

Methods for the Study of Ticks, Mosquitoes, and their Transmitted Pathogens: Toward a Greater Understanding of Vector Biology and Arthropod-Microbe Interactions

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Editorial

Vector-borne diseases (VBDs) are infections transmitted to humans and other animals by the bite of an infected arthropod vector¹. Vectors account for 20% of global tropical infectious diseases and place 80% of the world's human population at risk of VBDs². Mosquitoes and ticks, which are obligate blood-sucking arthropods, are responsible for significant increases in public health and economic burdens worldwide due to their direct feeding behaviors and transmission of a wide range of pathogens (parasites, bacteria, or viruses)³. Thus, this methods collection aims to introduce a broad range of essential and complementary approaches for studying these vectors through a variety of techniques based on the comprehensive knowledge of mosquito and tick biology. Studies on the fundamental aspects of mosquitoes and ticks (*in vitro* and *in vivo*), the microorganisms borne and transmitted by them (interactions among vectors, symbionts, pathogens, and vertebrate hosts), and vector control approaches are included in this Methods Collection.

The survival of arthropod vectors and their ability to maintain and transmit pathogens are closely linked to temperature⁴. Haziqah-Rashid et al. present a simple and low-cost method for determining the temperature preferences of mosquitoes⁵. Their apparatus consists of linked chambers with different temperatures and allows mosquitoes (and other flying insects) to fly freely to their preferred environment, thus enabling the determination of optimum insect temperatures. This will be useful for determining the effects of a variety of manipulations, such as pathogen infection, diet, or endosymbiont presence, on insects' temperature preferences, as well as for defining the parameters for use in modeling studies assessing the climatic suitability of regions for the establishment of disease vectors.

One invasive mosquito that is predicted to benefit from a warming climate and has spread around the world *via* international trade is the Asian tiger mosquito, *Aedes albopictus*⁶. As a vector of multiple viruses, this species represents a growing threat to human health, and novel control methods will be important in the fight against this

mosquito. In their article, Liang et al. describe the preparation of a novel plant-derived insecticide that shows high efficacy against *A. albopictus* larvae whilst showing no harmful effects on fish, which suggests low environmental toxicity⁷. Although only in the early stages of investigation, this botanical insecticide is a promising mosquito control candidate and may prove to be effective against other important mosquito species.

The maternally transmitted endosymbiont *Wolbachia* has shown promise for controlling the dengue virus in field populations of mosquitoes, thus demonstrating potential for deployment for dengue suppression^{8,9}. However, the mechanisms giving rise to this phenomenon are poorly characterized, and a greater understanding of the mosquito–endosymbiont–virus interactions is, therefore, required. To provide tools for the study of these interactions, Chen et al. describe a set of complementary nucleic acid-based and antibody-based methods for the detection and quantification of *Wolbachia* in mosquito cells that could also be applied to a variety of *Wolbachia*-harboring insects¹⁰.

The surveillance of mosquito-borne viruses is important for detecting circulating viruses that may pose potential threats to health, and this surveillance may also lead to the discovery of novel pathogenic or insect-specific viruses. Huang et al. report a cell culture-based method for the isolation and amplification of mosquito-associated viruses from field-collected mosquito samples¹¹, which can be followed by virus purification, identification, and characterization.

The success of an arthropod-borne pathogen in invading, colonizing, and being transmitted by a vector depends on its ability to overcome various physiological barriers, including

those in the salivary glands and midgut. Wang et al. present a protocol for assessing the vector competence of mosquitoes for viruses using two different infection methods¹². By comparing the dissemination and transmission of the dengue virus in *Aedes aegypti* after either oral or intrathoracic infection, this protocol highlights the impact that the salivary gland and midgut barriers play in reducing virus transmission within the mosquito and, therefore, determining vector competence.

The study of vectors such as ticks in the laboratory can be challenging because of their lengthy life cycle and requirement for feeding on multiple different host animals. The protocol by Khoo et al. details the setup of an artificial membrane feeder that can be used for short-term tick rearing in the lab without the use of animals and can be employed to study the effect of various controlled treatments on tick biology, such as pathogen infection and antibiotic ingestion¹³. Whilst this method is described for the feeding of all stages of *Ixodes scapularis*, the method can also be adapted to other tick species and applications.

Tick saliva contains many effectors that play important roles in host immune modulation and enhancing pathogen transmission during tick feeding. MicroRNAs, short non-coding sequences that regulate gene expression both at the tick–host interface and within the tick, are one such effector about which little is known. A method for isolating microRNAs from tick salivary gland cultures and extracellular vesicles (EVs) is presented by Leal-Galvan et al.¹⁴. By culturing *ex vivo* salivary glands, the authors were able to considerably reduce the number of ticks required for the production and isolation of EVs and were able to extract high-quality microRNAs sufficient for next-generation sequencing.

This method facilitates the isolation and study of tick EVs and should advance our understanding of these organelles in tick–host interactions.

Functional studies of pathogen genes are key to understanding their strategies for surviving and adapting to the contrasting host and vector environments. A major advancement for the study of intracellular *Rickettsia* was the development of plasmid shuttle vectors for the expression of genes of interest¹⁵. Wang et al. describe a protocol for the electroporation of purified *Rickettsia parkeri* for transformation with a shuttle vector, followed by the selection and visualization of the transformants in tick cells¹⁶. This transformation method can be adapted to introduce shuttle vectors or transposase mutagenesis systems into other *Rickettsia* species to improve the understanding of rickettsia–host–vector interactions.

The development of novel and effective tools to prevent VBD transmission depends on a solid knowledge of vector biology and the vector–microbe interface. The articles in this methods collection detail a range of laboratory techniques for studying ticks, mosquitoes, and the pathogens and symbionts they host, thus enabling multiple avenues of basic and applied research into these topics. Advances in vector research will facilitate the design of innovative solutions to control the spread of VBDs.

Disclosures

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