

Characterize the ability of *H. longicornis* to acquire and transmit *R. amblyommatis*

Ticks are blood-feeding ectoparasites capable of transmitting multiple human pathogens¹. Environmental changes have supported the expansion of ticks into new geographical areas that have become the epicenters of tick-borne diseases (TBDs)^{2,3}. *Haemaphysalis longicornis* (Asian longhorned tick) is native to East Asia and associated with diverse pathogens (e.g., *Anaplasma*, *Rickettsia*, *Ehrlichia*, *Borrelia*, *Babesia*, *Theileria*, and hemorrhagic fever viruses)⁴⁻⁶. Within the last decade, *H. longicornis* has rapidly invaded several parts of the world, including the Eastern United States⁷. The invasion of *H. longicornis* raises public health concerns as they can co-feed on shared vertebrate hosts (e.g., white-tailed deer), exchange tick-borne pathogens with other tick species established in the local environment (e.g., *Amblyomma americanum* and *Ixodes scapularis*), and contribute to the increasing number of TBDs⁸⁻¹¹.

Our recent longitudinal tick survey documented that while three established tick species (*D. variabilis*, *I. scapularis*, and *A. americanum*) continue to persist, *H. longicornis* has successfully invaded and is in the process of rapid expansion in Long Island, an endemic area of TBDs¹¹. Importantly, our work captured rapidly changing tick communities at this early stage of *H. longicornis* expansion and established a baseline for future tick surveys, providing a unique opportunity to study the presence of tick-borne pathogens in invading *H. longicornis*, alterations in tick populations in this insular environment, and their contributions to TBD transmission cycle. Prior work documented that the invading *H. longicornis* ticks in the United States preferentially feed on large or medium-sized animal hosts, such as white-tailed deer^{12,13}. The same animal species are also abundant in Long Island and have played a significant role in the invasion and expansion of native ticks. Thus, we hypothesize that these animal hosts have supported the rapid invasion and expansion of *H. longicornis*. Notably, we and others reported co-localization of *H. longicornis* and *A. americanum*, both spatially and vertically, implicating that these two tick species may share animal hosts and exchange pathogens during blood-feeding¹¹⁻¹³.

Rickettsia amblyommatis belongs to the spotted fever group (SFG) and infects *A. americanum*^{3,14}. Among the members of SFG *Rickettsia*, *R. rickettsii* causes Rocky Mountain spotted fever (RMSF), the most lethal TBD within the United States¹⁵. Cases of RMSF have been reported in the United States for over a century, transmitted by *D. variabilis*¹⁶. However, isolation of *R. rickettsii* from ticks has decreased, and recent studies suggest that novel species, such as *R. parkeri* and *R. amblyommatis*, are responsible for the increased number of cases of RMSF-like illnesses in both children and adults in the United States¹⁷⁻²³. In this application, we hypothesize that *H. longicornis* can acquire *R. amblyommatis* from the shared animal hosts and serve as a vector for *R. amblyommatis*. Thus, we propose to perform PCR to determine the presence of *R. amblyommatis* in *A. americanum* and *H. longicornis* and characterize *R. amblyommatis* infection in *A. americanum* and *H. longicornis* through the following Specific Aims:

Aim 1. Molecular detection of *R. amblyommatis* in *A. americanum* and *H. longicornis*. We will detect the presence of *R. amblyommatis* in *A. americanum* and *H. longicornis* by PCR to determine the pathogen spillover rates.

Aim 2. Characterization of *R. amblyommatis* infection in *A. americanum* and *H. longicornis*. We will infect *H. longicornis* with *R. amblyommatis* using the artificial tick-feeding system and characterize *R. amblyommatis* infections in various organ tissues in ticks.

The proposed work will provide significant insights into understanding the presence and potential exchanges of tick-borne pathogens between *A. americanum* and *H. longicornis* and examine whether *H. longicornis* can serve as a vector and reservoir for *R. amblyommatis*, representing a significant advance in understanding the role of the invasive *H. longicornis* ticks in exchanging tick-borne pathogens with native ticks and transmitting TBDs.