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ORIGINAL PAPER

Ecology of the interaction between *Ixodes loricatus* (Acari: Ixodidae) and *Akodon azarae* (Rodentia: Criceridae)

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Abstract The present study explores associations of different factors (i.e. host parameters, presence of other ectoparasites and [mainly biotic] environmental factors) with burdens of *Ixodes loricatus* immature stages in one of its main hosts in Argentina, the rodent *Akodon azarae*. For 2 years, rodents were trapped and sampled monthly at 16 points located in four different sites in the Parana River Delta region. Data were analysed with generalized linear mixed models with a negative binomial response (counts of larvae or nymphs). The independent variables assessed were (a) environmental: trapping year, presence of cattle, type of vegetation, rodent abundance; (b) host parameters: body length, sex, body condition, blood cell counts, natural antibody titers and (c) co-infestation with other ectoparasites. Two-way interaction terms deemed a priori as relevant were also included in the analysis. Most of the associations investigated were found significant, but in general, the direction and magnitude of the associations were context-dependent. An exception was the presence of cattle, which was consistently negatively associated with both larvae and nymphs independently of all other variables considered and had the strongest effect on tick burdens. Mites, fleas and *Amblyomma triste* were also significantly associated (mostly positively) with larval and nymph burdens, and in many cases, they influenced associations with environmental or host factors. Our findings strongly support that raising cattle may have

a substantial impact on the dynamics of *I. loricatus* and that interactions within the ectoparasite community may be an important—but generally ignored—driver of tick dynamics.

Keywords Parasite-host relationships · Disease ecology · Ixodidae · Rodents · Tick-borne diseases

Introduction

The distribution and burdens of parasites depend on interactions and processes occurring among parasites, hosts and the environment (e.g. Vaclav et al. 2008; Telfer et al. 2010; Cardon et al. 2011; Lutermann et al. 2015). Hence, the distribution and abundance of a given parasite depend on complex interactions and may result in complex dynamics. Knowing which factors are important for these dynamics is important to understand the ecology of parasite communities and diseases. In general, studies on parasite ecology have focused on single or few host-related or environment-related factors. Studies that integrate the joint influence of several relevant factors are rare (e.g. Krasnov et al. 2005; Cardon et al. 2011; Kiffner et al. 2011), although they might be important to unveil the web of causation behind the occurrence of a given parasite.

Ixodes loricatus Neumann, 1899 (Acari: Ixodidae) is a South American three-host tick whose adult stages are found in marsupials of the family Didelphidae, while immature stages parasitize cricetid rodents of the subfamily Sigmodontinae, preferring *Akodon azarae* (Rodentia: Cricetidae) in the southern range of its distribution (Nava et al. 2004; Beldomenico et al. 2005; Lareschi 2010; Guglielmone et al. 2011; Colombo et al. 2013). Few previous studies have contributed to our knowledge of the ecology of this tick species finding an absence of seasonality, an influence of host body size and contradictory results on gender bias

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(Lareschi et al. 2003; Nava et al. 2003, 2004; Beldomenico et al. 2005; Navone et al. 2009; Lareschi 2010; Colombo et al. 2014). Multivariable approaches that take into account potential confounders and effect modifiers have been undertaken by a single study that assessed seasonality (Colombo et al. 2014).

At the study area, *I. loricatus* immature stages share their sigmodontinae hosts with the ixodid tick *Amblyomma triste* (Nava et al. 2011; Colombo et al. 2013). This is a three-host tick whose adults parasitize large mammals, like *Blastocerus dichotomus*, *Hydrochoerus hydrochaeris*, wild and domestic carnivores, cattle, goats, horses and humans (Szabó et al. 2003; Guglielmone et al. 2006; Venzal et al. 2008; Nava et al. 2011). This tick is of public health importance since it transmits the human pathogen *Rickettsia parkeri* in the southern cone of South America (Venzal et al. 2004; Pacheco et al. 2006; Silveira et al. 2007; Nava et al. 2008).

There is a paucity of studies integrally evaluating environmental and host factors. Here, we offer, for the first time, a multivariable analysis that explores associations between burdens of immature stages of the tick *I. loricatus* and different variables on its main host in Argentina, the wild rodent *A. azarae*, under the hypothesis that host and/or environmental factors interact to influence *I. loricatus* distribution and burdens. The factors evaluated included those known to have the potential to influence ectoparasite burdens (i.e. host sex, age, physiological condition and immune response; season; host abundance) (Hughes and Randolph 2001; Soliman et al. 2001; Brunner and Ostfeld 2008; Kiffner et al. 2011; Nava et al. 2011; Anderson et al. 2013), environmental variables that result from dominant human activities in the study area (cattle and implanted forests), and also the often ignored accompanying ectoparasite community.

Materials and methods

Study area

The study was conducted in Campana (34° 11 S, 58° 50 W) in the Estación Experimental Agropecuaria Delta, Instituto Nacional de Tecnología Agropecuaria (INTA), Argentina. The site is characterized by levees that surround dry areas as well as temporarily or permanently flooded marshes with the dominance of graminoids and *Erythrina crista-galli* forests (Kandus et al. 2003). Also, the site has areas with *Cortadeira* spp., *Cynodon* spp. and commercial forestations of *Populus* spp. and *Salix* spp. The site is located in the lower Parana River Delta region, which is the southern extension of the Paranense Province of the Amazonic Phytogeographic Dominion (Cabrera 1994). The climate is temperate with a mean annual temperature of 16.7 °C and a mean annual rainfall of 1000 mm with an undefined rainy season (Kandus and Malvárez 2004). The most important economic activities are extensive cattle raising and salicaceae afforestation (Zoffoli et al. 2008). In the study area, there is a herd of beef cattle consisting of 21 Aberdeen Angus at a density of approximately one cow per hectare.

Data collection

Rodents were captured from November 2010 through October 2012 in three-night trapping sessions carried out every 5 weeks. Four trapping grids were set out at four different sites, each grid with an interval of at least 200 m from each other and consisting of squares with 12 Sherman-type live traps in the corners (192 per night) and two Ugglan-type live traps in the middle of the square (32 per night), baited with pelleted dog food. Within a site, two of the grids were located in places with natural grassland and the other two with implanted forest (*Populus* spp.). Half of the sites were located in extensive cattle raising lands and the other half in areas where cattle were absent. Rodent procedures, sampling and laboratory methods were carried out as described in Colombo et al. (2014). Rodents captured were later identified to the species level by assessing cranium morphology. In addition to the recovery of larvae (LL) and nymphs (NN) of *I. loricatus*, other ectoparasites were also recovered and counted separately by group: *A. triste* (the other tick species found in the studied rodents), mites (Laelapidae and/or Macronyssidae), lice (Hoplopleuridae) and fleas (Stephanocircidae and/or Ropalophsyllidae). Ticks were determined following Marques et al. (2004) and Estrada-Peña et al. (2005). All procedures were carried out 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.

Reproductive status of rodents was classified as "active", i.e. males with scrotal testicles and well developed seminal glands, females with signs of oeustrus, evidence of present or recent pregnancy or lactation, or "inactive" (no signs of the above). Blood samples were taken by heart puncture and collected in heparin-coated capillary tubes and eppendorf tubes without anticoagulant. White blood cells (WBC) and red blood cells (RBC) were counted and its concentration in blood estimated (cells/microlitre) following hematological methods used by Beldomenico et al. (2008). Natural antibodies (NAbs) were determined using a hemagglutination assay as described by Racca et al. (2014). RBC, WBC and NAb were used as proxies of the physiological condition of the hosts. RBC levels are indicative of poor aerobic capacity and result mainly from deficient nourishment and infection or parasitism (Beldomenico et al. 2008). WBC counts were used as a proxy of investment in cellular immunity (Beldomenico et al. 2008) and NAb as indicators of humoral immunity (Racca et al. 2014).

Statistical analysis

The analyses were conducted with generalized linear mixed models (GLMMs) with negative binomial responses, i.e. counts of LL or NN, using the *glmmADMB* package of the statistical software R (R Foundation for Statistical Computing, http://www.r-project.org). LL and NN abundance was considered separately. To control for the lack of independence of observations from the same trapping grid, we included the random intercept "grid ID". Kappa (κ) index (Hudson et al. 2002) was estimated in which 0 represents a total aggregation and 1 a non-aggregation of the ticks. The morphometric measures were used to create an estimate of body condition as a residual index (Green 2001), modified to adjust for distortions in the size/weight relationships caused by pregnancy. This index was calculated with a linear regression of body mass (response variable) against total length and pregnancy status (four-level factor: non-pregnant—which includes males—early pregnancy, mid-term pregnancy, advanced pregnancy). The independent variables of interest were as follows: (a) *environmental*: trapping year (1: Nov. 10–Oct. 11, 2: Nov. 11–Oct. 12); cattle (present or absent); type of vegetation (natural grassland or implanted forest); rodent abundance (total number of rodents captured in the same grid during the trapping session); season was assessed in a separate study (Colombo et al. 2014), but here, it was included in the analysis to control for potential confounding and effect modification; (b) *host parameters*: body length (proxy of age); sex; body condition (residual index); RBC; WBC; NAb; reproductive status (active-inactive); (c) *other ectoparasites* (total counts): other stage of *I. loricatus*; *A. triste*; lice; mites; fleas. Two-way interaction terms deemed a priori as relevant were also included in the analysis. Relevance was judged according to the biological sense of the hypothesis that the interaction term was testing. For example, the interaction "body condition × *A. triste*" evaluates the hypothesis that the influence of having *A. triste* on the infestation by *I. loricatus* is greater in individuals in poor condition than in those in good condition, which might be expected if the interaction between both ticks originates from facilitation via impaired defences. This multivariable approach examines the effect of each variable of interest while adjusting for all other independent variables included in the model—e.g. the effect of season for equal values of sex, body condition, etc., and in addition, it assesses relevant interaction terms. Because the breeding season spans from October to March, reproductive status (active/inactive) was assessed in each final model using a subset of data that excluded inactive months. The initial maximum model (model with all independent variables and selected two-way interactions) was then restricted by stepwise elimination of unimportant terms. The criterion used to remove the interaction term was Akaike information criteria (AIC) (Akaike 1974) or, when overdispersion was present, Quasi-

Akaike information criteria (QAIC) (Richards 2008). A single term was not retained in the model if its inclusion did not reduce AIC by at least 2 units.

Results

The number of *A. azarae* analysed was 388. The overall prevalence of *I. loricatus* was 19.5 %, being 11.5 and 11 % of LL and NN, respectively. The maximum number of LL collected from a single host was 10 (attached), whereas for NN it was 8. Tick counts showed aggregated distributions for LL ($\kappa=0.09$) and NN ($\kappa=0.11$). Regarding other ectoparasites, 33 % had *A. triste* (LL and/or NN), 37 % had lice (Hoplopleuridae), 48 % fleas (Stephanocircidae and/or Ropalophsyllidae) and 86 % had mites (Laelapidae and/or Macronyssidae). The final models for LL and NN are presented in Tables 1 and 2, respectively.

Regarding the host variables analysed, we found a male-biased parasitism for *I. loricatus* LL. Their burdens were greater in males than in females, especially among individuals with null or low mite counts (Table 1, Fig. 1), while this association was not observed for NN. On the other hand, the effect of body length on *I. loricatus* burdens depended on the presence of other ectoparasites and was different for LL and NN. For LL, the association was strongly negative, but only among *A. azarae* with high burdens of fleas (Table 1, Fig. 2). Regarding NN, burdens were greater the larger the rodent, especially when *A. triste* counts were high (Table 2, Fig. 3).

Another host factor found associated with *I. loricatus* burdens was WBC: There was a negative association between WBC and NN, but only significant when *A. triste* was present at moderate to high burdens (Table 2, Fig. 3). *A. azarae* with *A. triste* had lower *I. loricatus* NN as levels of WBC increased, and this relationship was stronger the greater the *A. triste* burden. This association was not observed for LL.

Concerning environmental variables, the presence of cattle had a strong negative influence on both LL and NN burdens (Tables 1 and 2), independently of other studied variables. Mean larval burdens were over 80 times higher in sites without cattle when compared with sites where cattle were present, and mean nymph burdens were over 20 times higher.

Also, we found that rodent abundance was associated with *I. loricatus* burdens. This relationship depended on season (for LL) and on *A. triste* burdens (for NN). LL increased with rodent abundance in spring, summer and autumn, but decreased in winter (Table 1, Fig. 4). NN burdens were negatively correlated with rodent abundance, especially in those rodents with low *A. triste* counts (Table 2, Fig. 5).

Finally, the presence of other ectoparasites showed a significant association with *I. loricatus* burdens. Mean larval

Table 1 Generalized linear mixed models showing the association between *I. loricatus* larval mean abundance per host and environmental variables, host parameters and other ectoparasites as independent variables

Model 1:
Response=*Ixodes loricatus* larval abundance
Independent variables=season+cattle+sex+rodent abundance+body length+fleas+mites+*A. triste*+rodent
abundance×season+*A. triste*×season+sex×mites+body length×fleas
Random intercept="grid ID"

Term	Coefficients	Standard error	*P* value	ΔAIC*
Intercept	−14.275	4.132	5.0e-04	–
Season[spring a]	0.472	1.309	0.718	–
Season[summer a]	1.011	1.389	0.466	–
Season[winter a]	2.726	1.125	0.015	–
Cattle[b]	−4.420	1.212	2.0e-04	16
Sex[c]	1.643	0.619	0.008	–
Rodent abundance	0.425	0.216	0.049	–
Body length	1.012	0.385	0.008	–
Fleas	5.502	2.303	0.016	–
Mites	0.201	0.073	0.006	–
A. triste	0.708	0.300	0.018	–
Rod. abun×season[spring a]	−0.285	0.274	0.297	2.2
Rod. abun×season[summer a]	−0.089	0.323	0.782	2.2
Rod. abun×season[winter a]	−0.641	0.281	0.022	2.2
A. triste×season[spring a]	−0.679	0.299	0.023	3.4
A. triste×season[summer a]	−0.998	0.343	0.003	3.4
A. triste×season[winter a]	−0.716	0.314	0.022	3.4
Sex[c]×mites	−0.209	0.085	0.015	4
Body length×fleas	−0.552	0.234	0.018	4

Reference levels:
[a] Autumn
[b] Absent
[c] Female
*AIC value increment if the single term is dropped

abundance was greater the more mites were co-infecting female hosts, but this association was not observed for males (Table 1, Fig. 1). In addition, we found that in autumn, as burdens of *A. triste* increased, mean *I. loricatus* larval abundance also increased, whereas a negative correlation occurred in summer. No association was found between *A. triste* and *I. loricatus* LL in winter and spring (Table 1, Fig. 4). Regarding *I. loricatus* NN, the association with *A. triste* was positive and especially strong among hosts that were large and/or had low WBC and at high rodent abundances (Table 2, Figs. 3 and 5). Lastly, a positive correlation was observed between LL and flea burdens, which was especially strong among smaller rodents (Table 1, Fig. 2).

We failed to find association between *I. loricatus* burdens and body condition, RBC, NAb, reproductive status, other stage of *I. loricatus*, lice, type of vegetation and trapping year.

Discussion

The extant data on *I. loricatus* ecology is scant. It is known that *A. azarae* is one of the main hosts for its immature stages in the southern range of its distribution, being found in other rodents like *Oxymycterus rufus*, *Oligoryzomys nigripes* and *Scapteromys aquaticus* (Nava et al. 2004; Beldomenico et al. 2005; Lareschi 2010; Colombo et al. 2013). On the other hand, two works assessed the seasonality of this tick. Barros-Battesti et al. (2000) found that larvae and nymphs were more abundant from April to September. Later, by means of multivariable analyses, Colombo et al. (2014) showed that apparent seasonal patterns resulted from ignoring potential confounders. When those confounders were taken into account, no seasonality was found. Regarding host factors, Beldomenico et al. (2005) failed to find association with age and sex, whereas Lareschi (2000, 2010) found a male-biased parasitism.

Table 2 Generalized linear mixed models showing the association between *I. loricatus* nymph mean abundance per host and environmental variables, host parameters and other ectoparasites as independent variables

Model 2:

Response=*Ixodes loricatus* nymph abundance

Independent variables=cattle+rodent abundance+*A. triste*+body length+WBC+*A. triste*×rod abund+*A. triste*×body length+*A. triste*×WBC

Random intercept="grid ID"

Term	Coefficients	Standard error	P value	ΔAIC*
Intercept	−9.960	2.790	4.0e-04	–
Cattle[a]	−3.260	0.848	1.0e-04	16.7
Rodent abundance	−0.152	0.091	0.096	–
A. triste	0.953	0.280	6.6e-04	–
Body length	0.885	0.257	5.7e-04	–
WBC	−1.2e-06	3.1e-05	0.968	–
A. triste×rod. abund.	0.037	0.012	2.0e-03	7.2
A. triste×body length	−0.076	0.023	1.1e-03	8.6
A. triste×WBC	−2.4e-05	8.6e-06	5.1e-03	6.0

Reference levels:

[a] Absent

*AIC value increment if the single term is dropped

Gender-biased parasitism is controversial. It is not ubiquitous neither consistent. It depends on biological features of a particular host and/or parasite species, varies spatially or temporally and is mediated by environmental factors (Krasnov et al. 2012). Many studies found higher burdens of ectoparasites in males, attributed to different behaviour, size and levels of steroid hormones (Hughes and Randolph 2001; Soliman et al. 2001; Krasnov et al. 2005; Boyard et al. 2008; Harrison et al. 2010; Lutermann, et al. 2015). However, others have reported female-biased parasitism by ectoparasites (Krasnov et al. 2005). Here, we found that males had higher burdens of

I. loricatus larvae than females, in agreement with Lareschi (2010), but this association depended on mite burdens. A strong male-biased parasitism was observed among individuals with null or low mite counts, whereas no association with sex was present among mice with higher mite burdens. These findings provide additional indication of the complexity of this issue and suggest that there might be a sex-dependent interaction between ectoparasites that deserves further analysis.

Host age and body size are other host factors that have been found associated with ectoparasite burdens (Soliman et al. 2001; Hudson et al. 2002; Brunner and Ostfeld 2008; Vaclav
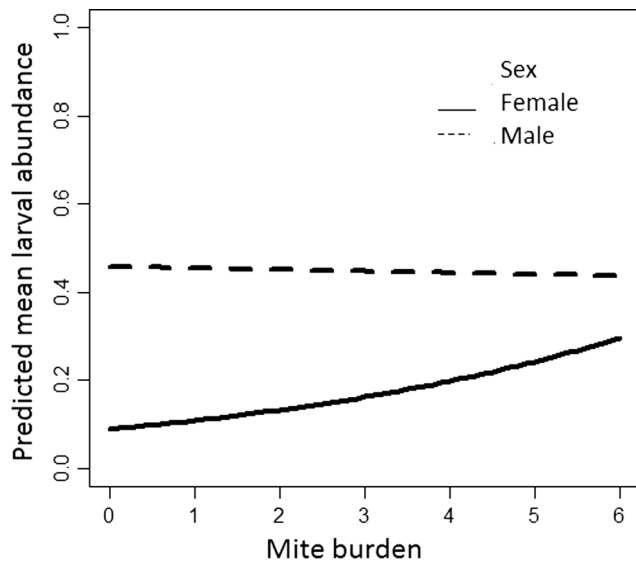

Fig. 1 Mean *Ixodes loricatus* larval abundance as predicted by a GLMM, showing the interaction between sex and mite burdens. (Prediction for a rodent in summer, at a site without cattle. All other variables were set at average or modal levels)
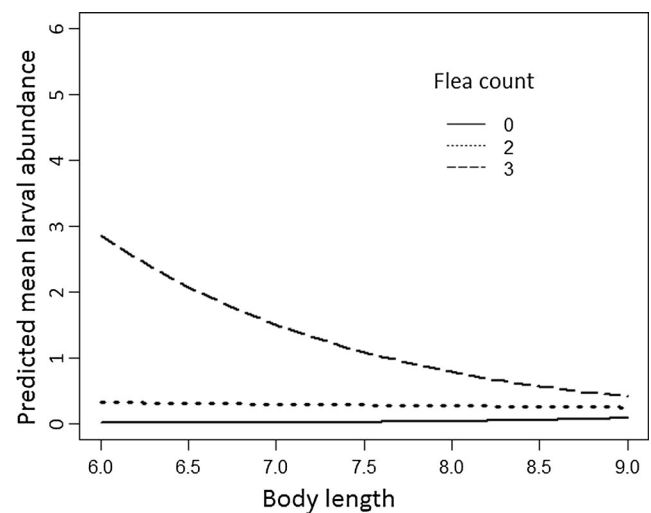

Fig. 2 Mean *Ixodes loricatus* larval abundance as predicted by a GLMM, showing the interaction between fleas and host body length. (Prediction for a male in summer, at a site without cattle. All other variables were set at average or modal levels)
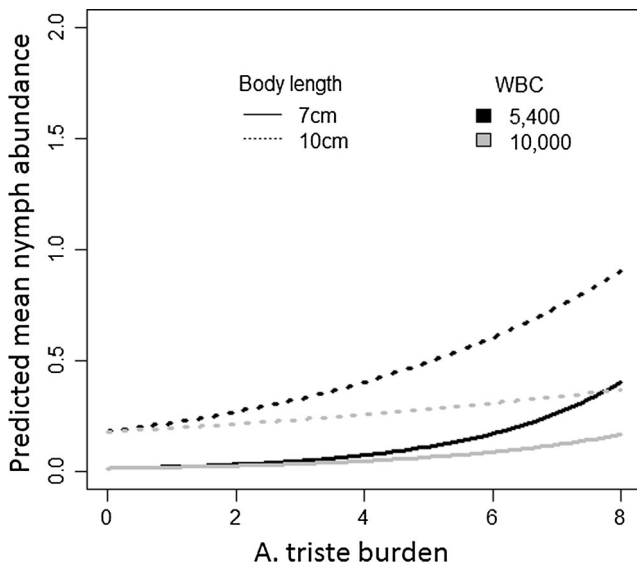
Fig. 3 Mean *Ixodes loricatus* nymph abundance as predicted by a GLMM, depicting the effect of *A. triste* burden for different values of body length and white blood cell counts. (Prediction for a male in summer, at a site without cattle. All other variables were set at average or modal levels)
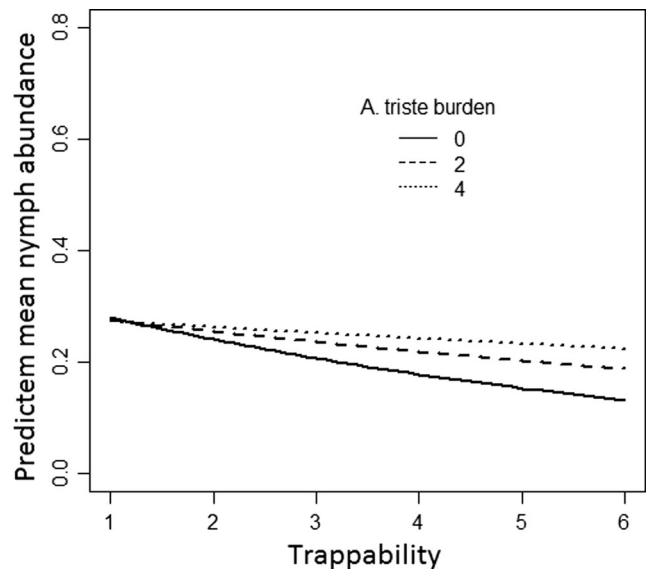

Fig. 5 Mean *Ixodes loricatus* nymph abundance as predicted by a GLMM, showing the interaction between *Amblyomma triste* burdens and rodent abundance (trappability). (Prediction for a male in summer, at a site without cattle. All other variables were set at average or modal levels)

et al. 2008; Cardon et al. 2011). The single previous study that tested this hypothesis for *I. loricatus* failed to find a significant association (Beldomenico et al. 2005). Previous studies on other *Ixodes* spp. immature stages on Sigmodontinae hosts found larger/older rodents more prone to have higher burdens (Beldomenico et al. 2004; Brunner and Ostfeld 2008). Here,

Fig. 4 Mean *Ixodes loricatus* larval abundance as predicted by a GLMM, showing the interaction between season and rodent abundance (trappability) for different burdens of *Amblyomma triste*. (Prediction for a male at a site without cattle. All other variables were set at average or modal levels)

we found a significant but inconsistent influence of body length on tick burdens. The association was negative for LL and positive for NN and again conditional on the parasitism by other ectoparasite. Small/young individuals with greater flea burdens had much higher *I. loricatus* LL counts than large/older individuals with similar flea loads. Larger/older mice with high *A. triste* burdens had higher *I. loricatus* NN. It has been hypothesized that larger/older hosts have greater parasite burdens because of a cumulative effect (Hawlena et al. 2006; Roulin et al. 2007; Vicente et al. 2007; Hayward et al. 2009) and because they offer a greater body surface for a higher number of parasites (Arneberg et al. 1998; Bandilla et al. 2008). On the other hand, Hudson et al. (2002) described that parasite burdens reach a plateau and subsequently decline in larger/older animals, probably as a result of an acquired immune response. Our results show that the phenomenon is highly complex in the studied system and suggest that the patterns observed for LL and NN are driven by different mechanisms and are influenced by other co-occurring ectoparasites. The host-acquired immune response might be important for resistance against LL but less so for NN.
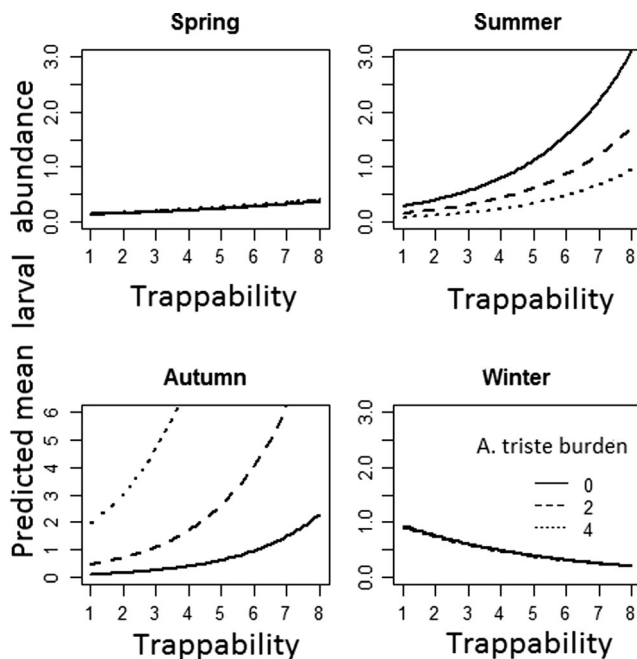
Measures of the immune function have seldom been causally related to ectoparasite burdens and rarely for a tick species on a wild host (Anderson et al. 2013). However, it is expected that a weakened immune function of hosts affects positively parasite fitness (Krasnov et al. 2005; Beldomenico and Begon 2010; Anderson et al. 2013). Here, a negative association between *I. loricatus* NN and WBC became apparent in individuals with moderate to high burdens of *A. triste*. Individuals with low WBC counts and important burdens of *A. triste* were especially prone to be infested by *I. loricatus* NN. Increased

WBC counts may be indicative of inflammatory response, and inflammatory responses are known to negatively impact blood feeding ectoparasites (Owen et al. 2010). Furthermore, tick salivary proteins suppress inflammatory immune responses locally by neutralizing chemokines that recruit cells of the immune system (Déruaz et al. 2008) and disrupt migration and phagocytosis by macrophages (Bowen et al. 2010; Kramer et al. 2011). Thus, it makes sense that individuals with suboptimal inflammatory response are more parasitized by ticks.

Turning to environmental factors, characteristics such as rodent abundance may correlate negatively with mean ecto-parasite abundance (Sorci et al. 1997; Stanko et al. 2002; Brunner and Ostfeld 2008), as with many hosts collecting ectoparasites, its burden is expected to decrease by dilution. On the other hand, this association may be positive under the hypothesis that ectoparasite productivity increases at high host densities (Zhonglai and Yaoxing 1997; Krasnov et al. 2002). It is not surprising then that the association between immature ticks and host abundance has been found inconsistent, in particular as their distribution on their hosts is linked to the density of questing ticks too (Schmidt et al. 1999), which, in turn, depends on prior densities of hosts. In this study, the association between LL and rodent abundance was inconsistent and depended on season. It was positive except in winter, when it became negative. Regarding NN, burdens were negatively correlated with rodent abundance, especially in those rodents with lower *A. triste* counts. The complexity of these results shows that further studies are needed to better establish the pattern and find the underlying mechanisms.

Presence of cattle was the factor with the greatest effect size, having a strong negative influence on both LL and NN independently of other studied variables. Adults and immature stages of *I. loricatus* ticks are exclusively associated to marsupials (Didelphidae) and small rodents (Cricetidae), respectively (Guglielmone et al. 2011). In the study area, cows are hosts to adults of *A. triste* but not to *I. loricatus* (Guglielmone et al. 2011; Nava et al. 2011). Our findings suggest that the presence of cattle might influence the abundance of *I. loricatus* by altering negatively the favourable habitat for marsupials, the principal host for *I. loricatus* adults. However, this hypothesis should be tested by further studies because the interaction between cattle, rodents and marsupials has not been explored. Whatever the explanation, it is noteworthy that raising cattle may have a substantial impact on *I. loricatus*.

Interspecific interactions within parasite communities can be important drivers of parasite dynamics (Telfer et al. 2010), and this holds when considering ectoparasite communities alone (Lutermann et al. 2015). These interactions could be synergistic or antagonistic (Lello et al. 2004; Pedersen and Fenton 2007; Lutermann et al. 2015). The former could be a result of facilitation mediated by impaired defences (Beldomenico and Begon 2010; Ulrich and Schmid-Hempel

2012), parasite-induced selective immunosuppression or trade-offs within the immune system (Jolles et al. 2008; Ezenwa and Jolles 2011; Pathak et al. 2012), while the latter could be a product of competition or cross-effective immune responses (Pedersen and Fenton 2007; Pollock et al. 2012). Concomitant infestation with other ectoparasites was strongly associated with both LL and NN, but the patterns were consistently dependent on other variables. The associations with the other tick species parasitizing *A. azarae*, *A. triste*, were in general positive (except for LL in summer). An experimental study that investigated interactions within the ectoparasite community of *Elephantulus myurus* found facilitating interactions between different tick species (Lutermann et al. 2015), which they interpreted to be likely mediated by the host's immune function. Interestingly, we found a positive association between *A. triste* and *I. loricatus* NN among individuals that had low WBC (hence, were investing less in cellular immune response), but the association between tick species tended to disappear in hosts with higher WBC levels, suggesting that the immune function is playing a role in this interaction between ectoparasites. The significant associations between *I. loricatus* and other parasite taxa, mites and fleas, were also generally positive, but they depended on host factors, sex and body length, respectively. Mites were positively associated with *I. loricatus* LL only in female hosts, and the association between fleas and LL was stronger the smaller the rodent. In contrast, the flea and tick removal experiment conducted by Lutermann et al. (2015) found predominantly antagonistic interaction between intertaxon ectoparasites, chiggers and ticks, and fleas and ticks, although they found less antagonistic interactions than expected. It is noteworthy that some of the antagonistic interactions found were not apparent when only considering cross-sectional data, for which in our system, antagonistic interactions should not be ruled out. Our results, as those reported by Lutermann et al. (2015), suggest strongly that interactions between ectoparasites may be more important for parasite dynamics than has so far been appreciated.

Ticks are distributed in an aggregated way on their hosts, where few individuals are infested with most of ticks (Ostfeld et al. 1996; Shaw et al. 1998; Perkins et al. 2003). This ubiquitous distribution may result from differential exposure to environmental factors or contrasting traits or physiological condition between more parasitized and less parasitized hosts (Perkins et al. 2003; Brunner and Ostfeld 2008; Kiffner et al. 2011; Debárbora et al. 2014). Also, hosts are generally co-infested with more than one parasite species (Petney and Andrews 1998; Behnke et al. 2001; Cox 2001), implying potential interactions within the host parasite community. On the other hand, in our study, except for the presence of cattle, all the associations found were influenced by another variable, highlighting the complexity of ectoparasite ecology and warning about the limitation of single-factor studies. This complexity might explain the frequent contradictions found in

ectoparasite ecology studies, especially when considering that a generally ignored factor when studying the ecology of an ectoparasite is the rest of the ectoparasite community.

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