



Tick host specificity: An analysis based on host phylogeny and tick ecological features using *Amblyomma triste* and *Amblyomma tigrinum* immature stages

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ABSTRACT

Here we have evaluated tick host specificity with two different methodological approaches considering *Amblyomma tigrinum* and *Amblyomma triste* immatures as targets. Firstly, the Std* index was applied, which considers host phylogenetic relationships weighted by the prevalence of the parasite; followed by generalized linear models to explore associations between different host species and burdens and prevalence of larvae and nymphs, independently. The Std* index showed that *A. tigrinum* larvae and nymphs infest host species belonging to different orders and classes, respectively; while *A. triste* immatures fed on hosts that belong to different tribes, showing that both tick species have low specificity and thus, a generalist behavior. When analyzing prevalence and burdens, we found that both tick species infest some hosts more heavily compared with the rest. Even though immature stages of *A. triste* and *A. tigrinum* are generalists, the level of infestation within the range of usual hosts of these two species is uneven. This shows that a generalist behavior may result in dissimilar levels of infestation across a range of usual hosts.

1. Introduction

Host specificity is one of the most important properties of a parasite species. It has profound implications in population dynamics, in the probability of extinction of a parasite and also determines the chances of a parasite to successfully adjust to a new host and to a new geographical area, potentially carrying and disseminating pathogens (Holt et al., 2003; Koh et al., 2004; Poulin, 2007). Considering that the prevalence or abundance of infection by a particular parasite usually varies widely across its group of host species, a true measure of host specificity should take into account how heavily and how frequently host species are parasitized by a given species (Poulin, 2007). Besides, some of the host species used by a parasite are likely to be closely related phylogenetically, whereas others are distantly related. So, to evaluate parasite specificity, host phylogenetic relationships should be taken into account (Poulin, 2007). A few studies have analyzed tick host specificity considering ecological variables in the analysis, finding different levels of specificity according to the tick species and the developmental stage studied (Cumming, 2002; Nava and Guglielmone, 2013; Espinaze

et al., 2015; Esser et al., 2016; Miller et al., 2016).

Amblyomma triste and *Amblyomma tigrinum* are members of the *Amblyomma maculatum* group (Nava et al., 2017). *Amblyomma triste* is widely distributed from USA to Argentina, while the distribution of *A. tigrinum* is restricted to South American countries (Nava et al., 2017). The distribution of *A. triste* is mostly restricted to wetlands and flooding areas; whereas *A. tigrinum* shows ecological plasticity and can colonize regions with different climate and vegetation characteristics (Nava et al., 2017). In the southern range of its distribution in Argentina and Uruguay, *A. triste* develops a one-year life cycle where the highest abundances are reached from late winter to mid-spring for adult stages and during summer for immatures (Venzal et al., 2008; Nava et al., 2011; Monje et al., 2016). In certain areas of Argentina, it was hypothesized that *A. tigrinum* could develop more than one generation per year (Nava et al., 2009) and its seasonal distribution shows that it can be found all year round (Guglielmone et al., 2000). However, adults are more abundant during summer (Guglielmone et al., 2000) while immature stages show a peak of abundance from late spring to middle autumn (Nava et al., 2009). Both tick species have a three-host life

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cycle. *Amblyomma triste* immatures are mainly associated with rodents (Caviidae and Cricetidae), although there are also records on marsupials (Didelphidae), carnivores (Felidae, Canidae), birds (Passeriformes) and bats (Chiroptera) (Nava et al., 2017). The deer *Blastocercus dichotomus* is considered to be the primary host of adult *A. triste*, but mammals of the families Bovidae, Suidae, Equidae, Tapiridae, Felidae, Canidae, Caviidae and Didelphidae have also been found to be parasitized with *A. triste* adults (Szabó et al., 2007; Nava et al., 2017). The principal host for immature stages of *A. tigrinum* are rodents (Caviidae and Cricetidae) and birds (several families of different orders) (Nava et al., 2017). Adults feed principally on wild and domestic Canidae, but were also found on Felidae, Equidae, Cervidae, Tayassuidae and Leporidae (Nava et al., 2017). Adults of both tick species can parasitize humans (Guglielmone et al., 2006); a fact that is of public health importance because these tick species are potential vectors of *Rickettsia parkeri*, a member of the spotted fever group (Venzal et al., 2004; Nava et al., 2008; Lado et al., 2014; Romer et al., 2014; Herrick et al., 2016). Furthermore, adults of *A. tigrinum* were found infected with an *Ehrlichia* strain closely related to *Ehrlichia chaffeensis* (Cicuttin et al., 2017).

The host range of a parasite is of profound importance when the parasite acts as a vector. This kind of information is the basis of knowing the factors that account for the variations in vector burdens, helping to predict when, where or which hosts will have the largest tick burdens (Brunner and Ostfeld, 2008). As the level of specificity of a tick species decreases, the probability of human contact with that tick species increases, enhancing the chances of tick-borne diseases (McCoy et al., 2013). For a given vector-borne pathogen, host competences may vary greatly across the range of species that can be infected (Gervasi et al., 2015). Therefore, establishing host specificity of vectors becomes an essential issue for developing predictive models of disease risk (McCoy et al., 2013).

In the present work we evaluated tick host specificity with two different methodological approaches considering *A. tigrinum* and *A. triste* immatures as targets. The Std* index was used to measure the average taxonomic distinctness of all host species used by a parasite species weighted by its prevalence in their different hosts (Poulin and Mouillot, 2005), and generalized linear models (GLM) were used to explore associations between different host species and burdens and prevalence of larvae and nymphs, independently.

2. Materials and methods

2.1. Data collection

The samples used in this study were collected from two different sites. Larvae and nymphs of *A. tigrinum* were monthly collected from rodents and birds from November 2005 to December 2006 in one grid (30°26'S 64°16'W) located in the semi-dry western Chaco phytogeographical province as defined by Cabrera (1994) in north-western Córdoba, Argentina. One hundred to 300 Sherman live-traps (baited with food pellets and seeds) and 50–150 Tomahawk live-traps (baited with carrot and orange) were set up in transects with 50 traps 5 m apart for 2 successive days every month. Birds were caught every month during one day in the morning and sunset with a 12 m high mist net and released after examination for ticks around the eyes, neck, beak and ear opening. Larvae and nymphs of *A. triste* were collected on rodents and birds on the vicinity of Campana (34°11'S, 58°50'W), Buenos Aires Province, Argentina. The site is located in the banks of the Parana River Delta region, which is the southern extension of the Paranense Province of the Amazonic Phytogeographic Dominion (Cabrera, 1994). Two sets of sampling sessions were performed: i) rodents of the families Caviidae and Cricetidae were monthly trapped from February 2008 to January 2010 during 2 days trapping sessions using 4 linear grids, each one with 25 Sherman live-traps set at 5 m intervals (baited with food pellets and seeds) and one trapping linear grid with 30 Tomahawk live-traps (baited with carrot) set at 5 m interval. Additionally, passerine birds

were trapped only during summer in March 2009 and January 2010 because it is the season when immature stages of *A. triste* prevail. Birds were caught with a 12 m high mist net during a two-day trapping session and released after examination for ticks around the eyes, neck, beak and ear opening.

ii) Only Cricetidae rodents were trapped every 5 weeks from November 2010 to October 2012 in 3 night trapping sessions. Four trapping quadrants (at least 200 m apart) were set out at each of 4 sites, each quadrant consisting of 3 Sherman live-traps in each corner and 2 Ugglan live-traps in the middle of the square, baited with dog food. Considering the 4 trapping sites, 16 quadrants were set in each trapping session.

Samples of rodents and birds were processed following methods described in Nava et al. (2006) for *A. tigrinum* and Nava et al. (2011) and Colombo et al. (2014) for *A. triste*. Rodents captured were euthanized under the approval of the Dirección de Flora y Fauna de la Provincia de Buenos Aires and the Ethic and Biosafety Committee of the Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral. Rodents of the family Caviidae were identified to the species level following Redford and Eisenberg (1992) and Díaz (2000), while Cricetidae rodents were determined with the collaboration of Dr. U.F.J. Pardiñas (Centro Nacional Patagónico, Puerto Madryn, Argentina). Bird species were determined following Narosky and Yzurieta (2003). Each rodent and bird was examined with a magnifying lens to recover ectoparasites. Santiago Nava counted and determined ticks to species level by keys and descriptions of both stages following Estrada-Peña et al. (2005), Martins et al. (2014) and Nava et al. (2017) and by comparison with known laboratory-reared material deposited in the tick collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela (INTA Rafaela), Argentina. Both tick species, collected from different hosts species, were easily differentiated by morphology and by genetic differences as previously done (Nava et al., 2006; Guglielmone et al., 2013; Nava et al., 2017). Also, both *A. tigrinum* and *A. triste* could be easily differentiated by morphology with the other tick species present in the study sites (Nava et al., 2017). Ticks were deposited in the tick collection of Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral and in the Tick collection of INTA Rafaela, Argentina.

2.1.1. Data analysis

Firstly, we calculated the Std* index separately for larvae and nymphs of *A. tigrinum* and *A. triste* in order to measure the average taxonomic distinctness in host species of each tick species weighted by its prevalence (Poulin and Mouillot, 2005). For both larvae and nymph stages, we considered in the analyses four and two species of rodents and birds (six host species in total) for *A. tigrinum*, respectively, and five species of rodents and two of birds (seven host species in total) for *A. triste*. The average taxonomic distinctness is the mean number of steps up a taxonomic hierarchy that must be taken to reach a taxon common to two host species, computed across all possible pairs of host species, and is then weighted by the product of the parasite's prevalence in each host species in a pair (Poulin, 2007), as follows:

$$S_{TD*} = \frac{\sum_{i < j} \omega_{ij} (p_i p_j)}{\sum_{i < j} (p_i p_j)}$$

where the double summation is over the set $\{i = 1, \dots, s; j = 1, \dots, s\}$, such that $i < j$ and s is the number of host species used by a parasite; w_{ij} is the taxonomic distinctness between host species i and j , or the number of taxonomic steps required to reach a node common to both; p_i and p_j are the prevalence of the parasite in host species i and j , respectively (Poulin and Mouillot, 2005). Thus, more weight is given to the taxonomic distance between two host species if the parasite achieves high prevalence values in these hosts than if the parasite occurs infrequently. This index was calculated with the program Borland

C++ Builder 6.0 (available online at <http://www.otago.ac.nz/zoology/downloads/poulin/TaxoBiodiv2>). The value of the Std* index is inversely proportional to specificity. A high index value means that the host species most frequently used by a parasite are, on average, not closely related, which means that its specificity is low. The taxonomic hierarchy used was: Phylum, Class, Order, Family, Tribe, Genus and Species. If the index reaches its maximum value (6) host species belong to different classes, if it reaches its minimum value (1) host species belong to the same genus. Taxonomic classification of hosts was made following Wilson and Reeder (2005) for rodents and Clements (2007) for birds. For this analysis we considered only host species represented by 60 specimens or more. This threshold sample size was estimated with the equation described in Arya et al. (2012) considering a 95% level of confidence, 20% of expected prevalence and 10% of allowable error. As a result of this decision, some host species, in special bird hosts of *A. tigrinum*, were not included in the analyses, probably underestimating the number of relevant host species; but the inclusion of reliable prevalence and burden values was prioritized.

Considering that tick host burdens might be influenced by densities of host-seeking ticks (Brunner and Ostfeld, 2008), we tested if the period of the year could have confounded or biased results on Std* by calculating the Std* value for all the seasons of the year together and another Std* for the period of high tick prevalence (Period H), i.e. from December through May for *A. tigrinum* and August through April for *A. triste*, and low tick prevalence (Period L: the remaining months). Birds trapped in Buenos Aires were sampled only in summer, so we included them only in the annual Std* index.

Secondly, not all the hosts species studied were included in the statistical models developed (see next paragraph). So, in order to be able to compare and discuss *A. triste* and *A. tigrinum* prevalence among all host species included in the Std* index (rodents and birds) we carried out a Chi-square analysis.

Finally, we evaluated the association between *A. tigrinum* and *A. triste* prevalence and burdens and their host species with generalized linear models (GLMs), including sex and period of the year (Period H and L) to control for potential confounding. Only adult and sub-adult rodents were included in the analyses. Eight different models were developed according to the response variables: 1) *A. tigrinum* larvae prevalence 2) *A. tigrinum* nymph prevalence 3) *A. triste* larvae prevalence 4) *A. triste* nymph prevalence 5) *A. tigrinum* larvae burdens 6) *A. tigrinum* nymph burdens 7) *A. triste* larvae burdens 8) *A. triste* nymph burdens. Generalized linear models were conducted with binomial and negative binomial responses for tick prevalence and burdens, respectively, using the statistical software R (R Core Team, 2014). For *A. triste* we developed a mixed model (GLMM) using the *glmmADMB* package, in order to control for the lack of independence of observations from the same trapping grid including the random intercept “quadrant ID”. For *A. tigrinum* we used the *MASS* package. The initial maximum model (model with all independent variables) was then restricted by stepwise elimination of unimportant terms. The criterion used to remove terms was Akaike information criteria (AIC) (Akaike, 1974) or, when overdispersion was present, Quasi-Akaike information criteria (QAIC) (Richards, 2008). Because data of bird sex was not available and the sampling methods of the two *A. triste* sampling groups was not homogeneous, we included in the models only data of rodents and data of *A. triste* obtained from November 2010 to October 2012. For *A. tigrinum* nymph analyses we did not include *Akodon dolores* (a cricetid rodent) data because burdens found were zero.

3. Results

A total of 604 and 783 rodents and birds were included in the analyses of *A. tigrinum* and *A. triste*, respectively. Host species, values of prevalence and burdens of larvae and nymphs of *A. tigrinum* and *A. triste* overall and by H and L periods are shown in Table 1. In the overall analysis we found that the cricetid rodents *A. dolores*, *Calomys venustus*,

Graomys centralis and the bird *Saltator multicolor* did not have different prevalences of *A. tigrinum* larvae ($p > 0.05$), but their prevalences were higher than those of the caviid *Galea musteloides* and the bird *Columbina picui* ($p < 0.01$). For *A. tigrinum* nymphs, *G. musteloides* and *S. multicolor* had the highest prevalence values ($p < 0.01$), followed by *C. venustus* and *G. centralis* and lastly, *A. dolores* and *C. picui* ($p < 0.01$). Regarding *A. triste*, the cricetid rodents *Oxymycterus rufus* and *Scapteromys aquaticus* had the highest larvae prevalences ($p < 0.01$), followed by the cricetid rodents *Akodon azarae*, *Oligoryzomys flavescens* and *O. nigripes* with similar prevalence values ($p > 0.05$), and lastly, the lowest prevalences were recorded in the birds *Zonotrichia capensis* and *Sporophila caerulescens*. For *A. triste* nymphs, *S. aquaticus* had the highest prevalence values ($p < 0.01$), followed by *O. nigripes*, then the remaining host species studied had similar lower values, except for *S. caerulescens*, which showed the lowest *A. triste* nymphs prevalence of all ($p < 0.01$).

Std* values are shown in Table 2. Values of *A. tigrinum* were comprised between 4.6 and 5.7 and were higher for nymphs than for larvae: 19%, 17% and 21% higher considering the whole year, H and L period, respectively. On average, Std* values were 4.6 for larvae and 5.6 for nymphs. This means that *A. tigrinum* immature stages have low specificity, especially nymphs, and that on average larvae and nymphs infest hosts species belonging to different orders and classes, respectively. Regarding *A. triste*, we found that values were comprised between 2.3 and 2.8 and that there were no differences of Std* values between larvae and nymphs within the same period. On average, Std* value was 2.5 for both larvae and nymphs, meaning that *A. triste* immature infests hosts of different tribes.

When testing if the period of the year has confounded or biased results on Std* values, we found that the periods studied were only slightly different (Table 2).

The models (Table 3) that evaluated the association between *A. tigrinum* larva and nymph prevalence with rodent host species, showed that *A. dolores* and *C. venustus* were the species with highest *A. tigrinum* larva prevalence. Whereas *G. musteloides* had the highest values of prevalence of *A. tigrinum* nymphs. Regarding *A. triste*, the models (Table 3) showed highest *A. triste* larvae prevalence for *S. aquaticus* and *O. rufus*. Whereas, *S. aquaticus* presented higher *A. triste* nymph prevalence than all other host species. The models (Table 4) that evaluated the association between rodent species and burdens of *A. tigrinum* larvae and nymphs, showed that *A. dolores* and *C. venustus* had the highest burdens of *A. tigrinum* larvae. Whereas, *G. musteloides* was by far the species with highest *A. tigrinum* nymph burdens. Regarding *A. triste*, the models (Table 4) showed that *O. rufus* and *S. aquaticus* were the species with greater larvae burdens. While *S. aquaticus* had also the highest burdens of *A. triste* nymphs.

Of the potential confounders assessed, period of the year was associated with larvae and nymphs of both tick species (Tables 3 and 4); tick prevalence and burdens were greater from December through May for *A. tigrinum* and August through April for *A. triste*. Regarding sex, the association found was not consistent; while for *A. tigrinum* larvae prevalence and burdens were higher in males, for *A. triste* the same association was found but for nymphs.

4. Discussion

This is the first work to conduct a specificity analysis considering both the taxonomic distinctness of host species and ecological features (host and environmental variables studied) of tick immature stages. The Std* index showed that *A. tigrinum* and *A. triste* larvae and nymphs are rather generalist. Nava and Guglielmono (2013) found that *A. tigrinum* and *A. triste* immature stages use host species that belong to different orders and different families as hosts, respectively; showing low host specificity. This is in agreement with what we found in the present study, with the exception of *A. tigrinum* nymphs infesting host species belonging to different classes, showing a slightly lower specificity, and

Table 1

Host species, total number of each host species in parentheses, prevalence (P) and mean abundance (MA) for larvae (LL) and nymphs (NN) of *Amblyomma tigrinum* and *Amblyomma triste* of all trapping year and differentiated in the period of high (period H) and low (period L) tick prevalence are shown.

Host species	All year				Period H				Period L			
	P (%)		MA		P (%)		MA		P (%)		MA	
	LL	NN	LL	NN	LL	NN	LL	NN	LL	NN	LL	NN
<i>A. tigrinum</i>												
Rodents												
<i>Akodon dolores</i> (110)	55	0	3.4	0	71	0	3.2	0	53	0	3.5	0
<i>Calomys venustus</i> (58)	50	10	6.5	0.12	77	8	11.6	0.2	38	6	4.5	0.1
<i>Graomys centralis</i> (117)	43	3	2.2	0.02	58	3	5.1	0.03	36	3	0.8	0.02
<i>Galea musteloides</i> (219)	17	55	1.5	4.2	32	68	4.1	6.3	11	51	0.4	3.3
Birds												
<i>Columbina picui</i> (66)	9	0	0.3	0	6	0	0.5	0	8	0	0.2	0
<i>Saltatricula multicolor</i> (59)	65	61	3.5	1.4	75	23	3.8	0.3	58	70	3.5	1.7
<i>A. triste</i>												
Rodents												
<i>Akodon azarae</i> (452)	25	11	2	0.2	31	15	3	0.3	11	2	0.2	0.04
<i>Oligoryzomys flavescens</i> (135)	22	16	2.7	0.5	34	28	5	1	6	2	0.3	0.04
<i>Oligoryzomys nigripes</i> (65)	19	34	1.2	1.3	24	19	1.5	1.7	0	0	0	0
<i>Oxymycterus rufus</i> (190)	40	16	11	0.4	50	21	14	0.5	15	2	0.5	0.04
<i>Scapteromys aquaticus</i> (62)	50	51	7	3	60	63	7.4	4	26	26	6	0.6
Birds												
<i>Sporophila caeruleascens</i> (59)	2	0	0.2	0	2	0	0.2	0	–	–	–	–
<i>Zonotrichia capensis</i> (63)	6.3	4	0.1	0.06	6.3	4	0.1	0.06	–	–	–	–

Table 2

Values of the specificity index Std* proposed by Poulin and Mouillot (2005) for larvae (LL) and nymphs (NN) of *Amblyomma tigrinum* and *Amblyomma triste* of all trapping year and differentiated in the period of high (Period H) and low (Period L) tick prevalence.

	All year	Period H	Period L
Std* <i>A. tigrinum</i>			
LL	4.7	4.6	4.7
NN	5.6	5.4	5.7
Std* <i>A. triste</i>			
LL	2.8	2.5	2.3
NN	2.8	2.5	2.3

A. triste immature found on hosts of different tribes, showing a slightly higher specificity. These differences could be attributed to the fact that in Nava and Guglielmone (2013) larvae and nymphs were analyzed together and considering the whole Neotropical region as a location, whereas other authors have found that tick species are globally generalists but can present higher specificity at a local scale (McCoy et al., 2013; Esser et al., 2016).

The Std* index showed that nymphs of *A. tigrinum* are more generalist than larvae, with Std* values between 17 and 21% higher in the former. Caviidae were found to be important hosts for *A. tigrinum* nymphs but not for larvae (see below), possibly producing the Std* differences. This was not observed between *A. triste* larvae and nymphs, which could be attributed to the fact that, while for *A. tigrinum* rodents hosts of the family Cricetidae and Caviidae were included in the analyses, Caviidae were not included for *A. triste* because of the low number of specimens captured (only 11 animals). Half of them were parasitized with *A. triste* larvae and 30% with nymphs (Nava et al., 2011). So, the incorporation of this host family to the analysis of *A. triste* nymph specificity could probably increase its Std* value. These findings emphasize the importance of studying the specificity of ticks in their different life stages.

Amblyomma triste immatures showed lower Std* values than *A. tigrinum*, suggesting that the former infest hosts with less taxonomic distances. Ticks with greater ecological plasticity are distributed in environmental settings with different conditions, which entails that they may be exposed to a wider diversity of hosts. It is noteworthy that although *A. tigrinum* is more restricted than *A. triste* in terms of

geographical distance, *A. tigrinum* shows ecological plasticity and can colonize regions with different characteristics of climate and vegetation, while the distribution of *A. triste* is mostly restricted to wetlands and flooding areas (Nava et al., 2017) being exposed to a lower diversity of hosts than *A. tigrinum*. The differences in the Std* index found between tick species, therefore, might be attributed to this point, and also to the fact that birds are common hosts of *A. tigrinum*, whereas they are occasional hosts of *A. triste* (Guglielmone et al., 2000; Nava et al., 2006; Nava et al., 2011; Flores et al., 2014), and that Caviidae was only included in the *A. tigrinum* analysis.

Regarding Std* index values obtained in the different periods of the year (period H and L), we did not find enough evidence to conclude that the densities of host-seeking ticks might be related to seasonal and host behavioral patterns.

When analyzing associations between host species and *A. tigrinum* and *A. triste* immature prevalence and burdens, we found that both tick species infest some hosts more heavily than others. Even though we found that immature stages of *A. triste* and *A. tigrinum* are generalist ticks, the level of infestation within the usual host range of these two species is uneven for both immature stages. This means that a generalist behavior does not directly imply similar levels of infestation across a range of usual hosts. This uneven parasitism might be the result of different phenomena, including environmental characteristics (Brunner and Ostfeld, 2008), host and tick behavior and host resistance (Fourie and Kok, 1992). Nava et al. (2006) found that bird species that feed on forested areas were more frequently parasitized with *A. tigrinum* immature than birds that feed in open areas and birds that do not feed on the ground. Maroli et al. (2015) found that *A. azarae*, *O. rufus* and *S. aquaticus* have different habitats and movement patterns at our study area. *Akodon azarae*, here found with low *A. triste* burdens, showed a broad horizontal and vertical use of space, climbing plants up to one meter in height, moving above the vegetation; whereas *O. rufus* and *S. aquaticus*, both with high *A. triste* burdens in our study, used space horizontally and always at ground level, probably facilitating encounter with *A. triste* immatures. On the other hand, different host species could develop different immune responses in light of tick infestation, with different levels of efficiency on tick removal. For example, some host species or lineages are resistant to *Rhipicephalus sanguineus* infestation, by developing a cellular immune response predominantly of basophils, whereas in less resistant hosts neutrophils and eosinophils prevail and

Table 3

Generalized linear model showing the association between *A. tigrinum* larva (model 1) and nymph (model 2) prevalence, *A. triste* larva (model 3) and nymph (model 4) prevalence, and host species as independent variables, sex and/or period of the year as confounding factors.

Model 1: Response = <i>Amblyomma tigrinum</i> larval prevalence Independent variables = Host species + Sex + Period of the year			
Term	Coefficients	Standard error	P-value
Intercept	-0.191	0.254	0.451
<i>C. venustus</i> ^a	-0.385	0.404	0.343
<i>G. centralis</i> ^a	-0.886	0.312	0.005*
<i>G. musteloides</i> ^a	-2.275	0.303	6.4e-14*
Sex ^b	0.658	0.229	0.004*
Period ^c	1.329	0.229	5.4e-07*

Model 2: Response = <i>Amblyomma tigrinum</i> nymph prevalence Independent variables = Host species + Period of the year			
Term	Coefficients	Standard error	P-value
Intercept	-2.739	0.613	5.0 e-06*
-	-	-	-
<i>G. centralis</i> ^a	-1.086	0.839	0.195
<i>G. musteloides</i> ^a	2.817	0.618	5.2e-06*
-	-	-	-
Period ^c	0.672	0.289	0.020*

Model 3: Response = <i>Amblyomma triste</i> larvae prevalence Independent variables = Host species + Period of the year Random intercept = "Grid ID"			
Term	Coefficients	Standard error	P-value
Intercept	-2.796	0.419	2.5 e-11 [†]
<i>O. flavesc</i> ^a	0.147	0.321	0.647
<i>O. nigripes</i> ^a	-0.119	0.464	0.797
<i>O. rufus</i> ^a	0.849	0.250	6.7 e-4 [*]
<i>S. aquaticus</i> ^a	1.514	0.445	6.6 e-4 [*]
-	-	-	-
Period ^c	2.013	0.298	1.5 e-11 [†]

Model 4: Response = <i>Amblyomma triste</i> nymph prevalence Independent variables = Host species + Sex + Period of the year. Random intercept = "Grid ID"			
Term	Coefficients	Standard error	P-value
Intercept	-4.758	0.525	< 2 e-16*
<i>O. flavesc</i> ^a	0.691	0.344	0.045*
<i>O. nigripes</i> ^a	0.704	0.495	0.155
<i>O. rufus</i> ^a	0.370	0.291	0.204
<i>S. aquaticus</i> ^a	3.324	0.495	1.9e-11*
Sex ^b	0.606	0.242	0.012*
Period ^c	2.566	0.411	4.3e-10*

Reference levels: Model 1: ^a *A. dolores*.
 Model 2: ^a *C. venustus*.
 Model 3 and 4: ^a *A. azarae*.
 Model 1–4: ^b Female, ^c Low.

higher burdens of ticks are found on them than in the former (Szabó et al., 1995; Ferreira et al., 2003; Veronez et al., 2010). Variation of grooming habits across species could be another factor influencing the asymmetric tick burdens found, grooming being a way of reducing burdens of immature ticks documented on rodents (Shaw et al., 2003) and other mammal species (Mooring et al., 1996).

On the other hand, we found that the main hosts for larvae are not the same as for nymphs, with the exception of the association *A. triste* – *S. aquaticus*. Tick preferences to feed on different host species depending on their life stage have been reported by other studies (e.g. Eisen et al., 2004; Nava et al., 2006; Boyard et al., 2008) but the reasons underlying this phenomenon remain unclear. These findings encourage the study of tick – host associations taking into account their life stage.

Our study has limitations related to the representativeness of some

Table 4

Generalized linear model showing the association between *A. tigrinum* larva (model 5) and nymph (model 6) burdens, *A. triste* larva (model 7) and nymph (model 8) burdens, and host species as independent variables, sex and/or period of the year as confounding factors.

Model 5: Response = <i>Amblyomma tigrinum</i> larvae burdens Independent variables = Host species + Sex + Period of the year			
Term	Coefficients	Standard error	P-value
Intercept	0.209	0.277	0.450
<i>C. venustus</i> ^a	0.514	0.431	0.233
<i>G. centralis</i> ^a	-0.801	0.340	0.018*
<i>G. musteloides</i> ^a	-1.596	0.277	9.3e-08*
Sex ^b	0.905	0.230	8.6e-05*
Period ^c	1.778	0.228	5.5e-13*

Model 6: Response = <i>Amblyomma tigrinum</i> nymph burdens Independent variables = Host species + Period of the year			
Term	Coefficients	Standard error	P-value
Intercept	-2.310	0.533	1.5e-05*
-	-	-	-
<i>G. centralis</i> ^a	-1.535	0.797	0.054
<i>G. musteloides</i> ^a	3.605	0.539	2.3e-11*
-	-	-	-
Period ^c	0.533	0.252	0.035*

Model 7: Response = <i>Amblyomma triste</i> larvae burdens Independent variables = Host species + Period of the year Random intercept = "Grid ID"			
Term	Coefficients	Standard error	P-value
Intercept	-3.011	0.482	4.1e-10*
<i>O. flavesc</i> ^a	0.689	0.324	0.033*
<i>O. nigripes</i> ^a	0.040	0.501	0.936
<i>O. rufus</i> ^a	1.486	0.258	8.0e-09*
<i>S. aquaticus</i> ^a	1.405	0.413	6.7e-04*
-	-	-	-
Period ^c	3.294	0.304	< 2.0e-16*

Model 8: Response = <i>Amblyomma triste</i> nymph burdens Independent variables = Host species + Sex + Period of the year Random intercept = "Grid ID"			
Term	Coefficients	Standard error	P-value
Intercept	-4.507	0.485	< 2.0e-16*
<i>O. flavesc</i> ^a	0.776	0.316	0.014*
<i>O. nigripes</i> ^a	1.258	0.409	0.002*
<i>O. rufus</i> ^a	0.250	0.277	0.366
<i>S. aquaticus</i> ^a	2.492	0.354	1.8e-12*
Sex ^b	0.570	0.225	0.011*
Period ^c	2.497	0.346	5.0e-13*

Reference levels: Model 5: ^a *A. dolores*.
 Model 6: ^a *C. venustus*.
 Model 7 and 8: ^a *A. azarae*.
 Model 5–8: ^b Female, ^c Low.

host species, potentially modifying the levels of specificity we found. For example, although *A. tigrinum* immatures were found on 23 bird species (Nava et al., 2006), we have included only two in the analyses because of the low number of captures of each bird species. As has been described in the material and methods section, the analysis of reliable prevalence and abundance values was prioritized. The fact that *A. tigrinum* hosts encompass different classes (Mammalia and Aves), preclude the hypothetical findings of lower Std* values when including more bird species in *A. tigrinum* analysis, no matter how high the prevalence values of the new bird species included would be. Besides, to truly understand the importance of different host species on tick ecology, specificity analyses should consider not only burden and prevalence values but also the number of ticks that successfully feed and engorge on each host species. Further studies should overcome the

limitations of this work by including other potential confounding factors, such as host body condition, body length, environmental variables, and considering host behavior and immune response, that may help to understand the reasons for the uneven host-use patterns of ticks in their various host species. In addition, studies in other systems and circumstances should test the hypothesis posited here to establish the generality of the pattern presented in this study.

5. Conclusions

In the present study we found that different tick immature stages might have different levels of specificity and preferences to feed on different host species, showing the importance of studying the specificity of ticks distinguishing their life stage.

Besides, we found that both tick species infest some hosts more heavily over others despite their generalist behavior. This shows that a generalist behavior may result in dissimilar levels of infestation across a range of usual hosts. In this context, distinguishing tick life stages in the investigation of local host patterns is necessary for an understanding of tick ecology globally.

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Conflict of interest

None

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