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Experimental infection and vector competence of Amblyomma patinoi, a member of the Amblyomma cajennense species complex, for the human pathogen Rickettsia rickettsii

Heidy-C Martínez-Diaz [a], Elkin Forero-Becerra [b], Marylin Hidalgo [a], Marcelo B. Labruna [c,*]

[a] Grupo de Enfermedades Infecciosas, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, D.C., Colombia
[b] Estancia postdoctoral – Convocatoria Colciencias 784/2017, Laboratorio de Bacteriología Especial, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, D. C., Colombia
[c] Departamento de Medicina Veterinária Preventiva e Saúde Animal, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo-USP, Av. Prof. Orlando Marques de Paiva, 87, São Paulo, SP 05508-270, Brazil

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ABSTRACT

Amblyomma patinoi ticks infected with *Rickettsia rickettsii* are present in Colombia, but its vector competence is unknown. Hence, we evaluated the vector competence of *A. patinoi* with *R. rickettsii* under laboratory conditions. Experimental guinea pigs and rabbits (males and females) were separated in the infected group (IG) and the control group (CG). In the IG, the filial 1 (F1) larvae (*R. rickettsii*-free) from Colombian *A. patinoi* engorged female specimens were exposed to *R. rickettsii* (ITU strain) by feeding on infected guinea pigs. Next, F1 nymphs and adults, and F2 larvae were allowed to feed on uninfected guinea pigs or rabbits and tested by qPCR targeting the *gltA* rickettsial gene. All animals used to feed the IG F1 ticks became febrile and had *R. rickettsii* infection (89% fatality rate) detected through serological or molecular techniques. After the F1 larvae ticks became *R. rickettsii* infected, subsequent IG tick stages were able to maintain the rickettsial infection by transstadial maintenance to all infested animals, indicating *A. patinoi* vector competence. Subsequently, almost 31% of the F1 female egg masses and only 42% of their F2 larvae were infected. Less than 50% of the infected females transmitted *R. rickettsii* transovarially, and only a part of the offspring were infected. This study demonstrated that *A. patinoi* might not be able to sustain *R. rickettsii* infection by transovarial transmission for successive tick generations without horizontal transmission via rickettsemic hosts. This condition might result in low *R. rickettsii*-infection rates of *A. patinoi* under natural conditions.

1. Introduction

The bacterium *Rickettsia rickettsii* is the causative agent of Rocky Mountain spotted fever, a life-threatening illness also known as Brazilian spotted fever in Brazil (Labruna, 2009) or Tobia spotted fever in Colombia (Patino et al., 1937). This pathogen is restricted to the Americas, where it has been reported in Argentina, Brazil, Canada, Colombia, Costa Rica, the United States, Mexico, and Panama (Dumler and Walker, 2005; Labruna et al., 2011). In many of these countries, the detection of rickettsial agents was carried out during the first half of the 20th century. After a long period of epidemiological silence, rickettsial infections have re-emerged as a public health problem in the last decade of the 20th century and the beginning of the 21st century (Dumler and Walker, 2005).

In Colombia, several studies have been carried out aiming to understand tick-borne diseases, including rickettsioses, since the 1930s (Patino et al., 1937). In the 21st century, several outbreaks of human rickettsiosis by *R. rickettsii* occurred in different regions of the country: Villeta (Cundinamarca) in 2004; Necoclí (Antioquia) in 2006; Los Córdobas (Córdoba) in 2007; and Turbo (Antioquia) in 2008 (Acosta et al., 2006; Hidalgo et al., 2011, 2007a; Pacheco et al., 2008).

Currently, different tick species have been involved as vectors of

Abbreviations: RH, relative humidity; *gltA*, rickettsial citrate synthase gene; IG, infected group; CG, control group; F1, first laboratory generation; F2, second laboratory generation; CEI, Conversion Efficiency Index; TOT, Transovarial Transmission Rate; FIR, Filial Infection Rate; dpi, days post-infestation; IFA, Indirect Immunofluorescence Assay; SP, São Paulo (Brazil).

* Corresponding author.
E-mail address: labruna@usp.br (M.B. Labruna).

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R. rickettsii in different geographical regions: *Dermacentor andersoni* in the western and *Dermacentor variablilis* in the midwestern and eastern of the United States (Ammerman et al., 2004; Demma et al., 2005; Labruna, 2009), *Rhipicephalus sanguineus* sensu lato (s.l.) ticks in a few areas of the state of Arizona in the United States and Northern Mexico (Castillo- Martínez et al., 2015; Demma et al., 2005), and *Amblyomma aureolatum* in the metropolitan area of São Paulo (SP, Brazil) and *Amblyomma sculptum* in southeastern Brazil (Katz et al., 2009; Labruna, 2009).

Ticks of the *Amblyomma cajennense* species complex are also main vectors of *R. rickettsii* in some areas of Central and South Americas (Labruna, 2009). This complex is composed by six morphologically and genetically related species distributed in specific geographic niches from southern Texas (U.S.A.) to northern Argentina, where they feed on a variety of vertebrate hosts, mostly domestic and wild medium- to large-sized mammals (Estrada-Peña et al., 2014; Nava et al., 2014). This species complex contains some of the most important human-biting ticks in the Neotropical region (Guglielmone and Robbins, 2018).

The species *Amblyomma patinoi* is a member of the *A. cajennense* complex. In 2015, *R. rickettsii* was reported in this tick species considering it at that time, the main vector of *R. rickettsii* under natural conditions in Villeta (Cundinamarca), Colombia (Faccini-Martínez et al., 2015). RMSF-confirmed cases (Hidalgo et al., 2007b; Patino et al., 1937) and seroprevalence for *R. rickettsii* in domestic animals (Hidalgo et al., 2009) and humans (Hidalgo et al., 2007c) have been reported in Villeta (Cundinamarca), where natural infestations by *A. patinoi* has been reported on domestic animals (Faccini-Martínez et al., 2017). *Amblyomma patinoi*, together with *Amblyomma mixtum*, are the only two species of the *A. cajennense* complex known to occur in Colombia (Nava et al., 2014; Rivera-Paez et al., 2016), but so far, the competence to transmit *R. rickettsii* to susceptible hosts has not been experimentally established.

Vector competence is a term used to describe the ability of an arthropod to serve as vector of a given pathogen and is related to the intrinsic susceptibility of the arthropod species and the pathogen capacity for replication within the vector and the transmission to a susceptible host (Cox et al., 2011). Therefore, this study aimed to evaluate the susceptibility and vector competence of *A. patinoi* to transmit *R. rickettsii* under laboratory conditions.

2. Materials and methods

2.1. Ticks

Amblyomma patinoi engorged females were collected on cattle (*Bos taurus*) in a farm at Villeta municipality (Cundinamarca, Colombia), and taken to the laboratory and incubated at a temperature of 25 °C, >95% relative humidity (RH), and photoperiod 0:24 (light: dark) until obtaining larvae to start the tick colony that was used in the present study. Throughout the present study, free-living tick stages were always held in a single incubator under the same conditions.

To establish that these *A. patinoi* larvae were free of rickettsial infection, we tested the progenitor females at the end of oviposition and a pool of eggs from each offspring (1 egg pool containing 20–30 eggs/female) by real-time PCR (qPCR) targeting the rickettsial citrate synthase (*gltA)* gene, following the instructions described in Soares et al. (2012).

2.2. Rickettsia rickettsii strain

Rickettsia rickettsii Itu strain was used in this study. This strain was originally isolated from *A. sculptum* (another member of the *A. cajennense* complex) from the municipality of Itu in the state of São Paulo, southeast Brazil, through the inoculation of guinea pigs with tick pool homogenates (Krawczak et al., 2014; Labruna et al., 2014). Only the guinea pig lineage of Itu strain (with no in vitro passage) was used in the experimental infections.

The inoculum preparation was performed according to Soares et al. (2012). Briefly, fragments of cryopreserved organs (spleen, liver, lung) of *R. rickettsii*-infected guinea pigs were thawed at room temperature, macerated in a mortar with brain-heart infusion broth, and filtered through sterile gauze. Subsequently, the filtrate was inoculated intraperitoneally in experimental guinea pigs (Soares et al., 2012).

2.3. Tick infestation protocols

Male and female of both New Zealand white rabbits (*Oryctolagus cuniculus*) and Hartley guinea pigs (*Cavia porcellus*) were used to feed adults and immature ticks, respectively. All animals were obtained from an animal facility, and each selected animal was used to feed a single tick stage. Animals were fed with commercial pellets, drinking water ad libitum, and they were not subjected to antibiotics or acaricide treatments during the experiment. Larvae, nymphs or adults, 10 to 15 days old, were used for animal infestations. Larval infestations consisted of approximately 1000 – 2000 larvae per guinea pig; nymphal infestations consisted of approximately 300 nymphs per guinea pig, and adult infestations consisted of 20 to 25 couples per rabbit. For the tick infestations on guinea pigs or rabbits, plastic feeding chambers were glued onto the shaved dorsum of each animal, as previously described (Horta et al., 2009; Pinter et al., 2002). Feeding chambers were opened daily, and the detached engorged ticks were removed, counted, and immediately taken to the incubator (25 °C and >95% RH) for molting (for engorged larvae and nymphs) or oviposition (for engorged females).

The study protocol was approved by the Animal Ethical Commission of the Faculty of Veterinary Medicine of the University of São Paulo (protocol nos. 5948070314, 3104/2013).

3. Experimental protocol

In this study two groups were formed, the infected group (IG) and the control group (CG). Initially, three guinea pigs were each inoculated intraperitoneally with *R. rickettsii* for acquisition feeding of IG ticks, and three other uninfected guinea pigs were used to feed the ticks of the CG. Then both IG and CG guinea pigs were infested by *Rickettsia*-free *A. patinoi* F_1 larvae (first laboratory generation) on day two post-inoculation. Engorged larvae retrieved from both infected and control groups were separately taken to the incubator for molting.

A portion of the resultant F_1 nymphs were used to infest six new uninfected guinea pigs (300 nymphs/host): three animals were infested with IG ticks, and the remained three animals were infested with CG ticks. Engorged F_1 nymphs retrieved in these infestations were separately taken to the incubator for molting.

A portion of the subsequent F_1 adults were used to infest six uninfected rabbits (20 to 25/rabbit) in a similar way to the previous nymphal stage (3 hosts for each experimental group). Engorged females retrieved were individually taken to the incubator until oviposition for obtaining the second larval generation (F_2).

Following the same methodology, the resultant F_2 larvae fed on six new uninfected guinea pigs following the same experimental protocol for the infected and control groups.

In summary, only the filial 1 (F_1) larvae of the IG were exposed to *R. rickettsii* by feeding on guinea pigs that had been inoculated intraperitoneally with *R. rickettsii* (acquisition feeding). The subsequent stages (F_1 nymphs and adults, and F_2 larvae) were allowed to feed on uninfected guinea pigs or rabbits in order to test their vector competence and to establish if *A. patinoi* larvae could acquire and maintain *R. rickettsii* infection by transstadial and transovarial perpetuations until the end of the experiment, the filial 2 (F_2) larvae.

3.1. Tick biological parameters

Throughout the experiments, the following tick biological parameters were evaluated: infection rates (for unfed F_1 nymphs, adults, and F_2

larvae); feeding period (number of days from releasing unfed ticks inside feeding chamber to detachment of engorged ticks); molting success (the proportion of engorged larvae or nymphs that successfully survived to the next stage in relation to the total number of engorged larvae or nymphs); oviposition success (proportion of engorged females that successfully oviposited); and the percentage of egg hatching (the proportion hatched larvae in relation to the total egg mass), which was visually estimated as previously determined (Labruna et al., 2000). Because of the large number of F_1 engorged larvae that were recovered from guinea pigs, molting success in this case was calculated for a random sample of 300 viable engorged larvae from each guinea pig.

All engorged females were individually weighed in an electronic balance (precision of 0.1 mg) in the day of detachment from host. The total egg mass deposited by each female was weighed at the end of oviposition and used to calculate the conversion efficiency index (CEI = mg of egg mass/mg of engorged female x 100). CEI measures the efficiency to which an engorged female converts its body weight into eggs (Drummond and Whetstone, 1970). This was determined for each female that successfully oviposited.

Additionally, it was calculated the transovarial transmission rate (TOT) of rickettsiae, based on the molecular analyses of ticks for rickettsial DNA, as follows: TOT = No. females with PCR-positive eggs / No. PCR-positive females at the end of oviposition x 100. TOT measures the proportion of infected females that gave rise to at least one infected egg (Gerardi et al., 2019; Soares et al., 2012). Also, the filial infection rate (FIR) was calculated for each female with positive TOT, as follows: FIR = No. PCR-positive larvae / No. tested larvae x 100. FIR means the proportion of infected larvae in relation to the total number larvae derived from each infected egg mass (Gerardi et al., 2019; Soares et al., 2012).

3.2. Evaluation of experimental animals

All infested animals had their rectal temperature measured from 0 to 21 days post-infestation (dpi). Guinea pigs were considered febrile when rectal temperatures were >39.5 °C (Monteiro, 1931); rabbits were considered febrile when rectal temperatures were >40.0 °C (Monteiro, 1933). The occurrence of scrotal reactions was observed as a characteristic of acute rickettsial infection in these animals (Monteiro, 1933).

Blood samples were collected intracardially from guinea pig, and ear vein from rabbits at 21 dpi. These samples were collected under anesthesia (25 mg/kg ketamine and 5 mg/kg de xylazine). Approximately 0.5–1.0 ml of blood were obtained from each animal and centrifuged at 2500 x *g* for 10 min to obtain sera in order to test for the presence of anti-*R. rickettsii* reactive antibodies (protocol described below). All animals that died before 21 dpi were subjected to necropsy and a spleen sample was collected and evaluated by qPCR for rickettsial infection.

3.3. Molecular analysis

Individual samples of unfed larvae, nymphs, and adults, as well as individual engorged females at the end of oviposition, egg pools (20–30 eggs/female), and guinea pig and rabbit spleen samples were tested by qPCR for detection of rickettsial DNA. DNA extraction of nymphs, adults, and eggs was performed using guanidine thiocyanate (Sangioni et al., 2005). For larvae, the boiling method was used (Horta et al., 2005), and spleen samples were processed by the DNeasy tissue kit (Qiagen, Chatsworth, CA, United States). Blank tubes were always included in the DNA extraction procedures, as negative controls of this step.

All DNA samples were processed individually using a Taqman real-time PCR assay (qPCR) using primers CS-5 (5-GAG AGA AAA TTA TAT CCA AAT GTT GAT-3) and CS-6 (5-AGG GTC TTC GTG CAT TTCTT-3), which amplify a 147-bp fragment of the *gltA* gene (Labruna et al., 2004). A fluorogenic probe specific for the genus *Rickettsia* [5′−6-FAMd (CAT TGT GCC ATC CAG CCT ACG GT) BHQ-1 3′] (Integrated DNA Technologies, Inc., San Diego, CA, U.S.A.) was used following the instructions described in Soares et al. (2012).

Each qPCR run included at least two control wells, negative (DNAse free water) and positive control (*Rickettsia vinii* DNA). Tick DNA samples that were negative for rickettsial DNA were tested in a conventional PCR protocol using forward (5′-CCG GTC TGA ACT CAG ATC AAG T-3′) and reverse (5′-GCT CAA TGA TTT TTT AAA TTG CTG T-3′) primers, which amplify a 460 bp-fragment of the tick 16S rRNA mitochondrial gene, as previously described (Mangold et al., 1998). If this PCR assay did not amplify amplicon of expected size, it was considered that DNA extraction was not efficient, and the sample was not included in the results.

3.4. Serological analysis

Guinea pig and rabbit serum samples were individually tested by the indirect immunofluorescence assay (IFA) using crude antigens derived from *R. rickettsii* Taiaçu strain, as previously described (Labruna et al., 2007). Each serum was diluted in two-fold increments with PBS starting from the screening dilution (1/64) to the endpoint titer (Labruna et al., 2007). A commercial fluorescein isothiocyanate-labeled goat anti-rabbit IgG whole molecule (Sigma Diagnostics, St. Louis, MO, United States) or rabbit anti-guinea pig IgG whole molecule (Sigma Diagnostics) was used. In each slide, a negative control (a serum previously known to be non-reactive, titer < 64), and positive control (a known reactive serum with endpoint titer ≥ 64) were included at the 1/64 dilution. The reactive and non-reactive sera of guinea pigs and rabbits derived from a previous study where these two host species were experimentally infected with *R. rickettsii* (Soares et al., 2012).

4. Statistical analyses

Molting and oviposition success values were compared between IG and CG ticks by using the Chi-square test. The parameters of engorged female weight, egg mass weight, CEI, and egg percentage of hatching were compared between IG and CG groups by the t-Student *t*-test when the data presented a normal distribution, or Mann–Whitney test when the data presented an abnormal distribution using GraphPad Prism version 7.0 for Windows (GraphPad Software, United States). Variables were considered significantly different if $P<0.05$.

5. Results

No rickettsial DNA was detected in the field-collected engorged females or their egg pools; therefore, the F_1 offspring of *A. patinoi* was considered to be free of rickettsial infection at the beginning of this study. The intraperitoneal inoculation of the three guinea pigs with *R. rickettsii* inocula (for acquisition feeding) resulted in fever between 40.1 °C to 40.4 °C that started four days after inoculation. The three guinea pigs of the IG that were inoculated with *R. rickettsii* presented lethargy characterized by decreased movement and consumption of food. Additionally, it was observed scrotal reactions and death on the day 6th or 9th-day post-inoculation. Their spleen samples were qPCR-positive for the rickettsial *gltA* gene. During acquisition feeding on these animals, larval feeding overlapped with the febrile period, ensuring that these ticks fed during the rickettsemic period, and therefore, were exposed to *R. rickettsii*. The guinea pigs used in the CG did not present any clinical alteration until the 21st day, when they were seronegative for *R. rickettsii*. Details of the clinical data of all phases of the trials are summarized in Table 1.

Engorged F_1 larvae and nymphs were detached from guinea pigs 6 to 9 dpi and 3 to 12 dpi, respectively, while engorged F_1 females detached from rabbits 7–19 dpi, and F_2 larvae detached from guinea pigs 3 to 9 dpi. All nine animals (six guinea pigs and three rabbits) that were used to feed IG F_1 ticks became febrile and their rickettsial infection was attested by detection of rickettsial DNA in a spleen sample collected from eight animals that died during the febrile period (89% fatality rate), or

Table 1
Clinical data of guinea pigs (GP1 to GP18) and rabbits (R1 to R6) that were exposed to *Rickettsia rickettsii* via inoculation or tick infestations (IG group) or to uninfected ticks (CG group) of the species *Amblyomma patinoi*.

Tick stage	Group	Source of infection	Hosts	Number ticks/host	Fever period [#]	Death[#]	Scrotal reaction	Diagnostic test*	
								qPCR	IFA titer
Larva (F_1)	IG	Intraperitoneal inoculation	GP1	1000–2000	4–7	9	Yes	Positive	n.a.
			GP2		4	6	Yes	Positive	n.a.
			GP3		4–7	9	Yes	Positive	n.a.
	CG	Uninfected control	GP4	1000–2000	No	No	No	n.a.	<64
			GP5		No	No	No	n.a.	<64
			GP6		No	No	No	n.a.	<64
Nymph (F_1)	IG	F_1 larvae exposed to *R. rickettsii*	GP7	300	6–9	12	No	Positive	n.a.
			GP8		5–9	11	Yes	Positive	n.a.
			GP9		5–8	11	Yes	Positive	n.a.
	CG	Uninfected control	GP10	300	No	No	No	n.a.	<64
			GP11		No	No	No	n.a.	<64
			GP12		No	No	No	n.a.	<64
Adult (F_1)	IG	F_1 larvae exposed to *R. rickettsii*	R1	20–25 couples	6–13	No	No	n.a.	32,768
			R2		6–9	12	Yes	Positive	n.a.
			R3		12–16[&]	19	Yes	Positive	n.a.
	CG	Uninfected control	R4	20–25 couples	No	No	No	n.a.	<64
			R5		No	No	No	n.a.	<64
			R6		No	No	No	n.a.	<64
Larva (F_2)	IG	F_1 larvae exposed to *R. rickettsii*	GP13	1000–2000	No	No	No	n.a.	<64
			GP14		5–9	No	Yes	n.a.	2048
			GP15		No	No	No	n.a.	<64
	CG	Uninfected control	GP16	1000–2000	No	No	No	n.a.	<64
			GP17		No	No	No	n.a.	<64
			GP18		No	No	No	n.a.	<64

[#] Numbers refer to days post-infestation with ticks, or in the case of GP1 to GP3, to days post-inoculation with *R. rickettsii*.

*Diagnostic test of rickettsial infection relied on the detection of rickettsial DNA by real-time PCR (qPCR) in the spleen of animals that died during the febrile period, or on the serological detection of anti-*R. rickettsii* endpoint titers by immunofluorescence assay (IFA) 21 days after tick infestation (n.a.: not available).

[&] Delay in the onset of the fever because the infected ticks took approximately six days to attach to host skin.

through seroconversion to *R. rickettsii* at 21 dpi (endpoint titer of 32,768) in one rabbit that did not die (Table 1).

Molecular analyses of ticks revealed that none of the CG ticks contained rickettsial DNA. Regarding the IG ticks, rickettsial DNA was detected in 28.3% and 83.3% of the F_1 nymphs and adults, respectively (Table 2). These results indicate that after larval acquisition feeding on *R. rickettsii*-infected guinea pigs, part of the IG ticks were able to maintain the rickettsial infection to subsequent stages by transstadial perpetuation. In addition, this perpetuation was followed by successful vector competence, because all guinea pigs and rabbits that were infested with these ticks became infected (Table 1).

Molting success of engorged larvae to nymphs was always high and statistically similar ($P>0.05$) for CG ticks (97.2%) and IG ticks (99.2%) (Table 3). For engorged nymphs to adults, molting success was also high for IG ticks (overall rate: 84.8%); however, due to logistic problems, we did not take note of this rate for CG engorged nymphs. The oviposition success of engorged females was also similar ($P>0.05$) between CG (100%) and IG (98.4%) groups (Table 3).

Right after detachment, the weight of IG engorged females (mean: 684.8 mg) was significantly higher ($P = 0.0012$) than the weight of CG engorged females (mean: 600.6 mg). A similar trend was observed for egg mass weight (383.8 mg for IG versus 328.7 mg for CG; $P = 0.0014$). Because the conversion efficiency index (CEI) values of the IG (55.5%) and CG (54.3%) females were not significantly different ($P = 0.3254$), females of both groups proportionally converted body weight into eggs at similar rates (Table 4). On the other hand, the percentage of the egg mass hatching of CG females (mean: 85.7%) was significantly higher ($P<0.0001$) than those of IG females (mean: 61.4%) (Table 4; Fig 1).

All 66 (100%) engorged females of the IG group yielded rickettsial DNA at the end of oviposition (Table 2). However, rickettsial DNA was detected in eggs pools of only 11.8% to 52.2% of these females, indicating an overall TOT rate of 30.8% for the 65 females that oviposited (Table 4). For all females that yielded *Rickettsia*-infected eggs, F_2 larvae

Table 2
Infection rates by rickettsiae, determined by qPCR, in *Amblyomma patinoi* ticks of the infected group (IG) and control group (CG) of the present study, after being exposed to *Rickettsia rickettsii* acquisition feeding during the F_1 larval stage.

Host of the latest feeding (see Table 1)	Tick stage tested by PCR	Number of infected ticks/ Number of tested ticks (% infection)
GP1 (larval feeding)	Unfed nymph (F_1) after molting	1/20 (5)
GP2 (larval feeding)		15/20 (75)
GP3 (larval feeding)		1/20 (5)
Total		17/60 (28.3)
GP4 (larval feeding)	Unfed nymph (F_1) after molting	0/10 (0)
GP5 (larval feeding)		0/10 (0)
GP6 (larval feeding)		0/10 (0)
Total		0/30 (0)
GP7 (nymphal feeding)	Unfed adult (F_1) after molting	5/10 (50)
GP8 (nymphal feeding)		10/10 (100)
GP9 (nymphal feeding)		10/10 (100)
Total		25/30 (83.3)
GP10 (nymphal feeding)	Unfed adult (F_1) after molting	0/10 (0)
GP11 (nymphal feeding)		0/10 (0)
GP12 (nymphal feeding)		0/10 (0)
Total		0/30 (0)
R1 (adult feeding)	Engorged female (F_1) at the end of oviposition	25/25 (100)
R2 (adult feeding)		24/24 (100)
R3 (adult feeding)		17/17 (100)
Total		66/66 (100)

Table 3
Molting and oviposition success of *Amblyomma patinoi* ticks of the control group (CG) and infected group (IG).

Group	Replicates *	No. larvae that molted to nymphs/No. engorged larvae	No. nymphs that molted to adults/ No. engorged nymphs	No. females that oviposited/No. engorged females
		(% molting success)	(% molting success)	(% oviposition success)
CG	1	291/300 (97)	Not evaluated	23/23 (100)
	2	292/300 (97.3)	Not evaluated	20/20 (100)
	3	292/300 (97.3)	Not evaluated	25/25 (100)
	Total	875/900 (97.2)		68/68 (100)
IG	1	298/300 (99.3)	160/261 (61.3)	25/25 (100)
	2	296/300 (98.6)	175/175 (100)	23/24 (95.8)
	3	299/300 (99.6)	229/229 (100)	17/17 (100)
	Total	893/900 (99.2)	564/665 (84.8)	65/66 (98.4)

* each replicate refers to engorged larvae or nymphs or females collected from an individual host.

were tested individually to calculate the FIR, the proportion of infected larvae in each infected egg mass. The overall FIR was 42.2% among a total of 180 F_2 larvae that were tested individually (Table 4).

Infestations of three guinea pigs with IG F_2 larvae resulted in rickettsial disease in only one animal (GP14), which seroconverted to *R. rickettsii* at an endpoint of 2048 at 21 dpi. The other two guinea pigs (GP13, GP15) did not show clinical alterations nor seroconversion to *R. rickettsii,* similarly to the three guinea pigs (GP16, GP17, GP18) that were infested with CG F_2 larvae (Table 1). These results are in accordance with the above results of TOT and FIR, which demonstrated that overall, <50% of the infected females transmitted *R. rickettsii* transovarially, and when they did, it was to only a portion of the larval offspring.

6. Discussion

This study demonstrated that *A. patinoi* larvae were competent at maintaining *R. rickettsii* infection through transstadial perpetuation after exposure to rickettsemic animals under experimental conditions. Likewise, part of the females that were first exposed to *R. rickettsii* as larvae were capable of transmitting the infection by the transovarial route to their progeny. Moreover, *A. patinoi* ticks were able to transmit *R. rickettsii* to susceptible hosts and generate disease, most of time with a fatal outcome. Thereby, we confirmed the vector competence of this tick species for *R. rickettsii*, which resulted in a fatality rate of 89% for hosts infested with F_1 nymphs and adults. These fatality rates are within the range of other studies that evaluated the vector competence of *A. sculptum* and *Amblyomma tonelliae* (another species of the *A. cajennense* complex) for *R. rickettsii* by using the same methods of the present study (Gerardi et al., 2019; Soares et al., 2012; Tarragona et al., 2016).

After larval acquisition feeding, the overall infection rate with *R. rickettsii* was 28.3% in F_1 nymphs and 83.3% in F_1 adults, which transmitted *R. rickettsii* through feeding to susceptible hosts. The different infection rates between unfed nymphs and adults was likely related to guinea pig- developed rickettsemia, which overlapped, at least in part, with the nymphal feeding period, resulting in a higher infection rate in the subsequent adult ticks. This transmission mechanism, called horizontal transmission (Harris et al., 2017), has long been considered an essential mechanism in the ecology of *R. rickettsii* (Labruna, 2009; Philip, 1959). This statement is corroborated by the 100% infection rate of engorged females, whose feeding period might also have overlapped with the rickettsemic period of the febrile rabbits.

The infection rates reported in this study are higher than those described for other species of the *A. cajennense* complex, such as 0% to 16% for nymphs and adults, respectively, reported with *R. rickettsii* Taiaçu strain (Soares et al., 2012). In the same way, *A. tonelliae* had infection rates from 8.8% to 66.9% for nymphs and adults, respectively, using the *R. rickettsii* ITU strain (Tarragona et al., 2016). In both studies, infection rates were higher in unfed adults than in unfed nymphs similarly to the present study. Also, in both previous studies authors evaluated the acquisition feeding in the larval stage with the same methods here employed.

Gerardi et al. (2019) performed a comparative evaluation on the susceptibility of six *A. sculptum* populations to *R. rickettsii* infection, with non-autochthonous and autochthonous strains of *R. rickettsii*. The authors found that infection rates with the *R. rickettsii* autochthonous strains were 8% to 16% for nymphs and 33 to 47% for adults. Also, they showed infection rates of 0% to 3% for nymphs, and 0% for adults with the non-autochthonous strain. Interestingly, that study showed that *A. sculptum* ticks might have a distinct infection susceptibility for different *R. rickettsii* strains.

Although the Colombian *A. patinoi* specimens were infected with a non-autochthonous *R. rickettsii* strain, they showed higher infection rates than those observed with other species of the *A. cajennense* complex, when infected with native Brazilian *R. rickettsii* strains. Such

Table 4
Biological and reproductive parameters of *Amblyomma patinoi* engorged females and their egg masses from the control group (CG) and infected group (IG).

Group	Repli-cates	Engorged female weight (mg)*	Egg mass weight (mg) per female*	CEI (%)*	% egg hatching*	TOT (%)	FIR (%)
CG	1	617.1 ± 88.1 (394.0 - 826.4)	355.0 ± 57.1 (238.6 – 510.5)	57.0 ± 2.7 (52 - 62)	77.4 ± 21.5 (20 - 100)		
	2	716.5 ± 133.3 (478.9 – 917.7)	369.4 ± 112.5 (104.3 – 516.7)	50.8 ± 10.5 (18 - 61)	92.7 ± 13.4 (50 - 100)		
	3	489.3 ± 134.5 (277.8 – 745.7)	273.1 ± 93.1 (79.2 – 433.7)	54.8 ± 7.0 (29 - 62)	87.8 ± 19.3 (15 - 100)		
	Total	600.6 ± 151.4 (277.8 – 917.7)	328.7 ± 98.7 (79.2 – 516.7)	54.3 ± 7.6 (18 - 62)	85.7 ± 19.4 (15 - 100)		
IG	1	642.8 ± 118.1 (407.0 – 966.5)	357.3 ± 81.2 (178.8 – 498.6)	55.5 ± 7.0 (35 - 62)	56.8 ± 25.4 (1 - 99)	6/25 (24.0)	0/50 (0)
	2	745.4 ± 152.0 (400.3 – 989.8)	430.2 ± 102.2 (195.4 – 559.8)	56.5 ± 6.0 (36 - 66)	72.2 ± 27.3 (5 - 95)	12/23 (52.2)	75/110 (68.2)
	3	661.4 ± 138.3 (438.4 – 893.8)	360.0 ± 83.9 (250.5 – 509.6)	54.3 ± 4.3 (48 - 61)	53.5 ± 19.0 (10 - 90)	2/17 (11.8)	1/20 (5.0)
	Total	684.8 ± 142.2 (400.3 – 989.8)	383.8 ± 95.0 (178.8 – 559.8)	55.5 ± 6.0 (35 - 66)	61.4 ± 25.6 (1 - 99)	20/65 (30.8)	76/180 (42.2)

CEI: Conversion efficiency index = egg mass weight / engorged female weight x100.
TOT: transovarial transmission rate (number females with PCR-positive eggs/number PCR positive females at the end of oviposition x 100).
FIR: Filial infection rate (number infected larvae/number tested larvae x 100).
* Values presented as mean ± standard deviation (range in parenthesis).

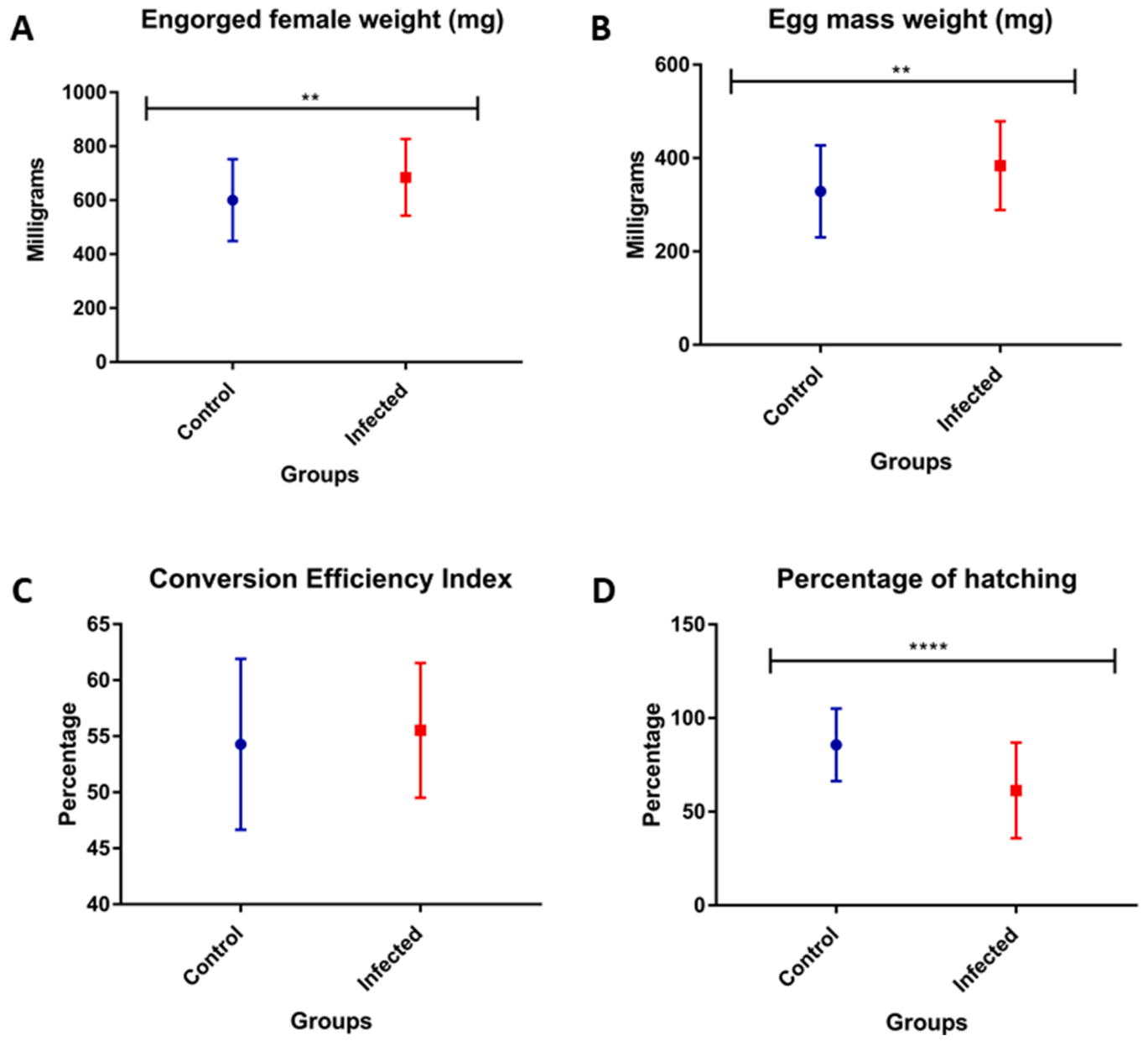

Fig 1. Biological and reproductive parameters of *Amblyomma patinoi* engorged females of the control group (uninfected) and infected group (females infected by *Rickettsia rickettsii*). Values are presented as mean ± standard deviation. (*) $P = < 0.05$ for statistical significance.
A. Engorged female weight. B. Egg mass weight C. Conversion efficiency index. D. Percentage of hatching.

differences might be related to a higher susceptibility of *A. patinoi* than *A. sculptum* to *R. rickettsii,* as it has already been demonstrated for *A. aureolatum* and *R. sanguineus* s.l., which are much more susceptible than *A. sculptum* to *R. rickettsii* infection (Labruna et al., 2008). The exact mechanisms or causes for these different susceptibilities are not yet established. However, it might be attributed to the different intrinsic genetic factors of the tick that affect its ability to transmit a pathogen, as well as to the tick microbiome that can be involved in tick-pathogen interactions (de la Fuente et al., 2017). Moreover, differences in the infection rates might not be attributed to *R. rickettsii* virulence or genetic polymorphism, which seem to not vary among South American isolates of *R. rickettsii* (Labruna et al., 2014).

The present TOT percentages ranged from 11.8% to 52.2%, similarly to those values reported for *A. sculptum* (Gerardi et al., 2019; Soares et al., 2012) and *A. tonelliae* (Tarragona et al., 2016), two other members of the *A. cajennense* species complex (Nava et al., 2014). It has been established that the efficiency of rickettsial transovarial transmission in ticks depends on the degree of rickettsial development in the ovarian tissues (Burgdorfer and Brinton, 1975). In the same way and following previous results in several *A. cajennense* complex species, the most effective transovarial transmission of *R. rickettsii* occurred when the primary infection was acquired during the nymphal feeding. In our study, the primary acquisition feeding was on the larval stage; however, we cannot exclude additional acquisition feeding during the nymphal stage, whose feeding period overlapped with the febrile period (possibly rickettsemic) guinea pigs.

While high rates of molting and oviposition success were observed for both uninfected and infected groups of ticks in the present study, there were some significant differences in the reproductive performance, with slightly higher weights of engorged females and eggs masses for the infected group, in contrast to a more marked difference in the egg hatching percentage, being significantly lower for the infected group. Such lower egg viability could be related to rickettsial infection, as previously reported for *A. sculptum* (Gerardi et al., 2019; Soares et al.,

2012).

This study demonstrated that *A. patinoi* ticks were partially refractory to *R. rickettsii* infection. Moreover, the overall TOT and FIR rates were <50% for the infected females, indicating that *A. patinoi* might not be able to sustain *R. rickettsii* infection by transovarial transmission for successive tick generations, without horizontal transmission via rickettsemic hosts (amplifying hosts). This condition might result in low *R. rickettsii*-infection rates of *A. patinoi* under natural conditions (Faccini-Martínez et al., 2015). Unfortunately, amplifying hosts for *R. rickettsii* in Colombia have never been reported, in contrasting to the situation of southeastern Brazil, where capybaras (*Hydrochoerus hydrochaeris*), and opossums (*Didelphis* spp.) to a lesser extent, are recognized as efficient amplifying hosts of *R. rickettsii* for *A. sculptum* ticks (Horta et al., 2009; Ramírez-Hernández et al., 2020).

Finally, human rickettsiosis is not a notifiable disease in Colombia despite several reports of active and fatal cases, along with seroprevalence in humans and animals in the same region. Therefore, more studies are required in Colombia on this topic for *A. patinoi* in order to strengthen and complement the knowledge of the epidemiology of rickettsiosis in the country.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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References

Acosta, J., Urquijo, L., Díaz, A., Sepúlveda, M., Mantilla, G., Heredia, D., Gonzalez, M., Parra, E., Rey, G., Munera, G., Hidalgo, M., Castañeda, E., Segura, O., Bolivar, J., Galeano, A., Giraldo, M., Rodriguez, E., Cedeño, N., Perez, C., Bayard, V., Hernandez, I., Villalobos, R., 2006. Brote de rickettsiosis en Necoclí, Antioquia, febrero-marzo de 2006. Inst. Nac. Salud 11, 177–186. https://www.ins.gov.co/buscador/IQEN/IQEN%20vol%2011%202006%20num%2012.pdf (accessed 3 March 2021).

Ammerman, N.C., Swanson, K.I., Anderson, J.M., Schwartz, T.R., Seaberg, E.C., Glass, G. E., Norris, D.E., 2004. Spotted-fever group Rickettsia in Dermacentor variabilis. Maryland. Emerg. Infect. Dis. 10, 1478–1481. https://doi.org/10.3201/eid1008.030882.

Burgdorfer, W., Brinton, L.P., 1975. Mechanisms of transovarial infections of spotted fever rickettsiae in ticks. Ann. N. Y. Acad. Sci. 266, 61–72. https://doi.org/10.1111/j.1749-6632.1975.tb35088.x.

Castillo- Martínez, A., Cueto-Medina, S.M., Hernández-Rodríguez, S., Gallegos-Robles, M.Á., Valdés-Perezgasga, M.T., Sánchez-Ramos, F.J., Ortega-Morales, A.I., 2015. Detección de Rickettsia sp. en la garrapata café del perro Rhipicephalus sanguineus (Acari: ixodidae) en Matamoros, Coahuila, México. Acta Zool. Mex. 31, 80–83. https://doi.org/10.21829/azm.2015.311509.

Cox, J., Brown, H.E., Rico-Hesse, R., 2011. Variation in vector competence for dengue viruses does not depend on mosquito midgut binding affinity. PLoS Negl. Trop. Dis. 5, e1172. https://doi.org/10.1371/journal.pntd.0001172.

de la Fuente, J., Antunes, S., Bonnet, S., Cabezas-Cruz, A., Domingos, A.G., Estrada-Peña, A., Johnson, N., Kocan, K.M., Mansfield, K.L., Nijhof, A.M., Papa, A., Rudenko, N., Villar, M., Alberdi, P., Torina, A., Ayllón, N., Vancova, M., Golovchenko, M., Grubhoffer, L., Caracappa, S., Fooks, A.R., Gortazar, C., Rego, R.O. M., 2017. tick-pathogen interactions and vector competence: identification of molecular drivers for tick-borne diseases. Front. Cell. Infect. Microbiol. 7, 1–13. https://doi.org/10.3389/fcimb.2017.00114.

Demma, L.J., Traeger, M.S., Nicholson, W.L., Paddock, C.D., Blau, D.M., Eremeeva, M.E., Dasch, G.A., Levin, M.L., Singleton, J., Zaki, S.R., Cheek, J.E., Swerdlow, D.L., McQuiston, J.H., 2005. Rocky mountain spotted fever from an unexpected tick vector in Arizona. N. Engl. J. Med. 353, 587–594. https://doi.org/10.1056/NEJMoa050043.

Drummond, R.O., Whetstone, T.M., 1970. Oviposition of the Gulf Coast Tick. J. Econ. Entomol. 63, 1547–1551. https://doi.org/10.1093/jee/63.5.1547.

Dumler, J.S., Walker, D.H., 2005. Rocky Mountain spotted fever–changing ecology and persisting virulence. N. Engl. J. Med. 353, 551–553. https://doi.org/10.1056/NEJMp058138.

Estrada-Peña, A., Tarragona, E.L., Vesco, U., Meneghi, D.de, Mastropaolo, M., Mangold, A.J., Guglielmone, A.A., Nava, S., 2014. Divergent environmental preferences and areas of sympatry of tick species in the Amblyomma cajennense complex (Ixodidae). Int. J. Parasitol. 44, 1081–1089. https://doi.org/10.1016/j.ijpara.2014.08.007.

Faccini-Martínez, Á.A., Costa, F.B., Hayama-Ueno, T.E., Ramírez-Hernández, A., Cortés-Vecino, J.A., Labruna, M.B., Hidalgo, M., 2015. Rickettsia rickettsii in Amblyomma patinoi ticks. Colombia. Emerg. Infect. Dis. 21, 537–539. https://doi.org/10.3201/eid2013.140721.

Faccini-Martínez, Á.A., Ramírez-Hernández, A., Barreto, C., Forero-Becerra, E., Millán, D., Valbuena, E., Sánchez-Alfonso, A.C., Imbacuán-Pantoja, W.O., Cortés-Vecino, J.A., Polo-Terán, L.J., Yaya-Lancheros, N., Jácome, J., Palomar, A.M., Santibáñez, S., Portillo, A., Oteo, J.A., Hidalgo, M., 2017. Epidemiology of spotted fever group rickettsioses and acute undifferentiated Febrile Illness in Villeta, Colombia. Am. J. Trop. Med. Hyg. 9, 782–788. https://doi.org/10.4269/ajtmh.16-0442.

Gerardi, M., Ramírez-Hernández, A., Binder, L.C., Krawczak, F.S., Gregori, F., Labruna, M.B., 2019. Comparative susceptibility of different populations of Amblyomma sculptum to Rickettsia rickettsii. Front. Physiol. 10, 1–12. https://doi.org/10.3389/fphys.2019.00653.

Guglielmone, A.A., Robbins, R.G., 2018. Hard Ticks (Acari: Ixodida: Ixodidae) Parasitizing Humans. A Global Overview. Springer, Cham, Switzerland, p. 314.

Harris, E.K., Verhoeve, V.I., Banajee, K.H., Macaluso, J.A., Azad, A.F., Macaluso, K.R., 2017. Comparative vertical transmission of Rickettsia by Dermacentor variabilis and Amblyomma maculatum. Ticks Tick Borne Dis 8, 598–604. https://doi.org/10.1016/j.ttbdis.2017.04.003.

Hidalgo, M., Lizarazo, D., Ovalle, M., Castañeda, E., Heredia, D., Zambrano, P., Mantilla, G., Parra, E., Vera, M., Porras, A., Gaines, A., Múnera, G., Carrillo, P., Orejuela, L., Valbuena, G., 2007a. Brote de rickettsiosis en Los Córdobas, departamento de Córdoba, febrero-marzo 2007. Inst. Nac. Salud 12, 371–378. https://www.ins.gov.co/buscador/IQEN/IQEN%20vol%2012%202007%20num%2024.pdf (accessed 3 March 2021).

Hidalgo, M., Miranda, J., Heredia, D., Zambrano, P., Vesga, P., Lizarazo, D., Mattar, S., Valbuena, G., 2011. Outbreak of Rocky Mountain spotted fever in Córdoba, Colombia. Mem. Inst. Oswaldo Cruz, Rio de Janeiro 106, 117–118. https://doi.org/10.1590/S0074-02762011000100019.

Hidalgo, M., Orejuela, L., Fuya, P., Carrillo, P., Hernandez, J., Parra, E., Keng, C., Small, M., Olano, J.P., Bouyer, D., Castaneda, E., Walker, D., Valbuena, G., 2007b. Rocky Mountain spotted fever. Colombia. Emerg. Infect. Dis. 13, 1058–1060. https://doi.org/10.3201/eid1307.0605.

Hidalgo, M., Sánchez, R., Orejuela, L., Hernández, J., Walker, D., Valbuena, G., 2007c. Prevalence of antibodies against spotted fever group rickettsiae in a rural area of Colombia. Am. J. Trop. Med. Hyg. 77, 378–380. https://doi.org/10.4269/ajtmh.2007.77.378.

Hidalgo, M., Vesga, J.F., Lizarazo, D., Valbuena, G., 2009. Short report: a survey of antibodies against Rickettsia rickettsii and Ehrlichia chaffeensis in domestic animals from a rural area of Colombia. Am. J. Trop. Med. Hyg. 80, 1029–1030. https://doi.org/10.4269/ajtmh.2009.80.1029.

Horta, M.C., Moraes-Filho, J., Casagrande, R.A., Saito, T.B., Rosa, S.C., Ogrzewalska, M., Matushima, E.R., Labruna, M.B., 2009. Experimental infection of opossumsDidelphis aurita by Rickettsia rickettsii and evaluation of the transmission of the infection to ticks Amblyomma cajennense. Vector-Borne Zoonotic Dis. 9, 109–117. https://doi.org/10.1089/vbz.2008.0114.

Horta, M.C., Pinter, A., Cortez, A., Soares, R.M., Gennari, S.M., Schumaker, T.T.S., Labruna, M.B., 2005. Rickettsia felis (Rickettsiales: rickettsiaceae) in Ctenocephalides felis felis (Siphonaptera: pulicidae) in the State of São Paulo. Brazil. Arq. Bras. Med. Vet. Zootec. 57, 321–325. https://doi.org/10.1590/S0102-09352005000300008.

Katz, G., Neves, V.L.F.de C., Angerami, R.N., Nascimento, E.M.M.do, Colombo, S., 2009. Situação epidemiológica e importância da febre maculosa no Estado de São Paulo. Bol. Epidemiológico Paul. 6, 4–13. http://periodicos.ses.sp.bvs.br/pdf/bepa/v6n69/v6n69a01.pdf (accessed 3 March 2021).

Krawczak, F.S., Nieri-Bastos, F.A., Nunes, F.P., Soares, J.F., Moraes-Filho, J., Labruna, M. B., 2014. Rickettsial infection in Amblyomma cajennense ticks and capybaras (Hydrochoerus hydrochaeris) in a Brazilian spotted fever-endemic area. Parasit. Vectors 7, 2–7. https://doi.org/10.1186/1756-3305-7-7.

Labruna, M., Mattar, V., S., Nava, S., Bermudez, S., Venzal, J., Dolz, G., Abarca, K., Romero, L., de Sousa, R., Oteo, J., Zavala Castro, J., 2011. Rickettsiosis en América Latina, el Caribe, España y Portugal TT - Rickettsioses in Latin America, Caribbean, Spain and Portugal. Rev. MVZ Córdoba 16, 2435–2457. https://doi.org/10.21897/rmvz.282.

Labruna, M.B., 2009. Ecology of Rickettsia in South America. Ann. N. Y. Acad. Sci. 1166, 156–166. https://doi.org/10.1111/j.1749-6632.2009.04516.x.

Labruna, M.B., Horta, M.C., Aguiar, D.M., Cavalcante, G.T., Pinter, A., Gennari, S.M., Camargo, L.M.A., 2007. Prevalence of Rickettsia infection in dogs from the urban and rural areas of Monte Negro Municipality, Western Amazon, Brazil. Vector-Borne Zoonotic Dis. 7, 249–255. https://doi.org/10.1089/vbz.2006.0621.

Labruna, M.B., Leite, R.C., Faccini, J., Ferreira, F., 2000. Life cycle of the tick Haemaphysalis leporis-palustris (Acari: ixodidae) under laboratory conditions. Exp. Appl. Acarol. 24, 683–694. https://doi.org/10.1023/A:1010768511790.

Labruna, M.B., Ogrzewalska, M., Martins, T.F., Pinter, A., Horta, M.C., 2008. Comparative susceptibility of larval stages of Amblyomma aureolatum, Amblyomma cajennense, and Rhipicephalus sanguineus to Infection by Rickettsia rickettsii. J. Med. Entomol. 45, 1156–1159 doi:10.1603/0022-2585(2008)45[1156:CSOLSO]2.0.CO;2.

Labruna, M.B., Santos, F.C.P., Ogrzewalska, M., Nascimento, E.M.M., Colombo, S., Marcili, A., Angerami, R.N., 2014. Genetic identification of Rickettsial isolates from fatal cases of Brazilian spotted fever and comparison with Rickettsia rickettsii isolates from the American continents. J. Clin. Microbiol. 52, 3788–3791. https://doi.org/10.1128/JCM.01914-14.

Labruna, M.B., Whitworth, T., Horta, M.C., Bouyer, D.H., McBride, J.W., Pinter, A., Popov, V., Gennari, S.M., Walker, D.H., 2004. Rickettsia species infecting Amblyomma cooperi ticks from an area in the state of São Paulo, Brazil, where Brazilian spotted fever is endemic. J. Clin. Microbiol. 42, 90–98. https://doi.org/10.1128/JCM.42.1.90–98.2004.

Mangold, A.J., Bargues, M.D., Mas-Coma, S., 1998. Mitochondrial 16S rDNA sequences and phylogenetic relationships of species of Rhipicephalus and other tick genera among Metastriata (Acari: ixodidae). Parasitol. Res. 84, 478–484. https://doi.org/10.1007/s004360050433.

Monteiro, J.L., 1933. Comportamento experimental do Coelho aos virus do typho Exanthemático de São Paulo e da febre maculosa das Montanhas Rochosas. Mem. Inst. Butantan 8, 41–47. https://bibliotecadigital.butantan.gov.br/arquivos/76/PDF/4.pdf (accessed 3 March 2021).

Monteiro, J.L., 1931. O "Typho exanthematico de S. Paulo" e suas relações com a febre maculosa das montanhas rochosas, á luz das provas de immunidade cruzada. Mem. Inst. Butantan 6, 209–220. https://bibliotecadigital.butantan.gov.br/arquivos/76/PDF/18.pdf (accessed 3 March 2021).

Nava, S., Beati, L., Labruna, M.B., Cáceres, A.G., Mangold, A.J., Guglielmone, A.A., 2014. Reassessment of the taxonomic status of Amblyomma cajennense (Fabricius, 1787) with the description of three new species, Amblyomma tonelliae n. sp., Amblyomma interandinum n. sp. and Amblyomma patinoi n. sp., and reinstatement of Amblyomma mixtum Koch, 1. Ticks Tick Borne Dis 5, 252–276. https://doi.org/10.1016/j.ttbdis.2013.11.004.

Pacheco, O., Giraldo, R., Hidalgo, M., Galeano, A., Echeverry, I., Echevarria, L., 2008. Estudio de brote febril hemorrágico en el corregimiento de Alto de Mulatos - Distrito Especial Portuario de Turbo, Antioquia, enero de 2008. Inst. Nac. Salud 13, 145–156. https://www.ins.gov.co/buscador-eventos/IQEN/IQEN%20vol%2013%202008%20num%2010.pdf (accessed 3 March 2021).

Patino, L., Afanador, A., Paul, J.H., 1937. A spotted fever in Tobia, Colombia: preliminary report. Am. J. Trop. Med. 17, 639–653. https://doi.org/10.4269/AJTMH.1937.S1-17.639.

Philip, C., 1959. Some epidemiological considerations in Rocky Mountain spotted fever. Public Health Rep. 74, 595–600. https://doi.org/10.2307/4590519.

Pinter, A., Labruna, M.B., Faccini, J.L.H., 2002. The sex ratio of Amblyomma cajennense (Acari: ixodidae) with notes on the male feeding period in the laboratory. Vet. Parasitol. 105, 79–88. https://doi.org/10.1016/S0304-4017(01)00650-1.

Ramírez-Hernández, A., Uchoa, F., Serpa, M.C., de, A., Binder, L.C., Souza, C.E., Labruna, M.B., 2020. Capybaras (Hydrochoerus hydrochaeris) as amplifying hosts of Rickettsia rickettsii to Amblyomma sculptum ticks: evaluation during primary and subsequent exposures to R. rickettsii infection. Ticks Tick Borne Dis 11, 101463. https://doi.org/10.1016/j.ttbdis.2020.101463.

Rivera-Paez, F., Labruna, M., Martins, T., Sampieri, B., Camargo-Mathias, M., 2016. Amblyomma mixtum Koch, 1844 (Acari: ixodidae): first record confirmation in Colombia using morphological and molecular analyses. Ticks Tick Borne Dis 7, 842–848. https://doi.org/10.1016/j.ttbdis.2016.03.020.

Sangioni, L.A., Horta, M.C., Vianna, M.C.B., Gennari, S.M., Soares, R.M., Galvão, M.A.M., Schumaker, T.T.S., Ferreira, F., Vidotto, O., Labruna, M.B., 2005. Rickettsial infection in animals and Brazilian spotted fever endemicity. Emerg. Infect. Dis. 11, 265–270. https://doi.org/10.3201/eid1102.040656.

Soares, J.F., Soares, H.S., Barbieri, A.M., Labruna, M.B., 2012. Experimental infection of the tick Amblyomma cajennense, Cayenne tick, with Rickettsia rickettsii, the agent of Rocky Mountain spotted fever. Med. Vet. Entomol. 26, 139–151. https://doi.org/10.1111/j.1365-2915.2011.00982.x.

Tarragona, E.L., Soares, J.F., Costa, F.B., Labruna, M.B., Nava, S., 2016. Vectorial competence of Amblyomma tonelliae to transmit Rickettsia rickettsii. Med. Vet. Entomol. 30, 410–415. https://doi.org/10.1111/mve.12189.