ILLINOIS NATURAL HISTORY SURVEY

BIENNIAL REPORT, 2020-2022

WASTE TIRE AND EMERGENCY PUBLIC HEALTH FUNDS

LEGISLATIVE MANDATE:

"TO PROVIDE FOR RESEARCH ON DISEASE VECTORS ASSOCIATED WITH USED AND WASTE TIRES AND THE DISEASES THEY SPREAD."

ILLINOIS
Illinois Natural History Survey
PRAIRIE RESEARCH INSTITUTE

REPORT TO GOVERNOR J. B. PRITZKER AND THE ILLINOIS GENERAL ASSEMBLY

SUMMARY OF ACCOMPLISHMENTS BY THE MEDICAL ENTOMOLOGY LABORATORY

Chris M. Stone, Ph.D.
Principal Investigator, Medical Entomology Laboratory
Illinois Natural History Survey
Prairie Research Institute

Eric M. Schauber, Ph.D.
Director, Illinois Natural History Survey
Prairie Research Institute
University of Illinois at Urbana-Champaign
1816 S Oak St, Champaign, IL 61820

Cover photo by Joseph L. Spencer.

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Summary of Accomplishments

Overview:

- The Medical Entomology Program at the Illinois Natural History Survey has served the State of Illinois for over 30 years by providing applied field and laboratory research on mosquito vectors so as to effectively detect, prevent, and control vector-borne diseases and invasions in Illinois. Our world is characterized by rapid change – our changing climate, land use, and demographics pose serious challenges with regards to vector control. Our collective risk of zoonotic spillover and vector-borne disease occurrence has increased over the past half-century as humans continue to encroach on wild habitats, populate urban and suburban areas in higher densities, and facilitate introductions of invasive species. Vector-borne disease outbreaks are evergreen occurrences – it's not a matter of whether we will experience another outbreak, such as Zika, but when. The primary mission of the INHS Medical Entomology Lab is to anticipate vector-borne disease risks in Illinois, work towards preventing them, and be prepared to rapidly respond when outbreaks occur in our state. In doing so, our goal is to make Illinois a recognized leader at the national and international levels in vector-borne disease surveillance, research, and prevention.

- We maintain state-of-the-art facilities, including Biosafety Level 2 and Arthropod Containment Level 2 areas, a rapid-turnaround molecular lab, an arsenal of mosquito traps, and environmental chambers with long-term colonies of mosquitoes for research on vector potential and control. We can collect, process, and identify field-collected vectors; identify their disease agents and study their genetics and insecticide resistance status; and experimentally infect vectors to ask questions about behavior, risk, and control. In addition, we use laboratory and field experiments and mathematical modeling approaches to improve and innovate vector surveillance and control strategies.

- Understanding how vector distributions are changing in our state, where and why populations are resistant to commonly-used insecticides, and developing novel vector surveillance and control methods are matters of urgency in Illinois. Current concerns include insecticide resistance in the major West Nile virus vectors, the increase in abundance and spread of the Asian tiger mosquito and several tick species (for which currently no (cost-)effective control methods exist) and the emergence or incipient threat of several vector-borne diseases. The INHS Medical Entomology Program is uniquely situated and expertly staffed for tackling these challenges on multiple fronts, from vector and disease agent detection and control, to collaborative research and outreach, and stakeholder engagement and education.

Activities for the 2020 - 2022 period:

Collaborations and funding

- We continue to collaborate closely with the Illinois Department of Public Health on statewide monitoring and surveillance programs for 1) insecticide resistance in mosquitoes, 2) mosquito and arbovirus diversity, and 3) ticks and tick-borne pathogens. As a result of our collaboration with IDPH, in 2021 the State of Illinois received funds for tick surveillance through the CDC Enhanced Laboratory Capacity (ELC) program for the first time and was recognized at the national CDC VectorWeek meeting as the top state submitting records through Arbonet and the second most likely state to conduct tick surveillance. In 2020, our collaboration on mosquito surveillance expanded to document the diversity and abundance of mosquitoes throughout the state, and test them for neglected and emerging viruses of public health concern, such as Eastern Equine Encephalitis virus, to enhance emergency preparedness for the state.

- During FY2020-22 we maintained, initiated, and extended collaborations (including field-training in vector surveillance) with an array of local public health, mosquito abatement, and municipal or state parks and forest preserve districts across Illinois. Collaborations regarding the impact of climate change on vector-borne disease transmission were initiated and/or strengthened with the IL State Climatologist and BRACE-IL at the University of Illinois Chicago, the Nature Conservancy, and Carle Illinois College of Medicine. Work on environmental justice and vector-borne diseases in urban green spaces continues with the Urban Wildlife Institute at the Lincoln Park Zoo. A new project, BiteMap, was supported with seed funding from the UIUC National Center for Supercomputing Applications (NCSA) – this project is nearing completion and will allow us to build a number of citizen-science based projects related to monitoring mosquito and tick activity and risk throughout the state. Collaborations also continue with Southern Illinois University, Eastern Illinois University, IL Extension and the Dixon Springs Agricultural Center, USDA ARS (Peoria, IL), the UIUC Institute for Sustainability, Energy, and Environment (iSEE), the Tyson Research Center at the University of Washington in St. Louis, and the Centers for Disease Control and Prevention (CDC). We worked with the Prairie Research Institute to develop web resources for the UIUC Division of Research Safety on mosquito and tick fieldwork safety and hazard awareness. We

also provided instrumental leadership towards establishing a research and public health coordination network for vector-borne diseases across the seven states comprising the Ohio River Valley.

- Five graduate (2 PhD, 3 MSc) students in the University of Illinois Urbana-Champaign Department of Entomology began or completed their research in the lab. We also provided opportunities for undergraduate research through the School of Integrative Biology, including through their Access and Achievement Program, and by hiring undergraduates and recent graduates as hourly and seasonal technicians. This student involvement in the lab increases the technical capacity of the State of Illinois for monitoring and studying arthropod vectors of disease, builds knowledge expertise, and contributes to workforce development in public health entomology. Our expertise in educating and training students was recognized by the University of Illinois Urbana-Champaign with an invitation to teach a course, "Vector-Borne Diseases" every two years on campus.

Research Outputs

- Members of the Medical Entomology Program published 14 peer-reviewed publications, four technical reports, and 3 fact sheets over the 2020–2022 period. Research outputs were presented at various national and regional conferences. Members of the lab are active in national groups and organizations, for instance the NEON Tick Working Group, and in professional societies such as the Illinois Mosquito and Vector Control Association. At the Entomological Society of America's Annual Meeting in 2020 we presented the invited talk on annual highlights in Medical Entomology [1].

[1] C. M. Stone. Highlights of Medical Entomology, 2020. *Journal of Medical Entomology*, 58(5):2006–2011, 2021

- In 2020, we initiated a new mosquito surveillance program throughout the state in collaboration with IDPH. In the first two years of this program we have detected a new West Nile virus vector in Illinois, detected the primary vector of Eastern equine encephalitis virus, *Culiseta melanura* in three additional counties (including Cook County), and found evidence of La Crosse encephalitis virus in Asian tiger mosquitoes in the state (p. 19).

- We documented the range extension of the Asian tiger mosquito throughout Illinois, explored how their abundance relates to land use, and their population genetics to understand patterns of introduction and spread, and investigated how blood-feeding patterns of this species varies in neighborhoods of varying socioeconomic conditions (p. 20).

- We explored the blood-feeding behavior and ecology of the northern house mosquito (a major West Nile virus vector), explored how vegetation management practices in stormwater detention basins can affect mosquito abundance, and performed a study on the overwintering behavior of this species (p. 21), which opens the door for novel control methods targeting mosquitoes.
- We found that the northern house mosquito (a major West Nile virus vector) is becoming more resistant to commonly-used insecticides, and that resistant populations occur throughout the state. Current and future research is focused on developing methods to combat insecticide resistance (p. 22).
- We developed a mathematical modelling framework to assess how decision makers can navigate the trade-offs between public demands for insecticide use to control mosquitoes and environmental concerns regarding insecticide use (p. 23).
- We contributed to an assessment of the impacts of climate change on Illinois by The Nature Conservancy and performed studies on the impact of variation in temperature and other environmental factors on invasive mosquito traits that impact their disease transmission potential (p. 24).
- We identified two phytochemicals that occur in nectar of some plants that greatly expand the lifespan of mosquitoes and identified genes that are involved in mosquito longevity, which will allow for genetic methods to indicate age of mosquitoes. We also explored a novel type of trap that could broaden the range of mosquitoes easily sampled in surveillance programs at public health and mosquito abatement districts (p. 25).
- We investigated how the microbiomes of different mosquito species is affected by environmental factors such as habitat type (e.g., treeholes or waste tires) and environmental pesticide contamination. These are important first steps to manipulating mosquito microbiomes to turn them into less effective vectors (p. 26).
- With funding provided by IDPH, we are conducting statewide surveillance for ticks and tick-borne disease agents. This work is leading to a better understanding of the abundance and distribution of different tick species, and their prevalence of infection with pathogens of public health importance, such as those causing Lyme disease, or emerging diseases such as Heartland virus or Tidewater Spotted Fever (p. 27). Maps with the results of our tick surveillance work are available to the public.

INHS Medical Entomology Program

Mosquito-borne diseases continue to pose a significant public health threat globally as well as within the United States. Concerns about arboviral epidemics[2] are particularly relevant for Illinois, which over the period 2004-2016 fell within the top 10 (at the state-level) of reported disease cases due to mosquito-borne diseases (see Figure 1). To a large extent, this is due to local transmission of West Nile virus, with numbers of disease cases spiking in certain years, as well as other locally-transmitted mosquito-borne viruses (e.g., La Crosse and St. Louis encephalitis viruses). These viruses are likely underreported as they often lead to relatively mild symptoms in humans; however, severe, neuroinvasive, and fatal cases occur in Illinois each year. Other endemic viruses, such as the highly lethal Eastern Equine Encephalitis virus, have been increasing in the Northeastern U.S. and neighboring states (Indiana, Michigan) in recent years, suggesting a need for increased preparedness and surveillance activities, which our lab is addressing.

[2] due to an arthropod-borne virus

Travel-related or imported cases are also of concern, in particular for those pathogens which have a locally-established competent vector[3]. This was brought into focus by the recent Zika virus epidemic. In 2016, 41,680 cases of Zika infection in humans were reported to the National Notifiable Disease Surveillance System.[4] Most of these cases were acquired during travel to South America or within U.S. Territories, with active transmission occurring in Florida and Texas. However, the large volume of international travelers to our state could introduce arboviruses such as Zika, dengue, or chikungunya. One of the major vectors of these viruses is the Asian tiger mosquito, *Aedes albopictus*. This invasive mosquito has spread across the United States over the past three decades and now appears to be firmly established in the southern and central parts of Illinois where it is rapidly increasing in abundance. Our research indicates the range of this mosquito in Illinois is still expanding. This highlights the need for robust outbreak preparedness and continued improvements in vector surveillance and control methods, which our lab is addressing.

[3] an organism, such as a mosquito or tick, which transfers a pathogen from one infected human or animal to another

[4] R. Rosenberg, N. P. Lindsey, M. Fischer, C. J. Gregory, A. F. Hinckley, P. S. Mead, G. Paz-Bailey, S. H. Waterman, N. A. Drexler, G. J. Kersh, et al. Vital signs: Trends in reported vectorborne disease cases-United States and Territories, 2004–2016. *Morbidity and Mortality Weekly Report*, 67(17):496, 2018

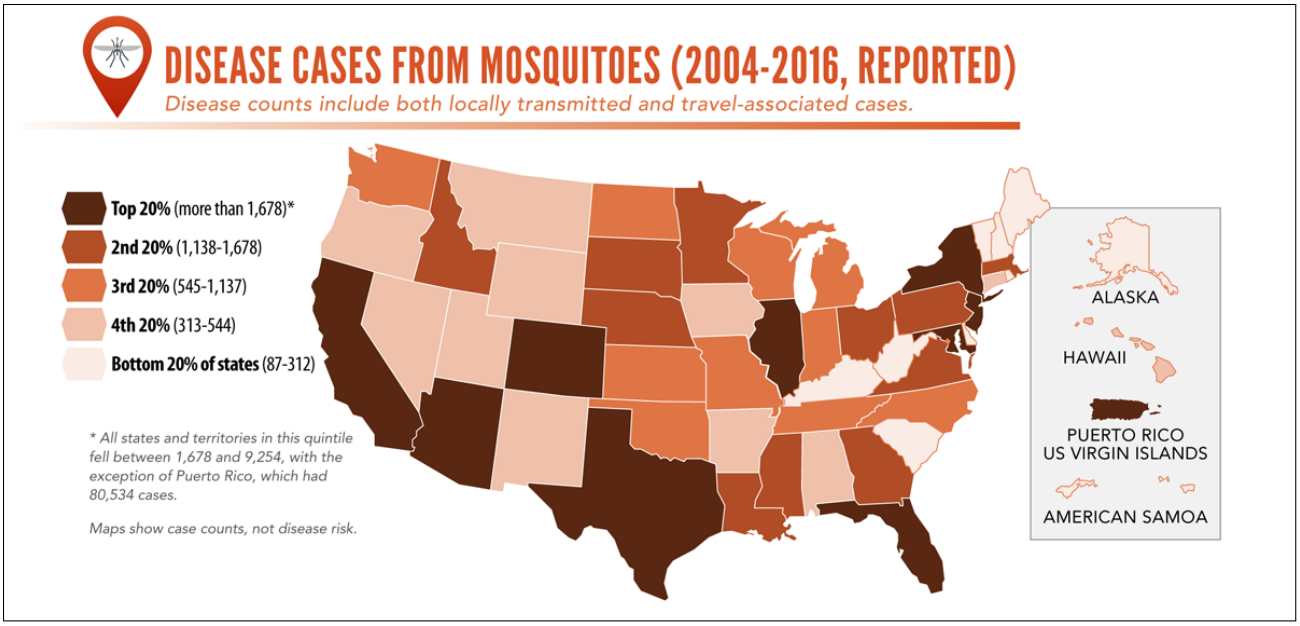

Figure 1: The number of reported mosquito-borne disease cases per state and territory over the period 2004-2016. With 2,582 reported disease cases over this period, Illinois is among the states with the highest mosquito-borne disease burdens in the United States. Source: CDC Vital Signs, May, 2018.

The Medical Entomology Program at the Illinois Natural History Survey (INHS) conducts research on vectors associated with used and waste tires and the disease agents they spread. More than 14 million waste tires are generated annually within Illinois and are considered a serious public health issue because they serve as ideal incubators for a wide range of mosquito vectors. Mosquitoes that thrive in waste tires notably include several important disease vectors: the Asian tiger mosquito *Aedes albopictus*, the Asian rock pool mosquito *Aedes japonicus*, the eastern tree-hole mosquito *Aedes triseriatus*, the northern house mosquito *Culex pipiens*, and the white-dotted mosquito *Culex restuans*. These mosquito species are considered threats to human and wildlife health because of their ability to transmit locally present dog heartworm, Eastern Equine Encephalitis virus, La Crosse encephalitis virus, St. Louis Encephalitis virus, West Nile virus, and introduced (exotic) dengue, chikungunya and Zika viruses.

Figure 2: Used tires, when discarded in discrete, vegetated areas are ideal breeding sites for invasive mosquitoes, such as the Asian tiger mosquito, and provide a means for vectors to spread throughout the state.

In 1989, Illinois passed the Waste Tire Act (ILCS 5/53 to 55.7a) to address issues associated with these solid waste hazards. Funds associated with this legislation are devoted to (a) the removal of used and waste tire dumps to reduce threats to public health, (b) mosquito surveillance and abatement, and (c) research on mosquitoes associated with waste tires and the disease agents they spread.

This led to the establishment of the Medical Entomology Program at the INHS to conduct research on the ecology, genetics, and biosys-

Figure 3: Neglected tire pile containing thousands of developing larvae in woods (left) and newly emerged *Aedes japonicus* female resting inside a tire (right) with active immature mosquito development, Marshall County, Illinois – Spring 2022. Photos: Dr. H.C. Tuten/INHS Medical Entomology Lab.

tematics of mosquito species associated with waste tires, establish and monitor their susceptibility to insecticides, evaluate alternative control methods in the laboratory and field, and contribute to surveillance and monitoring of invasive mosquito vectors and emerging pathogens. The Program developed into a multidisciplinary group of research scientists, technical personnel, students, and hourly academic staff, who conduct research on a broad range of questions regarding the biology and behavior of mosquito vectors, the transmission and management of mosquito-borne pathogens, and their impact on human health. More recently, with funding provided by IDPH, the Program has leveraged its surveillance and research capacity to document and investigate tick distributions and tick-borne disease agent in Illinois. In addition to our research and on-going surveillance we provide critical functions for the state by providing broad outreach materials (e.g., such as this webinar for IL-Extension) and the capacity and expertise needed to rapidly respond to vector-borne disease outbreaks or cases (e.g., tick-borne Heartland virus) [5].

[5] H. C. Tuten, K. L. Burkhalter, K. R. Noel, E. J. Hernandez, S. Yates, K. Wojnowski, J. Hartleb, S. Debosik, A. Holmes, and C. M. Stone. Heartland virus in humans and ticks, Illinois, USA, 2018–2019. *Emerging Infectious Diseases*, 26(7):1548–1552, 2020

Research capacity and future directions

The Medical Entomology Program is uniquely suited to tackle the major challenges confronting vector-borne disease surveillance and control in Illinois. The Program has built up a roster of expert vector-borne disease biologists and an extensive set of scientific facilities and equipment devoted to surveillance and research of vectors. This allows the Program to leverage its existing resources to address emerging and shifting priorities in the vector-borne disease landscape in Illinois.

Our research priorities are understanding variation in risk of hu-

man exposure to vector-borne diseases, and the development of cost-effective surveillance, prevention, and control methods. These efforts are informed by a detailed understanding of the biology and behavior of vectors, application of cutting-edge molecular methods, and mathematical modeling.

Research directions in progress include: assessing how the changing landscape of Illinois affects the presence and abundance of mosquito and tick species and disease agents; assessing the capacity of invasive mosquitoes to transmit established and possibly emerging viruses; monitoring insecticide resistance in IL mosquito populations and developing strategies to prevent insecticide resistance; developing new control methods and traps based on our understanding of vector behavior and attraction to hosts; and conducting statewide tick and tick-borne pathogen surveillance, diagnostics, and developing novel tick surveillance and control strategies, and providing outreach and education to the public on tick-bite prevention.

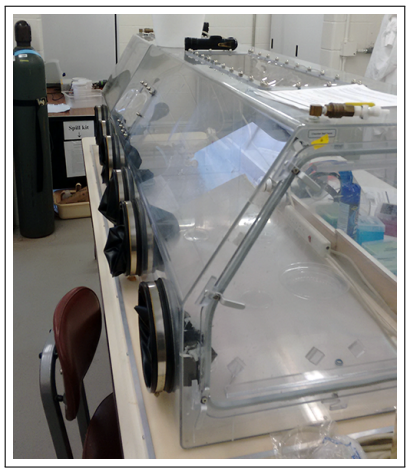

Figure 4: The Medical Entomology Lab includes Biological Safety Level 2 and Arthropod Containment Level 2 spaces where studies with infected vectors can be performed. Handling of infected vectors occurs within a glove box (pictured).

Surveillance and diagnostics capacity in the Lab consists of a wide range of field-collection equipment (e.g., traps) and innovation of new surveillance methods, state-of-the-art microscopes to allow for rapid identification/documentation and dissection of specimens, and ultralow freezers for long-term storage of specimens and samples without degradation of (bacterial) DNA or (viral) RNA.

The Lab is Biosafety Level 2 and Arthropod Containment Level 2 certified, which provides the capacity to screen vectors for pathogens, determine hosts by DNA sequencing of bloodmeals of collected vectors, as well as test the capacity of invasive or native vectors to transmit circulating or emerging pathogens. Additionally, the Lab has capacity for high-throughput detection of vector-borne pathogens (e.g., mosquito- and tick-borne viruses or bacteria).

Figure 5: An INHS Medical Entomology Lab member setting up a resting trap to collect blood-fed mosquitoes.

Collaborations of the Program include local public health departments, mosquito abatement districts, the Illinois Department of Public Health, and the Centers for Disease Control and Prevention (CDC). We also work closely with other local, national, and international vector-borne disease research groups and networks. These include collaborative pursuits supported by the National Socio-Environmental Synthesis Center (SESYNC) on modelling optimal mosquito control efforts, and a Critical Conversation, organized by lab members and colleagues from iSEE which brought together stakeholders from industry, governments, NGOs, and academia to discuss how to improve risk assessment and regulation of genetically-modified mosquitoes.

The Medical Entomology Program has a long history of service

to the State of Illinois, for instance by offering a pathogen screening service to aid districts lacking testing capacity for certain pathogens or requiring confirmation of test results. The Program has also long been involved with the Illinois Mosquito and Vector Control Association and its annual meetings, and two lab members currently serve on the Executive Board, to facilitate communication with and uptake of research findings by public health and mosquito abatement districts.

In the past two years, we have provided outreach talks for University of Illinois Extension, at the Kane Co., Macon Co., and Mercer Co. local public health depts., and for the Champaign Co. Forest Preserve District. We provided field training in tick collection methods to representatives from the Illinois Department of Public Health, and Mercer, LaSalle, and Kankakee local health departments, and shared electronic training materials and supplies with various other local health departments, mosquito abatement districts, and other stakeholders. We have also performed tick identifications or confirmations for IDPH and local health departments.

The Medical Entomology Program is situated, through historical associations, research, and current capacity, to fulfill a unique and much-needed role on the investigation and mitigation of a wide range of vector-borne diseases in Illinois. We have developed capacity for the collecting and testing of ticks for a suite of pathogens of public health concern, data which are informing IDPH maps of this emerging area of public health concern. We are also testing mosquitoes, through surveys and collections by the lab and public health partners, for a suite of emerging viruses.

Figure 6: Horseshoe Lake, IL, contains suitable habitat for the main Eastern Equine Encephalitis virus vector, *Culiseta melanura*, and is included in our mosquito and arbovirus surveillance program which started in 2020. Photo by J. Yan.

Overview of Research Outputs

Figure 7: The primary vector of Eastern equine encephalitis virus, *Culiseta melanura*, was found through the INHS-MEL surveillance efforts to have a broader range in IL than previously realized, including being present in the Greater Chicago area. Photo by C. Cora.

Surveillance for invasive mosquitoes and emerging arboviruses

The worldwide range expansion over recent decades of important mosquito vectors, such as the yellow fever mosquito *Aedes aegypti*, the Asian tiger mosquito *Aedes albopictus*, and the Asian rock pool mosquito *Aedes japonicus* poses a serious threat to public health. With their introduction and expansion, these tire-breeding species have altered the epidemiology of vector-borne diseases through their capacity to transmit native and introduced pathogens. Public concerns about these invasive mosquitoes and pathogens transmitted by them have only been intensified by the recent epidemics of Zika virus in Central and South America. Likewise, as temperatures, precipitation, and land use continue changing throughout Illinois, West Nile transmission will change as well. We have performed a number of field studies that inform our understanding of the changing landscape of mosquito-borne disease in Illinois.

A new multiyear program and collaboration between IDPH and the INHS-MEL started in 2020, which focuses on the documentation of mosquito species throughout the state and on surveillance of emerging viruses of public health concern, such as Eastern Equine Encephalitis, Jamestown Canyon virus, and La Crosse encephalitis virus, as well as West Nile virus. This work consists of targeted surveys and testing performed by our lab specialists in each of the IDPH regions in Illinois, and via testing of samples provided by collaborating local health and abatement districts, such as City of Chicago Public Health and the Macon Mosquito Abatement District. Through this work we are updating our knowledge of mosquito species presence and distribution throughout the state, which will provide a critical baseline through which to assess changes in risk of mosquito-borne diseases. A new species we have recorded in parts of southern Illinois through this effort is *Culex nigripalpus*, an important vector of West Nile virus in the Southeastern United States. Whether and how the presence of this species will alter West Nile virus transmission is an area of ongoing research. We also detected the primary vector

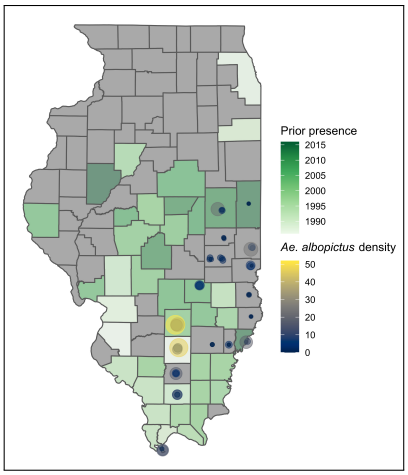

Figure 8: Previous records of *Aedes albopictus* occurrence for counties in Illinois (in green) and detection of this species in additional counties as a result of our surveillance efforts.

of Eastern equine encephalitis virus, *Culiseta melanura* (Figure 7), in three additional counties in Illinois, including for the first time in Cook County (Mackay et al., submitted). Eastern equine encephalitis is a rare, but highly fatal disease, which has been increasing in incidence and range in recent years in the United States and human cases have occurred in neighboring states. By improving our knowledge of where these mosquitoes occur, and testing them for the presence of this concerning arbovirus, our work supports emergency public health preparedness.

In a recent paper, we analyzed the spread of the Asian tiger mosquito over the last 30 years in Illinois and performed genetic analyses on specimens from 10 locations in the state collected through our surveillance efforts with IDPH or by collaborating mosquito abatement and local health departments [6]. Through this and more recent work by our lab we are documenting the geographic spread throughout southern and central Illinois (Figure 8), exploring how this species co-occurs with other mosquito species and its dependence on urbanized areas [7], and we are tracking the genetic lineages present in Illinois, including in the Greater Chicago area, as this species is establishing there (Stone et al., in preparation). This work will aid in understanding where this species is being introduced from and inform mitigation measures. As part of our surveillance program, we have found females of this species infected with La Crosse encephalitis virus in the Peoria region, an area that historically has seen consistent cases of this disease.

Another ongoing area of study focuses on the Asian tiger mosquito in residential settings, to better understand how the increase in abundance in south and central Illinois in recent years affects people's risk of exposure to arboviruses. We performed a study that explores whether there are differences in mosquito population abundance, age structure, and biting behavior between neighborhoods that vary in socioeconomic status (SES). We analyzed the hosts these mosquitoes were biting, by molecular determination of the origins of blood meals found within them, and found that humans were more likely to be bitten in wealthier neighborhoods, while other vertebrates like Eastern cottontail rabbits, were more likely to be fed on in poorer neighborhoods. This suggests that the impacts of socioeconomic status on vector-borne disease risk due to this invasive species will vary for zoonoses such as La Crosse virus and potential transmission of viruses maintained by humans (e.g., chikungunya). The work is currently being analyzed and prepared for publication (Mackay et al., in preparation).

[6] C. M. Stone, Z. Zuo, B. Li, M. Ruiz, J. Swanson, J. Hunt, C.-H. Kim, and R. L. Smith. Spatial, temporal, and genetic invasion dynamics of *Aedes albopictus* (Diptera: Culicidae) in Illinois. *Journal of Medical Entomology*, 67(17):496, 2020

[7] V. Trivellone, Y. Cao, M. Blackshear, C.-H. Kim, and C. Stone. Landscape composition affects elements of metacommunity structure for Culicidae across south-eastern illinois. *Frontiers in Public Health*, page 924, 2022

Figure 9: An INHS Medical Entomology Lab technician performs a landing catch of the Asian tiger mosquito in a backyard in Urbana, IL.

Ecology and control of West Nile virus vectors in Illinois

We performed a large-scale field study along an urban-rural transect spanning Cook, DuPage, and Will Counties to understand how the risk of exposure to West Nile virus depends on the level of green and developed space in an area, and how the blood feeding preferences of mosquitoes shift along such a transect (Mackay et al., in preparation). To look at this, we performed bird counts at three time points through the season, used an established network of camera traps to provide information on the presence of medium- and large-sized mammals at the same locations, and collected mosquitoes with a variety of traps at a weekly basis from May-October. Mosquitoes were identified to species in the lab, and *Culex* species have been tested for West Nile virus using molecular methods. The origin of blood meals in all captured mosquitoes is being determined via sequencing. The data are being analyzed and readied for publication. We also collaborated with the Macon Mosquito Abatement District to determine blood hosts of West Nile vectors collected in their district in 2020, to understand whether there are locations where bites on humans are more likely to occur. This showed that the vast majority of blood meals taken by this species came from a handful of bird species: American robins, house sparrows, northern cardinals, and European starlings. Understanding how mosquito biting favors more or less competent reservoir hosts depending on environmental characteristics could improve our understanding of West Nile virus and our ability to target environments that contribute more strongly to transmission.

Members of our lab were coauthors on a study with the Allan lab (UIUC) that investigated how vegetation management practices (mowing and management of cattails and turfgrass) in surface storm water management structures such as detention basins affects West Nile virus vector populations. Mowing frequency was shown to lead to a greater abundance of mosquitoes, particularly for turf grass. The results also highlighted that *Culex pipiens* and *Culex restuans* responded differently to the type of litter. This work highlights how mosquito ecology and West Nile risk as a result of environmental management differs between species, and suggests ways to improve control of these species in storm water habitats using integrated vector management practices [8].

A recent direction in our lab has focused on the behavior and ecology of *Culex pipiens* during parts of the season where surveillance and control have traditionally not considered them, during Fall, Winter and early Spring. During Fall, females of this species prepare for their overwintering diapause by engaging in nectar gluttony, before

[8] A. J. Mackay, E. J. Muturi, E. M. Moen, M. Holland, and B. F. Allan. Influence of vegetation and vegetation management on *Culex* mosquitoes in surface stormwater habitats. *Wetlands Ecology and Management*, pages 1–16, 2021

overwintering in protected places such as cellars, storm water pipes or culverts, or other structures. Targeting female mosquitoes with sugar bait stations during these periods may offer a previously unexplored option for West Nile virus control. We are performing a number of studies to explore this potential Achilles' heel in more detail. We have performed field studies to understand when this species starts appearing in and using their overwintering habitats, and when in the late summer and Fall their nectar-feeding behavior becomes more pronounced. We have performed a lab study that explored why some *Culex pipiens* females emerge in a prediapause state in late summer, while others don't until later in the autumn, and what the consequences of this could be for their energetic reserves and overwintering success (Wilson, in preparation). Another study has looked at the impact of temperature and variation in the quantity and quality of larval food resources on the likelihood of female mosquitoes entering diapause (Mackay et al., in preparation). Additional research to explore the potential to target these mosquitoes during autumn, and whether this can successfully inhibit West Nile virus transmission the following year, is planned.

Insecticide resistance monitoring

Application of insecticides to reduce the numbers of adult, biting, and potentially infective female mosquitoes can possibly play an important role in reducing the risk of exposure to West Nile virus, and as an emergency measure in the case of an outbreak of a rare or emerging arbovirus. A concern is that over time and with repeat applications, mosquito populations can evolve insecticide resistance, and become less sensitive to the chemicals. Because the development of insecticide resistance in mosquito populations potentially threatens our ability to quickly kill infected mosquitoes, it is critical to know where and how frequently such resistance occurs and what may be causing it. Our lab currently conducts statewide insecticide-resistance monitoring of mosquito populations in Illinois to characterize where and how frequently it occurs.

In collaboration with IDPH, the INHS Medical Entomology Lab has been obtaining baseline data on the insecticide resistance status of Illinois mosquito species since 2017. Through collaborations with local health and mosquito abatement districts, we collect mosquito eggs across the state in locations of interest, rear them to adulthood in temperature- and humidity-controlled incubators in our lab, and test them following CDC guidance on insecticide resistance monitoring, using known insecticide-sensitive mosquito colonies for mortality control comparisons, to allow for direct comparisons between

populations from different regions.

A main takeaway of our surveillance is that there appears to be widespread resistance or tolerance to commonly used permethrin-based insecticides in Illinois *Culex pipiens* populations, while in other species (*Culex restuans*, *Aedes albopictus*), we have detected less evidence of resistance. For *Culex pipiens*, a common vector of West Nile virus, 82% of sampled populations showed signs of resistance to permethrin. Organophosphate insecticides such as malathion are nowadays rarely used in Illinois, but could be in case of an emergency response. Surprisingly, 61% of *Culex pipiens* did show resistance to this insecticide as well. Based on our genetic investigations, we found that 55% of insecticide-resistant *Culex pipiens* specimens sequenced to date contain a point mutation associated with resistance. For *Culex restuans*, another common vector of WNV, 44% of sampled populations tested showed resistance to permethrin. For *Aedes albopictus*, a possible vector for a wide range of invasive and established viruses, including chikungunya and La Crosse encephalitis virus, 28% of sampled populations showing signs of resistance to permethrin.

Related projects that we have worked on in the past two years include an investigation of resistance patterns in relation to pesticide usage with the Wheaton Mosquito Abatement District; an investigation of the role environmental variables play in the expression of insecticide resistance (we found evidence for both genetic and environmental components to the expression of resistance, the latter suggests that what happens in nature may be quite different and more difficult to predict than what we observe under controlled laboratory conditions); and we have measured enzyme levels to obtain insight into the physiological mechanisms that are causing resistance in these mosquitoes, which is an important step to being able to develop an insecticide resistance management plan.

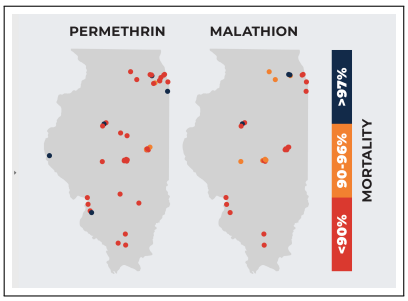

Figure 10: Outcomes from controlled insecticide-resistance CDC bottle bioassays at the INHS Medical Entomology Lab highlight that resistance (less than 90% average mortality, or mosquitoes killed when exposed to insecticide, in a controlled assay) to permethrin (left panel) and malathion (right panel) is prevalent throughout Illinois in mosquito vectors of West Nile virus.

Mathematical modelling to optimize vector control interventions

Mathematical models of vector-borne disease transmission and control can aid in the design of integrated vector management programs by exploring how combinations of interventions, or the spatial arrangement of control interventions, affect disease outcomes. One study we participated in resulted a workshop held at the National Socio-Environmental Synthesis Center (SESYNC) with a variety of experts in mosquito biology and infectious disease modelling. Public health agencies often have to strike a balance between demand for an intervention (for instance as a result of news coverage or concern regarding an ongoing outbreak), which can take the form of a chemical insecticide, and opposition from the public for the use of

such insecticides due to environmental or other health concerns. The results suggest that public risk perception of both the virus and insecticide use can drastically alter the course of an outbreak, pointing to the need for robust public outreach [9]. Future work building on this framework will allow us to make concrete recommendations to decision makers who need to address public concerns about pesticide use while maintaining effective arboviral control.

[9] G. P. Suarez, O. Udiani, B. F. Allan, C. Price, S. J. Ryan, E. Lofgren, A. Coman, C. M. Stone, L. K. Gallos, and N. H. Fefferman. A generic arboviral model framework for exploring trade-offs between vector control and environmental concerns. *Journal of Theoretical Biology*, page 110161, 2020

Effects of changing temperatures and environments on mosquitoes

Temperature and the density of mosquito larvae in aquatic habitats are two factors that change seasonally and are likely to change in the future as summers become increasingly hot and invasive mosquito species become more abundant throughout the state. Understanding how these factors impact virus transmission by mosquitoes in temperate areas such as Illinois is an important step to understanding and mitigating risk of arboviral transmission.

We contributed to an assessment of the impacts of climate change on public health and vector-borne diseases in Illinois for The Nature Conservancy [10]. Members of our lab have also initiated a collaboration with the State Climatologist as well as BRACE-Illinois (UIC) to develop outreach and informational materials related to vector-borne diseases and climate change. Our lab has also joined a national long-term collaboration led by researchers at the Tyson Research Center (Washington University in St. Louis) that will track changes in the first and last appearance of the Asian tiger mosquito and the northern house mosquito in Spring and Fall, to understand whether these mosquitoes are entering and exiting diapause earlier and later, and whether mosquito seasons are becoming longer in Illinois and how this compares to other locations in the United States.

[10] D. J. Wuebbles, J. Angel, K. Petersen, and Z. Zhang. An assessment of the impacts of climate change in Illinois. 2021

In a lab study, we studied the effects of temperature and larval diet on Asian tiger mosquito size, longevity, and their expression levels of genes involved in immunune responses, to develop a better understanding of their ability to transmit pathogens under these varying environmental conditions (Mackay *et al*, in preparation). The work informs our understanding of seasonal and potential future risk due to this invasive species.

Another species of concern is *Aedes aegypti*, the major vector of dengue and Zika – while currently established in the southern US, populations have been becoming established further north in recent years and habitat is predicted to be suitable for this species in parts of Illinois by 2050 [11]. We have been studying how factors such as temperature and nutrition levels affect this species' life history traits such as longevity and fecundity, which strongly influence their

[11] M. U. Kraemer, R. C. Reiner, O. J. Brady, J. P. Messina, M. Gilbert, D. M. Pigott, D. Yi, K. Johnson, L. Earl, L. B. Marczak, et al. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nature microbiology*, 4(5):854–863, 2019

population dynamics [12]. We have also performed extensive sets of experiments to investigate mosquito-virus interactions using dengue virus in our Arthropod Containment Laboratory 2 section of the lab. Through this work we have found that the rate of infection and viral titer in mosquitoes is strongly influenced by both larval and adult nutrition, and we have identified a number of immune genes that are affected by these same environmental factors (Yan *et al*, in preparation). This work helps us understand the types of environmental conditions that support intense dengue transmission, allowing for better targeting of control methods. It also ensures that Illinois has the capacity to perform vector competence studies with arboviruses, and future work is planned to understand the vector competence of native mosquito species for a number of potentially emerging viruses.

[12] J. Yan, R. Kibech, and C. M. Stone. Differential effects of larval and adult nutrition on female survival, fecundity, and size of the yellow fever mosquito, *Aedes aegypti*. *Frontiers in Zoology*, 18(1):1–9, 2021

Mosquito longevity, behavior, and development of novel traps

Mosquito longevity is one of the main drivers of arboviral transmission potential. Mosquito survival depends strongly on their ability to locate and use nectar sources, but little is known of the importance of different plant species in Illinois for mosquito nectar feeding. This is regrettable, because having this knowledge would offer potential for mosquito management via manipulation or management of plants and weeds. In a collaboration with Prof. May Berenbaum (UIUC, Entomology), we have studied the effect of nectar phytochemicals on mosquito life history traits and La Crosse virus competence. Asian tiger mosquitoes feeding on certain phytochemicals had substantially longer lifespans and a transcriptomic analysis of these mosquitoes revealed that these phytochemicals upregulate longevity-related and immune-related pathways [13]. This work sheds light on the genetic underpinnings of longevity of mosquitoes. Future work will explore the potential to exploit these insights for more accurate age-grading methods.

We performed work in collaboration with researchers at Penn State to develop and test the performance of a novel mosquito trap in comparison to mosquito traps traditionally used in West Nile surveillance. The advantage of this novel trap is that it is designed to result in less damage to mosquito specimens, making identifications easier, and that it can be configured to attract either host-seeking or oviposition-site seeking mosquitoes. A statistical analysis highlighted that the trap performed comparably to existing trap types, but attracted certain species of mosquitoes, such as *Culex salinarius*, at a higher rate (Hall *et al*, in preparation).

[13] T. M. Njoroge, B. Calla, M. R. Berenbaum, and C. M. Stone. Specific phytochemicals in floral nectar upregulate genes involved in longevity regulation and xenobiotic metabolism, extending mosquito life span. *Ecology and Evolution*, 11(12):8363–8380, 2021

Mosquito microbiomes

The microbial communities that reside in mosquitoes can impact transmission of mosquito-borne pathogens, for instance by making mosquitoes more or less susceptible to infection, or by altering their life span. An improved understanding of the diversity of mosquito microbiomes, leading to a deliberate alteration of those microbiomes, can lead to the development of novel vector control methods. This burgeoning field is at the forefront of vector biology, though by far most of the work done to date has focused on vectors of global health concern (e.g., malaria and dengue vectors). Several studies performed at the INHS Medical Entomology Lab have begun characterizing the microbiomes of important species in Illinois.

Work published recently on such topics include studies that investigated whether the microbiome composition differs between two important West Nile virus vectors, *Culex pipiens* and *Culex restuans*, and showed that bacterial richness was higher in the former species [14]. In a different study, we explored how different larval habitats, used tires and treeholes, affect the microbiome composition of the vectors *Aedes japonicus* and *Aedes triseriatus*, showing that mosquito species as well as habitat type structure mosquito microbiomes [15]. We further explored how exposure to pesticides in nature can change the microbial composition of mosquitoes' guts [16]. These insights provide important baseline information for future studies on microbial control of mosquitoes.

[14] E. O. Juma, C.-H. Kim, C. Dunlap, B. F. Allan, and C. M. Stone. *Culex pipiens* and *Culex restuans* egg rafts harbor diverse bacterial communities compared to their midgut tissues. *Parasites & Vectors*, 13(1):1–12, 2020

[15] E. O. Juma, B. F. Allan, C.-H. Kim, C. Stone, C. Dunlap, and E. J. Muturi. The larval environment strongly influences the bacterial communities of *Aedes triseriatus* and *Aedes japonicus* (Diptera: Culicidae). *Scientific reports*, 11(1):1–15, 2021

[16] E. O. Juma, B. F. Allan, C.-H. Kim, C. Stone, C. Dunlap, and E. J. Muturi. Effect of life stage and pesticide exposure on the gut microbiota of *Aedes albopictus* and *Culex pipiens* l. *Scientific Reports*, 10(1):1–12, 2020

Risk assessments of genetically-modified mosquitoes

Following a "Critical Conversation on Genetically Modified Mosquitoes", whereby national and international experts in community engagement, regulation, bioethics, and mosquito biology gathered in Chicago in 2019 to discuss issues relevant to the use of GM technologies to combat vector-borne diseases, an opinion piece summarizing the main takeaways from the gathering was published [17]. Members of the INHS Medical Entomology Lab in 2020 participated in a workshop at UC San Diego funded by the DARPA Safe Genes program on the value of a global gene drive project registry. A manuscript detailing the outcomes is being prepared.

[17] B. Allan, C. Stone, H. Tuten, J. Kuzma, and N. Kofler. Genetically modified mosquitoes could be released in Florida and Texas beginning this summer – silver bullet or jumping the gun? *The Conversation*, 2020

Tick and tick-borne pathogen surveillance for Illinois

Until 2019, Illinois lacked a statewide program for active surveillance of ticks and tick-borne pathogens. Since 2019, in collaboration with and funded by the Illinois Department of Public Health, and since

2021 with additional support from the CDC Enhanced Laboratory Capacity program, the Illinois Natural History Survey Medical Entomology Lab has leveraged expertise and technical capacity in the lab to perform year-round statewide surveys for ticks and tick-borne disease agents of public health concern and conduct special investigations following human tick-borne disease cases. The INHS-MEL also provides a diagnostic service for submission of samples and photos of arthropods of public health concern, including ticks.

The collections and testing of ticks follow CDC guidelines, and results are reported to IDPH and CDC. IDPH is publishing our surveillance data in real time on their interactive online maps to provide the public and physicians with the most up-to-date information on a county by county basis on the presence of tick species of public health concern and their prevalence of infection with pathogens causing human illness, such as Lyme borreliosis.

The focus of our Illinois Statewide Tick Surveillance Program is to determine the distribution of the tick species of greatest public health concern in IL, and obtain estimates of the density of infected ticks in different areas in the state. A significant focus has been on the Lyme disease vector *Ixodes scapularis*. This species and its associated pathogens is expanding across Illinois from north to south, with considerable variation in abundance among regions (Figure 11). Our spatial analysis of abundance patterns has linked this variation to several environmental parameters, with significant factors mainly related to forest fragmentation [18]. Adult female blacklegged ticks were tested in our lab for a suite of pathogens (*Borrelia burgdorferi*, *Borrelia miyamotoi*, *Borrelia mayonii*, *Babesia microti*, *Anaplasma phagocytophilum*, *Ehrlichia muris eauclairensis*). In addition to finding high rates of infection with *Borrelia burgdorferi* s.s., the causative agent of Lyme Disease, and documenting expansion of *Anaplasma phagocytophilum* and *Borrelia miyamotoi*, we found the first evidence of the presence of *Babesia microti* in blacklegged ticks in Illinois (Figure 12).

Another area of focus is to improve our understanding of the distribution of the Gulf Coast tick, *Amblyomma maculatum*, which we had previously found to be established in Illinois [19]. In 2020 and 2021 we performed extensive targeted surveillance for this species in southern and central Illinois and found that this species is much more broadly distributed within the state than previously realized. Since May 2020 we have collected over 800 specimens of this species, and are finding an infection rate of ca. 20%)1 in 5 ticks) within them for the agent of Tidewater spotted fever (*Rickettsia parkeri*) (Philips et al., in preparation). We also collaborated with the Allan lab (UIUC) on a habitat suitability model of this species which made use of our data, and our model predicts a broader range of suitable habitat

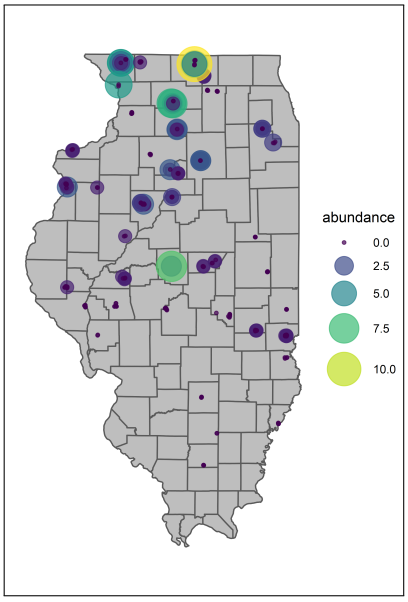

Figure 11: Collection sites and abundance of adult female blacklegged ticks collected during Fall 2019 and 2020 per 150m transect, highlighting variation in abundance throughout the state. Other collections have focused on adult and nymphal blacklegged ticks during Spring, as well as the vectors of Spotted Fever Group Rickettsioses and other emerging pathogens.

[18] E. Cimo-Dean. Environmental factors affecting the range expansion of *Ixodes scapularis*, the blacklegged tick, (Acari: Ixodidae) in Illinois. Master's thesis, 2021

[19] V. C. Phillips, E. A. Zieman, C.-H. Kim, C. M. Stone, H. C. Tuten, and F. A. Jiménez. Documentation of the expansion of the gulf coast tick (*Amblyomma maculatum*) and *Rickettsia parkeri*: first report in Illinois. *Journal of Parasitology*, 106(1):9–13, 2020

under current climate conditions, including around the shores of the Great Lakes. One of the main drivers of suitable habitat was found to be the minimum temperature in the coldest month, suggesting that with further climate change, this species' range should continue to increase northwards [20].

We are also leveraging collections made through our surveillance program to enrich collaborations with other researchers. These include national studies on population genetics of blacklegged ticks and Gulf Coast ticks in which Illinois specimens have been included, with collaborators at the University of Georgia and Old Dominion University. We also collaborated on a study investigating how interactions between tick microbiota may be involved in the development of Alpha-Gal Syndrome in people following long star tick bites [21].

Through our public tick submission program we documented the first record of the primary vector of Powassan virus, *Ixodes cookei*, biting a human and the first occurrence record of the Gulf Coast tick in northern DuPage County.

In addition to training participating local health and abatement districts, we collaborate with a variety of researchers in the state and make training and informational materials available to lower the bar for public health professionals and other researchers to become involved in tick collections. We have recently completed a checklist of ticks that have been reported in Illinois, as well as host species they have been associated with, using data from a variety of sources including natural history collections. A key to the nymphal ticks of Illinois has been created, as a traditional dichotomous key, a pictorial key, and an online matrix-based key (Struckhoff et al., in preparation). These resources, based on research for which our student won several awards, will soon be published and aid greatly in establishing broad interdisciplinary capacity for tick surveillance and research in the state.

[20] J. M. Flenniken. Environmental drivers of gulf coast tick range expansion in the United States. Master's thesis, 2021

[21] D. Kumar, S. R. Sharma, A. Adegoke, A. Kennedy, H. C. Tuten, A. Y. Li, and S. Karim. Recently evolved *Francisella*-like endosymbiont outcompetes an ancient and evolutionarily associated *Coxiella*-like endosymbiont in the lone star tick (*Amblyomma americanum*) linked to the alpha-gal syndrome. *Frontiers in Cellular and Infection Microbiology*, page 425, 2022

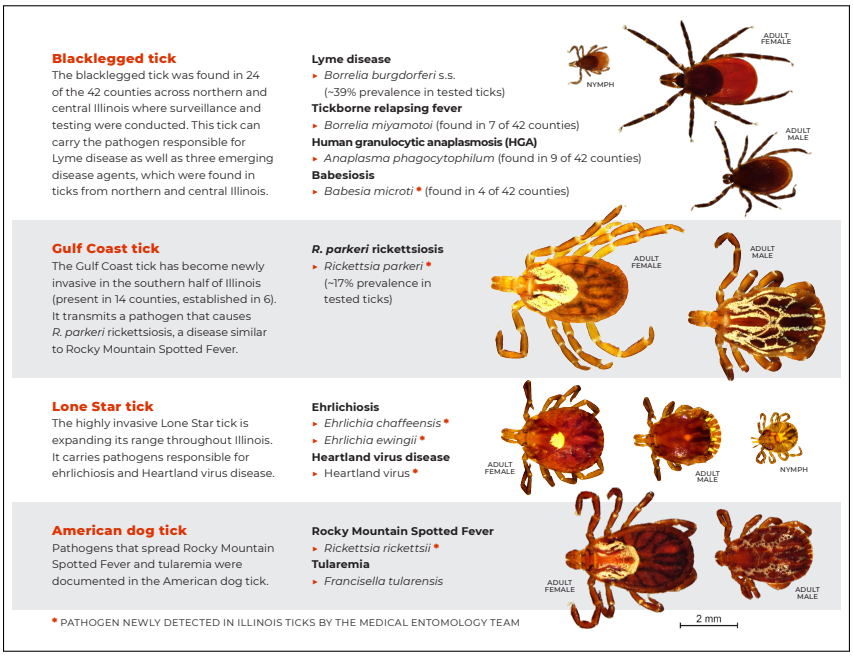

Figure 12: An overview of the main tick species of human health concern that are tracked as part of our ongoing tick surveillance program with IDPH, and information on pathogens found in them (per 2021). Images by E. Struckhoff.

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americanum) linked to the alpha-gal syndrome. *Frontiers in Cellular and Infection Microbiology*, page 425, 2022.

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