



THE LABYRINTH
Indiana State Department of Health Laboratories Newsletter



Chikungunya Virus

By Marta Cruz, ISDH Microbiologist



Source: CDC/ J. Gathany

Do you plan to travel soon? Beware. You do not want to bring back home the Chikungunya Virus (CHIKV) as a souvenir.

CHIKV is not a new virus, but what is new is that it recently found the perfect ingredients to spread in the Americas. Add one infected person, an area with competent mosquito vectors, and a susceptible human population and you end up with local transmission. Now

add millions of travelers (vacation trips, business, mission trips and visiting relatives) and you have importation of the virus throughout the Western Hemisphere. The CDC estimates that nine million U.S. residents travel to the Caribbean each year. With the recent CHIKV outbreaks in the Caribbean and Central and South America, the probability of infected people returning to the U.S. from these newly affected areas is higher, increasing the chances of sporadic local transmission in areas where the vector is present, and Indiana is one of those areas.

CHIKV is an arthropod-borne alphavirus transmitted to humans by an infected *Aedes* species mosquito, with infection resulting in an acute febrile illness and severe joint pain. CHIKV was first recognized in the 1950's in Tanzania, Africa (1), and the name Chikungunya (meaning "to become contorted") derives from the Kimakonde language (7). After a large outbreak in Kenya in 2004, CHIKV spread to India, Asia, the islands of the Indian Ocean, and Europe (1, 2). In 2006, an increase in the number of CHIKV cases in La Reunion, a French island in the Indian Ocean, caused by a secondary vector, *Aedes albopictus*, revealed a possible mutation in the virus that increased its transmissibility (1, 3).

The main vectors of the disease are *Aedes aegypti* (5) and *Aedes albopictus* (1), with only an infected female being capable of transmitting the virus (7). Both species can be found in the southeastern and limited parts of the southwestern United States. *Aedes albopictus* is also found along the east coast, through the Mid-Atlantic States and in the lower Midwest, including Indiana (8, 15). Established populations of *Aedes albopictus* (the Asian "tiger mosquito") were first discovered in Texas, in 1985. It is believed that this highly invasive mosquito came to the U.S. in shipments of used tires with dormant eggs inside from Asia, where the mosquito is abundant. The mosquito was then widely dispersed via highways and major roadways to tire-related businesses (4, 6). Between 2006 and 2013, an average of 28 persons per year tested positive for recent CHIKV infection in the U.S., all of them travelers visiting or returning from affected areas mostly in Asia. None of these cases triggered a local outbreak in the U.S. In December 2013, the first local transmission in the Western Hemisphere was reported in the French part of the Caribbean island of Saint Martin. Since then, Chikungunya has spread throughout the Caribbean and into Central and South America (2). In July 2014, Florida reported the first local transmission of the virus in the U.S. (8).

(Continued on next page)

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Our Mission:

The Indiana State Department of Health Laboratories partners with other public health agencies to provide timely and accurate information needed for surveillance and outbreak investigations to protect and improve Hoosier health.

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Chikungunya Virus (continued):

As of January 2015, a total of 2,344 Chikungunya cases for the year 2014 have been reported to ArboNET (the arbovirus surveillance system that collects data on arboviral infections) from states in the U.S. Eleven locally-transmitted cases have been reported from Florida, with all other cases reported from travelers returning from affected areas in the Americas (2,306), Asia (15), or the Pacific Islands (12). Of the 2,333 travel-associated cases, 23 are from Indiana (9), and the first in-state CHIKV case was detected in Allen County in June 2014 (14).

After an infected mosquito bites a healthy individual, symptoms can appear in 3-7 days. Fever of 102° F (39° C), arthralgia/arthrititis, backache, and headache are the most frequent clinical manifestations of disease. Viremia (presence of high levels of virus in the blood) can last for 4-6 days to a maximum of 12 days (1). In the majority of patients, symptoms resolve in 1-3 weeks, but for some, severe or incapacitating joint pain persists for weeks, months or even years. Mortality is rare but occurs mostly in older adults (>65 years of age) or people with medical conditions (e.g., high blood pressure, diabetes, heart disease). Maternal-fetal transmission has been documented during pregnancy when a woman is viremic at the time of delivery; complications for the baby include neurological disease and myocardial disease, among others (11). Infected individuals should avoid contact with mosquitoes while viremic to prevent spreading the virus (10). If a non-infected mosquito bites a viremic host it will be able to transmit the virus to a healthy individual after approximately 10 days (13).

There is no vaccine or medication to prevent Chikungunya fever. Treatment consists of ingesting fluids, resting, and taking analgesics for the joint pain (7). Use of aspirin is not recommended until Dengue virus is ruled out due to risk of bleeding. Prevention relies on reducing the number of natural and artificial mosquito habitats, staying in rooms with a/c or screens, using clothing which minimizes skin exposure and applying repellent containing DEET or picaridin (among others) to exposed skin (10). Infection is thought to provide lifelong immunity (8).

Laboratory diagnosis is accomplished by testing serum or plasma to detect virus, viral nucleic acid, or virus-specific immunoglobulin (IgM) and neutralizing antibodies. The specific test performed will depend on the collection time of the specimens and the onset of illness. Viral culture may detect virus in the first 3 days of illness, and the Chikungunya virus should be handled under biosafety level (BSL) 3 conditions. During the first 8 days after onset of illness, Chikungunya viral RNA can often be identified in serum. Chikungunya virus IgM antibodies are generally detectable at 4 or more days after onset of illness and can persist for months; however serum collected within 8 days from onset of illness may not have detectable IgM antibodies, and testing should be repeated on a convalescent phase sample to rule out infection (12). Fever with or without arthralgia is a common symptom of many diseases. Differential diagnosis for acute febrile illness of a patient is of extreme importance and should take into account place of residence, travel history, and exposure. Some of the diseases that should also be considered in Chikungunya virus differential diagnosis are Dengue fever, malaria, leptospirosis, and meningitis among others. Patients with suspected Chikungunya virus should be managed as Dengue virus until dengue is ruled out to reduce the risk of complications and improve outcome (10).

Chikungunya virus testing is performed at the Centers for Disease Control (CDC), a few state health departments, and one commercial laboratory in California (12). The ISDH lab sends Chikungunya suspected samples to CDC for testing but is currently evaluating a commercially available test for detection of Chikungunya IgG and IgM antibodies that provides sensitivity comparable to that of the CDC protocol (12). Validation of a real-time RT-PCR assay for mosquito pools and serum samples is also planned for the near future.

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Taxonomic Identification of Bats Tested by the Indiana State Department of Health (ISDH) Rabies Lab (Part Two)

By Erica Vecchio, ISDH Microbiologist

Rabies is a viral disease that is found worldwide, particularly among the children of poor, rural populations of undeveloped nations with little available healthcare (3, 8). Without proper post-exposure treatment, infection with rabies is almost always fatal (1, 2), and over 55,000 human deaths occur annually (3, 8). The causative agent, an RNA virus in the *Lyssavirus* genus (2, 3), affects the central nervous system, and is transmitted primarily through bites or exposure to infected saliva from rabid animals (1, 3, 6). Due to vaccination of both domestic and wild animals and the prevalence of post-exposure treatment, human rabies infections in the United States are rare, with an average of 1 to 2 cases per year (3). Although they are not the most frequently tested mammals submitted to the Indiana State Department of Health (ISDH) Rabies Lab, bats are the most common vectors in animal to human transmissions in the United States (1, 2, 3, 4, 6, 8). Of 23,370 bats tested for rabies nationwide in 2011, 1380 bats of varying species, or approximately 5.9% of those submitted, were positive for the virus (2). From 1965 to 2014, over 9000 bats have been tested by ISDH, and over 630 have been confirmed as rabid (4, 6, rabies lab). This equates to about 5-6% of those specimens tested having an infection with the virus, a percentage similar to the national average in 2011 (5.9%). Since the bats submitted for testing often display erratic, motor-impaired behavior, they represent a biased sampling of the overall population of bats, of which less than 1% are estimated to be rabid (1, 2, 6).

Taxonomic Identification of Bats (continued)

More than 8 distinct bat variants of rabies, each generally associated with a specific species, have been discovered in the United States. Much less is understood about bat variants of rabies than about variants affecting terrestrial mammals. For example, there have been instances in which bat rabies variants have displayed host switching from bats to foxes and bats to skunks (1, 2). As such, the Centers for Disease Control and Prevention (CDC) requests from all state rabies labs information on specimens tested, including species, county from which collected, and dates collected/tested, in order to monitor existing reservoirs of rabies and to investigate possible host switching events (2). The ISDH Rabies Lab identifies submitted bats to the species level through the use of taxonomic keys.

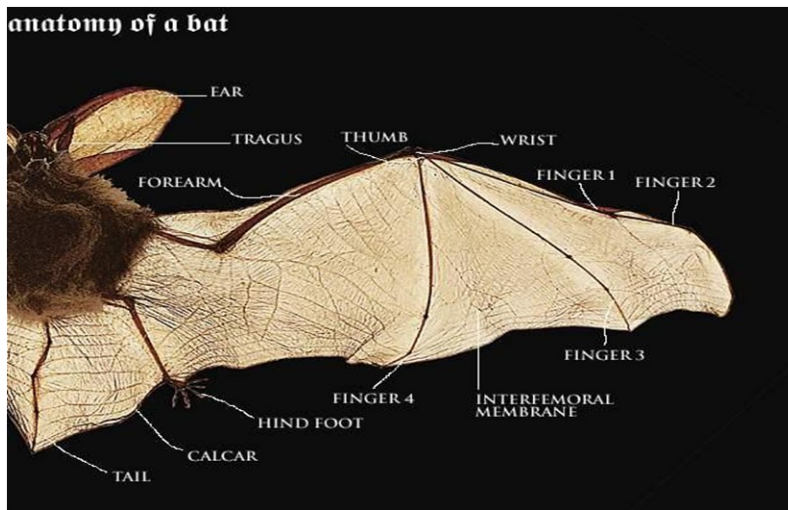
Generally speaking, taxonomic keys are used to determine the identity of unknown specimens by using paired statements describing particular characteristics (often physical) of organisms. The investigator must make a choice between the statements, which will either lead to identification of the specimen or another set of paired choices. This process continues until the investigator is usually able to discover the identity of the subject. Taxonomic keys may range from very broad (kingdom level) to highly specific (species or subspecies level). The keys utilized most commonly in the ISDH Rabies lab are found in [Bats of Indiana](#) by Whitaker, et al, and they are used to identify bats to genus then species (7).

Although 12 species of bats have been definitely identified in the state through the years, only 10 may currently be found in Indiana; the remaining two are rarely or never observed. *Corynorhinus rafinesquii* (the big-eared bat) is believed to be native to Kentucky and transported only by accident to Indiana, and *Myotis austroriparius* (the southeastern myotis) is likely extirpated from the state. The bats remaining in Indiana, listed in order by relative abundance, are *Eptesicus fuscus* (big brown bat), *Myotis lucifugus* (little brown myotis), *Lasiurus borealis* (Eastern red bat), *Perimyotis subflavus* (Eastern pipistrelle), *Myotis septentrionalis* (Northern myotis), *Myotis sodalis* (Indiana myotis), *Lasiurus cinereus* (hoary bat), *Lasiorycteris noctivagans* (silver-haired bat), *Nycticeius humeralis* (evening bat), and *Myotis grisescens* (gray myotis) (7). By far, the bats most commonly submitted to the rabies lab for testing are *E. fuscus*, big brown bats; individuals of this species account for over 90% of all bat specimens identified by the lab (5). Since this is the case, personnel in the rabies lab generally use the Whitaker keys slightly out-of-sequence and first look at the fur color and teeth of submitted bats.

Big brown bats are larger animals (forearm length averaging over 42 mm) that are dark brown in color. Additionally, they do not have a small space behind the canine teeth. The presence of this space in some species is due to the reduced size of the teeth following the canines (7).

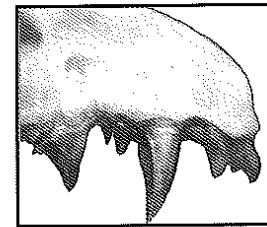
If the specimen is not readily recognized as a big brown bat, the Whitaker keys are again utilized. Identification of genus is the first step: ear size, furring of the dorsal aspect of the interfemoral membrane (part of the wing membrane), fur color, dentition, forearm length, and size/shape of the tragus (a small projection of skin from the external ear that partially covers the opening of the ear) are examined in turn. When the genus has been successfully identified, the species may be readily apparent due to prior knowledge of which bats are present in Indiana, or other characteristics may need to be examined in order to distinguish between species of bats belonging to the genera *Myotis* and *Lasiurus*. *Myotis* bats are often difficult to speciate. Dorsal hair color, forearm length, ear length, size/shape of the tragus, toe hair length, keeling of the calcar, and presence/absence of the sagittal crest are examined to determine the species. The calcar is a small structure composed of cartilage that projects from the ankle in some bats and is used to spread the interfemoral membrane of the wing. The keel is a tiny piece of skin, variable in shape according to bat species, which is attached to the calcar. The sagittal crest is a bony protuberance of the midline of the skull. By contrast, bats of the genus *Lasiurus* are very distinctive in appearance; they are readily distinguished from other bats and one another by size and fur color. *L. borealis* (the Eastern red bat) is small in size (total length 91-112 mm) and has fur that is deep red or orange frosted with white. *L. cinereus* (the hoary bat) is larger in size (total length 134-140 mm) and has a coat that is brown tipped with white (7).

The following figures display morphology and definition used in the identification of bats in the rabies laboratory.

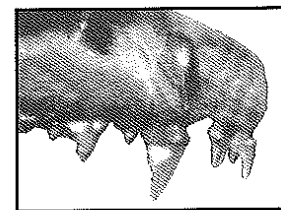


Above: Figure 1. Bat morphology, including interfemoral membrane, forearm, tragus, and calcar (Source: Digitaltutors.com)

Graphics by Linda Castor



Toothrows of bats showing space behind canine with two small teeth in *Myotis* (a)



Space with one small tooth in *Pipistrellus* (b)



and no space, simply a large tooth behind canine in *Eptesicus* (c)

Right: Figure 2. Dentition of selected genera of Indiana bats (Source: *Bats of Indiana*)

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Food Microbiology and Chemistry Labs' Journey towards Accreditation

By Megan Teachout

Have you seen the "ISO" logo and a string of numbers on a document from a testing lab or supply company? What's the big deal anyway? That logo is a measure of quality based on requirements, or standards, established by a group of representatives that make up the International Organization of Standardization (ISO). This group has developed many standards to use as quality benchmarks for various manufacturing processes and product testing; including chemical and biological testing that we perform at ISDH Labs. By meeting the requirements listed in the testing laboratory standard known as ISO 17025, a laboratory shows that it is meeting the ISO guidelines and is competent to perform specific tests. These standards do not just pertain to us as a testing lab—other standards you may have heard of include ISO Guide 34 for preparing certified reference materials and ISO 17043 for providing proficiency tests.

Testing laboratories throughout the world have worked towards meeting the long list of requirements to become accredited. In 2011, President Obama worked with the US Food and Drug Administration (FDA) to create the Food Safety Modernization Act (FSMA), stating that State and Federal labs testing this country's food supply must work towards becoming accredited over the next several years. ISDH Labs has been fortunate enough to be able to work with a network of already accredited labs and labs seeking accreditation, including the FDA, the United States Department of Agriculture (USDA), Michigan Department of Agriculture and Rural Development, and Ohio Department of Agriculture, to meet the requirements of ISO 17025 and FSMA. Their assistance helped direct us throughout this project.

A significant challenge to becoming accredited was coordinating many small projects for the different areas of ISDH Labs. Our "mentor" labs were able to give us ideas and provide a sequence of items to be accomplished to achieve our goal. This involved the quality assurance coordinators creating procedures for performing preventive maintenance on equipment, setting requirements for calibrating instruments, and performing internal audits. The lab supervisors over the Food Microbiology and Food Chemistry Labs had to revise procedures, create forms to record quality control results, and document corrective actions when necessary in the lab. The Media Prep Lab was also required to meet ISO 17025 standards by ensuring all media made for the Food Micro Lab met documentation requirements proving quality and sterility. The ISDH Labs IT/STARLIMS team was also quite involved in this process. With their help, the Food Microbiology and Food Chemistry Labs are now able to record results, track quality control parameters, and report out results using STARLIMS.

Our goal of accreditation is within sight. We chose an accrediting body called L-A-B, which is a third-party vendor who assesses our progress towards accreditation. In late 2014, Chris Grimes, ISDH Labs Quality Assurance Director, took the lead with filling out a lengthy application and gathering all important documentation for L-A-B to review. Assessors must review the application, along with laboratory records, procedures, and manuals to evaluate our request. A L-A-B assessor came onsite January 20-22, 2015 to review the extensive ISO 17025 checklist, looking in detail at our quality management system, including our incident management program (IMP), document control system (iPassport), laboratory information management system (STARLIMS), and technical records and practices.

At the end of the visit, our assessor gave us a formal report stating our non-conformances. These non-conformances must be addressed within 30 days of the onsite assessment. Once L-A-B has reviewed our response, they have 30 days to determine if we are accredited to perform very specific tests in the Food Microbiology and Food Chemistry Labs.

Accreditation is an on-going process; once it has been achieved, the status must be maintained. Our commitment to quality means that we will have to continuously check records, review procedures, hold regular meetings with our customers, and maintain our equipment. Over the next several years, the goal for ISDH Labs will be to add additional tests to our scope of accreditation, including tests outside of the Food Microbiology and Food Chemistry Labs.

ISDH Outreach and Training Team Elected to New SCACM Board Positions

By Jyl Madlem and Shelley Matheson



The South Central Association for Clinical Microbiology (SCACM) is a not-for-profit organization directed by volunteers from all levels of clinical microbiology. This organization has members from at least six states, including Michigan, Illinois, Indiana, Ohio, Kentucky, and West Virginia. The organization's primary goal is to provide high quality, low cost, continuing education opportunities for clinical microbiologists throughout the Midwest.

Shelley Matheson, Omar Perez, and Jyl Madlem won best vendor booth at the 2014 SCACM Spring Meeting (left). The theme for the vendor hall opening evening was "Clue"; our staff had all the weapons from the classic Clue board game and dressed the part.

Jyl Madlem and Shelley Matheson have served as SCACM elected board members for 3 and 4 years respectively. Jyl has been a member for 3 years, and served as the Board Secretary since joining the organization. Shelley has been a member for 11 years, and has served as the Indiana State Director for the last 4 years.

This year, Shelley and Jyl were elected to new positions on the SCACM Board of Directors. Shelley is the new President-Elect, and as such will be relinquishing her seat as the Indiana State Director. Jyl has just been elected the new Indiana State Director, and will be stepping aside as the Board Secretary.

SCACM hosts a Spring Meeting for the entire organization, with Fall State meetings held in each of the 6 member states. Join us this October for the Indiana Fall meeting to be held in Nashville, Indiana. See Jyl or Shelley for more details. Opportunities always exist for folks interested in becoming involved in the planning of meetings, finances, exhibits, publications, etc. To be a part of this fun and active group, fill out our [Willingness to Serve Form](#), or speak with Jyl or Shelley.



About The LAByrinth

The LAByrinth is published quarterly by the editorial staff of Indiana State Department of Health Laboratories.



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www.in.gov/isdh/24567.htm