment of human arbovirus cases (see “Environmental Assessment “ Form.)
 - b. As able, the state (Public Health Entomologist and/or interns) will assist local health department staff during the environmental assessments of human

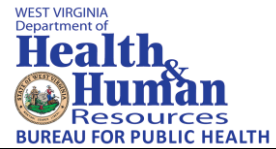
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- arbovirus cases and provide guidance and training for environmental assessments of human cases of novel or emerging mosquito-borne diseases (dengue fever, chikungunya, ZIKV disease).
- c. Maintain and provide education material to local health departments that can be given to citizens during environmental assessments.
3. Conduct active adult mosquito surveillance from May through October. Location of trapping sites will be determined by the Public Health Entomologist in consultation with local health department and other interested parties prior to active adult mosquito surveillance season.
 - a. Hire summer mosquito surveillance interns to assist with mosquito surveillance by March 2018 and ensure appropriate training and occupational health and safety plans are in place. Record mosquito collection information on the "Mosquito Surveillance Collection" Form in the Appendix.
 - b. Provide training to parties conducting mosquito surveillance in their jurisdiction prior to the start of vectorborne disease season. Mosquito surveillance trainings involve mosquito species identification, mosquito biology, operation of mosquito trapping equipment, and proper mosquito submission to OLS.
 - c. Identify mosquito samples within **three business days** of collection.
 - i. Prepare mosquito samples for testing.
 1. Keep adult mosquitoes frozen at -80°C until arboviral testing.
 2. Pool mosquitoes into sets of 10 to 50 adult mosquitoes of the same genus from the same location and same end collection date. (The Asian tiger mosquito, *Aedes albopictus*, will be maintained in their own testing pools separate from other *Aedes* species). To prevent the accumulation of untested mosquito pools with < 10 mosquito specimens and increase the percentage of mosquito samples screened for arbovirus, mosquitoes of different genera with few mosquito specimens from the same collecting locality and same end collection date will be combined into the same mosquito pool.
 3. Complete a "West Virginia OLS Arbovirus Laboratory Animal Specimen Submission" Form for each mosquito pool sample. This form is available at <http://www.wvdhhr.org/labservices/forms/index.cfm>
 4. Ensure mosquito samples collected from local health departments and state health department are submitted to OLS for future testing of mosquitoes for human arbovirus.
 - d. Evaluate mosquito surveillance activities annually utilizing historical human arboviral disease surveillance data to identify new trapping site.

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- e. Provide mosquito trapping equipment to parties conducting mosquito surveillance to provide standardized collecting methodology across the state.
4. Test Arboviral testing of mosquito samples
 - i. Test adult mosquitoes based on mosquito genus using real-time reverse transcription polymerase chain reaction (PCR) within **five business days** of receipt. Results may be delayed due to microbiologist staff shortages or unforeseen events requiring emergency laboratory testing. Under special circumstances, arboviral testing may be conducted based upon species (ex. the Asian tiger mosquito *Aedes albopictus*). All mosquitoes will be tested for La Crosse virus, ZIKV, WN virus, and St. Louis encephalitis virus.
 - ii. Real-time reverse transcription polymerase chain reaction allows a targeted sequence of viral RNA to be transcribed into cDNA which is then amplified millions of times in a few hours. Real-time reverse transcription PCR is useful for detecting very low amounts of RNA molecules.
 - iii. Mechanical homogenization of mosquitoes will begin with two copper beads in each pool and will be lysed in guanidine isothiocyanate-containing RNA lysis buffer. Qiagen® QIAmp RNeasy Mini kit will be used to isolate viral RNA from mosquito tissue. AgPath-ID one-step RT-PCR with detection enhancer will be used for PCR amplification. Polymerase chain reactions will be run using ABI 7500FAST. Biosearch Technologies provides the primers and Taqman probes. The Centers for Disease Control and Prevention provide controls for validation.
 - iv. OLS reports mosquito test results to the Zoonotic Disease Program (WVZDProgram@wv.gov) and the local health department in the county where the mosquitoes were collected.
 - v. OLS maintains mosquito arboviral test results electronically.
 - b. Use mosquito test results to calculate the WN virus MIR for each collection week.
 - c. Provide WNV MIR data and arboviral infection information to local health departments through the "West Virginia Vectorborne Disease Surveillance Report"
 - d. Use weekly WN virus MIR data to monitor trends in mosquito viral activity and determine if additional prevention measures need to be implemented
5. Feedback mosquito surveillance data
 - a. Report arboviral disease positive mosquito pools (and associated collection information) to the Centers of Disease Control and Prevention (CDC) through ArboNET. Mosquito pools without viral infection will be reported to ArboNET before December 31, 2018.
 - b. Report *Aedes aegypti* and *Aedes albopictus* activity (and associated collection

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- information) to the CDC through MosquitoNET.
- c. Provide regular surveillance updates to public health partners via dissemination of the “West Virginia Vectorborne Disease Surveillance Report.” Depending on vector and disease activity, this report may be sent weekly or every two weeks. This report summarizes human and non-human mosquito and tickborne disease surveillance data.
- d. Summarize yearly mosquito surveillance data in either a zoonotic disease annual report or mosquito surveillance annual report.
- e. Reports will be emailed and posted electronically on the DIDE website.
- 6. Maintain mosquito surveillance data
 - a. Ensure that the current Epi Info 7 database is complete and accurate for data collected through both state and local mosquito surveillance efforts. Data should be reviewed on a weekly basis during the season.

Disease Control Objectives

1. Perform or increase mosquito control activities when increased arboviral activity in mosquitoes is detected in an area.
2. Provide or increase public education when increased arboviral activity in mosquitoes is detected in an area.

Disease Prevention Objectives

1. Reduce disease risk through:
 - a. Public education regarding personal protective measures (i.e. mosquito bite prevention).
 - b. Integrated pest management of areas harboring mosquitoes infected with human arbovirus based upon mosquito surveillance results.
2. Use mosquito surveillance data to provide timely notification to the public, health care providers, and local health departments of arboviral activity in mosquitoes.

Disease Surveillance Objectives

1. Identify mosquito infected with arboviral diseases in areas where humans are at risk for infection.
2. Determine the arboviral infection rate in the mosquito populations based upon species and geographic distribution.
3. Use mosquito distribution, population density, and seasonal phenology information to identify geographic localities susceptible to establishment of novel or travel-associated

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arboviral diseases such as dengue, chikungunya, and ZIKV disease.

4. Identify new or invasive mosquito species not previously identified in West Virginia that could be capable of transmitting disease.
5. Provide early notification of increased arboviral disease activity in mosquitoes through arboviral testing of mosquitoes within two weeks of mosquito collection.

Occupational Health

It is important to prevent exposure to mosquito-transmitted diseases when conducting environmental assessments and mosquito surveillance. Individuals should cover their skin as much as possible. Wear long-sleeved shirts, long pants, socks, and a hat. Head nets may be necessary in areas with high mosquito activity. Different chemicals can be used to deter mosquitoes from biting people. Repellents are substances that help people avoid mosquito bites. Repellents are formulated for use on bare skin and are sold as aerosols, creams, solids, sticks, pump sprays, and liquids. Repellents do not kill mosquitoes and other insects, but repellents will deter these insects from biting people. Unlike repellents, permethrin will kill mosquitoes. Since permethrin is an insecticide, it is recommended for use only on clothing, shoes, bed nets, and camping gear – never on the skin.

For routine mosquito surveillance activities for monitoring arboviral activity in the mosquito populations, surveyors must have authority and permission from the property owner before collecting mosquito samples from the property. Supervisors must know the locations where mosquito surveyors are collecting mosquitoes each day. Mosquito surveyors working in the field must have means to communicate with their supervisors in the event of an emergency. For training and safety purposes, mosquito surveyors are encouraged to work in teams.

Accidents and injuries accrued during mosquito collection will be reported immediately to the supervisor. Vehicular accidents or vehicle mechanical problems will also be reported immediately to the supervisor. Mosquito surveyors are encouraged to be up-to-date on tetanus vaccinations.

Public Health Significance

After its introduction into New York City in 1999, WN virus expanded its territory across the United States, reaching California by 2002. The WN encephalitis epidemic called attention to the weakened public health infrastructure for arbovirus surveillance in the United States. Due to this concern, federal money was allocated to improve public health infrastructure, including laboratory diagnosis and medical entomology capacity.

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In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed ZIKV infection in Brazil and on Feb. 1, 2016, the World Health Organization (WHO) declared ZIKV disease a public health emergency of international concern. Local transmission of ZIKV occurred in Florida, Texas, Puerto Rico, the U. S. Virgin Islands, and American Samoa in 2016 and 2017. The immense growth of cities in southeast Asia, Central and South America and Caribbean Islands and downgrading of mosquito control programs in these areas in recent decades has provided a suitable habitat for yellow fever and dengue fever transmission. Yellow fever outbreaks occurred in port cities in the continental United States as recently as late 19th and early 20th century.

The occurrence of arboviral disease outbreaks is unpredictable; therefore, public health officials should remain vigilant for increased activity during the summer months, which coincides with increased vector activity. SLE and EEE can occur in sometimes dramatic outbreaks at lengthy intervals with little or no apparent transmission in intervening years. With the emergence of viruses that can be transmitted by autochthonous transmission, public health officials should remain vigilant for local transmission of emerging arboviral infections during the summer months and counsel patients with suspected or confirmed infections to avoid mosquito bites to prevent transmission to others. Public health conducts various surveillance activities focused on detection and monitoring of arboviral diseases:

In West Virginia, the major locally-transmitted arbovirus of concern is LAC; however, 10 cases of WN virus infection were reported in humans during the nationwide epidemic of 2012. Birds positive for EEE were identified in 2002 in West Virginia. Twelve human cases of SLE were reported from West Virginia between 1964 and 2017, with majority of cases occurring in 1975 during a national SLE epidemic. The last case was reported in 1997. Two travel-associated cases of chikungunya were reported in West Virginia in 2014. In West Virginia, 1-2 travel-associated cases of dengue fever and malaria are reported each year.

Etiologic Agent

SLE virus, WN virus, and ZIKV belong to the Flaviviridae family. La Crosse virus belongs to the California encephalitis complex of the Bunyaviridae.

Reservoir

Table I outlines selected medically significant arboviruses transmitted by mosquito, reservoir, and additional modes of transmission.

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Table I: Arboviral Diseases Under Mosquito Surveillance in West Virginia

Virus (abbreviation)	*Mosquito Vector	Mosquito Activity	Amplifying Hosts; i.e., Reservoir Species ¹	Dead-end Hosts ²	Human to Human Transmission
La Crosse Encephalitis (LAC)	** <i>Aedes triseriatus</i> , ** <i>Aedes albopictus</i> , ** <i>Aedes japonicus</i>	Daytime biters; Deciduous forests; Container breeders	Small rodents (chipmunks, squirrels), transovarial transmission	Humans	No
St. Louis Encephalitis (SLE)	** <i>Culex species</i>	Dusk and dawn; Container breeders, Permanent or semi-permanent pools, ponds	Birds	Humans, Domestic mammals	No
West Nile (WN) Encephalitis	** <i>Culex species</i>	Dusk and dawn; Container breeders, Permanent or semi-permanent pools, ponds	Birds	Humans, Domestic mammals	Yes
Zika virus (ZIKV) Disease	<i>Aedes aegypti</i> , ** <i>Aedes albopictus</i>	Day time biters; Container breeders	n/a	n/a	Yes

*Mosquitoes that feed on humans or mammals. Mosquitoes that feed mostly on the amplifying host are not included.

**Mosquito species found in West Virginia.

¹Amplifying host: species that allows replication of the virus. The arbovirus can rise to high levels in the bloodstream of an amplifying host. A mosquito that takes a blood meal from an amplifying host picks up enough

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virus so that the mosquito can transmit the arbovirus the next time it bites a human. The amplifying host is the reservoir species.

²Dead-end host: species that does not allow replication of the virus to high levels. Arbovirus does NOT rise to high levels in the bloodstream of a dead-end host. A mosquito that takes a blood meal from a dead-end host CANNOT transmit the virus the next time it bites a human.

Mode of Transmission

Mosquitoborne arboviruses are primarily spread from the bite of an infected female mosquitoes. Although comparatively rare, individuals can become infected with an arbovirus through additional modes of transmission. WN virus can be transmitted through blood transfusions and organ transplants. Sexual transmission of ZIKV has been documented. Arbovirus can be transmitted from the mother to her child during fetus development (ZIKV), through delivery (WN virus and ZIKV), and during breast feeding (WN virus). Laboratory technicians have been infected with WN virus and ZIKV in laboratory settings.

Incubation Period

A mosquito undergoes a complete metamorphosis, passing through four successive stages in its development: egg, larva, pupa, and adult (Fig. 1)

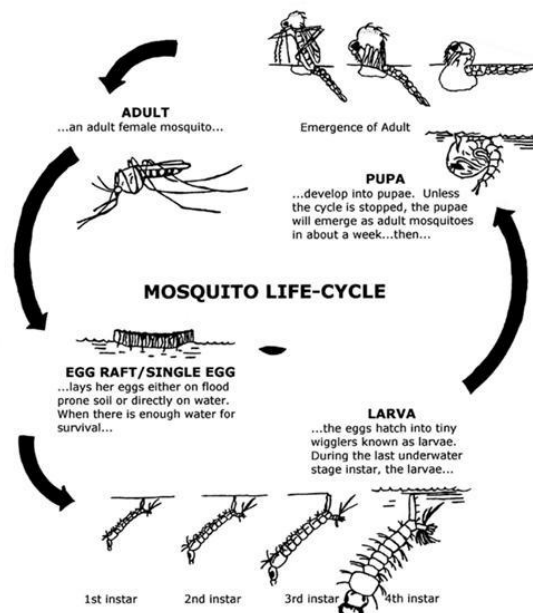


Fig. 1. Mosquito Life Cycle

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Outbreak Recognition

There are many ways to discuss infection information collected through arboviral testing of mosquito pools. For example, detection of virus in a mosquito pool demonstrates infection in the mosquito species collected from a locality. Although valuable as an indicator of arboviral infection in the locality sampled, this method does not ascertain the prevalence of infection in the mosquito population. Discussing arboviral infection as either a function of the number of infected mosquito pools or the percentage of infected mosquito pools relates infection incidence to mosquito population size; however, the number of mosquito pools and the number of mosquito specimens per pool must remain constant for any comparative value.

Infection rates provide the most accurate definition of infection prevalence in the mosquito population. Mosquito infection rate is a better indicator of virus incidence in the vector population because this value relates incidence of infection with a population indicator. Mosquito infection rates do not require the number of mosquito pools or the mosquito pool size to remain constant. The minimum infection rate (MIR):

$$MIR = \frac{\text{virus positive mosquito pools}}{\text{total number of mosquitoes tested}} \times 1,000$$

Due to its simplicity and historical value amongst many mosquito surveillance programs, MIR will be the measure of infection rate used by the West Virginia Mosquito Surveillance Program.

The level of arboviral infection in the mosquito population will determine the course of mosquito-borne disease management. A statewide MIR > 5 per 1000 *Culex* spp. (the primary WN virus vector) mosquitoes for two consecutive weeks may be an indicator of increased human WN virus infection in the following weeks. It is difficult to quantify and relate infection rates among other mosquito species to future WN virus activity in humans in West Virginia. In some years of low human WN encephalitis incidence, the WN virus MIR was greater than 5 per 1000 *Culex* spp; however, these high infection rates did not continue through the following weeks. Examining data across two or more weeks will accommodate for fluctuations caused by low mosquito yield (attributed to local weather or trap mishaps). Early seasonal peaks in WN virus infection in *Culex* spp. could be exaggerated when low numbers of mosquitoes are collected. Since MIR is the ratio of the number of positive mosquito pools to the total number of mosquitoes tested, a small number of infected mosquitoes could result in a high calculated infection rate. Therefore, it is recommended to view infection rates during the early season (when the *Culex* mosquito populations are starting to rise) with caution when the weekly *Culex* spp. count is less than 200

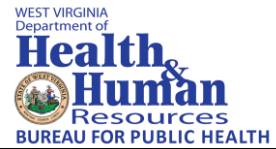
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and infection is limited to a single *Culex* spp. pool.

Preventive Interventions

Prevention activities as they relate to mosquito surveillance can be divided into two categories: Integrated pest management and response to elevated viral activity in mosquitoes derived from MIRs.

Integrated Pest Management

Integrated pest management (IPM) programs are a sustainable approach to managing mosquitoes by combining biological, cultural, physical, and chemical tools in a way that minimizes economic, health, and environmental costs. IPM programs use current, comprehensive information on mosquito species biology and their specific interactions with the environment. This information, in combination with available pest control methods, is used to manage mosquito populations by the most economical means and with the least possible hazard to people, property, and the environment. See the IPM document provided by DIDE at: <http://www.dhhr.wv.gov/oeps/disease/Zoonosis/Mosquito/Pages/IPM.aspx>

The following activities can be used as part of an IPM approach to control mosquito populations in West Virginia:

- Remove/modify mosquito breeding habitats by emptying and removing any water-holding containers, filling tree holes with thermal insulation, and performing land modifications that allow for proper water drainage.
- Participate in community tire drives that remove old tires from an area. The West Virginia Department of Environmental Protection Rehabilitation Environmental Action Plan (REAP) was created to rid the state of unsightly litter and provides collection events where West Virginia citizens can dispose of tires without individual cost of disposal. Additional information about REAP, PPOD, and local tire collecting events is available on their website (<http://www.dep.wv.gov/dlr/oer/reap/tires/Pages/TireCollectionEvents.aspx>).
- Use biological pesticides for managing mosquito larvae. *Bacillus thuringiensis israelensis* (*Bti*) and *Bacillus sphaericus* (*Bs*) are available commercially as 'biorational' larvicides. Since their active ingredient is only triggered when ingested by target insects, *Bti* and *Bs* are safe to handle and a special pesticide applicator license is not required for application.

Recommendations for Responding to Increased Viral Activity in Mosquitoes

The level of human risk can be categorized based on mosquito viral activity as calculated by MIRs. The following section outlines various levels based on mosquito WN virus activity and provides appropriate prevention actions to take for each level. Actions implemented in previous levels should be maintained through the subsequent level (ex. responses at the 'low human risk level')

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should continue through the 'medium human risk level.')

Level 1: Low human risk for WN encephalitis

Conditions

- No WN virus infection in mosquito pools (often seen during late spring and early summer)

Action

- Routine public education (general symptoms of WN virus infection, general mosquito integrated pest management, personal protection against mosquitoes)
- Routine trainings to local health departments about mosquito-borne disease epidemiology, surveillance results from previous year, and case ascertainment and investigation
- Routine press release notices (general symptoms of WN virus infection, general mosquito integrated pest management, personal protection against mosquitoes)
- Routine notifications to physicians and veterinarians (upcoming mosquito season, preferred diagnostic test methods, promoting OLS services)
- Routine reminder to laboratories to send human samples to OLS
- Routine memo to local health departments about resources available West Virginia Department of Health & Human Resources
- Routine mosquito and arbovirus surveillance activities
- Continue to notify public about arbovirus surveillance results
- Maintain mosquito larval control
- Inventory pesticides and pesticide control equipment

Level 2: Moderate human risk for WN encephalitis

Conditions

- WN virus infection in some mosquito pools in the county but statewide MIR < 5 / 1000 *Culex* spp. or statewide MIR > 5 / 1000 *Culex* spp. for only one week

Action

- Enhance public health education message (signs and symptoms of encephalitis, encourage patients to seek medical care if required, inform the public about pesticide applications)
- Ensure notification of key agencies of WN virus activity including local offices of emergency services
- Conduct case site investigations associated with human or equine cases
- Increase adult mosquito surveillance in surveyed areas
- Increase number of mosquito pools tested for WN virus

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- Increase surveillance and control of mosquito larvae
- Conduct localized, limited chemical control of adult mosquitoes based upon consultation with State Public Health Entomologist
- Contact commercial applicators in anticipation of large scale adulticide application

Level 3: High human risk for WN encephalitis

Conditions

- Statewide MIR > 5 / 1000 *Culex* spp. for two continuous weeks

Action

- Conduct full scale media campaign (general symptoms of WN virus infection, encourage patients to seek medical care if required, general mosquito integrated pest management, personal protection against mosquitoes, and mosquito-borne disease surveillance activities)
- Alert physicians, veterinarians, and local health departments about the impending severity through Health Alert Network
- Enhance information to healthcare providers
- Conduct active human case investigation
- Continue case site investigations of human and equine cases
- Increase geographic coverage of adult mosquito surveillance
- Continue enhanced larval surveillance and control of mosquito larvae
- Initiate more extensive adult mosquito control
- Consider localized aerial adulticide application in rural environments

Conditions for each risk level may change based upon other surveillance indicators. The relationship between some surveillance indicators (ex. human cases, equine cases, WN virus positive birds) and human risk is not completely understood for West Virginia. For example, a county with a human case of WN virus infection but no infected mosquito pools (possibly because the county does not have an active mosquito surveillance program) would probably be considered at medium risk for WN encephalitis. Or having multiple human cases of WN virus infection and infected mosquito pools in a county but a statewide MIR < 5 / 1000 *Culex* spp. mosquitoes would warrant the county as being at high risk for WN encephalitis.

Surveillance Indicators

1. Time between date of mosquito collection by local health department partners and date of mosquito sample submission to Public Health Entomologist
2. Time between date of mosquito sample submission to Public Health Entomologist and date of electronic storage of data associated with mosquito sample

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3. Time between date of mosquito sample submission to OLS and date of receipt of arboviral test results from mosquito samples
4. Time between date of mosquito collection and date of receipt of arboviral test results from mosquito samples
5. Proportion of state population with a county involved active mosquito surveillance each year
6. Proportion of environmental assessments conducted on confirmed and probable arboviral cases