

Original article

Immature hard ticks infected with *Rickettsia amblyommatis* on breeding birds from Pantanal

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ABSTRACT

Immature hard ticks from the genus *Amblyomma* feed on blood from a wide range of Neotropical avian hosts. They serve as vectors for pathogens of medical and veterinary importance, such as *Rickettsia* agents of the spotted fever group (SFG). Hence, determining ecological factors that increase encounter rates between immature ticks and their avian hosts may contribute to the understanding of tick-borne diseases transmission. Here, we used 720 individual birds from 96 species surveyed in the Brazilian Pantanal to test whether host breeding season influenced tick infestation probabilities. Additionally, collected ticks were screened for *Rickettsia* agents to describe new avian-tick-bacteria associations. Our models revealed that the probability of an individual bird being infested with immature ticks was similar during the breeding and pre-breeding season, but higher loads of immature tick stages were found during the breeding season. Host sex did not predict infestation probability, but *Rickettsia* agents recovered from ticks were more prevalent during the pre-breeding season. The new records of host usage by larvae and nymphs of *Amblyomma* in Pantanal and the growing body of tick surveys in Neotropical avian communities, suggest that immature ticks may benefit from avian blood sources during their annual cycle. The low number of infected ticks with *Rickettsia* agents on Pantanal birds suggest that this vertebrate group are likely not acting as reservoirs for these microorganisms. However, long-term surveys at the same site are imperative to determine which tick species are acting as reservoirs for *Rickettsia* agents in Pantanal and determine whether birds are playing a role in dispersing ticks and tick-borne pathogens.

1. Introduction

Hard ticks from the genus *Amblyomma* are widespread across the Neotropical region (Guglielmone et al., 2003; Nava et al., 2017) with adults of some species showing a certain degree of host specificity to large mammals (Esser et al., 2016). In contrast, the prevalence of immature hard ticks is highly variable within and across avian host communities (Ogrzewalska et al., 2009; Lugarini et al., 2015; Martínez-Sánchez et al., 2020; Fecchio et al., 2021). These differences in

infestation probability of immature ticks have been correlated with avian host behavior that enhance contact rates, thus increasing infestation (Newman et al., 2015; Fecchio et al., 2021). For example, it is expected that breeding birds have a higher probability of being infested with immature ticks than non-breeding birds because of increased movement of adult birds during nest-building and nestling feeding (Newman et al., 2015).

Several recent surveys have shown that immature hard ticks can feed on a wide range of avian hosts across Neotropical biomes (e.g.,

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Ogrzewalska et al., 2009, 2011, 2014; Lugarini et al., 2015; Esser et al., 2016; Fecchio et al., 2020; Martínez-Sánchez et al., 2020). For example, larvae and nymphs of *Amblyomma longirostre* (Koch, 1844) and *Amblyomma nodosum* Neumann, 1899 have been found mainly in passerine hosts, whereas all stages of *Amblyomma sculptum* Berlese, 1888 including adults, have also been found parasitizing several clades of wild birds, including non-passerines (Nava et al., 2017). This suggests that some hard tick species may rely upon avian blood sources during part of their life cycle, which in turn, might also influence the infection risk and spread of associated tick-borne pathogens they carry. In fact, wild birds hosting larvae of *Ixodes pacificus* Cooley and Kohls, 1943 have been shown to contribute to the enzootic maintenance of *Borrelia burgdorferi* sensu lato and that certain bird life history traits predict infection by these tick-borne spirochetes (Newman et al., 2015).

Here, we aim to report tick infestation on passerine and non-passerine wild birds in the Brazilian Pantanal. Then, we determined the tick species harboring *Rickettsia* agents to provide new avian host-tick-bacteria associations from a megadiverse region. Finally, we determined whether bird breeding behavior influences the prevalence and abundance of immature ticks on their avian hosts. Specifically, we tested whether birds defending territories and seeking nesting material are more infested by immature ticks than during the period when birds are laying eggs or feeding nestlings. We hypothesized that increased bird movement during the driest period in Pantanal, when larvae and nymphs are more abundant in the leaf litter (Ramos et al., 2014), could enhance infestation on birds prior to their breeding season. We accounted for host phylogenetic relationships and temporal effects in our analysis because tick abundance and diversity vary within host clades and seasonally in Pantanal (Ramos et al., 2014; Fecchio et al., 2020).

2. Materials and methods

2.1. Study site

The Brazilian Pantanal is an extensive wetland located in the central South America with grasslands, neotropical savannas, and monospecific stands of trees flooding between January to May, whereas dense gallery forests and patches of deciduous forest remain unflooded year-round (Pott and Pott, 1994). The avifauna in Pantanal is mainly influenced by four surrounding biomes: Amazonia, Atlantic Rainforest, Cerrado, and Chaco (Pinho et al., 2017). Annual rainfall is approximately 1200 mm, most of which occurs in December (Pinho and Marini, 2014). Regional climate is categorized as AW according to Köppen and Geiger (1928).

Fieldwork for this study was carried out between August to November 2019 at three sites in Northern Pantanal: 1) Fazenda Baía São João (municipality of Santo Antônio de Leverger, 16° 44'S, 55° 33' W); 2) Base Avançada de Pesquisa do Pantanal (municipality of Poconé, 16° 29'S, 56° 24' W); and 3) Fazenda Retiro Novo (municipality of Nossa Senhora do Livramento, 16° 15' S, 56° 22' W). According to precipitation patterns, this portion of Pantanal is characterized by two well-defined seasons: 1) the dry season, from April to September which overlaps with the non-breeding season for most understory birds but is the breeding season for aquatic and large migratory birds, and 2) the rainy season, from November to March, which also corresponds to the breeding season for most passerine birds (Pinho et al., 2017).

2.2. Bird sampling

Birds were captured using mist net or by firearms. Netted birds were either released or euthanized and prepared as museum specimens. Birds were captured, manipulated, and collected in accordance with corresponding permits in Brazil (license issued by Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio numbers: 72,548, 72,790) and vouchers are deposited at Ornithological Collection of Federal

University of Mato Grosso, Brazil. Bird species nomenclature follows the BirdTree project (Jetz et al., 2012).

Individual birds were grouped according to their breeding behavior in Pantanal (Pinho et al., 2017). Here we created two categories of breeding seasons: (1) pre-breeding, which includes birds that are actively defending territory or seeking material to build their nest (e.g., foraging on the ground, forest edges, bushes, and shrubs), and (2) breeding, for the period in which birds are attending nests to incubate eggs or feeding nestlings (Pinho and Marini, 2014).

2.3. Collection and identification of ticks

After inspection of birds, ticks were collected and stored in 95% ethanol until later processing. All nymphal ticks were morphologically identified to the species level based on the dichotomous keys of Martins et al. (2010, 2016). Additionally, a restricted number of *Amblyomma* larvae were identified molecularly at the species level as described below.

2.4. DNA extraction, molecular detection, and sequencing

DNA was extracted from identified ticks using guanidine isothiocyanate, as previously described (Sangioni et al., 2005). DNA extractions were screened for *Rickettsia* agents by polymerase chain reaction (PCR) using the primers CS-78 and CS-323 that amplify a ~401 base pair (bp) fragment of the citrate synthase gene (*gltA*) common to all *Rickettsia* species (Labruna et al., 2004). When a single larva or nymph was found on an individual host it was individually tested. If at least 3 larvae or nymphs were found on the same individual host, they were tested in pools of three ticks each. Positive samples underwent a subsequent PCR amplification using the primers Rr190.70p and Rr190.602n, which amplify a ~530-bp fragment of the 190-kDa outer membrane protein gene (*ompA*) present only in *Rickettsia* of the spotted fever group (SFG) according to Regnery et al. (1991). For each reaction, negative (nuclease-free water) and positive controls (*Rickettsia rickettsii* DNA) were included. To identify *Rickettsia*-positive tick larvae, DNA extractions underwent a third PCR targeting a ~460-bp fragment of the tick mitochondrial 16S rRNA gene, according to Mangold et al. (1998). PCR products were resolved in 1.5% agarose gels stained with GelRed™ Nucleic Acid Gel Stain (Biotium, Fremont, CA) and visualized in a ChemiDoc XRS system (Bio-Rad, Hercules, CA). Expected size amplicons were purified using the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Chicago, IL) and prepared for sequencing with BigDye™ kit (Applied Biosystems, Foster, CA). An ABI-PRISM 3500 Genetic Analyzer (Applied Biosystems, Foster, CA) was employed for sequencing procedures using the same primers used for PCR. In order to evaluate the quality of the sequences of *Rickettsia* and tick mitochondrial 16S rRNA, electropherograms were verified with CLC Genomics Workbench software (Qiagen®) and obtained sequences were then subjected to BLAST analyses (Altschul et al., 1990) to infer closest identities with organisms available in GenBank.

2.5. Statistical analyses

To investigate seasonal and taxonomic effects on the probability of tick infestation within avian hosts, we used a Bayesian phylogenetic mixed model (Hadfield, 2010). We accounted for phylogenetic relationships between species because of the assumed nonindependence of traits due to common ancestry (Felsenstein, 1985; Harvey and Pagel, 1991). We used the function MCMCglmm from the package 'MCMCglmm' (Hadfield, 2010) to create a linear mixed model using Bayesian Markov Chain Monte Carlo (Hadfield, 2012). To account for phylogenetic effects, we used 100 randomly selected phylogeny trees based on the Hackett backbone (Hackett et al., 2008) obtained from the Birdtree project (<http://birdtree.org>; Jetz et al., 2012). We applied the models to each of the trees and then extracted the mean density of the

Table 1
Number of immature ticks recorded on birds sampled in Pantanal. LA = larva, NY = nymph.

Avian taxa	Birds captured	Birds infested	<i>Amblyomma</i> sp. (LA)	<i>A. triste</i> (LA)	<i>A. longirostre</i> (NY)	<i>A. triste</i> (NY)	<i>A. nodosum</i> (NY)	<i>A. sculptum</i> (NY)
Accipitridae								
<i>Busarellus nigricollis</i>	2	0						
<i>Buteo magnirostris</i>	1	0						
<i>Buteogallus meridionalis</i>	1	0						
<i>Buteogallus urubitinga</i>	1	0						
Alcedinidae								
<i>Chloroceryle aenea</i>	1	0						
Cardinalidae								
<i>Saltator coerulescens</i>	18	3					3	
<i>Saltator similis</i>	2	0						
Coerebidae								
<i>Coereba flaveola</i>	7	0						
Columbidae								
<i>Columbina picui</i>	1	0						
<i>Columbina talpacoti</i>	9	0						
<i>Leptotila verreauxi</i>	11	0						
Corvidae								
<i>Cyanocorax cyanomelas</i>	2	0						
Cotingidae								
<i>Pachyrhamphus polychopterus</i>	1	0						
Cracidae								
<i>Crax fasciolata</i>	1	0						
Cuculidae								
<i>Coccyzua minuta</i>	2	0						
<i>Coccyzus melacoryphus</i>	1	0						
<i>Crotophaga ani</i>	7	2						7 ^a
<i>Guira guira</i>	2	0						
Dendrocolaptidae								
<i>Dendroplex picus</i>	6	0						
Emberizidae								
<i>Arremon flavirostris</i>	9	0						
<i>Coryphospingus cucullatus</i>	1	0						
<i>Paroaria capitata</i>	43	1	2					
<i>Sicalis flaveola</i>	4	0						
<i>Sporophila angolensis</i>	9	1	6			6 ^a		
<i>Sporophila caerulescens</i>	13	0						
<i>Sporophila collaris</i>	30	0						
<i>Sporophila leucoptera</i>	3	1	1					
<i>Volatinia jacarina</i>	12	1					1 ^a	
Furnariidae								
<i>Certhiaxis cinnamomeus</i>	35	0						
<i>Cranioleuca vulpina</i>	7	1			1 ^a			
<i>Furnarius leucopus</i>	10	1				1 ^a		
<i>Furnarius rufus</i>	12	1						1 ^a
<i>Pseudoseisura unirufa</i>	7	0						
<i>Synallaxis albilora</i>	13	0						
Galbulidae								
<i>Galbula ruficauda</i>	4	0						
Hirundinidae								
<i>Progne tapera</i>	4	0						
<i>Stelgidopteryx ruficollis</i>	8	0						
Icteridae								
<i>Agelasticus cyanoptus</i>	12	0						
<i>Cacicus cela</i>	12	0						
<i>Cacicus solitarius</i>	3	0						
<i>Gnorimopsar chopi</i>	2	0						
<i>Icterus cayanensis</i>	3	0						
Parulidae								
<i>Basileuterus flaveolus</i>	39	1		3 ^a				
<i>Basileuterus hypoleucus</i>	8	0						
Picidae								
<i>Picumnus albosquamatus</i>	2	0						
Pipridae								
<i>Antilophia galeata</i>	1	1			1			
<i>Pipra fasciicauda</i>	13	0						
Thamnophilidae								
<i>Cercomacra melanaria</i>	13	2					3 ^a	
<i>Formicivora rufa</i>	1	1					2	
<i>Herpsilochmus longirostris</i>	1	0						
<i>Hypocnemoides maculicauda</i>	8	1					1	
<i>Taraba major</i>	6	2					2	
<i>Thamnophilus doliatus</i>	1	0						
Thraupidae								

(continued on next page)

Table 1 (continued)

Avian taxa	Birds captured	Birds infested	<i>Amblyomma</i> sp. (LA)	<i>A. triste</i> (LA)	<i>A. longirostre</i> (NY)	<i>A. triste</i> (NY)	<i>A. nodosum</i> (NY)	<i>A. sculptum</i> (NY)
<i>Conirostrum speciosum</i>	1	0						
<i>Eucometis penicillata</i>	13	2					4	
<i>Ramphocelus carbo</i>	104	10			4		7	
<i>Thraupis palmarum</i>	2	0						
<i>Thraupis sayaca</i>	2	0						
Tinamidae								
<i>Crypturellus undulatus</i>	1	1						22 ^a
Trochilidae								
<i>Amazilia fimbriata</i>	1	0						
<i>Glaucis hirsutus</i>	1	0						
<i>Hylocharis chrysura</i>	1	0						
<i>Phaethornis nattereri</i>	3	0						
Troglodytidae								
<i>Campylorhynchus turdinus</i>	2	0						
<i>Cantorchilus leucotis</i>	7	0						
<i>Thryothorus genibarbis</i>	2	0						
Trogonidae								
<i>Trogon curucui</i>	2	0						
Turdidae								
<i>Turdus amaurochalinus</i>	7	1					1	
<i>Turdus hauxwelli</i>	3	0						
<i>Turdus leucomelas</i>	9	1					1	1
<i>Turdus rufiventris</i>	3	0						
Tyrannidae								
<i>Arundinicola leucocephala</i>	4	0						
<i>Casiornis rufus</i>	1	0						
<i>Cnemotriccus fuscatus</i>	9	0						
<i>Corythopis delalandi</i>	1	0						
<i>Elaenia albiceps</i>	6	0						
<i>Elaenia parvirostris</i>	1	0						
<i>Elaenia spectabilis</i>	1	0						
<i>Euscarthmus meloryphus</i>	3	0						
<i>Fluvicola albiventer</i>	9	0						
<i>Hemitriccus margaritaceiventer</i>	5	0						
<i>Hemitriccus striaticollis</i>	6	0						
<i>Legatus leucophaeus</i>	4	0						
<i>Leptopogon amaurocephalus</i>	4	0						
<i>Machetornis rixosa</i>	3	0						
<i>Myiarchus ferox</i>	6	0						
<i>Myiopagis viridicata</i>	1	0						
<i>Myiophobus fasciatus</i>	3	0						
<i>Myiozetetes cayanensis</i>	7	0						
<i>Pitangus sulphuratus</i>	25	1					1 ^a	
<i>Platyrinchus mystaceus</i>	1	0						
<i>Poecilotriccus latirostris</i>	18	0						
<i>Todirostrum cinereum</i>	3	0						
<i>Tyrannus melancholicus</i>	4	0						
Vireonidae								
<i>Hylophilus pectoralis</i>	3	0						
<i>Vireo olivaceus</i>	3	0						
Total	720	36	9	3	6	7	26	31

^a New records on avian hosts species.

combined posterior distribution using the package mulTree (Guillermo and Healy, 2014). Bird phylogeny was included as a random term in the model. To test effects of the explanatory variables on the probability of host infestation, we used a categorical mixed model using the logit link function. We set uninformative priors for both fixed and random effects of models. We used an uninformative inverse-Wishart prior distribution using parameter expansion with the prior means (alpha.mu) set as 0 and prior covariance matrix (alpha.V) set as 1000, using a residual variance fixed at 1 (Hadfield, 2010). To ensure model convergence, we ran each model for 500,000 iterations, with burnin set at 10,000 and stored samples every 100 iterations.

Within a subset of the data, we investigated how host sex, breeding season, and taxonomic group affected the probability of tick infestation, using a model with the same properties described above. The models resulted in comparable effective sample size for all factors (~1000). All chains were visually inspected to ensure proper mixing, and autocorrelation was checked using the command 'autocorr', with 0.1 used as a

target threshold. Fixed effects were considered statistically significant when the 95% credible interval did not overlap zero. We calculated the percentage of variance explained by each of the fixed effects only and the combination of fixed and random effects, using marginal and conditional R², respectively (Nakagawa and Schielzeth, 2013). For tick-infested birds, we also used Fisher's exact tests to investigate the association between breeding season and avian taxonomic group with the occurrence of tick rickettsiae infection. All the analyses were performed using R (ver. 4.2.0, www.r-project.org).

3. Results

Overall, 720 individuals representing 96 avian species and 27 avian families, were captured (Table 1). Around 92% of the birds were passerines and the most well sampled species were *Ramphocelus carbo* ($n = 104$), *Paroaria capitata* ($n = 43$), *Basileuterus flaveolus* ($n = 39$), *Certhiixis cinnamomeus* ($n = 35$), and *Sporophila collaris* ($n = 30$). A total of 82

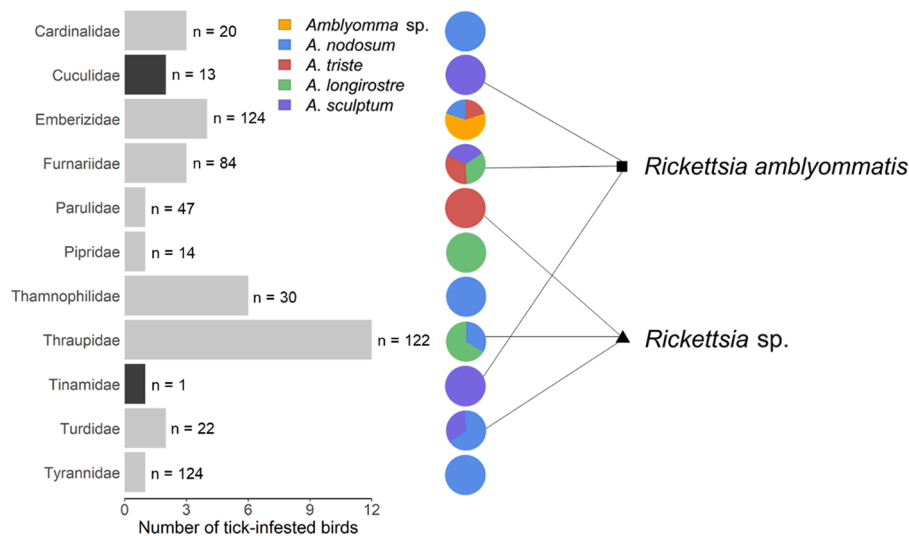


Fig. 1. Interaction between avian hosts (taxonomic family), ticks, and *Rickettsia*. Non-passerines are shown in dark-gray bars, whereas passerines are shown in light-gray bars. The number of sampled individuals is presented in the barplot. Pie charts represent the proportion of *Amblyomma* species found on each avian host family.

Amblyomma ticks (12 larvae and 70 nymphs) were collected from 21 avian species belonging to 11 avian families (Table 1; see Supplementary Table S1 for tick abundance on each bird host, locality, and time of year). Nymphs were identified by morphological characters into four *Amblyomma* species (number of nymphs): *A. longirostre* (6), *A. nodosum* (26), *A. sculptum* (31), and *Amblyomma triste* Koch, 1844 (7). One larval pool was identified to the species level as *A. triste* by sequencing, as described below, while nine larvae could only be identified to the genus *Amblyomma* (Table 1; Fig. 1). New records of nymphs are reported for the following tick and host species: *A. longirostre* on *Cranioleuca vulpina*; *A. nodosum* on *Cercomacra melanaria*, *Pitangus sulphuratus*, and *Volatinia jacarina*; *A. sculptum* on *Crotophaga ani*, *Crypturellus undulatus*, and *Furnarius rufus*; *A. triste* on *Furnarius leucopus* and *Sporophila angolensis* (Table 1). Additionally, larvae of *A. triste* were reported for the first time on *B. flaveolus*.

DNA from 79 *Amblyomma* ticks were screened by PCR for rickettsiae infection. Forty ticks [3 larvae and 37 nymphs (6 *A. longirostre*, 24 *A. nodosum*, 6 *A. sculptum*, and 1 *A. triste*)] were tested individually and 39 ticks [9 larvae and 30 nymphs (3 *A. nodosum*, 21 *A. sculptum*, and 6 *A. triste*)] were tested in pools of three ticks each.

Molecular data obtained from the 79 evaluated ticks, showed that at least six (7.59%) yielded amplicons for both the *gltA*-PCR and for *ompA*-PCR assays (Fig. 1). Partial *ompA* sequences were generated from three samples encompassing the following tick species: *A. longirostre* (one individual nymph) and *A. sculptum* (one individual and one pool of nymphs). Partial *ompA* sequences yielded two different haplotypes: sequences obtained from one *A. sculptum* nymph collected on *C. ani* matched 99% (459/460 bp) with *Rickettsia amblyommatis* (MG887828) detected in *Amblyomma auricularium* (Conil 1878) from Bahia state in the Brazilian Caatinga biome, here referred as *R. amblyommatis* isolate Pantanal_MT (ON951668); partial sequences of the *ompA* gene from *A. longirostre* and *A. sculptum* collected on *C. vulpina* and *C. undulatus*, respectively, were identical to each other and 100% (406/406 bp) identical to corresponding sequences of *R. amblyommatis* (MH818422) detected in ticks collected from wild birds of Panama, herein designated as *R. amblyommatis* isolate Pantanal2_MT (ON951669-70). Because of the quality of amplified DNA, it was not possible to sequence *ompA* amplicons obtained from two nymphs of *A. nodosum* collected on *Turdus amaurochalinus* and *Eucometis penicillata*, which were tested individually; and from one larval pool of *A. triste* (identified by molecular methods) collected on *B. flaveolus*. For all these samples, the *gltA* sequences were identical to each other and matched 99% (326/328 bp) with several SFG rickettsiae (KY753118, MK441839, MK720995), here

Table 2

Phylogenetic generalized linear mixed model results for the effects of season and taxonomic group on the probability of tick infestation in birds from Pantanal. The references for the categorical variables are: Season (Pre-breeding) and Order (Non-passerines). Estimates were produced averaging 100 models. Parameter estimates were considered statistically significant when 95% confidence intervals (CI) did not overlap zero. The dataset included 720 birds representing 96 species.

Variables	Estimate	95% CI	
		Lower	Upper
Fixed terms			
Season (Breeding)	0.63	-0.21	1.49
Order (Passeriformes)	1.62	-2.56	7.91
Random terms			
Phylogenetic variance	12.36	1.64	39.04

referred to as *Rickettsia* sp. haplotype Pantanal3_MT (ON951665-7). Because of the positive *gltA* and *ompA*-PCR results, we also generated one mitochondrial 16S rDNA partial sequence obtained from one larval pool, and the 16S rDNA haplotype (ON924756) was 100% (377/377 bp) identical to the homologous sequences of *A. triste* (MK059469, KU284991, KU284989).

Around 51% of the birds ($n = 720$) were sampled during the breeding season and 6.0% were infested by ticks. For the birds sampled during the pre-breeding season, 4.0% carried ticks. Despite the higher number of

Table 3

Phylogenetic generalized linear mixed model results for the effects of season, taxonomic group, and sex on the probability of tick infestation on birds from Pantanal. The references for the categorical variables are: Season (Pre-breeding), Order (Non-passerines) and Sex (Female). Estimates were produced averaging 100 models. Parameter estimates were considered statistically significant when 95% confidence intervals (CI) did not overlap zero. The dataset included 343 birds representing 83 species.

Variables	Estimate	95% CI	
		Lower	Upper
Fixed terms			
Season (Breeding)	0.76	-0.32	1.84
Order (Passeriformes)	3.70	-2.84	16.2
Sex (Male)	0.41	-0.64	1.45
Random terms			
Phylogenetic variance	24.9	0.12	131.57

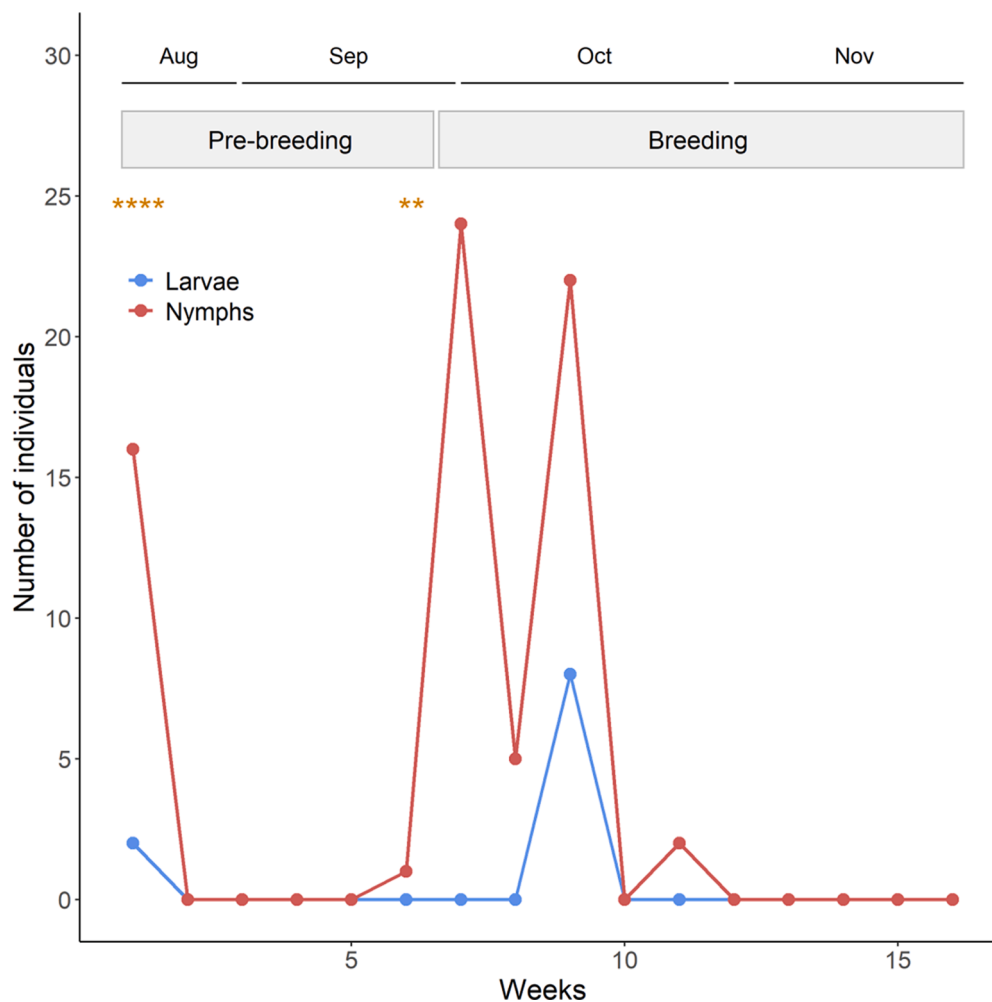


Fig. 2. Weekly variation in the number of ticks (larvae and nymphs) during the study period in relation to avian pre-breeding and breeding seasons. The first week begins on August 13th. Each asterisk depicts a single record of *Rickettsia*.

tick infesting passerines ($n = 33$) in comparison to non-passerines ($n = 3$), the percentage of infested individuals were similar 5.0% and 5.4%, respectively. Thus, tick-infestation probability was not significantly higher during either the pre-breeding season or for passerine species (marginal $R^2 = 0.02$, conditional $R^2 = 0.93$; Table 2). The phylogenetic signal was very strong in the data (total variance explained = 90%), demonstrating that tick infestation was influenced by phylogenetic relationships.

Considering the data subset for which we recorded the sex of the individuals ($n = 343$) we found ticks on 8.3% of the males, whereas tick-infestation was a little lower for females (5.3%). Despite that, we did not observe a significant effect of sex on tick-infestation probability (marginal $R^2 = 0.09$, conditional $R^2 = 0.97$; Table 3). Similar to the first model, breeding season and taxonomic group did not affect the probability of tick-infestation. The phylogenetic signal was also very strong (total variance explained = 87%).

Tick abundance varied seasonally, with a higher number of larvae and nymphs being found during the birds' breeding season (Binomial test; $P < 0.001$; Fig. 2). On average, parasitized birds hosted 2.5 ± 2.4 larvae (mean \pm SD; range: 1–6) and 2.1 ± 3.8 nymphs (mean \pm SD; range: 1–22). When we restricted the analysis to tick-infested individuals, we observed that rickettsiae infection were statistically more common in ticks infesting birds during the bird's pre-breeding season (Fisher's exact test; $P = 0.024$). Moreover, despite a minor trend, rickettsiae infection was not more commonly recorded in ticks collected from non-passerines (Fisher's exact test; $P = 0.072$).

4. Discussion

Our models showed that the probability of a bird being infested by immature ticks is similar during the pre-breeding and breeding seasons. Moreover, host sex also did not explain tick prevalence in Pantanal. These similarities in tick prevalence between sex and season were found for both passerine and non-passerine hosts. However, during the breeding season birds were more heavily infested by immature ticks and rickettsiae agents were more prevalent in ticks during the bird's pre-breeding season.

Counter to our hypothesis, increased host movement near the ground prior to their breeding season (which overlaps with late dry season in Pantanal) did not increase the probability of a bird being infested by immature ticks. This demonstrate that *Amblyomma* tick species found on Pantanal birds unlikely adjust their life cycle to account for the avian host reproductive cycle to assure transmission to nestlings. However, higher loads of immature ticks (mainly nymphs) found during the breeding season when birds are actively foraging to feed their nestlings, suggest that this bird behavior promoting differential contact to the immature stages of the tick's life cycle may explain temporal differences in tick distribution across avian hosts communities.

The complete annual cycle of behavioral diapause, host seeking, or questing behavior are unknown for most *Amblyomma* species in Brazil (Luz and Faccini, 2013; Nava et al., 2017), making it impossible to infer coevolutionary strategies of larvae and nymphs to encounter their intermediate vertebrate hosts. For example, adults of *A. triste* are thought

to be specialized to Marsh deer (*Blastocerus dichotomus*) with larvae and nymphs parasitizing wild rodents, but with some reports of immature ticks on passerine birds (Nava et al., 2017). Moreover, this tick species undergoes an annual cycle that is strongly associated with flooded environments (Nava et al., 2017). Here, we report three new records of immature *A. triste* infesting three passerine species (larvae on *B. flaveolus*; nymph on *F. leucopus* and *S. angolensis*), of which larvae were infected with a *Rickettsia* agent. It is difficult therefore, to infer whether this tick species uses passerine hosts only to feed opportunistically during part of its life cycle or if it may represent a stable host-parasite relationship in Pantanal. Nevertheless, passerine birds are great hosts for larvae and nymphs of *A. longirostre* and *A. nodosum* and were found during the late dry season in Pantanal when most passerine species are not breeding. Furthermore, we report here, in an unprecedented way, the first records of nymph of the tick species *A. longirostre* on *C. vulpine*, nymphs of *A. nodosum* on *C. melanaria*, *P. sulphuratus*, and *V. jacarina*, besides the nymphs of *A. sculptum* on *C. ani*, *C. undulatus*, and *F. rufus*. Although adults of these two tick species do not engage in behaviors to encounter avian hosts, their immature stages might benefit in questing on vegetation to attach to avian hosts and guarantee an alternative blood source during the harshest period in this seasonal biome.

Rickettsiae agents were found in three *Amblyomma* species parasitizing six resident avian host species during the late dry season in Pantanal. The low number of *R. amblyommatis* found in ticks parasitizing birds is consistent with previous surveys in Brazil (Ogrzewalska et al., 2008; Pacheco et al., 2012; Lugarini et al., 2015; Ramos et al., 2015). Although *R. amblyommatis* displayed a low prevalence in Pantanal avian communities, this bacteria species is able to infect a broad range of hard tick species associated with several vertebrate hosts, being one of the most widespread and generalist *Rickettsia* agents across the Neotropics (Estrada-Peña et al., 2021). Two out of three infections by *R. amblyommatis* in Pantanal were recorded from ticks parasitizing Cuculiformes and Tinamiformes, expanding the avian taxonomic host groups that carry hard ticks infected by SFG rickettsiae. Whether resident birds can act as amplifier hosts serving as infection sources capable of transmitting and infecting ticks and thus promoting horizontal transmission of *R. amblyommatis* in Pantanal needs additional research.

Limitations of our results should be acknowledged. First, we did not account for year-round variation in tick abundance. Second, we did not include in our analyses microclimatic and microhabitat variables to explain tick occurrence in their avian hosts. Third, our analyses are based on tick distribution across avian communities from three locations. Therefore, longitudinal studies conducted in the same location across the whole year and with an abundant host species would be imperative to determine temporal patterns of tick infestation and circulation of tick-borne pathogens in avian hosts.

CRedit authorship contribution statement

Alan Fecchio: Conceptualization, Writing – original draft, Resources. **Thiago Fernandes Martins:** Conceptualization, Writing – original draft, Validation. **Raphael I. Dias:** Conceptualization, Writing – original draft, Formal analysis. **Jeffrey A. Bell:** Resources. **João B. Pinho:** Resources, Funding acquisition, Supervision. **Victoria Luiza de Barros Silva:** Validation. **Richard de Campos Pacheco:** Conceptualization, Writing – original draft, Validation, Funding acquisition, Supervision.

Data availability

Raw dataset is available as supporting information.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ttbdis.2023.102121.

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