ABSTRACT
Ticks are the most important arthropod vectors capable of transmitting a diverse array of pathogens to humans. Environmental changes and reforestation have supported the expansion of ticks into new geographical areas that have become the epicenters of tick-borne diseases. Historically, the geographic range of *Amblyomma americanum* (Lone Star tick) was restricted to the southeastern United States. However, recent tick surveys identified the progressive northward expansion of the Lone Star tick, seemingly displacing native *Ixodes scapularis* (deer tick) and *Dermacentor variabilis* (American dog tick) populations in the northern United States. During the last few years, the Lone Star tick has become the most abundant tick species found on Long Island, New York. Sequencing analysis identified the polymicrobial nature of human pathogens that this tick transmits. These include *Borrelia lonestari* (southern tick-associated rash illness), *Ehrlichia chaffeensis* (human monocytic ehrlichiosis), *E. ewingii* (ehrlichiosis), and *Rickettsia amblyommatis* (rickettsiosis). In addition, the Lone Star tick has been implicated in a life-threatening allergic reaction to α-Gal, also known as meat allergy. In Suffolk County, cases of ehrlichiosis and Rocky Mountain Spotted Fever (RMSF)-like diseases have increased, particularly in children. We believe that these reports may be associated with *R. amblyommatis*.

R. amblyommatis belongs to the Spotted Fever Group (SFG) of *Rickettsia* that presents an obligate intracellular lifecycle. Among the members of SFG *Rickettsia*, *R. rickettsii* causes RMSF, the most lethal tick-borne disease within the United States. However, over the last decades, isolation of *R. rickettsii* from ticks have decreased, and recent serological and epidemiological studies suggest novel species of SFG *Rickettsia*, such as *R. parkeri* and *R. amblyommatis*, to be responsible for the increased number of cases of RMSF-like illnesses within the United States. While *R. parkeri* has been isolated from patients in southern states, there exists a significant gap in knowledge on the role of *R. amblyommatis* in *A. americanum* and its pathogenic potential to cause rickettsioses in humans.

The hallmark of rickettsial diseases is the invasion of *Rickettsia* into vascular endothelial cells, followed by intracellular replication, vascular pathologies, and bacteremia for transmission of *Rickettsia* into uninfected vectors. Progressive endothelial cell injury leads to the generation of characteristic erythematous rash, vasculitis, cutaneous necrosis, and life-threatening symptoms, including sepsis, making *Rickettsia* one of the most deadly pathogens. Recent analyses of rickettsial genomes and advances in genetic studies of *Rickettsia* provided insights into the biology of *Rickettsia* with the identification of conserved and unique virulence genes involved in the rickettsial lifecycle. However, limited studies are available to address the molecular basis for how *Rickettsia* co-opt and manipulate host genes at each step of the infectious lifecycle.

Previous work suggested that *R. amblyommatis* is non-pathogenic as two strains, WB-8-2 and North Texas, failed to cause clinical diseases in guinea pigs. In contrast, another *R. amblyommatis* strain 9-CC-3-1 caused vascular inflammation in guinea pigs. This is not an usual finding among the *Rickettsia* as strains of *R. rickettsii* displayed drastically different virulence in animal infection models. From our studies here, we describe that *R. amblyommatis* strain GAT-30V (CDC) from *A. americanum* replicates within the cytoplasm of microvascular endothelial cells with delayed cytopathogenesis and causes TLR4-dependent pathogenesis in mice, supporting clinical evidence that *R. amblyommatis* may cause disease manifestation in some patients. Having demonstrated our ability to work with *R. amblyommatis*, we propose to isolate and characterize *R. amblyommatis* strain SBU from Lone Star ticks (Suffolk County, New York), investigate virulence mechanisms, study host response to *R. amblyommatis* infection, and identify host genes responsible for rickettsial pathogenesis and host immunity. Our work will demonstrate, for the first time, whether *R. amblyommatis* is responsible for the increased number of RMSF-like illnesses in Suffolk County, New York.