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Vector competence of *Amblyomma americanum* (Acari: Ixodidae) for *Rickettsia rickettsii*

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Abstract

Rickettsia rickettsii – the etiologic agent of Rocky Mountain spotted fever (RMSF) – is widely spread across the Americas. In the US, *Dermacentor* spp. ticks are identified as primary vectors of *R. rickettsii* and *Rhipicephalus sanguineus* s.l. has been implicated in transmission of this pathogen in several locations in the Southwest. Conversely, ticks of the genus *Amblyomma* are recognized vectors of RMSF in Central and South America, but not in the US. *A. americanum* is one of the most aggressive human-biting ticks in the US, whose geographical range overlaps with that of reported RMSF cases. Despite sporadic findings of *R. rickettsii* DNA in field-collected *A. americanum* and circumstantial association of this species with human RMSF cases, its vector competence for *R. rickettsii* has not been appropriately studied. Therefore, we assessed the ability of *A. americanum* to acquire and transmit two geographically distant isolates of *R. rickettsii*. The Di-6 isolate of *R. rickettsii* used in this study originated in Virginia and the AZ-3 isolate originated in Arizona. Under laboratory conditions, *A. americanum* demonstrated vector competence for both isolates, although the efficiency of acquisition and transovarial transmission was higher for Di-6 than for AZ-3 isolate. Uninfected larvae acquired the pathogen from systemically infected guinea pigs, as well as while feeding side by side with *Rickettsia*-infected ticks on non-rickettsiemic hosts. Once acquired, *R. rickettsii* was successfully maintained through the tick molting process and transmitted to susceptible animals during subsequent feedings. Guinea pigs and dogs infested with infected *A. americanum* developed fever, scrotal edema and dermatitis or macular rash. *R. rickettsii* DNA was identified in animal blood, skin, and internal organs. The prevalence of infection within tick cohorts gradually increased due to side-by-side feeding of infected and uninfected individuals from 33 to 49% in freshly molted nymphs to 71–98% in engorged females. Moreover, *R. rickettsii* was transmitted transovarially by approximately 28% and 14% of females infected with Di-6 and AZ-3 isolates, respectively. Hence, *A. americanum* is capable of acquiring, maintaining and transmitting *R. rickettsii* isolates originating from two different geographical regions of the US, at least under laboratory conditions. Its role in ecology and epidemiology of RMSF in the US deserves further investigation.

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Keywords

Amblyomma americanum; Lone star tick; *Rickettsia rickettsii*; Rocky Mountain spotted fever; Vector competence

1. Introduction

Rickettsia rickettsii is a tick-borne pathogen that causes Rocky Mountain spotted fever (RMSF) and Brazilian spotted fever in North, Central, and South America. In the United States and Canada, the American dog tick (*Dermacentor variabilis*) and the Rocky Mountain wood tick (*Dermacentor andersoni*) are reputed as major vectors of this pathogen (Burgdorfer, 1975) while from Mexico to Argentina various ticks of the genus *Amblyomma* have been implicated in natural maintenance of *R. rickettsii* and transmission to humans. These include *A. aureolatum, A. cajennense* sensu lato, *A. dubitatum, A. tenellum* (published as *A. imitator*), *A. parvum* and *A. scultptum* (de Rodaniche, 1953; Guedes et al., 2005; Pinter and Labruna, 2006; Labruna et al., 2008; Labruna, 2009; Oliveira et al., 2010; Guedes et al., 2011; Labruna et al., 2011; Soares et al., 2011; Brites-Neto et al., 2013; Dzul-Rosado et al., 2013; Krawczak et al., 2014). In addition, brown dog ticks (*Rhipicephalus sanguineus* sensu lato) are involved in transmission of *R. rickettsii* in Mexico, the south-western US, and Brazil (Bustamante and Varela, 1947; Demma et al., 2005; de Almeida et al., 2013).

In the United States, the lone star tick (*Amblyomma americanum*) is one of the most aggressive human-biting ticks. Normally, larvae and nymphs of this species feed on a variety of medium-sized to large mammals and ground-feeding birds, while adult ticks prefer feeding on ungulates. However, all three life stages readily attach to and feed on humans, often in large numbers. *A. americanum* is a known vector of a number of bacterial agents including *Francisella tularensis*, *Ehrlichia chaffeensis*, *E. ewingii*, and *R. amblyommatis* (reviewed by (Childs and Paddock, 2003)), *E. ruminantium*-like organism referred to as the Panola Mountain *Ehrlichia* (PME) (Loftis et al., 2006, 2008) and Heartland virus (Savage et al., 2013). The geographical range of *A. americanum* has dramatically increased in the past 30 years, ostensibly due to expansion of white-tailed deer populations (Childs and Paddock, 2003). It is now distributed from west-central Texas, north to Iowa, and eastward spanning all the eastern United States. Along the Atlantic Coast, the range of this species extends from northern Mexico as far north as New York, New Hampshire and Maine (Springer et al., 2014) (http://www.cdc.gov/ticks/maps/lone_star_tick.pdf).

A large part of the *A. americanum* geographical range overlaps with the known distribution of both *D. variabilis* and reported cases of RMSF. The two tick species utilize the same species of vertebrate hosts as both *A. americanum* immatures and *D. variabilis* adults feed on medium-sized mammals. Therefore, there is a potential for *A. americanum* larvae and nymphs to become naturally exposed to *R. rickettsii* whether by acquiring blood meal from infected animals or while feeding side-by-side with infected *D. variabilis* adults.

Indeed in the pre-PCR era, Parker and coauthors described identification of the RMSF agent in questing *A. americanum* nymphs in Oklahoma (Parker et al., 1943). They also

summarized a dozen spotted fever cases recognized in Louisiana, Maryland, Missouri, Oklahoma and Texas between 1926 and 1942, for which available evidence suggested transmission by lone star ticks (Parker et al., 1943). More recently, *R. rickettsii* DNA was detected in several *A. americanum* collected in Kansas (Berrada et al., 2011). Moreover, at least one PCR- and sequence-confirmed human case of RMSF in North Carolina has been associated with the bite of an *A. americanum* tick (Breitschwerdt et al., 2011) suggesting that the lone star tick may indeed serve as a vector of *R. rickettsii*. Yet, vector competence of the lone star tick for *R. rickettsii* has not been properly evaluated in a controlled environment unlike that of several other *Amblyomma* spp. distributed from Mexico to South America. Therefore, we assessed the ability of *A. americanum* to acquire, maintain and transmit *R. rickettsii* under laboratory conditions.

2. Methods

2.1. Rickettsial isolates

Two isolates of *R. rickettsii* originating from different geographical regions of the US were used in this study. Isolate BSF-Di-6 was originally isolated in 1961 from a spleen and liver homogenate taken from a Virginia opossum (*Didelphis virginiana*) trapped in Hanover County, VA (Bozeman et al., 1967). Since then, the isolate was propagated and stored in yolk-sack culture for a total of five passages. The AZ-3 isolate was cultured in 2004 from blood of an Arizona patient who died of RMSF (Eremeeva et al., 2006). Prior to the current study, this isolate was propagated and stored in Vero cells for a total of three passages (Karpathy et al., 2007). According to a molecular analysis, BSF-Di-6 and AZ-3 belong to different genotypes within the Clade I of *R. rickettsii* isolates (Karpathy et al., 2007). In preliminary trials, both isolates have been shown to cause nonlethal clinical infection in guinea pigs when inoculated with dosages containing approximately 10^3 to 10^5 copies of rickettsial DNA. Survival of laboratory animals through the infection was necessary to ensure successful feeding of ticks. Our preliminary experiments demonstrated that guinea pigs infected with low dosages of either isolate exhibited self-limiting fever after incubation period of 5–9 days, scrotal edema with or without necrosis, erythematous dermatitis of ears, and swollen, hemorrhagic footpads.

2.2. Preparation of tissue homogenates

In advance of the study, groups of male guinea pigs were inoculated intraperitoneally with either of the two isolates. Each inoculum consisted of infected Vero cells containing approximately 10^5 copies of rickettsial DNA resuspended in 3 ml of in Snyder-1 media (sodium phosphate glutamate buffer). These guinea pigs were euthanized at the height of infection – 7–9 days post infection (DPI). Spleen and liver homogenates were prepared from individual guinea pigs by triturating tissues in SRM buffer (sucrose glutamate buffer (0.22 M sucrose, 0.1 M potassium phosphate, 0.005 M sodium L-glutamate, pH 7.0)) supplemented with 5 mM $MgCl_2$ and 1% Hypaque-76 (Nycomed, Inc., Princeton, NJ). The amount of pathogen in homogenates derived from individual animals was evaluated by quantitative PCR. A single homogenate with the highest amount of rickettsial DNA was chosen from among guinea pig groups infected with each of the two isolates and aliquoted to

contain 10^5 copies of *R. rickettsii* DNA per aliquot. These aliquots were stored in liquid nitrogen until used to infect the study animals.

2.3. Amblyomma americanum colony

A. americanum ticks used in this study were derived from *Rickettsia*-free colonies maintained at the CDC Medical Entomology Laboratory by feeding on pathogen-free naïve New Zealand white rabbits as previously described (Troughton and Levin, 2007). The colony originated from adult ticks collected from vegetation at a State Park 15 miles SW of Atlanta, Georgia and has been maintained in the laboratory for six generations. Absence of either pathogenic or endosymbiotic *Rickettsia* spp., as well as known tick-borne *Anaplasma, Borrelia, Ehrlichia* species and Heartland virus, in this colony has been assured in every generation by PCR-testing samples of larvae from progeny of every female and serological assessment of all rabbits used for tick feeding.

2.4. Model animals

Pathogen free and tick-naïve male guinea pigs 6–9 wks.-old were used as model animals. Throughout the study, guinea pigs were monitored daily for clinical signs of infection including fever (defined as body temperature ≥ 39.5 °C), scrotal edema, dermatitis and edema of ears and foot pads (Walker et al., 1977). Samples of skin from the ear and EDTA-treated whole blood from the ear vein were collected from experimental guinea pigs every 2–3 days using sterile 2mm-diameter ear punches (Kent Scientific Corporation, Torrington, CT) and 27-gauge needles respectively. Animals were euthanized at 10–14 days after infection using barbiturate overdose (Beuthanasia®-D,Intervet International B.V.). Samples of serum, liver, spleen, testis with epididymis, lung and heart were collected at the time of euthanasia. Blood and tissue samples were stored at −20° C until tested by PCR for the presence of *R. rickettsii* DNA and sera samples were refrigerated at 4° C for serologic testing.

In addition, two mix-breed (hound-type) 1 year-old male dogs were used to assess the ability of *R. rickettsii*-infected adult ticks to transmit infection to canine hosts. Throughout the study, the dogs were housed indoors – in a climate-controlled animal facility as described before (Levin et al., 2014a). Dogs were provided with water and a standard dry food diet (Laboratory Canine Diet 5006; Purina, Fairburn, GA, USA) ad libitum. The appetite, behavior, disposition, and level of activity of each dog were monitored twice daily throughout the study. Dogs exhibiting decreased appetite were fed soft canned dog food (Pedigree, McLean, VA, USA).

2.5. Assessment of vector competence

First, we evaluated the ability of *A. americanum* to become infected with *R. rickettsii* as a result of feeding together on the same host with infected *D. variabilis* ticks. This experiment was conducted with the Di-6 isolate of *R. rickettsii*, which originated from an area within the overlapping ranges of *D. variabilis* and *A. americanum.* Groups of approximately 200 uninfected *A. americanum* larvae were placed on three naïve guinea pigs together with 25 *Rickettsia*-infected (38.0 ± 6.9%) *D. variabilis* nymphs from the laboratory colony described

before (Schumacher et al., 2016). Engorged larvae were collected, allowed to molt and tested for the presence of *R. rickettsii* DNA.

Next, we assessed the ability of *A. americanum* larvae to acquire *R. rickettsii* from systemically infected model animals. Two groups of three guinea pigs were intraperitoneally inoculated with spleen/liver homogenates of either DI-6 or AZ-3 isolates of *R. rickettsii*. The inoculated animals were each infested with approximately 500 uninfected larvae of *A. americanum* on either the seventh or fifth day post-inoculation depending on the rickettsial isolate used so that tick feeding period would coincide with the peak of infection in the hosts. Engorged larvae were collected daily and allowed to molt. Twenty five freshly molted nymphs fed as larvae upon each needle-inoculated guinea pig were tested individually by PCR.

To evaluate the capability of infected nymphs for transmission of the pathogen to susceptible hosts, remaining nymphs from each of the inoculated guinea pigs were fed on three naïve guinea pigs in pools of 50. Simultaneously, uninfected *A. americanum* larvae were placed on the same guinea pigs for assessment of rickettsial transmission between infected and uninfected ticks as a result of side-by-side feeding and to propagate *R. rickettsii*-infected tick colonies. Engorged ticks were allowed to molt and 25 molted adults and 25 nymphs from each guinea pig were tested for the presence of rickettsial DNA.

For assessment of transovarial transmission (TOT), adult ticks derived from guinea pig-fed nymphs were allowed to feed on New Zealand white rabbits as previously described (Troughton and Levin, 2007; Levin and Schumacher, 2016). Rabbits were infested with 20 pairs (male/female) of *A. americanum* ticks from either Di-6- or AZ-3-infected cohorts. Engorged female ticks were kept in an incubator until they laid eggs and tested for the presence of *R. rickettsii* DNA soon after oviposition. Approximately 100 larvae from the progeny of each individual female were tested in a single pool by PCR to assess frequency of TOT.

We also tested the ability of *R. rickettsii*-infected adult ticks to transmit infection to canine hosts. Each of the two dogs was infested with 20 pairs of *A. americanum* ticks as described by Zemtsova et al. (2010). One dog was infested with ticks from the cohort infected with the DI-6 isolate of *R. rickettsii* and the other – with the AZ-3 isolate. The absence of antibodies to spotted fever group (SFG) rickettsiae in each dog was confirmed prior to enrollment into the study by the indirect immunofluorescence assay (IFA) as described below. Throughout the 2-wk study period, the body temperature of each dog was measured every morning; and temperatures ≥ 39.5 °C were defined as febrile (Aiello and Moses, 2016). Ear skin biopsies 2 mm diameter were collected aseptically from the ear twice per week under general anesthesia for PCR as skin biopsies appear to be significantly more useful in detecting rickettsial infection than blood-PCR (Levin et al., 2016). At 14 days post infestation, dogs were treated with oral doxycycline as previously described (Levin et al., 2014a) and transferred to an unrelated study.

2.6. PCR and serology

DNA extraction and PCR procedures were carried out in separate facilities. DNA was extracted from tick and tissue samples using the Qiagen DNEasy Blood & Tissue kit, whereas blood samples were processed with the Flexi Gene kit (Qiagen Inc., Valencia, CA) according to manufacturer's protocols. The presence of Rickettsial DNA was detected by PCR using primers RR190-547F and RR190-701R to amplify a 154-bp fragment of the *romp*A gene of *Rickettsia* spp. as described by Eremeeva et al. (2003). All samples were tested in duplicate. Two negative (distilled water) and two positive (*R. massiliae* plasmid) samples were included in each run. Samples demonstrating amplification prior to 40 cycles with appropriate amplicon melting temperature in both replicates were considered positive.

IFA was performed on guinea pig and dog sera, as previously described (Lennette et al., 1995), using FITC labeled goat anti-guinea pig and anti-dog IgG (γ) conjugates diluted per manufacturer's recommendations (KPL, Inc. Gaithersburg, Maryland, USA). Slides were spotted with whole cell *R. rickettsii* antigen, air-dried and fixed in acetone. Serum samples were initially screened at 1/16 and 1/256 dilutions, and positive samples were titrated to the endpoint in a two-fold dilution series. Serologic data are reported as the reciprocal of the last dilution showing positive fluorescence. Titers ≥ 32 were considered positive.

Prevalence of rickettsial infection in cohorts of ticks and frequencies of TOT were compared by Chi-squared and Fisher exact tests (2-tailed) at the 95% confidence level.

2.7. Ethics statement

The study was undertaken at a facility fully-accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Care and husbandry of animals were performed in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals 8th edition. All procedures of this study were pre-approved by the Centers for Disease Control Institutional Animal Care and Use Committee and monitored by a veterinarian stationed on-site. The US Animal Welfare Act and all associated regulations were strictly followed.

3. Results

A. americanum larvae fed on guinea pigs simultaneously with Di-6–infected *D. variabilis* nymphs were tested (25 per guinea pig) by PCR after they molted into the nymphal stage. In total, five (6.7 ± 2.9%) out of 75 tested ticks contained *R. rickettsii* DNA. As the rickettsial isolate used in this experiment originated from an area occupied by both tick species, results demonstrate that *R. rickettsii* infection in *A. americanum* can result from interspecies spill-over when the American dog tick and the lone star tick utilize the same vertebrate hosts.

Uninfected *A. americanum* larvae acquired *R. rickettsii* from guinea pigs needle-inoculated with either of the two isolates and transmitted the pathogen transstadially to the nymphal stage. Approximately 49% and 33% of freshly molted nymphs were PCR-positive after feeding as larvae upon animals infected with DI-6 and AZ-3 isolates, respectively (Table 1). The prevalence of infection was significantly different between the two cohorts ($p = 0.04662$).

According to our previous observations, clinical signs of rickettsial infection in guinea pigs infected with the Di-6 and AZ-3 isolates include fever (evident after 5–9 DPI), scrotal inflammation and dermatitis of ears and foot-pads. In addition, rickettsial DNA can be detected in guinea pig skin or venous blood starting 6–8 DPI (Levin et al., 2016). When *A. americanum* nymphs from infected cohorts were placed on naïve guinea pigs in the current study, the majority of animals displayed similar clinical signs of rickettsial infection. Of the nine guinea pigs infested with ticks infected with the isolate DI-6, seven developed fever above 39.5 °C starting on 7–9 DPI and lasting 2–4 days. The same seven animals had scrotal and foot-pad edema as well as ear and foot-pad dermatitis of varying severity. Additionally, rickettsial DNA was detected in skin, blood and internal organs of these seven guinea pigs (Table 2). Of the nine guinea pigs fed upon by AZ-3-infected nymphal ticks, five developed fever starting 6–8 DPI, but eight exhibited scrotal edema, as well as dermatitis and edema of ears and foot pads. Rickettsial DNA was also detected in skin, blood and internal organs of eight of the nine guinea pigs (Table 2). One of the animals exposed to *A. americanum* nymphs infected with AZ-3 isolate of *R. rickettsii* became morbidly ill by day 9 post-infestation and had to be euthanized. All 18 guinea pigs fed upon by *A. americanum* nymphs infected with DI-6 and AZ-3 isolates of *R. rickettsii* developed IgG antibodies reactive with *R. rickettsii* antigen within 14 days after placement of ticks, including the one euthanized on day 9 post infestation (Table 2). Reciprocal IFA titers ranged from 64 to 2048 indicating that all guinea pigs were exposed to the pathogen delivered via tick bite. Geometric mean titers in groups exposed to DI-6 and AZ-3 isolates were 362 and 420, respectively.

Uninfected *A. americanum* larvae fed side by side with infected nymphs acquired both DI-6 and AZ-3 isolates and transmitted the pathogen transstadially. The prevalence of infection in resulting nymphs varied significantly between individual guinea pigs – from 0% to 40% (as detected by testing 25 ticks per guinea pig). Overall, 11% and 9% of freshly molted nymphs were PCR-positive after feeding as larvae side by side with ticks infected with DI-6 and AZ-3 isolates, respectively (Table 1). The prevalence of infection in resulting nymphs was not significantly different between DI-6 and AZ-3 cohorts.

Infected *A. americanum* nymphs used in the co-feeding experiment were allowed to molt and 25 freshly molted adult ticks (12 males and 13 females per guinea pig) from the Di-6 and AZ-3 cohorts were tested by PCR. The prevalence of infection in unfed adult ticks was 68% and 52% in the 2 cohorts, respectively (Table 3) and did not differ between males and females. For both cohorts, prevalence of infection in unfed adult ticks was higher than in unfed nymphs (Tables 1 and 3), though the differences were not statistically significant ($p = 0.096$ and $p = 0.105$, respectively). Even higher percentages of adult ticks from the same cohorts tested positive after their feeding upon naïve New Zealand rabbits. In either cohort, 100% of males tested PCR-positive after feeding. Female ticks were tested at the end of oviposition. Among the 99 ovipositing females from the Di-6 cohort, 97 (98%) contained rickettsial DNA; and 65 out of 92 (70.7%) females were positive in the AZ-3 cohort (Table 3). The described step-by-step increase in the prevalence of rickettsial infection in cohorts of *A. americanum* from unfed nymphs to engorged adults also confirms efficient transmission of both *R. rickettsii* isolates between simultaneously feeding ticks at all stages of their life cycle.

Rickettsial DNA was also detected in larval batches produced by some of the infected females indicating successful TOT. A total of 27 (27.8%) out of 97 individual larval batches produced by females infected with the Di-6 isolate tested positive (Table 3). Of the 65 females infected with the AZ-3 isolate, 9 (13.8%) produced infected progeny. The frequency of TOT of *R. rickettsii* was significantly higher ($p = 0.036$) among ticks infected with the Di-6 isolate than those infected with AZ-3 isolate.

Dogs infested with adult ticks from either Di-6- or AZ-3-infected cohorts became infected and clinically ill. The body temperature rose above 39.5 °C on day 4 and 3 post-infestation, respectively, and peaked at 41.2 °C and 41.5 °C on day 7. Both dogs developed abundant petechiae on the gums, buccal mucosa, and in the conjunctiva of both eyes. Widespread macular skin rash appeared on all exposed areas of the skin including ears, limbs, testes and the abdomen. In addition, dogs became anorectic and lethargic within a week of tick placement. Taken together, clinical signs exhibited by dogs after infestation with adult *A. americanum* infected with either Di-6 or AZ-3 isolates of *R. rickettsii* were identical to those of canine RMSF (Levin et al., 2014a). In addition, *R. rickettsii* DNA was identified in skin biopsies from the dog exposed to Di-6 on days 7 and 9 post-infestation and the dog exposed to AZ-3 on days 5, 7, and 12.

4. Discussion

The geographical range of *R. rickettsii* includes most the North and South Americas, from Canada to Argentina. Some strains of *R. rickettsii* can cause up to 80% mortality in humans. Different tick species serve as vectors of *R. rickettsii* including *A. aureolatum, A. parvum, A. sculptum, D. andersoni, D. variabilis,* and *Rh. sanguineus*. In addition, varying degrees of vector competence have been demonstrated under laboratory conditions for *A. tenellum* (published as *A. imitator*), *D. occidentalis,* and *H. leporispalustris* (Parker et al., 1933, 1951; Freitas et al., 2009; Oliveira et al., 2010). In the United States, the Rocky Mountain wood tick – *D. andersoni* and the American dog tick – *D. variabilis* are the best known vectors of *R. rickettsii* while *Rh. sanguineus* s.l. acts as the primary vector in parts of the Southwest. Conversely, in Central and South America several species of the genus *Amblyomma* act as vectors and reservoirs of the pathogen (Bustamante and Varela, 1946; de Rodaniche, 1953; Lima et al., 1995; de Lemos et al., 1997; Pinter and Labruna, 2006; Labruna et al., 2008; Paddock et al., 2008; Labruna, 2009; Dzul-Rosado et al., 2013).

In the US, *A. americanum* is the predominant human-biting tick throughout the Southeast (Merten and Durden, 2000; Stromdahl et al., 2015). Its geographical range overlaps with that of RMSF in the southern and southeastern states, where it is often much more numerous than *D. variabilis* (Apperson et al., 2008). *A. americanum* represented 53% of all ticks recovered from humans in Mississippi from 1990 through 1999 and approximately 56% of ticks collected from humans between 2008 and 2014 in Texas (Goddard, 2002; Mitchell et al., 2016), while a study in Georgia and South Carolina found that 83% of ticks attached to 460 patients were lone star ticks (Felz et al., 1996). A recent survey in North Carolina showed that *A. americanum* accounted for 93–95% of ticks removed from the skin of outdoor workers (Lee et al., 2014). The potential role of *A. americanum* in transmission of *R. rickettsii* to humans has been suspected in Oklahoma and North Carolina (Cooley and

Kohls, 1944; Breitschwerdt et al., 2011) and the pathogen has been occasionally identified in questing lone star ticks (Parker et al., 1943; Heise et al., 2010; Berrada et al., 2011). However only scant experimental evidence has been published to support transmission of *R. rickettsii* by *A. americanum* ticks. For example, transmission of *R. rickettsii* to guinea pigs by *A. americanum* nymphs and adults was demonstrated under laboratory conditions (Maver, 1911), but the efficiency of acquisition or transmission could not be assessed. In 1933, Parker with coauthors reported that the lone star tick can occasionally transmit the agent of RMSF transovarially (Parker et al., 1933). Yet, follow-up experiments by Burgdorfer and Briton provided equivocal results as *A. americanum* infected with a highly virulent Sawtooth-♀2 strain of *R. rickettsii* time after time failed to maintain infection transovarially because of high mortality in both females and eggs (Burgdorfer and Brinton, 1975). With the discovery of other pathogenic and endosymbiotic bacteria transmitted by the lone star tick (Childs and Paddock, 2003) and associated obstacles to establishment and maintenance of conclusively uninfected tick colonies, the vector competence of this species for *R. rickettsii* has not been sufficiently studied.

To evaluate the ability of *A. americanum* for acquisition, maintenance and transmission of *R. rickettsii*, we used a specific pathogen-free (SPF) tick colony maintained in our laboratory for several generations. This colony is routinely tested in every generation to insure absence of known tick-borne bacteria including *Anaplasma, Borrelia, Ehrlichia*, and *Rickettsia* species. Use of SPF ticks and laboratory animals assured that any clinical and pathological signs in vertebrate hosts as well as all positive PCR results in either tick or animal samples can be unequivocally attributed to a single pathogen – *R. rickettsii*. Different isolates of a rickettsial pathogen can cause widely disparate effects on the survival of its vector (Levin et al., 2009). This suggested that the ability of a tick species to acquire, maintain and transmit a pathogen may vary between different strains circulating within or outside of the geographical range of that tick. Therefore, one of the rickettsial isolates used in this study (Di-6) originated from the area within the geographical range of *A. americanum* while the other isolate (AZ-3) was derived from Arizona – outside of the lone star tick range. Guinea pigs served as model animals for the bulk of the experiments as a less costly alternative to natural hosts of *A. americanum*. Yet, dogs were used for the final confirmation that infected *A. americanum* are capable of causing clinically recognizable infections in a natural host.

Our experiments demonstrate that *A. americanum* can be a competent vector for both of these two isolates of *R. rickettsii*, although the degree of competence differs somewhat between the isolates. Uninfected larval ticks are able to acquire the pathogen from either systemically infected (rickettsiemic) hosts or simultaneously feeding infected nymphs. Prior to this study, we determined that in naïve guinea pigs infected with Di-6 and AZ-3 isolates of *R. rickettsii* the first rise of body temperature occurs at 5–9 days post-infection (Levin et al., 2016) – at 5–7 DPI in needle-inoculated animals and 7–9 DPI in those infected by ticks. In addition, rickettsial DNA is not detectable in either the venous blood or skin biopsies of guinea pigs until 6–8 after placement of *R. rickettsii*-infected ticks (Levin et al., 2016). Meanwhile, *A. americanum* larvae and nymphs complete their engorgement on guinea pigs within 3–4 and 5–6 days, respectively. Thus, both larval and nymphal ticks in the current study detached from the hosts prior to either clinical or molecular manifestations of a systemic infection and rickettsemia. Based on this, we concluded that the actual sharing of

the pathogen between simultaneously feeding ticks in the present study took place prior to the development of a systemic infection and rickettsemia in the hosts – i.e. via the co-feeding route. The efficiency of rickettsial proliferation by this route is relatively low – fewer larvae acquire either isolate via co-feeding than from rickettsiemic hosts (11% vs 49% and 9% vs 33% for the Di-6 and AZ-3 isolates, respectively). Yet, additional sharing of *Rickettsia* takes place within both nymphal and adult cohorts of ticks during their feeding, which is demonstrated by the rising prevalence of infection from the unfed nymphs (33–49%) to blood-fed male ticks derived from those nymphs (100%).

Placement of nymphs infected with either rickettsial isolate upon naïve guinea pigs induced typical clinical infection and/or seroconversion confirming the competence of *A. americanum* nymphs in transmission of *R. rickettsii* to susceptible hosts. This corroborates results reported by Maria Maver a century ago (Maver, 1911). Likewise, two dogs infested with *Rickettsia*-positive adult *A. americanum* both developed canine RMSF. Thus lone star ticks acquiring *R. rickettsii* with larval or nymphal blood-meal successfully retain the pathogen transstadially and transmit it to susceptible hosts during subsequent feedings. Our results confirm vector competence of *A. americanum* for *R. rickettsii* under laboratory conditions, although the efficiency of pathogen acquisition is lower than what had been reported for some other vector species including *D. variabilis, A. aureolatum,* and *H. leporispalustris* (Labruna et al., 2008; Freitas et al., 2009; Schumacher et al., 2016).

Ticks are often referred to as both vectors and reservoirs of pathogenic rickettsiae due to their ability to maintain bacteria from generation to generation via TOT. In some vector species, TOT of *R. rickettsii* can be highly effective with up to 100% of *D. andersoni* and *A. aureolatum* progenies being infected under laboratory conditions (Burgdorfer, 1963; Burgdorfer and Brinton, 1975; Burgdorfer, 1988; Labruna et al., 2011). Conversely, rickettsial DNA was detected in larval samples from only nine (47.4%) progenies of the 19 *D. variabilis* females infected with the Di-6 isolate (Schumacher et al., 2016), and the frequency of TOT of *R. rickettsii* (Taiaçu strain) in *A. sculptum* (reported at the time of publication as *Amblyomma cajennense*) varied between 6.2% and 42.8% in cohorts of ticks exposed to the pathogen at different life stages (Soares et al., 2011). Unfortunately, these published TOT rates are not directly comparable to each-other because individual studies utilized different methods to infect ticks, different host species, and isolates of *Rickettsia* that were sympatric with the vector in some cases and allopatric in others. In our experiments, *R. rickettsii* DNA was detected in progenies of approximately 28% and 14% of *A. americanum* females infected with the Di-6 and AZ-3 isolates, respectively. Thus, an isolate originating from within the geographic range of *A. americanum* seems to be transmitted transovarially twice as often as an isolate circulating outside of that range. It must be pointed out, however, that an *A. americanum* female produces several thousand eggs and only 100 larvae were tested from each progeny. Therefore, the true frequency of TOT in our study might have been underestimated if the filial prevalence of infection was below 1%.

A number of previous studies have reported higher rates of TOT when ticks acquired *R. rickettsii* as larvae or nymphs, whereas low to no transmission was observed in those becoming infected only during the adult feeding (Parker et al., 1933; Burgdorfer and Brinton, 1975,; Piranda et al., 2011; Soares et al., 2011). It has been proposed that ticks

infected only at the adult stage may fail to transmit *R. rickettsii* transovarially because of the inability of the pathogen to sufficiently colonize the ovaries in females (Burgdorfer and Brinton, 1975). In our experiments, the prevalence of infection with either Di-6 or AZ-3 isolates in all cohorts of unfed freshly molted nymphal or adult *A. americanum* was always below 100%, and the prevalence of infection increased with every successive feeding due to sharing of the pathogen between simultaneously feeding ticks. Therefore, it was impossible to separate individual females, which acquired the *Rickettsia* during larval feeding and successfully maintained infection throughout the life cycle, from those acquiring bacteria during nymphal or adult feedings. Still, it is noteworthy that the proportion of infected *A. americanum* females producing infected progenies was approximately 2–3 times lower than the prevalence of infection in the preceding nymphs and approximately 4–5 times lower than in engorged adults. We infer that even among ticks acquiring *R. rickettsii* as larvae, only a fraction was capable of TOT. Overall, it appears that the ability of *A. americanum* to carry *R. rickettsii* from generation to generation is lower than those reported for *D. andersoni, D. variabilis,* and *A. aureolatum*, but comparable to that in *A. sculptum.*

In our experiments, we noted significant differences between the Di-6 and AZ-3 isolates in their infectivity for larval *A. americanum* as well as in the frequency of TOT. In both cases, ticks were more successful in acquiring and transmitting the eastern Di-6 isolate, which is derived from the area within the geographical range of *A. americanum*. Considering the continent-wide distribution of *R. rickettsii* correlating with molecular differentiation of its isolates (Karpathy et al., 2007), it is likely that diverse strains of the pathogen are adapted to different vectors. Conversely, different populations of a widely distributed tick species may vary in the vector competence for the different strains of a pathogen (Levin et al., 2009; Moraes-Filho et al., 2015).

Considering the relatively low frequency of TOT in this study, *A. americanum* may not be able to perpetually maintain the infection in the wild from generation to generation. However, new introductions of *R. rickettsii* into *A. americanum* populations are possible as a result of interspecies spill-over events when lone star ticks feed on already rickettsiemic hosts or side by side with infected *D. variabilis*, as demonstrated in this study. Moreover, the prevalence of *R. rickettsii* infection in all experimental cohorts of *A. americanum* steadily increased from larval to adult stage due to sharing of the pathogen between ticks feeding together. Although the current study was primarily performed in guinea pigs, existence and efficiency of the co-feeding route of rickettsial transmission had been demonstrated in natural hosts – dogs (Zemtsova et al., 2010). This co-feeding route can be realized even when ticks feed on insusceptible or previously immunized animals (Zemtsova et al., 2010; Levin et al., 2014b). The fact that two geographically and genetically distinct isolates of *R. rickettsii* were both transmitted between simultaneously-feeding ticks, which completed engorgement prior to development of rickettsemia in the hosts, indicates pervasiveness of the co-feeding route of rickettsial transmission in *A. americanum*.

Lone star ticks are notorious for attaching to their hosts in extremely high numbers. There are reports of over 3000 *A. americanum* including larvae, nymphs and adults being collected from a single jack rabbit in Texas, and white-tailed deer sampled in Arkansas had as many as 2550 *A. americanum* ticks per ear (Goddard, 1989; Goddard and McHugh, 1990). Such

dense infestations provide suitable conditions for transmission of pathogens – including *R. rickettsii* – between simultaneously feeding ticks. In addition, different life stages of this tick utilize the same host species as *D. variabilis* including eastern cottontail rabbits, dogs, feral swine, deer, horses, raccoons, and even squirrels (Koch, 1982; Demarais et al., 1987; Mock et al., 1991; Pung et al., 1994; Kollars et al., 2000; Allan et al., 2001; Goldberg et al., 2002; Cooney et al., 2005; Duell et al., 2013; Barrett et al., 2014). It is unknown how often these hosts become systemically infected with *R. rickettsii* but utilization of the same hosts and large numbers of simultaneously feeding ticks provide opportunity for *A. americanum* to acquire the pathogen from infected individual ticks of either the same or a different species. Once *R. rickettsii* is picked up by *A. americanum* with larval or nymphal blood-meal, it will be sustained transstadially and further transmitted to other ticks or susceptible hosts during the next feeding.

On the other hand, the prevalence of a pathogenic *Rickettsia* within a tick population may be diminished or even suppressed when a significant proportion of ticks is infected with an endosymbiotic *Rickettsia* species due to transovarial interference (Burgdorfer et al., 1981a, 1988; Macaluso et al., 2002). In 1973, W. Burgdorfer and colleagues identified a novel spotted fever group *Rickettsia* in questing lone star ticks, which was provisionally designated as "*Rickettsia amblyommii*" (Burgdorfer et al., 1981b; Stothard and Fuerst, 1995). This endosymbiotic bacterium, only recently formally named *Rickettsia amblyommatis* (Karpathy et al., 2016), has since been detected in most of the examined populations of *A. americanum* with prevalence of infection often exceeding 40% (reviewed by Karpathy et al., 2016). As transovarial maintenance of multiple infections of *Rickettsia* spp. in ticks is believed to be all but impossible, *A. americanum* is not currently considered as a vector of RMSF (Goddard and Norment, 1986; Weller et al., 1998; Childs and Paddock, 2003). However, recent studies identified dual and even triple rickettsial coinfections in questing ticks. A single *D. variabilis* tick collected in Ohio was found coinfected with *Rickettsia bellii, R. montanensis,* and *R. rickettsii* (Carmichael and Fuerst, 2010); two *A. americanum* collected in Kansas were concurrently infected by *R. rickettsii* and *R. amblyommatis* (Berrada et al., 2011); and two Peruvian *A. maculatum* were co-infected with both *Candidatus* R. andeanae and *R. parkeri* (Flores-Mendoza et al., 2013). In addition, laboratory-reared *A. dubitatum* were able to acquire and transmit *R. rickettsii* transstadially despite being 100% infected with *R. bellii* (Sakai et al., 2014). These findings indicate that presence of one *Rickettsia* in a tick does not automatically preclude acquisition or transstadial transmission of another bacterial species in nature. The ability of *R. amblyommatis* to interfere in acquisition, maintenance and transmission of *R. rickettsii* by the lone star tick needs to be verified and evaluated.

In conclusion, our results correspond with earlier deductions that the lone star tick may be an epidemiologically significant vector of Rocky Mountain spotted fever (Parker et al., 1943; Berrada et al., 2011; Breitschwerdt et al., 2011). We have demonstrated that *A. americanum* is a competent vector of *R. rickettsii*. It can acquire the pathogen from systemically infected hosts as well as from (simultaneously feeding) infected ticks of either the same or different species. Interspecies transmission of *Rickettsia* via co-feeding can allow new introductions of the pathogen into *A. americanum* populations as spill-over from the primary vectors. Ticks acquiring *R. rickettsii* during feeding successfully retain the

pathogen during the molting process from larval to nymphal and from nymphal to adult stages, and transmit it to susceptible hosts during the subsequent feedings. Despite a relatively low efficiency of TOT, the aggressive feeding behavior of *A. americanum*, all stages of which can feed on humans, and its tendency to cluster on a host in high numbers may allow the lone star tick to sustain the transmission cycle of *R. rickettsii* in nature. Its role in the ecology and epidemiology of RMSF in North America may be underestimated. A number of recent studies demonstrated paucity of *R. rickettsii* in *D. variabilis* – the reputed primary vector of the causative agent of RMSF in the eastern US (Ammerman et al., 2004; Moncayo et al., 2010; Stromdahl et al., 2011; Nadolny et al., 2014). These findings triggered a revision of vector competence of tick species other than *D. variabilis*, including the work present here. However, a comparative analysis of *A. americanum* and *D. variabilis* vector competence was not an objective of the current study, thus authors refrain from making any conclusions or speculations in that regard. A separate study is warranted to further elucidate relative roles of different tick species as vectors of *R. rickettsii* in nature and its transmission to humans.

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References

Aiello, SE., Moses, MA. The Merck Veterinary Manual. Merck & Co., Inc.; Whitehorse Station, NJ, USA: 2016. p. 3325

Allan SA, Simmons LA, Burridge MJ. Ixodid ticks on white-tailed deer and feral swine in Florida. J. Vector Ecol. 2001; 26:93–102. [PubMed: 11469190]

Ammerman NC, Swanson KI, Anderson JM, Schwartz TR, Seaberg EC, Glass GE, Norris DE. Spotted-fever group *Rickettsia* in *Dermacentor variabilis*, Maryland. Emerg. Infect. Dis. 2004; 10:1478–1481. [PubMed: 15496254]

Apperson CS, Engber B, Nicholson WL, Mead DG, Engel J, Yabsley MJ, Dail K, Johnson J, Watson DW. Tick-borne diseases in North Carolina: is *Rickettsia amblyommii* a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? Vector Borne Zoonotic Dis. 2008; 8:597–606. [PubMed: 18447622]

Barrett A, Little SE, Shaw E. *Rickettsia amblyommii* and *R. montanensis* infection in dogs following natural exposure to ticks. Vector Borne Zoonotic Dis. 2014; 14:20–25. [PubMed: 24359419]

Berrada ZL, Goethert HK, Cunningham J, Telford SR 3rd. *Rickettsia rickettsii* (Rickettsiales: Rickettsiaceae) in *Amblyomma americanum* (Acari: ixodidae) from Kansas. J. Med. Entomol. 2011; 48:461–467. [PubMed: 21485390]

Bozeman FM, Shiral A, Humphries JW, Fuller HS. Ecology of Rocky Mountain spotted fever II. Natural infection of wild mammals and birds in Virginia and Maryland. Am. J. Trop. Med. Hyg. 1967; 16:48–59. [PubMed: 6067061]

Breitschwerdt EB, Hegarty BC, Maggi RG, Lantos PM, Aslett DM, Bradley JM. *Rickettsia rickettsii* transmission by a lone star tick, North Carolina. Emerg. Infect. Dis. 2011; 17:873–875. [PubMed: 21529399]

Brites-Neto J, Nieri-Bastos FA, Brasil J, Duarte KM, Martins TF, Verissimo CJ, Barbieri AR, Labruna MB. Environmental infestation and rickettsial infection in ticks in an area endemic for Brazilian spotted fever. Rev. Bras. Parasitol. Vet. 2013; 22:367–372. [PubMed: 24142167]

Burgdorfer W, Brinton LP. Mechanisms of transovarial infection of spotted fever rickettsiae in ticks. Ann. N. Y. Acad. Sci. 1975; 266:61–72. [PubMed: 829476]

Burgdorfer, W., Hayes, SF., Mavros, AJ. Nonpathogenic rickettsiae in Dermacentor andersoni: a limiting factor for the distribution of Rickettsia rickettsii. In: Burgdorfer, W., Anacker, RL., editors. Rickettsiae and Rickettsial Diseases. Academic Press; New York: 1981a. p. 585-594.

Burgdorfer, W., Hayes, SF., Thomas, LA., Lancaster, JL. A new spotted fever group rickettsia from the lone star tick, *Amblyomma americanum*. In: Burgdorfer, W., Anacker, RL., editors. Rickettsiae and Rickettsial Diseases. Academic Press; New York: 1981b. p. 585-594.

Burgdorfer W. Investigation of "Transovarial transmission" of *Rickettsia rickettsii* in the wood tick, *Dermacentor andersoni*. Exp. Parasitol. 1963; 14:152–159.

Burgdorfer W. A review of Rocky Mountain spotted fever (tick-borne typhus), its agent, and its tick vectors in the United States. J. Med. Entomol. 1975; 13:269–278.

Burgdorfer, W. Ecological and epidemiological considerations of Rocky Mountain spotted fever and scrub typhus. In: Walker, DH., editor. Biology of Rickettsial Diseases. CRC Inc.; Boca Raton (FL): 1988. p. 33-50.

Bustamante ME, Varela G. III. Estudios de fiebre manchada en México. Hallazgo del *Amblyomma cajennense* na-turalmente infectado, en Veracruz. Rev. Inst. Salubr. Enferm. Trop. 1946; 7:75–78.

Bustamante ME, Varela G. IV: Estudios de fievre manchada en Mexico. Papel del *Rhipicepbalus sanguineus* en la transmision de la fiebre manchada en la Republica Mexicana. Rev. Inst. Salubr. Enferm. Trop. 1947; 8:138–140.

Carmichael JR, Fuerst PA. Molecular detection of *Rickettsia bellii*, *Rickettsia montanensis* and *Rickettsia rickettsii* in a *Dermacentor variabilis* tick from nature. Vector Borne Zoonotic Dis. 2010; 10:111–115. [PubMed: 19485770]

Childs JE, Paddock CD. The ascendancy of *Amblyomma americanum* as a vector of pathogens affecting humans in the United States. Annu. Rev. Entomol. 2003; 48:307–337. [PubMed: 12414740]

Cooley RA, Kohls GM. The genus amblyomma (Ixodidae) in the United States. J. Parasitol. 1944; 30:77–111.

Cooney JC, Burgdorfer W, Painter MK, Russell CL. Tick infestations of the eastern cottontail rabbit (*Sylvilagus floridanus*) and small rodentia in northwest Alabama and implications for disease transmission. J. Vector Ecol. 2005; 30:171–180. [PubMed: 16599149]

de Almeida RF, Garcia MV, Cunha RC, Matias J, e Silva EA, de Fatima Cepa Matos M, Andreotti R. Ixodid fauna and zoonotic agents in ticks from dogs: first report of *Rickettsia rickettsii* in *Rhipicephalus sanguineus* in the state of Mato Grosso do Sul mid-western Brazil. Exp. Appl. Acarol. 2013; 60:63–72. [PubMed: 23229491]

de Lemos ER, Machado RD, Pires FD, Machado SL, da Costa LM, Coura JR. Rickettsiae-infected ticks in an endemic area of spotted fever in the State of Minas Gerais, Brazil. Mem. Inst. Oswaldo Cruz. 1997; 92:477–481. [PubMed: 9361740]

de Rodaniche EC. Natural infection of the tick, *Amblyomma cajennense*, with *Rickettsia rickettsii* in Panama. Am. J. Trop. Med. Hyg. 1953; 30:696–699.

Demarais S, Jacobson HA, Guynn DC. Effects of season and area on ectoparasites of white-tailed deer *Odocoileus virginianus* in Mississippi USA. J. Wildl. Dis. 1987; 23:261–266. [PubMed: 3586204]

Demma LJ, Traeger MS, Nicholson WL, Paddock CD, Blau DM, Eremeeva ME, Dasch GA, Levin ML, Singleton J, Zaki SR, Cheek JE, Swerdlow DL, McQuiston JH. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. N. Engl. J. Med. 2005; 353:587–594. [PubMed: 16093467]

Duell JR, Carmichael R, Herrin BH, Holbrook TC, Talley J, Little SE. Prevalence and species of ticks on horses in central Oklahoma. J. Med. Entomol. 2013; 50:1330–1333. [PubMed: 24843940]

Dzul-Rosado K, Peniche-Lara G, Tello-Martín R, Zavala-Velázquez J, de Campos Pacheco R, Labruna MB, Sánchez EC, Zavala-Castro J. *Rickettsia rickettsii* isolation from naturally infected *Amblyomma parvum* ticks by centrifugation in a 24-well culture plate technique. Open Vet. J. 2013; 3:101–105. [PubMed: 26623321]

Eremeeva ME, Dasch GA, Silverman DJ. Evaluation of a PCR assay for quantitation of Rickettsia rickettsii and closely related spotted fever group rickettsiae. J. Clin. Microbiol. 2003; 41:5466–5472. [PubMed: 14662926]

Eremeeva ME, Bosserman E, Zambrano M, Demma L, Dasch GA. Molecular typing of novel Rickettsia rickettsii isolates from Arizona. Ann. N. Y. Acad. Sci. 2006; 1078:573–577. [PubMed: 17114781]

Felz MW, Durden LA, Oliver JH Jr. Ticks parasitizing humans in Georgia and South Carolina. J. Parasitol. 1996; 82:505–508. [PubMed: 8636862]

Flores-Mendoza C, Florin D, Felices V, Pozo EJ, Graf PCF, Burrus RG, Richards AL. Detection of *Rickettsia parkeri* from within Piura, Peru, and the first reported presence of candidatus *Rickettsia andeanae* in the tick *Rhipicephalus sanguineus*. Vector Borne Zoonotic Dis. 2013; 13:505–508. [PubMed: 23488453]

Freitas LHT, Faccini LH, Labruna MB. Experimental infection of the rabbit tick, *Haemaphysalis leporispalustris*, with the bacterium *Rickettsia rickettsii*, and comparative biology of infected and uninfected tick lineages. Exp. Appl. Acarol. 2009; 47:321–345. [PubMed: 19067185]

Goddard J, McHugh CP. Impact of severe tick infestation at Little Rock AFB, Arkansas on Volant Scorpion military training. Mil. Med. 1990; 155:277–280. [PubMed: 2122308]

Goddard J, Norment BR. Spotted fever group rickettsiae in the lone star tick, *Amblyomma americanum* (Acari: ixodidae). J. Med. Entomol. 1986; 23:465–472. [PubMed: 3772952]

Goddard, J. Brooks Air Force Base, Texas 78235-5301: Medical Entomology Section. Epidemiology Division, USAF; 1989. Ticks and tickborne diseases affecting military personnel; p. 140

Goddard J. A ten-year study of tick biting in Mississippi: implications for human disease transmission. J. Agromed. 2002; 8:25–32.

Goldberg M, Rechav Y, Durden LA. Ticks parasitizing dogs in northwestern Georgia. J. Med. Entomol. 2002; 39:112–114. [PubMed: 11931241]

Guedes E, Leite RC, Prata MC, Pacheco RC, Walker DH, Labruna MB. Detection of Rickettsia rickettsii in the tick Amblyomma cajennense in a new Brazilian spotted fever-endemic area in the state of Minas Gerais. Mem. Inst. Oswaldo Cruz. 2005; 100:841–845. [PubMed: 16444414]

Guedes E, Leite RC, Pacheco RC, Silveira I, Labruna MB. Rickettsia species infecting Amblyomma ticks from an area endemic for Brazilian spotted fever in Brazil. Rev. Bras. Parasitol. Vet. 2011; 20:308–311. [PubMed: 22166385]

Heise SR, Elshahed MS, Little SE. Bacterial diversity in *Amblyomma americanum* (Acari: Ixodidae) with a focus on members of the genus *Rickettsia*. J. Med. Entomol. 2010; 47:258–268. [PubMed: 20380308]

Karpathy SE, Dasch GA, Eremeeva ME. Molecular typing of isolates of *Rickettsia rickettsii* by use of DNA sequencing of variable intergenic regions. J. Clin. Microbiol. 2007; 45:2545–2553. [PubMed: 17553977]

Karpathy SE, Slater KS, Goldsmith CS, Nicholson WL, Paddock CD. *Rickettsia amblyommatis* sp. nov., a spotted fever group rickettsia associated with multiple species of *Amblyomma* ticks in North and South America. Int. J. Syst. Evol. Microbiol. 2016; 66(12):5236–5243. E-pub 09/14/2016. [PubMed: 27638476]

Koch HG. Seasonal incidence and attachment sites of ticks (Acari, Ixodidae) on domestic dogs in Southeastern Oklahoma and Northwestern Arkansas, USA. J. Med. Entomol. 1982; 19:293–298. [PubMed: 7120309]

Kollars TM Jr, Oliver JH Jr, Durden LA, Kollars PG. Host association and seasonal activity of *Amblyomma americanum* (Acari: Ixodidae) in Missouri. J. Parasitol. 2000; 86:1156–1159. [PubMed: 11128501]

Krawczak FS, Nieri-Bastos FA, Nunes FP, Soares JF, Moraes-Filho J, Labruna MB. Rickettsial infection in *Amblyomma cajennense* ticks and capybaras (*Hydrochoerus hydrochaeris*) in a Brazilian spotted fever-endemic area. Parasites Vectors. 2014; 7:7. [PubMed: 24387674]

Labruna MB, Ogrzewalska M, Martins TF, Pinter A, Horta MC. Comparative susceptibility of larval stages of *Amblyomma aureolatum, Amblyomma cajennense*, and *Rhipicephalus sanguineus* to infection by *Rickettsia rickettsii*. J. Med. Entomol. 2008; 45:1156–1159. [PubMed: 19058642]

Labruna MB, Ogrzewalska M, Soares JF, Martins TF, Soares HS, Moraes-Filho J, Nieri-Bastos FA, Almeida PA, Pinter A. Experimental infection of *Amblyomma aureolatum* ticks with *Rickettsia rickettsii*. Emerg. Infect. Dis. 2011; 17:829–834. [PubMed: 21529391]

Labruna MB. Ecology of *Rickettsia* in South America. Ann. N. Y. Acad. Sci. 2009; 1166:156–166. [PubMed: 19538276]

Lee S, Kakumanu ML, Ponnusamy L, Vaughn M, Funkhouser S, Thornton H, Meshnick SR, Apperson CS. Prevalence of Rickettsiales in ticks removed from the skin of outdoor workers in North Carolina. Parasites Vectors. 2014; 7:607. [PubMed: 25533148]

Lennette, EH., Lennette, DA., Lennette, ET. American Public Health Association. Washington, DC: 1995. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections; p. 633

Levin ML, Schumacher LBM. Manual for maintenance of multi-host ixodid ticks in the laboratory. Exp. Appl. Acarol. 2016; 7:343–367.

Levin ML, Killmaster LF, Zemtsova G, Grant D, Mumcuoglu KY, Eremeeva ME, Dasch GA. Incongruent effects of two isolates of *Rickettsia conorii* on the survival of *Rhipicephalus sanguineus* ticks. Exp. Appl. Acarol. 2009; 48:347–359.

Levin ML, Killmaster LF, Zemtsova GE, Ritter JM, Langham G. Clinical presentation, convalescence, and relapse of Rocky Mountain spotted fever in dogs experimentally infected via tick bite. PLoS One. 2014a; 9:e115105. [PubMed: 25542001]

Levin ML, Zemtsova GE, Montgomery M, Killmaster LF. Effects of homologous and heterologous immunization on the reservoir competence of domestic dogs for *Rickettsia conorii* (*israelensis*). Ticks Tick-Borne Dis. 2014b; 5:33–40. [PubMed: 24201056]

Levin ML, Snellgrove AN, Zemtsova GE. Comparative value of blood and skin samples for diagnosis of spotted fever group rickettsial infection in model animals. Ticks Tick-Borne Dis. 2016; 7:1029–1034. [PubMed: 27282078]

Lima VLC, Figueiredo AC, Pignatti MG, Modolo M. Febre maculosa no município de Pedreira, Estado de São Paulo, Brasil: Relação entre ocorrência de casos e parasitismo humano por ixodídeos. Rev. Soc. Bras. Med. Trop. 1995; 28:135–137. [PubMed: 7716327]

Loftis AD, Reeves WK, Spurlock JP, Mahan SM, Troughton DR, Dasch GA, Levin ML. Infection of a goat with a tick-transmitted *Ehrlichia* from Georgia, U.S A., that is closely related to *Ehrlichia ruminantium*. J. Vector Ecol. 2006; 31:213–223. [PubMed: 17249337]

Loftis AD, Levin ML, Spurlock JP. Two USA *Ehrlichia* spp. cause febrile illness in goats. Vet. Microbiol. 2008; 130:398–402. [PubMed: 18328644]

Macaluso KR, Sonenshine DE, Ceraul SM, Azad AF. Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. J. Med. Entomol. 2002; 39:809–813. [PubMed: 12495176]

Maver MB. Transmission of spotted fever by other than Montana and Idaho ticks. J. Infect. Dis. 1911; 8:324–326.

Merten HA, Durden LA. A state-by-state survey of ticks recorded from humans in the United States. J. Vector Ecol. 2000; 25:102–113. [PubMed: 10925803]

Mitchell EA, Williamson PC, Billingsley PM, Seals JP, Ferguson EE, Allen MS. Frequency and distribution of rickettsiae borreliae, and ehrlichiae detected in human-parasitizing ticks, Texas, USA. Emerg. Infect. Dis. 2016; 22:312–315. [PubMed: 26811941]

Mock DE, Brillhart DB, Fox LB, Upton SJ. Ticks (Acari: Ixodidae) from raccoons (*Procyon lotor*) in Eastern Kansas, USA, with a new State record for *Ixodes texanus*. J. Kansas Entomol. Soc. 1991; 64:344–345.

Moncayo AC, Cohen SB, Fritzen CM, Huang E, Yabsley MJ, Freye JD, Dunlap BG, Huang J, Mead DG, Jones TF, Dunn JR. Absence of *Rickettsia rickettsii* and occurrence of other spotted fever group rickettsiae in ticks from Tennessee. Am. J. Trop. Med. Hyg. 2010; 83:653–657. [PubMed: 20810834]

Moraes-Filho J, Krawczak FS, Costa FB, Soares JF, Labruna MB. Comparative evaluation of the vector competence of four South American populations of the *Rhipicephalus sanguineus* group for the bacterium *Ehrlichia canis*, the agent of canine monocytic ehrlichiosis. PLoS One. 2015; 10:e0139386. [Electronic Resource]. [PubMed: 26414283]

Nadolny RM, Wright CL, Sonenshine DE, Hynes WL, Gaff HD. Ticks and spotted fever group rickettsiae of southeastern Virginia. Ticks Tick-Borne Dis. 2014; 5:53–57. [PubMed: 24201057]

Oliveira KA, Pinter A, Medina-Sanchez A, Boppana VD, Wikel SK, Saito TB, Shelite T, Lucas B, Popov V, Teel PD, Walker DH, Galvao MAM, Mafra C, Bouyer DH. Amblyomma imitator ticks

as vectors of *Rickettsia rickettsii*. Mexico. Emerg. Infect. Dis. 2010; 16:1282–1284. [PubMed: 20678325]

Paddock CD, Fernandez S, Echenique GA, Sumner JW, Reeves WK, Zaki SR, Remondegui CE. Rocky mountain spotted fever in Argentina. Am. J. Trop. Med. Hyg. 2008; 78:687–692. [PubMed: 18385370]

Parker RR, Philip CB, Jellison WL. Rocky Mountain spotted fever: potentialities of tick transmission in relation to geographical occurrence in the United States. Am. J. Trop. Med. 1933; 8:341–348.

Parker RR, Kohls GM, Steinhous EA. Rocky Mountain spotted fever: spontaneous infection in the tick *Amblyomma americanum*. Public Health Rep. 1943; 58:721–729. [PubMed: 19315920]

Parker RR, Pickens EG, Lackman DB, Belle EJ, Thraikill FB. Isolation and characterization of Rocky Mountain spotted fever rickettsiae from the rabbit tick *Haemaphysalis leporis-palustris* Packard. Public Health Rep. 1951; 66:455–463. [PubMed: 14816519]

Pinter A, Labruna MB. Isolation of *Rickettsia rickettsii* and *Rickettsia bellii* in cell culture from the tick *Amblyomma aureolatum* in Brazil. Ann. N. Y. Acad. Sci. 2006; 1078:523–529. [PubMed: 17114770]

Piranda EM, Faccini JLH, Pinter A, Pacheco RC, Cançado PHD, Labruna MB. Experimental infection of *Rhipicephalus sanguineus* ticks with the bacterium *Rickettsia rickettsii*, using experimentally infected dogs. Vector Borne Zoonotic Dis. 2011; 11:29–36. [PubMed: 20569011]

Pung OJ, Durden LA, Banks CW, Jones DN. Ectoparasites of opossums and raccoons in southeastern Georgia. J. Med. Entomol. 1994; 31:915–919. [PubMed: 7815408]

Sakai RK, Costa FB, Ueno TE, Ramirez DG, Soares JF, Fonseca AH, Labruna MB, Barros-Battesti DM. Experimental infection with *Rickettsia rickettsii* in an *Amblyomma dubitatum* tick colony, naturally infected by *Rickettsia bellii*. Ticks Tick-Borne Dis. 2014; 5:917–923. [PubMed: 25108783]

Savage HM, Godsey MS Jr, Lambert A, Panella NA, Burkhalter KL, Harmon JR, Lash RR, Ashley DC, Nicholson WL. First detection of heartland virus (Bunyaviridae: Phlebovirus) from field collected arthropods. Am. J. Trop. Med. Hyg. 2013; 89:445–452. [PubMed: 23878186]

Schumacher L, Snellgrove A, Levin ML. Effect of *Rickettsia rickettsii* (Rickettsiales: Rickettsiaceae) infection on the biological parameters and survival of its tick vector-*Dermacentor variabilis* (Acari: Ixodidae). J. Med. Entomol. 2016; 53:172–176. [PubMed: 26494822]

Soares JF, Soares HS, Barbieri AM, Labruna MB. Experimental infection of the tick *Amblyomma cajennense*, Cayenne tick, with *Rickettsia rickettsii*, the agent of Rocky Mountain spotted fever. Med. Vet. Entomol. 2011; 26:139–151. [PubMed: 22007869]

Springer YP, Eisen L, Beati L, James AM, Eisen RJ. Spatial distribution of counties in the continental United States with records of occurrence of *Amblyomma americanum* (Ixodida: Ixodidae). J. Med. Entomol. 2014; 51:342–351. [PubMed: 24724282]

Stothard DR, Fuerst PA. Evolutionary analysis of the spotted fever and thyphus [sic] groups of *Rickettsia* using 16S rRNA gene sequences. Syst. Appl. Microbiol. 1995; 18:52–61.

Stromdahl EY, Jiang J, Vince M, Richards AL. Infrequency of *Rickettsia rickettsii* in *Dermacentor variabilis* removed from humans, with comments on the role of other human-biting ticks associated with spotted fever group rickettsiae in the United States. Vector Borne Zoonotic Dis. 2011; 11:969–977. [PubMed: 21142953]

Stromdahl EY, Nadolny RM, Gibbons JA, Auckland LD, Vince MA, Elkins CE, Murphy MP, Hickling GJ, Eshoo MW, Carolan HE, Crowder CD, Pilgard MA, Hamer SA. Borrelia burgdorferi not confirmed in human-biting *Amblyomma americanum* ticks from the southeastern United States. J. Clin. Microbiol. 2015; 53:1697–1704. [PubMed: 25788545]

Troughton DR, Levin ML. Life cycles of seven ixodid tick species (Acari: Ixodidae) under standardized laboratory conditions. J. Med. Entomol. 2007; 44:732–740. [PubMed: 17915502]

Walker DH, Harrison A, Henderson F, Murphy FA. Identification of Rickettsia rickettsii in a guinea pig model by immunofluorescent and electron microscopic techniques. Am. J. Pathol. 1977; 86:343–358. [PubMed: 402079]

Weller SJ, Baldridge GD, Munderloh UG, Noda H, Simser J, Kurtti TJ. Phylogenetic placement of rickettsiae from the ticks *Amblyomma americanum* and Ixodes scapularis. J. Clin. Microbiol. 1998; 36:1305–1317. [PubMed: 9574696]

Zemtsova G, Killmaster LF, Mumcuoglu KY, Levin ML. Co-feeding as a route for transmission of *Rickettsia conorii israelensis* between *Rhipicephalus sanguineus* ticks. Exp. Appl. Acarol. 2010; 52:383–392. [PubMed: 20589416]

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Table 1

Acquisition and transstadial transmission of *R. rickettsii* by *A. americanum* larvae.

Source of infection	Isolate of *R. rickettsii*	Molted nymphs	
		Positive/ Tested	% ± St. Err.
Needle-inoculated guinea pigs	DI-6	37/75	49.3 ± 5.8
	AZ-3	25/75	33.3 ± 5.5
Simultaneous feeding with infected nymphs	DI-6	25/225	11.1 ± 2.1
	AZ-3	21/225	9.3 ± 1.9

Table 2

Signs of rickettsial infection in guinea pigs after exposure to *A. americanum* nymphs infected with *R. rickettsii*.

Signs of infection	Exposed to nymphs infected with	
	DI-6	AZ-3
Fever	7/9	5/9
Edema and dermatitis	7/9	8/9
Rickettsial DNA in skin & blood	7/9	8/9
Rickettsial DNA in internal organs	7/9	8/9
Seroconversion	9/9	9/9

Table 3

Transovarial transmission of *R. rickettsii* by infected *A. americanum* females.

Isolate of *R. rickettsii*	Prevalence of infection in adult ticks (% ± St. Err.)		No infected females	No infected larval batches	Frequency of transovarial transmission (% ± St. Err.)
	Prior to feeding	Engorged females			
DI-6	68.0 ± 9.5	98.0 ± 1.4	97	27	27.8 ± 4.6
AZ-3	52.0 ± 10.2	70.7 ± 4.8	65	9	13.8 ± 4.3