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Highlights in Medical Entomology, 2019: Familiar Foes and New Frontiers

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Abstract

The 2019 Entomological Society of America annual meeting was held in St. Louis, Missouri, just blocks away from the iconic Gateway Arch. Representing a 'gateway to the West', this inspired the theme of the Highlights in Medical Entomology to reflect on the accomplishments of the past year as we move into a 'new frontier' of vector biology research. Papers were selected broadly across arthropods that influence public health, focusing on topics ranging from West Nile virus transmission, ticks and tick-borne disease, to advances in genetics and 'big data' studies. This included current perspectives on West Nile virus ecology and epidemiology, which has now been endemic in the United States for 20 yr. Additional topics such as the advantages of citizen science and the importance of scientific communication were also discussed. Together, these papers demonstrate the achievements of the vector community while emphasizing the challenges that we collectively face to reduce the burden of vector-borne disease.

Key words: medical entomology, vector-borne pathogen

Medical entomology is a diverse field defined as the study of insects and arachnids (collectively referred to as arthropods) that influence human health. This includes mosquitoes (Diptera: Culidae), ticks (Ixodida: Ixodidae), or other blood-feeding arthropods and the respective pathogens that they transmit. To focus the 2019 Highlights in Medical Entomology from the highly variable interests that shape the vector biology field, a theme of 'Familiar Foes and New Frontiers: The Path Forward in Vector-borne Disease Research' was derived from the iconic Gateway Arch in the host city of St. Louis. Relevant papers were selected from July 2018 to November 2019 across this theme to celebrate the accomplishments and current challenges in vector-borne disease research.

Twenty Years of West Nile Virus in the United States (1999–2019)

2019 marked 20 yr since the initial detection of West Nile virus in North America (Hadfield et al. 2019, Kramer et al. 2019). At the passage of this grim milestone, endemic transmission of the virus has resulted in over 48,000 cases, approximately 2,300 deaths, and an estimated 7 million total human infections over the last two decades (Center for Disease Control and Prevention 2019, Ronca et al. 2019). However, with each passing year, we understand more about West Nile virus transmission dynamics through the collection of stronger entomological, epidemiological, and climate-based data that enable more robust analysis of the factors that influence West Nile virus transmission.

Several papers published in 2019 that utilized long-term data sets to gain a more thorough understanding of regional West Nile virus transmission patterns. A 15-yr study (2002–2016) of West Nile virus in Iowa examined the temporal and spatial distribution of human cases, sentinel chicken seroprevalence, and mosquito abundance/infection data, defining seasonal patterns of infection and a distinct gradient in West Nile virus cases across the state (Dunphy et al. 2019). Human West Nile virus incidence strongly correlated to *Culex tarsalis* Coquillett abundance, that when combined with higher mosquito infection rates and prevalence of human-derived blood meals, implicate *Cx. tarsalis* as the primary West Nile virus vector in the state (Dunphy et al. 2019). Additional studies also have capitalized on long-term surveillance data sets to better understand how climate conditions drive West Nile virus transmission. The first focused on 15 yr of mosquito surveillance and infection data from Harris County, Texas to determine whether temperature and rainfall could have predictive functions to infer mosquito abundance and mosquito infection rates (Poh et al. 2019). Although predictive modeling did not accurately estimate

mosquito abundance, an infection rate model relying on winter temperatures performed well, providing a valuable resource for public health officials to proactively monitor and initiate interventions to reduce potential disease risks (Poh et al. 2019). A second study based in New York and Connecticut similarly examined the effects of climate on mosquito infection rates and human West Nile virus cases to improve predictive models (Keyel et al. 2019). By examining a large number of climate, host, and environmental variables, the authors identified that summer temperatures and soil moisture had the greatest predictive function, able to account for 45 and 72% of the respective variation in mosquito infection rates and human cases (Keyel et al. 2019).

With the 20-yr anniversary of West Nile virus, a special collection of articles was published in the *Journal of Medical Entomology*, highlighting our current understanding of West Nile virus epidemiology and the outstanding questions that remain for this endemic virus. The special collection included an overview of how West Nile virus spread and established a cycle of endemic virus transmission in North America (Kramer et al. 2019) and a summation of the clinical outcomes and impacts on public health infrastructure since its introduction (Petersen 2019). Also included was an overview of West Nile virus mosquito vectors present in North America, highlighting the important differences in vector competence and ecology that shape regional patterns of virus transmission (Rochlin et al. 2019). To date, a total of 65 different mosquito species have tested positive for West Nile virus, yet *Culex spp.* comprise more than 97% of West Nile virus-positive mosquito pools, demonstrating their integral role as primary vectors (Rochlin et al. 2019). Surveillance data demonstrated that regional differences in the distributions of *Culex* vector species and their respective host preferences largely shape regional transmission dynamics (Rochlin et al. 2019). These local differences have created a major challenge in the use of forecasting/prediction models to estimate West Nile virus intensity prior to detection, where models developed from meteorological and land-use variables are often unique to a specified region and are limited by spatial scales that typically do not extend beyond the county or state level (Barker 2019). While prediction models have been implemented in California and South Dakota to serve as early-warning systems to aid public health decisions, the majority of forecasting efforts have not been applied by public health agencies, providing a significant challenge to improve the utility of these models to guide interventions (Barker 2019).

In addition, recent publications have summarized how West Nile virus has evolved since its initial introduction, where the original strain (NY99) has been displaced by two distinct virus lineages, WN02 and SW03 (Duggal et al. 2019, Hadfield et al. 2019). This evolution of West Nile virus has been driven by its interactions with its avian and mosquito hosts that likely have caused purifying selection of the virus (Duggal et al. 2019), and is supported by infection studies that demonstrate that WN02 is more efficiently transmitted by *Culex* mosquitoes and reaches higher viremias in passerine birds (Duggal et al. 2019, Hadfield et al. 2019). Through viral sequences isolated from mosquito pools or bird samples collected from West Nile virus surveillance, the spread of these West Nile virus genotypes can be visualized using the online interface Nextstrain (Hadfield et al. 2019), providing an increased understanding of how West Nile virus has adapted to the American continent. From these data, it appeared that West Nile virus is now maintained at local or regional levels after a period of initial rapid spread, suggesting that unique selective pressures influence the stability and emergence of new West Nile virus strains (Hadfield et al. 2019). These approaches in genomic epidemiology enable the possibility to follow West Nile virus strains over time and space to better understand how virus spread contributes to localized or regional outbreaks, thus having the potential to inform and maximize vector control efforts to limit human disease cases.

Ticks and Tick-Borne Disease

2019 also marked the first recorded human bite in the United States by the invasive Asian Longhorn tick, *Haemaphysalis longicornis* Neumann (Wormser et al. 2019). This known vector of human and animal pathogens was recently detected in the United States in 2017 and has since established itself in 13 states. A recent report has dispelled the concern that the Asian Longhorn tick could potentially transmit the Lyme disease spirochete in the United States through vector competence experiments with *Borrelia burgdorferi* (Breuner et al. 2020, online in October 2019). Although *B. burgdorferi* was initially detected in larvae by polymerase chain reaction (PCR) 1 d after feeding on an infected mouse, bacteria were not detected in nymphs at 1–2 wk or 5–6 wk after molting (Breuner et al. 2020, online in October 2019). This provides strong evidence that *B. burgdorferi* cannot be passed transtadially to the nymphal stage, therefore indicating that *H. longicornis* is unlikely to contribute to the transmission of Lyme disease in the United States (Breuner et al. 2020, online in October 2019).

A recent study of infected *Ixodes scapularis* Say in New York, NY challenged many of our preconceived notions of Lyme disease ecology by demonstrating that areas at high risk for Lyme disease transmission can be found in urban environments (VanAcker et al. 2019). *Ixodes scapularis* were detected in 17 of 24 parks surveyed in New York City, with 9 of 10 parks examined on Staten Island displaying established tick populations and a prevalence of *B. burgdorferi* infection as high as 40% (VanAcker et al. 2019). This indicates that urban green spaces can serve as viable locations for Lyme disease transmission, an important implication since ~80% of people in North America reside in urban centers (VanAcker et al. 2019).

New data also have provided insight into the transmission of another tick-borne disease, Rocky Mountain Spotted Fever, caused by the bacteria *Rickettsia rickettsia*. In experiments with infected American Dog ticks, *Dermacentor variabilis* Say, infectious *Rickettsia* were transmitted to guinea pigs almost immediately after tick attachment (Levin et al. 2020, online in November 2019). While attachment of less than 30 min was able to initiate host infection resulting in mild symptoms, even attachments of less than 8 h produced clinical outcomes (Levin et al. 2020, online in November 2019). These results reiterate the importance of the prompt removal of attached ticks to prevent the transmission of tick-borne pathogens.

Innovative Approaches to Study Vector-Borne Disease

Due to the challenges in the study of vector-borne disease, oftentimes, 'outside-the-box' thinking may lead to exciting new advances to better understand and potentially disrupt vector-borne disease transmission. In my opinion, two studies exemplified this creativity and the use of innovative new approaches. The first, repurposed a commonly prescribed anti-malarial drug, atovaquone, to block malaria parasite transmission (Paton et al. 2019). Either prior to an infectious bloodmeal or shortly thereafter, surface contact with atovaquone at multiple concentrations prevented *Plasmodium*

falciparum development in *Anopheles gambiae* Giles (Paton et al. 2019). These data support the integration of atovaquone into the production of bed nets and indoor residual spraying to further increase their effectiveness in blocking malaria transmission (Paton et al. 2019).

The second study, performed pilot experiments using radio telemetry to examine the movements of kissing bugs, *Triatoma* spp. (Hemiptera: Reduviidae) (Hamer et al. 2018). Using small transmitters (0.2 g) mounted to either *Triatoma gerstaeckeri* (Stål) or *Triatoma sanguisuga* (Leconte), 11 individual kissing bugs were tracked for an average of 5 d (Hamer et al. 2018). Finding multiple cryptic resting sites, this pilot study demonstrated the ability of radio telemetry to study fine-scale insect movements that can be used to better understand insect behavior and inform vector control practices (Hamer et al. 2018).

Advances in Vector Genetics

The last year brought significant advancements in vector genetics, with the publication of new and improved genomes of important vectors of human disease. This includes the first comparative analysis of Tsetse fly (Diptera: Glossinidae) genomes, where six species were sequenced across each of the three sub-genera (*Morsitans*, *Palpalis*, and *Fusca*) that serve as vectors of human African trypanosomiasis (Attardo et al. 2019). Comparative analysis to other Diptera defined both similarities and differences, with conservation of the X chromosome and the enrichment of specific genes unique to *Glossina* spp. in addition to those involved in lactation (Attardo et al. 2019). Of interest, significant variation in the photoreceptors that distinguish color could account for differences in Tsetse fly attraction to different trap types and methods used for Tsetse vector control (Attardo et al. 2019).

Matthews et al. (2018) brought significant improvements to the *Aedes aegypti* (L.) genome (Matthews et al. 2018), anchoring contigs to the three *Ae. aegypti* chromosomes and overcoming many of the challenges of its highly repetitive genome that had plagued the previous assembly (Nene et al. 2007). Through advances in sequencing technology, the authors were able to reduce the number of contigs by 93%, generating an assembly supported by physical and cytological maps with a complete re-annotation (Matthews et al. 2018), greatly enhancing the utility of this reference genome for the scientific community studying mosquitoes.

Two recent papers have demonstrated the utility of the CRISPR-Cas9 system in *Culex quinquefasciatus* Say by creating knockouts in eye pigmentation genes to produce a heritable 'white-eye' phenotype (Anderson et al. 2019, Li et al. 2020, online in November 2019). These proof-of-principle experiments demonstrated the application of gene-editing technologies in an important yet often neglected mosquito vector (Anderson et al. 2019, Li et al. 2020, online in November 2019). Importantly, these studies overcome difficulties in *Culex* microinjection that have previously limited *Culex* genetic modification to a single study (Allen et al. 2001), therefore representing a significant advancement for *Culex* genetics.

Further applications of CRISPR-Cas9 technologies in mosquitoes have featured quite prominently over the last year, with improvements in genetic modification (Chaverra-Rodriguez et al. 2018) and engineered resistance to Zika virus (Buchman et al. 2019). A recent paper targeting an olfactory receptor in *Ae. aegypti* may have the most potential to prevent mosquito bites. By targeting an olfactory receptor, Ir8a, via CRISPR-Cas9 involved in recognition of human lactic acid, mosquito attraction to humans was significantly impaired (Raji et al. 2019). Although the *Ir8a* mutation did not completely prevent human recognition, these experiments provide support for approaches to genetically alter olfactory components to reduce human biting and mosquito-borne disease transmission.

Genetic Epidemiology

New advances in genetic sequencing have enabled the rapid emergence of the field of genomic epidemiology. Through the use of virus sequence and phylogenetics, virus outbreaks can be monitored historically or in 'real-time' to aid public health decisions for reducing disease spread or the identification of the origin of introduction (Grubaugh et al. 2019a, Pollett et al. 2020). This approach to epidemiology has been instrumental in identifying a hidden Zika virus (ZIKV) outbreak in Cuba in 2017 that had previously been undetected (Grubaugh et al. 2019b). By piecing data from travel-associated cases of Zika, the authors identified transmission on the island of Cuba well after outbreaks in the rest of the Caribbean, tracing this undetected transmission to at least three independent introductions of ZIKV (Grubaugh et al. 2019b). Similar approaches have informed the spread of West Nile virus in the Americas using existing viral sequences (Hadfield et al. 2019), further demonstrating the application of these genetic approaches to the epidemiological monitoring of mosquito-borne viral disease.

Citizen Science in Medical Entomology

In an increasingly connected world aided by the accessibility of information from the internet and social media, the application of citizen science is beginning to play a larger role in the collection of arthropod vector samples and public health surveillance. This was exemplified by the engagement of a Maryland community to deploy a network of Gravid *Aedes* traps to reduce populations of *Aedes albopictus* (Skuse) (Johnson et al. 2018). Through individual 'buy-in' and ownership by the community, the program was able to achieve high trap coverage for sustained periods throughout the mosquito season that significantly reduced *Ae. albopictus* abundance, demonstrating that citizen science can achieve long-term and sustainable methods of mosquito control (Johnson et al. 2018).

Similarly, the engagement of citizen science also led to the acquisition of human behavioral data associated with tick exposure (Fernandez et al. 2019). Through the use of a smartphone application, The Tick App (https://thetickapp.org/) enrolled almost 1,500 users to collect information from May to September of 2018 regarding location, pet ownership, and the engagement in outdoor activities (Fernandez et al. 2019). This initial survey provided tick identification services and a diary function for users to record their daily functions that led to tick encounters (Fernandez et al. 2019). Through this interface, the analysis of these human activities could better inform public health interventions to reduce the risk of tick-borne disease.

Future Challenges

The study of medical entomology and vector-borne disease is a continually changing field, influenced by the ongoing movements of arthropod vectors and their respective pathogens in an increasingly globalized society. The current speed of shipping and air travel have led to continual threats of introduction and spread of vector species and the potential for disease transmission. Unfortunately, most of our surveillance activities are performed in isolation at the county- or state-level, yet arthropod vectors do not respect these traditional

boundaries. This creates a major challenge for the control of vector-borne disease, raising the importance of open data sharing and collaborations within the vector community.

This is evident by mosquito abundance data collected in the United States, where greater than 1,000 mosquito control agencies perform surveillance, yet mosquito abundance data is largely unavailable (Rund et al. 2019). At present, only a handful of agencies publicly share data, creating the need for a national open-access data repository of mosquito surveillance data (Rund et al. 2019). Through this type of data sharing, mosquito control and public health agencies could maximize mosquito control efforts, as well as provide the necessary infrastructure to assess regional disease transmission risk (Rund et al. 2019). Moreover, these data-sharing opportunities easily can extend and similarly benefit other arthropod vectors of human disease.

An additional challenge for the vector community is the accurate and proper communication of scientific findings to the general public. The influence of social media in our world of today is critical to any vector control program, where clear messaging is essential for the general reader. As a case in point, the recent article by Evans et al. (2019) examined the genetics of *Ae. aegypti* before and after the release of a transgenic suppressing strain of *Ae. aegypti* in Brazil. Providing evidence that the mating suppression was incomplete, the accompanying introgression of DNA into the natural population was instead misconstrued by the general public as the spread of a transgene into the natural population through poor word choice and a lack of clarity. This is of increasing importance where genetic technologies and their respective applications become more feasible scientifically, and the success of their deployment is increasingly dependent on public sentiment.

Summary

In summary, the Highlights of Medical Entomology for 2019 capture the exciting advances over the previous year in our understanding of arthropod biology and vector-borne disease transmission. In many ways, this body of work represents a new frontier as we collectively usher in many novel techniques, the use of genetic information, and incorporating citizen science to combat both new and familiar foes that contribute to vector-borne disease. As we move to confront future challenges, transparent scientific communication is critical to engage the public and stakeholders alike, to ensure success in reducing vector-borne disease transmission.

Acknowledgments

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