NEVBD QUARTERLY DIGEST

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NEVBD

NORTHEAST REGIONAL CENTER FOR EXCELLENCE IN VECTOR-BORNE DISEASES

UPCOMING EVENTS & ANNOUNCEMENTS

- Asian Longhorned Tick Webinar Available Online!
  Visit: http://neregionalvectorcenter.com/online-programs

- Integrated Tick Management Pesticide Applicator Certification Course Coming in 2019.
  Pesticide Management Education Program of New York State.
  Visit: http://moodle.cce.cornell.edu/

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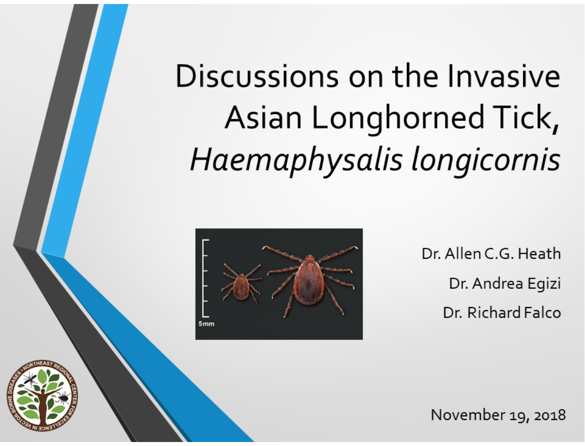
NEVBD ONLINE PROGRAMS

Interactive Webinar on the Invasive Asian Longhorned Tick

NEVBD hosted a webinar on the invasive Asian longhorned tick (*Haemaphysalis longicornis*) on November 19, 2018. This webinar featured commentary from Dr. Allen C.G. Heath, Dr. Andrea Egizi, and Dr. Richard Falco on the biology, ecology, and distribution of this invasive species in both New Zealand and the United States. Their discussions also covered approaches to tick control for this species, and experiences on the discovery and surveillance of the tick in New Jersey and New York during the 2017 and 2018 seasons.

Close to 300 individuals joined us for this webinar, including audience members from across the United States and Canada. Stay tuned for additional publications on this invasive tick from NEVBD partners at Rugters University Center for Vector Biology and Columbia University in the coming months.



Materials from this webinar are now available on the NEVBD wesbite at **http://neregionalvectorcenter.com/online-programs**. You can access a recording of the webinar, a transcript of the question-answer discussion period, and select slides from the presentation.

TRAINING & CAREER RESOURCES

Vector Biology Boot Camp - Spring 2019

NEVBD will be hosting its second annual Vector Biology Boot Camp in Spring 2019. This program provides hands-on training in key elements of vector surveillance program operations through lectures, lab exercises and field collections. This program covers tick and mosquito species of medical importance to the Northeastern United States, and is specifically targeted to vector-borne disease professionals working in this region.

Stay tuned for announcements on the next application cycle to the Vector Biology Boot Camp in February 2019 at http://neregionalvectorcenter.com/vector-biology-boot-camp.

Who should attend the Vector Biology Boot Camp?

Ideal candidates for the Vector Biology Boot Camp include professionals with limited experience in key components of vector surveillance and/or control for ticks and/or mosquitoes. Previous applicants included individuals recently hired into a vector surveillance program, individuals developing new surveillance programs in their agencies, and individuals expanding their professional capacity to conduct vector surveillance and control in their organization.

When is the next Vector Biology Boot Camp?

The next Vector Biology Boot Camp will be held in May of 2019 at Fordham University's Louis Calder Center. NEVBD is evaluating the program schedule to align with ongoing professional programs being held this spring to limit time constraints for attendance.

NEVBD TEAM MEMBER SPOTLIGHT

Maria Onyango, PhD - Postdoctoral Associate, Wadsworth Center Arbovirus Laboratory

Maria Onyango is a postdoctoral associate working with Dr. Laura Kramer in the Griffin Laboratory at the New York State Department of Health Wadsworth Center. Originally from Kenya, Maria earned both her undergraduate and Master of Science degrees from the University of Nairobi. Maria pursued her doctoral thesis at the Australian Animal Health Laboratories, CSIRO, on the population genetics of two species of biting midges, *Culicoides imicola* and *Culicoides brevitarsis* She joined the Yale School of Public Health for postdoctoral training, studying how *Spiroplasma* - a microbial taxon recently isolated from the tsetse fly - impacts the genetic structuring of the tsetse fly in Uganda.

Maria Onyango, PhD

Recognizing the impact that climate change has already begun to have on the personal lives of Kenyans (like dry taps in the city and severe droughts that prevent people from affording three meals a day), Maria felt driven to engage in research to understand the impact changing temperatures will have on disease vectors and their capacity to transmit pathogens. This passion led her to Dr. Kramer's lab, where she is currently working to understand the impact of temperature and Zika virus on the capacity for disease transmission of both the Yellow Fever mosquito (*Aedes aegypti*) and Asian tiger mosquito (*Aedes albopictus*).

Key Terms:

- **Vector competence** is the ability of a host (like a mosquito) to get, maintain, and transmit microbial agents (like the Zika virus).
- **Vectorial capacity** is a measure of how well, or how efficiently, a vector transmits a disease

Maria Onyango Describes Her Work

Motivation for the project: Both Zika virus and its *Aedes* mosquito host species have experienced unprecedented geographical range expansions in recent years. This expansion may be influenced by increasing temperatures due to climate change. Significant knowledge gaps exist on the impact of temperature variation, host microbiome, and genotypes on the vector competence of *Aedes* mosquitoes for Zika virus.

What we hope to understand: Through our work, we hope to understand the impact that temperature will have on the factors that contribute to the vectorial capacity of the Yellow Fever mosquito and Asian tiger mosquito for Zika virus. We are also curious about how the Zika virus interacts with the gut microbiota of the Asian tiger mosquito during the process of infection.

How we are doing it: We have three populations for these two *Aedes* mosquito species reared under diurnal temperature regimens. The mosquitoes are exposed to the Zika virus, after which transmission is assessed. The guts of the mosquitoes are also collected before and after blood meals to identify microbial taxa.

What the data can tell us: Results of our study show that increases in temperature lowered the vectorial capacity of our mosquito populations. These higher temperatures reduced the life span of the mosquitoes and lowered their rate of blood feeding. Asian tiger mosquitoes reared at the lowest temperature showed the highest vectorial capacity. These results suggest that future climate change may reduce the survival and reproduction of these mosquitoes, and their ability to transmit disease. Our study results also showed that infection with Zika virus reduced the diversity of the mosquito gut microbiota, but higher temperature increased the diversity of the gut microbiota. It is clear from these results that the impact of future climate change is likely to be different for individual species and individual pathogens.

NEVBD PESTICIDE RESISTANCE MONITORING NETWORK

BY JAMES BURTIS, PhD, CORNELL UNIVERSITY DEPARTMENT OF ENTOMOLOGY

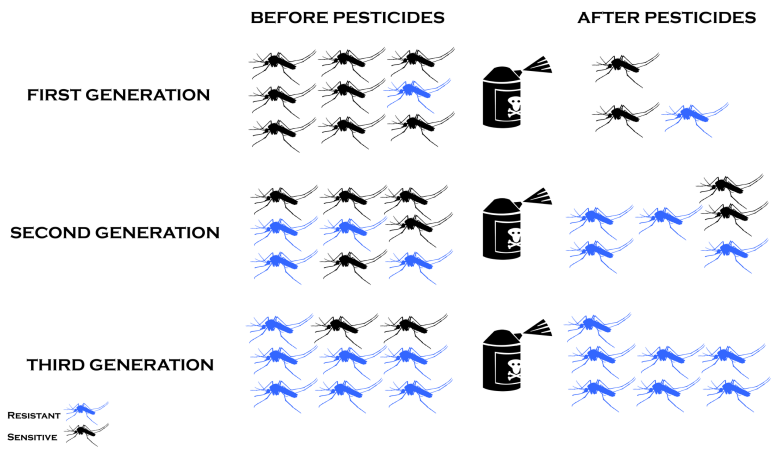
Importance of Detecting Pesticide Resistance

Pesticide resistance is an emerging global issue for many mosquitoes and some ticks, including those that transmit vector-borne diseases to humans. **Pesticide resistance is defined as a heritable reduction in sensitivity to a pesticide**. It occurs when a pesticide causes high mortality in a vector population, but does not kill the entire population. The few surviving individuals reproduce and pass on resistant genetic trait(s) to their offspring. Over time, more resistant individuals survive to reproduce, and out-compete the individuals that are sensitive to the pesticide. This eventually causes a dramatic reduction in the effectiveness of pesticide control methods. Resistance may be hard to recognize initially as it takes multiple generations of the pest population to spread.

This is a particularly urgent issue relating to the control of mosquito and tick populations, as there are relatively few commercially available pesticides registered for use on vectors. Often, the operational response to this lower effectiveness of pesticides on resistant populations is to increase the number of times the product is applied in order to achieve the same level of control. This not only raises the financial cost of using pesticides, but can also cause environmental damage by negatively affecting organisms not targeted by the pesticide. This phenomenon, called the “pesticide treadmill”, can be difficult to stop or “get off”.

How Does Pesticide Resistance Happen?

- A pesticide is used to kill a target pest, like mosquitoes. But, not all of the pests die. Some individuals are able to survive contact with the pesticide.
- The surviving pests will pass on their ability to survive contact with this pesticide to their offspring. The number of these **resistant** individuals increases in the pest population.
- The next time that pesticide is used, there are more resistant individuals in the group who can survive. They also pass this ability to their offspring, increasing the number of resistant individuals in the population.
- After a few generations, a large portion of the pest population is resistant to the pesticide, making that product less effective at controlling pest populations.



Process of Pesticide Resistance Development

Monitoring Pesticide Resistance in the United States

Currently, there is no established network for detecting pesticide resistance in mosquito or tick populations in the United States; this is largely due to logistical and financial costs. As a result, detection of resistance has been patchy. Additionally, the primary pesticides used by government agencies and companies involved in vector control change across jurisdictions and are dependent on product availability. This makes it difficult to determine which pesticides are appropriate to target for resistance monitoring.

Public health officials across the nation are well-aware of this resistance monitoring gap. According to a survey conducted in 2017 by the National Association of County & City Health Officials (NACCHO), only 14% of mosquito control agencies in the USA currently collect pesticide resistance data, with 98% of survey respondents agreeing that resistance testing programs are in need of improvement. (You can access the full *Mosquito Control Capabilities in the U.S.* report at www.naccho.org.)

The CDC recently developed initiatives for national resistance reporting through their MosquitoNet reporting platform.

NEVBD Efforts to Address the Pesticide Resistance Monitoring Gap

NEVBD is working to assist the CDC and collaborators throughout the Northeastern United States to address the resistance monitoring gap. Our resistance monitoring program is taking the following steps to address this pressing need:

- **Pesticide Use and Resistance Survey:** A pesticide use and resistance survey will be launched in late January 2019. The survey is designed to reveal the extent of current resistance monitoring operations in the region, determine priorities for NEVBD assistance to the public health community, and increase awareness of the monitoring program. The survey will also ask questions about current vector surveillance and control activities to ensure that NEVBD's efforts to detect pesticide resistance are relevant to ongoing field operations. Any individual working in a non-federal agency or organization in the Northeastern USA is eligible and encouraged to participate in this survey by signing up at: http://neregionalvectorcenter.com/resistance.

- **Educational Resources:** In collaboration with our partners, NEVBD is developing educational resources to enable public health practitioners throughout the region to independently conduct pesticide resistance monitoring for mosquito populations in their jurisdiction. These resources will include webinars and clear protocol guidelines, made available for free through the NEVBD website as they are developed.

- **Specimen Submission System:** Starting in May 2019, NEVBD will establish a specimen submission system, through which public health practitioners in the region can submit *Culex pipiens* and *Aedes albopictus* eggs to assess for resistance. Submitted specimens will be reared at Cornell University and tested for pesticide resistance. Specimen collection guidelines and submission forms will be posted on the NEVBD website in spring 2019. Mosquito egg collection kits will also be available to those who require them.

CDC Bottle Bioassay to test for resistance (Photo credit CDC)

- **Adult & Larval Mosquito Testing:** NEVBD will test adult and larval mosquitoes collected from the region using the CDC bottle bioassay and NEVBD larvicidal resistance assay. Pesticide resistance data collected at Cornell University will be shared publicly on the NEVBD website and posted on MosquitoNET as part of an ongoing effort to construct a national pesticide resistance monitoring network.

- **Pesticide Resistance in Tick Populations:** Pesticide resistance must be monitored in tandem with the development and use of new tick control methods. In collaboration with Dr. Scott Campbell at the Suffolk County Department of Health Services, NEVBD is beginning to test tick populations on Long Island to determine whether long-running control operations have reduced their vulnerability to commonly used pesticides. NEVBD will also be testing the sensitivity of lab-based tick colonies to build baseline resistance metrics for two medically significant vectors: the blacklegged tick (*Ixodes scapularis*) and the lone star tick (*Amblyomma americanum*).

This is a new and rapidly growing NEVBD program, aimed at addressing the emerging issue of pesticide resistance at a regional scale. Participation and feedback from the community of practice throughout the Northeastern region is essential.

For further information and program updates, please visit http://neregionalvectorcenter.com/resistance. Questions about our Resistance Monitoring Program can be addressed directly to Dr. James Burtis (jb766@cornell.edu)!

INSECTICIDE RESISTANCE MONITORING EFFORTS AND RECENT SUPPORT FOR SAME IN NEW JERSEY

BY SCOTT C. CRANS, JACK PETERSEN, TADHGH RAINEY, AND NICK INDELICATO

Development of Professional Training Programs in Insecticide Resistance Monitoring

Testing local mosquito populations for resistance to commonly used insecticides is an extremely important step in maintaining control of larval and adult mosquito populations. The importance of insecticide resistance screening has been recognized for a long time in New Jersey (NJ). For many years, NJ's Mosquito Control Commission supported a program at the NJ Agricultural Experiment Station (NJAES, Rutgers) to investigate insecticide formulations used in NJ's airspray program. Unfortunately, this program ended in the mid-2000s.

The NJ Office of Continuing Professional Education coordinated a training opportunity for local programs in 2006, in an effort to support ongoing insecticide resistance monitoring in the state. This professional training opportunity was supported by the Mosquito Research and Control Unit (MR&C) at Rutgers Entomology, and made available to local NJ mosquito control programs. Janet McAllister (CDC) led a two-day workshop using the CDC bottle bioassay, covering the process of insecticide resistance development and how to screen for it using the CDC bottle bioassay. Primary goals of this first workshop were hands-on demonstrations of the bottle bioassay, providing necessary training to encourage local program adoption. The workshop was a success, with just over 30 individuals participating. Several additional workshops were provided over the following years. However, few local programs adopted this tool for mosquito resistance monitoring.

The key to a successful resistance monitoring program lies in the use of standardized protocols as well as the participation of all mosquito control agencies in the State and region over a long-time frame. To address low adoption rates, the NJ Office of Mosquito Control Coordination coordinated additional resistance monitoring workshops in July 2017 and September 2018, in a continued effort to support a statewide program. These workshops were a collaborative effort between the NJ State Mosquito Control Commission, NJ Mosquito Control Association, Mercer County Mosquito Control, and Hunterdon County Mosquito & Vector Control Program. NJ mosquito control programs accepting CDC ELC funding were required to have at least one individual attend. The free program was also open to surrounding states.

Methods & Materials for the 2018 Training Workshop

Each participating county ordered a CDC bottle bioassay test kit from CDC. Specific insecticides were requested according to the needs and interests of each individual county. Technical grade organophosphate [malathion] and pyrethroid insecticides [etofenprox, sumithrin, and permethrin] were ordered and tested.

Aedes albopictus mosquito (Photo credit: CAES)

Mosquitoes for testing were collected from the field by participating counties using oviposition (egg) traps, targeting Asian tiger mosquitoes (*Aedes albopictus*). Egg papers were stockpiled over the 2018 summer season, and then reared to adults two to three weeks before the workshop. Ideally, 3-5 day old females should be used for the bottle bioassay. Our mosquitoes were both younger and older than this age range, and two other species captured in the egg traps were included in testing: Eastern tree hold mosquito (*Aedes triseriatus*) and Asian bush mosquito (*Aedes japonicus*).

Participants in the 2017 workshop

Bottle bioassays (using 250 ml bottles) were performed on the second day of the workshop. Mosquitoes were exposed to the diagnostic dose of insecticide, following CDC protocols. Our team prefers to wait 60 minutes to assess the efficacy of our insecticides, which differs from the CDC instructions.

Mosquito mortality was recorded every 15 minutes until all mosquitoes in the bottle were dead. We were encouraged by the results of our testing, as all mosquitoes tested for resistance were dead within 60 minutes. These results were reported to CDC through the MosquitoNET Insecticide Resistance database.

Summary of Our Experience

Workshop results from both the 2017 and 2018 workshops were reviewed, including a review of the data that was collected, discussions of problems we experienced, and remedies to improve the process moving forward. In general, this renewed statewide/regional training effort was successful in introducing the CDC bottle bioassay procedure to 19 out of 21 NJ mosquito control programs. Additionally, programs from New York, Pennsylvania, and Delaware sent staff to participate in the workshop. Supplies were given to any programs looking to attempt bottle bioassays on their own later in the season, provided they shared their data.

One of the greatest challenges for these workshops was coordinating a synchronized hatch of mosquito larvae and then rearing them to properly-aged adults in time for the workshop. We held several meetings to address this issue, reviewing techniques different mosquito control programs used successfully leading up to the 2018 workshop. These meetings helped fellow scientists share their methods and increased everyone’s chances of having testable mosquitoes in time for future workshops.

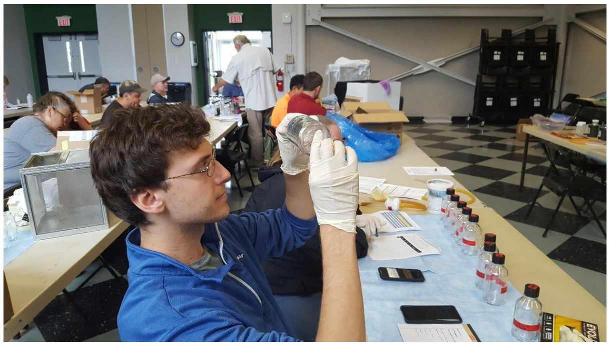
Future Directions

A single bottle bioassay is only a snapshot of resistance. What is truly needed is a full-length motion picture, a feature production. Two issues are central to this argument: (1) mosquitoes collected from the field need to be compared to standard, lab-reared mosquitoes that are fully sensitive to insecticides, and (2) field-collected mosquitoes need to be tested repeatedly over time (preferably years) to get a real idea of the true level of sensitivity to insecticides of wild populations. Clearly, we have our work cut out for us.

We will continue to build a standardized insecticide resistance program in the State of New Jersey, sharing our data and making our workshops available to other programs.

Working together in a group setting makes this process much more clear. When done a few times in this hands-on way, the bottle bioassay is very doable for nearly any mosquito control program with the will to try.

Active ingredient supplied by CDC



Reading a bottle, 2018 workshop

All busy enjoying the activity

Dr. Petersen reviewing the day's agenda

UNDER THE MICROSCOPE:

Biosketch of a Vector Villain

Northern House Mosquito

Culex pipiens

Culex pipiens adult (Photo credit K. Loeffler)

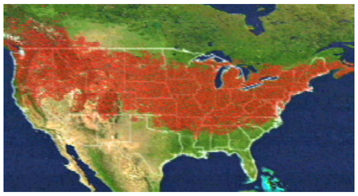
The Northern House Mosquito is a medium-sized, plain mosquito that is usually brown or grayish brown, with pale bands on its abdomen. You might see this mosquito referred to as *Culex pipiens* form pipiens, but most people call it *Culex pipiens*.

This mosquito is a vector of several viruses and other pathogens that impact humans, livestock, and pets in the Northern part of the USA, including:

- West Nile virus
- Eastern equine encephalitis
- St. Louis encephalitis
- Dog heartworm

Where do they live?

Globally, you can find these mosquitoes in temperate climates. This includes the northern half of the USA and parts of Canada. The Northern House Mosquito is part of a group of mosquitoes that look very similar, but have important differences in behavior and biology. This group is called the *Culex pipiens* complex, and includes the Southern House Mosquito (*Culex quinquefasciatus*) and a hybrid species (*Culex pipiens* form molestus) that live in other areas of the USA.



Culex pipiens distribution
(Photo credit NASA/Goddard Space Flight Center Scientific Visualization Studio)

When do they bite?

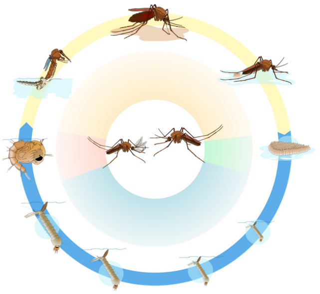
The Northern House Mosquito bites at night, most often in the first few hours after sunset. These mosquitoes prefer feeding outside on birds, but will also enter homes and feed on people. This is why the Northern House Mosquito is an important vector of diseases.

What times of the year are they active?

Adult female Northern House Mosquitoes will hunker down for the winter in dry, warm shelters, such as barns, basements, caves, or tree hollows. These females will come out in the spring, feed, and lay their eggs. Several generations of mosquito can live each season, meaning you can find adult Northern House Mosquitoes from late spring into late fall.

Where do they lay eggs?

A female Northern House Mosquito can lay eggs multiple times in her life. Females will lay a "raft" of 100-200 eggs on the surface of foul or dirty stagnant water. This standing water can be found in containers, catch basins, ditches, ground pools, clogged gutters, neglected bird feeders, and other areas that contain organic waste materials.



Culex mosquito life cycle
(Photo credit M.R. Villarreal)

Tips on how to avoid the Northern House Mosquito:

- ***Remove standing water from your yard*!** This includes making sure your rain gutters are clean, your bird bath is fresh, and there are no places where water can pool after rain, like folds in plastic tarps or open buckets.
- Use a CDC-recommended insect repellent, and consider wearing long sleeves and pants to prevent bites.
- Use screens on your open doors and windows to keep these mosquitoes out of your home.

- Andreadis, T., Thomas, M.C., and J. Shepard. 2005. Identification Guide to the Mosquitoes of Connecticut. The Connecticut Agricultural Experiment Station. Bulletin No. 966. https://www.ct.gov/caes/lib/caes/documents/publications/bulletins/b966b996.pdf
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- Walter Reed Biosystematics Unit. *Culex (Cux.) pipiens*. http://www.wrbu.org/mqID/mq_medspc/AD/CXpip_hab.html