



Factors affecting patterns of *Amblyomma triste* (Acari: Ixodidae) parasitism in a rodent host



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ABSTRACT

Here we offer a multivariable analysis that explores associations of different factors (i.e., environmental, host parameters, presence of other ectoparasites) with the interaction of *Amblyomma triste* immature stages and one of its main hosts in Argentina, the rodent *Akodon azarae*. Monthly and for two years, we captured and sampled rodents at 16 points located at 4 different sites in the Parana River Delta region. The analyses were conducted with Generalized Linear Mixed Models with a negative binomial response (counts of larvae or nymphs). The independent variables assessed were: (a) *environmental*: trapping year, season, presence of cattle; type of vegetation (natural grassland or implanted forest); rodent abundance; (b) *host parameters*: body length; sex; body condition; blood cell counts; natural antibody titres; and (c) *co-infestation with other ectoparasites*: other stage of *A. triste*; *Ixodes loricatus*; lice; mites; and fleas. Two-way interaction terms deemed *a priori* as relevant were also included in the analysis. Larvae were affected by all environmental variables assessed and by the presence of other ectoparasites (lice, fleas and other tick species). Host factors significantly associated with larval count were sex and levels of natural antibodies. Nymphs were associated with season, presence of cattle, body condition, body length and with burdens of *I. loricatus*. In most cases, the direction and magnitude of the associations were context-dependent (many interaction terms were significant). The findings of greater significance and implications of our study are two. Firstly, as burdens of *A. triste* larvae and nymphs were greater where cattle were present, and larval tick burdens were higher in implanted forests, silvopastoral practices developing in the region may affect the population dynamics of *A. triste*, and consequently the eco-epidemiology of *Rickettsia parkeri*. Secondly, strong associations and numerous interactions with other ectoparasites suggest that co-infestations may be more important for tick dynamics than has so far been appreciated.

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1. Introduction

Amblyomma triste (Acari: Ixodidae) is a tick species mainly associated with marshlands and distributed in the Neotropical and Nearctic regions (Guglielmone et al., 2003, 2013; Guzmán-Cornejo et al., 2006; Mertins et al., 2010). It has a one year cycle and uses three hosts (Nava et al., 2009, 2011). Adults parasitize large mammals, preferentially the deer *Blastocerus dichotomus*, but also the large rodent *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).

Immature stages feed on mice (Sigmodontinae) and cavies (Caviidae) (Nava et al., 2009, 2011; Guglielmone and Nava, 2011; Martins et al., 2014; Colombo et al., 2013). 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).

Akodon azarae (Rodentia: Cricetidae) is one of the main hosts for immature stages of this tick species in Argentina (Nava et al., 2009, 2011; Colombo et al., 2013). This rodent can be found in diverse habitat types, such as natural grasslands, crop areas and scrublands, from southern Brazil to central Argentina (Zuleta et al., 1988; Redford et al., 2011).

Little is known about the ecology of *A. triste*, but it appears to be more complex than expected (Guglielmone et al., 2013). Host-parasite dynamics result from the interplay of several host-related

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and parasite-related factors, including parameters intrinsic to the environment, the host and the parasite (Hudson et al., 2002; Vaclav et al., 2008; Cardon et al., 2011; Lutermann et al., 2015), and even interactions with other members of the parasite community (Telfer et al., 2010). Here we offer a multivariable analysis that explores associations of different factors (i.e., environmental, host parameters, presence of other ectoparasites) with the interaction between *A. triste* immature stages and the rodent *A. azarae*.

2. Materials and methods

2.1. Study area

The study was conducted in the Estación Experimental Agropecuaria Delta, Instituto Nacional de Tecnología Agropecuaria (INTA), Campana (34°11'S, 58°50'W), Buenos Aires, 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 *Cortaderia* 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). In the study area there is a herd of beef cattle consisting of twenty-one Aberdeen Angus cows maintained at a density of approximately one cow per hectare.

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).

3. Data collection

Rodents were captured from November 2010 through October 2012 in 3-night trapping sessions carried out every 5 weeks. Four trapping grids were set out at 4 different sites, each grid consisting of squares with 12 Sherman-type live-traps in the corners and 2 Ugglan-type live-traps in the middle of the square, baited with pelleted food. Within a site, the grids were at least 200 m apart from each other. Two of the grids were located in places with natural grassland and the other 2 with implanted forest (*Populus* spp.). Half of the sites were located in extensive cattle raising lands and the other half in areas where cattle was absent. Every morning traps were inspected, trapped rodents were transported to a field lab, anesthetized by inhalation of Isoflurane, sacrificed by cervical dislocation and then conserved in individual plastic bags with ethanol 96%. Blood samples were taken by heart puncture and collected in heparin-coated capillary tubes and eppendorf tubes without anticoagulant. Rodents were later identified to the species level by assessing cranium morphology. Although other rodent species (Cricetidae: Sigmodontinae) were trapped during this study (namely, *Oryzomys rufus*, *Oligoryzomys flavescens*, *Oligoryzomys nigripes*, *Scapteromys aquaticus*, and *Deltamys kempi*, as described by Colombo et al., 2013), only *A. azarae* was the abundant enough to carry out the multivariable analyses desired. In addition, this rodent species is frequently used as host by *A. triste* immature stages at the study area (Nava et al., 2011; Colombo et al., 2013).

4. Ectoparasites

Each rodent identified as *A. azarae* was examined in the laboratory with a magnifying lens to recover ectoparasites. Ticks were counted and determined following Estrada-Pena et al. (2005), Martins et al. (2010) and also compared with material deposited

in the tick collection of INTA, Estación Experimental Agropecuaria Rafaela, Argentina. 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, Argentina. Other ectoparasites were also recovered and counted separately by group: *Ixodes loricatus* (the other tick species found in the studied rodents), mites, lice and fleas. *I. loricatus* ticks were determined following Marques et al. (2004).

5. Host parameters

After sacrifice, rodents were weighed, measured and data of their sex and reproductive status were taken. Reproductive status was classified as “active”, i.e., males with scrotal testicles and well developed seminal glands, females with signs of oestrus, evidence of present or recent pregnancy, or lactation; or “inactive” (no signs of the above). The morphometric measures were used to create an estimate of body condition as a residual index adjusted to control for distortions in the size/weight relationships caused by pregnancy (Green, 2001). 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).

White blood cells (WBC) and red blood cells (RBC) were counted and its concentration in blood estimated (cells/microlitre) following haematological methods used by Beldomenico et al. (2008). Eppendorf tubes were centrifuged and the serum obtained was transported in liquid nitrogen and stored at –20 °C until processed in the laboratory. Natural antibodies (NAb) were determined using a hemagglutination assay as described by Racca et al. (2014). Titres were recorded as the logarithm of the last dilution showing clear evidence of agglutination.

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 infestation 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).

6. Statistical analysis

Larvae (LL) and nymphs (NN) abundance was considered separately for the analysis. The analyses were conducted with Generalized Linear Mixed Models (GLMM) 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>). To control for the lack of independence of observations from the same trapping grid, we included the random intercept “Grid ID”.

The independent variables used were as follows. (a) *Environmental*: trapping year (1 = Nov'10–Oct'11; 2 = Nov'11–Oct'12), season (as determined by solstices and equinoxes as follows: summer, autumn, winter and spring), 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); (b) *host parameters*: body length (proxy of age); sex; body condition (residual index); RBC; WBC; NAb; (c) *other ectoparasites* (total counts): other stage of *A. triste*; *Ixodes loricatus*; lice; mites; fleas. Two-way interaction terms deemed *a priori* as relevant were also included in the analysis (e.g. “body condition × lice” can evaluate the hypothesis that the influence of having lice on the infestation by *A. triste* is greater for individuals in poor condition

Table 1Generalized linear mixed models showing the association between larval *A. triste* burden per host and environmental variables, host parameters and other ectoparasites.

Model: Response = Larval <i>Amblyomma triste</i> burden Independent variables = Trapping year + Season + Cattle + Vegetation + Rodent Abundance + <i>I. loricatus</i> + Sex + Nab + Lice + Fleas + Cattle*Rodent abundance + Sex*Lice + Lice* Fleas + <i>I. loricatus</i> Vegetation + Trapping year *Season Random intercept = "Grid ID"				
Term	Coefficients	Standard error	P-value	ΔAIC [*]
Intercept	−2.350	0.918	0.010	–
Trapping year ^a	−1.182	0.592	0.045	–
Season spring ^b	0.174	0.957	0.855	–
Season summer ^b	1.401	0.597	0.019	–
Season winter ^b	0.002	0.567	0.996	–
Cattle ^c	2.432	0.971	0.012	–
Vegetation type ^d	−1.299	0.911	0.153	–
Rodent abundance	−0.007	0.085	0.931	–
<i>I. loricatus</i> burden	0.005	0.048	0.908	–
Sex ^e	0.193	0.284	0.497	–
Lice	0.183	0.067	0.006	–
Fleas	0.228	0.101	0.024	–
Nab	0.215	0.084	0.010	5.15
Cattle ^c * Rod. Abund.	−0.381	0.131	0.003	6.15
Sex ^b * Lice	−0.189	0.066	0.004	8.56
Lice* Fleas	−0.066	0.022	0.003	10.2
Vegetation type ^d * <i>I. loric.</i>	1.004	0.375	0.007	8
Trapp. Year ^a * Season spring ^b	2.897	1.092	0.008	5.5
Trapp. Year ^a * Season summer ^b	1.586	0.781	0.042	5.5
Trapp. Year ^a * Season winter ^b	−0.723	−0.723	0.517	5.5

^a Reference levels: Year 1.^b Autumn.^c Absent.^d Natural grassland.^e Female.^{*} AIC value increment if the single term is dropped.

than in those in good condition). 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 vegetation type for equal values of sex, body condition, etc.

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 over-dispersion 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.

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.

7. Results

The total number of *A. azarae* captured in all trapping sessions was 389, 163 females and 226 males. Although at lower numbers than *A. azarae*, other rodent species were captured in this study; namely, *Oxymycterus rufus*, *Oligoryzomys flavescens*, *Oligoryzomys nigripes*, *Scapteromys aquaticus*, *Deltamys kempi*. A descriptive analysis of the ectoparasites found on them was offered by Colombo et al. (2013). The overall prevalence of *A. triste* (LL and NN) in *A. azarae* was 33%, 27% of the rodents had LL and 12% had NN. The maximum number of LL collected from a single host was 138 (attached), whereas for NN the maximum record was 8. Regarding other ectoparasites, 19% had *I. loricatus* (LL and/or NN), 37% had lice (Hoplopleuridae), 48% fleas (Stephanocircidae and/or Ropalophyllidae) and 86% had mites (Laelapidae and/or Macronyssidae).

The final models for LL and NN are presented in Tables 1 and 2, respectively, including effect sizes and statistical significances. LL were associated with all the environmental variables and strongly

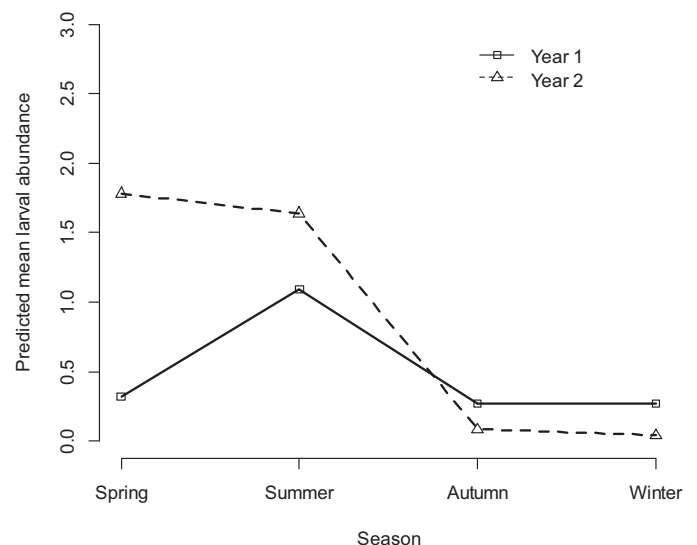


Fig. 1. Mean *Amblyomma triste* larval abundance as predicted by a GLMM, showing the seasonal pattern for both sampling years. (Prediction for a male in a site with cattle and natural grassland. All other variables were set at average or modal levels).

associated with the presence of other ectoparasites (except mites). On the other hand, sex and Nab were the only host factors significantly associated with larval count. Nymphs were associated with cattle, season, body condition, body length and burdens of *I. loricatus*, but the latter significantly interacted with season, body length and body condition.

Season had a strong influence for both LL and NN (Tables 1 and 2), with lowest records in autumn and greatest in summer (Figs. 1 and 2), although in the second year the larval *A. triste* burden was slightly greater in spring (Fig. 1).

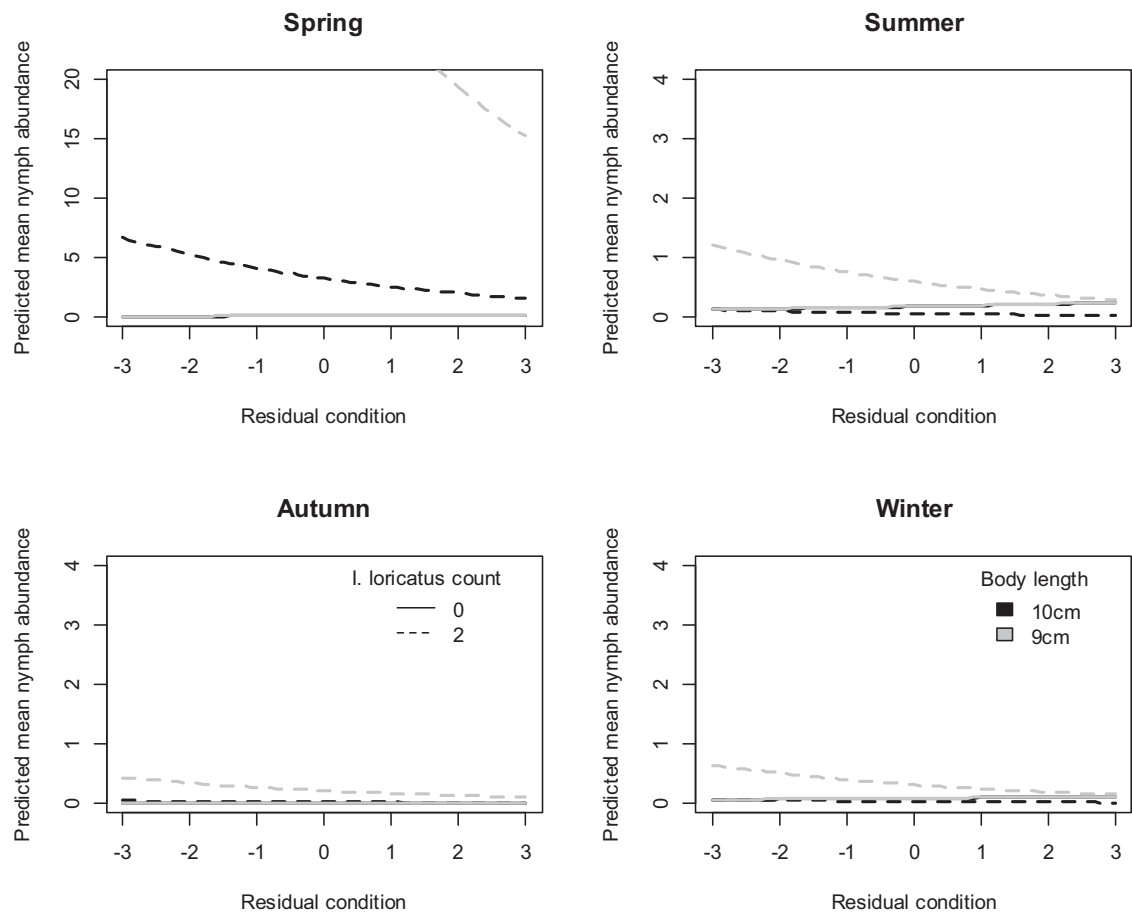


Fig. 2. Mean *Amblyomma triste* nymphal abundance as predicted by a GLMM, showing the interaction of *I. loricatus* with season, body condition and body length. (Prediction for a male in a site with natural grassland and without. All other variables were set at average or modal levels).

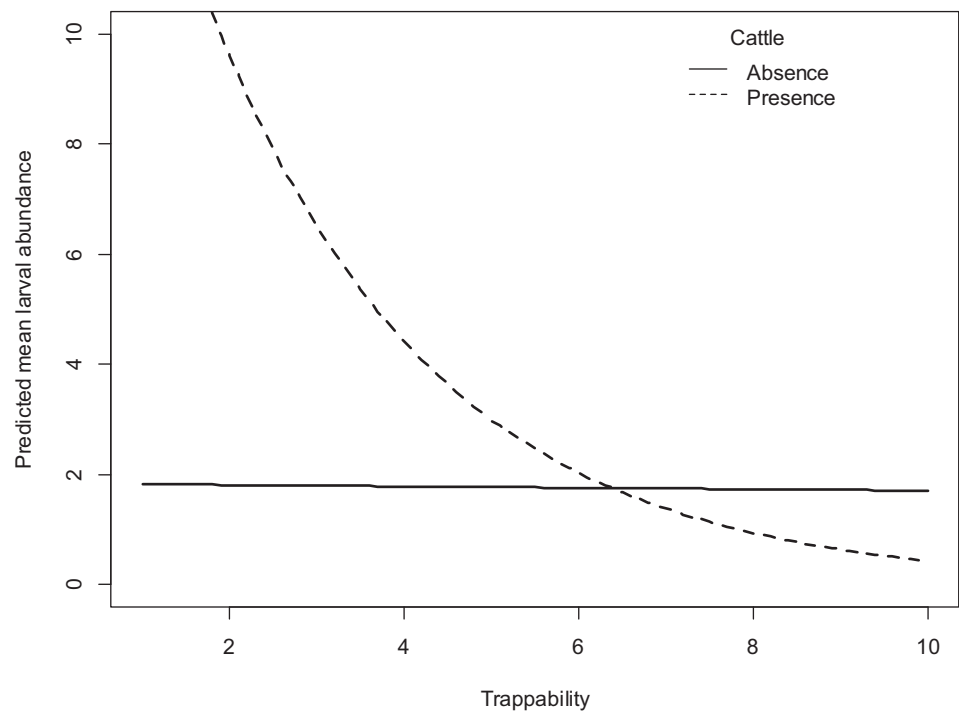
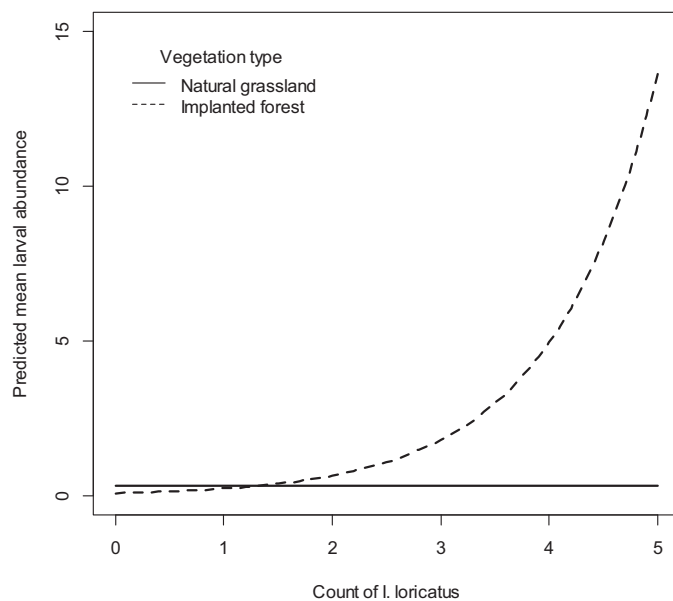


Fig. 3. Mean *Amblyomma triste* larval abundance as predicted by a GLMM, depicting the effect of different rodent abundances in sites with and without cattle. (Prediction for a male in a site with natural grassland during the second trapping year. All other variables were set at average or modal levels).

Table 2Generalized linear mixed models showing the association between nymphal *A. triste* burden and environmental variables, host parameters and other ectoparasites.

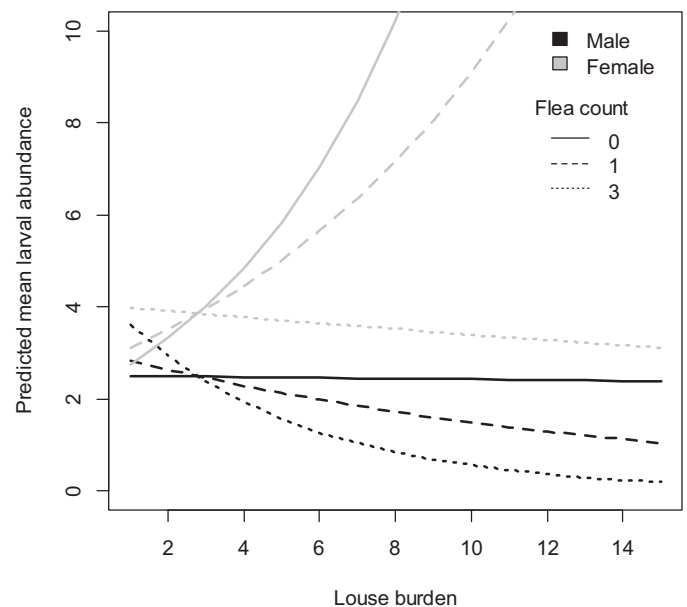
Model 2: Response = Nymphal <i>Amblyomma triste</i> burden Independent variables = Season + Cattle + <i>I. loricatus</i> + Body Condition + Body length + <i>I. loricatus</i> *Body Condition + <i>I. loricatus</i> *Season + <i>I. loricatus</i> *Body length Random intercept = "Grid ID"				
Term	Coefficients	Standard error	P-value	ΔAIC [*]
Intercept	−4.814	3.208	0.133	–
Season spring ^a	2.674	0.856	0.002	–
Season summer ^a	3.266	0.734	8.6e−06	–
Season winter ^a	2.546	0.693	0.0002	–
<i>I. loricatus</i> burden	11.820	5.062	0.019	–
Body condition	0.106	0.072	0.138	–
Body length	−0.018	0.323	0.954	–
Cattle ^b	1.287	0.639	0.044	2.5
<i>I. loric.</i> *Body cond.	−0.173	0.091	0.058	3.8
<i>I. loric.</i> *Season spring ^a	1.156	0.769	0.132	5.6
<i>I. loric.</i> *Season summer ^a	−1.118	0.520	0.032	5.6
<i>I. loric.</i> *Season winter ^a	−1.080	0.451	0.016	5.6
<i>I. loric.</i> *Body length	−1.122	0.501	0.025	4.5

^a Reference levels: Autumn.^b Absent.^{*} AIC value increment if the single term is dropped.**Fig. 4.** Mean *Amblyomma triste* larval abundance as predicted by a GLMM, showing the interaction between vegetation type and *I. loricatus* burdens. (Prediction for a male in a site without cattle natural grassland during the first trapping year. All other variables were set at average or modal levels).

Larval *A. triste* burdens were generally greater in sites with cattle than in those without, but this effect was largely dependent on rodent abundance (Table 1). When the number of rodents was low, the difference was very large, but it rapidly decreased as rodent abundance increased, to the point of larval abundance becoming greater at sites without cattle at the highest rodent abundance records (Fig. 3). Independently of other studied variables, NN showed significantly greater mean abundance in sites with cattle.

Larval *A. triste* burdens were greater in sites with implanted forests than in natural grasslands, but this was only observed in individuals that also had *I. loricatus* (Table 1, Fig. 4). The difference became more significant and exponentially larger the more *I. loricatus* larvae were co-parasitizing the host (Fig. 4).

Females had greater larval *A. triste* burdens than males, but this difference was only observed among individuals parasitized by lice. The more lice were co-infecting the host the greater the female-biased parasitism (Table 1, Fig. 5).

**Fig. 5.** Mean *Amblyomma triste* larval abundance as predicted by a GLMM, showing the interaction between lice and fleas by sex. (Prediction for a site with natural grassland and with cattle during the first trapping year. All other variables were set at average or modal levels).

Natural antibodies were positively correlated with LL (Table 1).

Body length was strongly negatively correlated with nymphal tick burden, but only for individuals that were parasitized with *I. loricatus* (Table 2, Fig. 2). The larger the *I. loricatus* burden, the stronger the negative correlation. Body condition was also associated with nymphal *A. triste* burden, but again this depended on co-infestation with *I. loricatus* (Table 2, Fig. 2).

Fleas and lice were associated with larval *A. triste* abundance, but the magnitude and direction of their associations depended on each other (Table 1, Fig. 5). As flea burdens increased, LL also increased, but this was reversed if lice were present (Fig. 5). Similarly, but only in females, louse burden was strongly positively associated with *A. triste* LL, but especially when no or few fleas were present (Fig. 5). In individuals with larger flea burdens the association reversed. In males, lice were not associated with LL, unless they had fleas, in which case the association was negative (Fig. 5). *Ixodes loricatus* was positively associated with LL, but only in implanted forests

(Table 1, Fig. 4). Regarding NN, *I. loricatus* was found to be strongly associated and explained much of the variability in nymphal tick abundance. It also interacted with three of the other significant independent variables (see significant interaction terms in Table 2). As *I. loricatus* burdens increased, the mean abundance of NN was higher, especially in spring, and in small (hence young) individuals.

Reproductive status, when assessed in each final model with a subset of data that only included relevant dates, was not associated with LL or NN burdens.

8. Discussion

The ecology of *A. triste* does not appear to be simple. Along the Parana River, a corridor where ecological conditions for *A. triste* seem to be favorable, its distribution is not consistent with what might be predicted; the tick was present in unexpected localities, and absent in many areas where its presence was presumed (Guglielmone et al., 2013). To better understand *A. triste* ecology, we assessed diverse factors associated with this tick occurrence in one of the main hosts of immature stages, *A. azarae*, in an area where the tick is known to occur.

Host factors may be important drivers of the population dynamics of ticks, as they can influence the probability of encounter with a questing tick and/or affect the performance of the parasite on a host (Brunner and Ostfeld 2008). It has been found that, in rodents, testosterone depresses the innate and acquired resistance to *Ixodes ricinus*, which could result in sex-biased parasitism (Hughes and Randolph, 2001). This may explain that the patterns reported for ixodid tick parasitism in rodents are in general male-biased (Hughes and Randolph 2001; Soliman et al., 2001; Krasnov et al., 2005; Boyard et al., 2008; Harrison et al., 2010; Krasnov et al., 2012; Lutermann et al., 2015). However, field data from *A. azarae* failed to find a difference between *I. loricatus* burdens of males and females (Beldomenico et al., 2005; Colombo et al., 2014a,b). Moreover, here we found no effect of sex among individuals with null or very low louse infestations, whereas females infected with lice had greater burdens of *A. triste* than males with similar louse burdens, which runs counter the hypothesis of immunological handicap of males. This is further supported by the positive association between LL and NAb. It is worth mentioning that a raw analysis (uni-variable), comparing males and females, produces an apparent male-biased result: mean larval abundance in males was 2.7, but only reached 1.2 in females. This highlights the importance of controlling for potential confounders and effect modifiers when conducting analysis of field data.

The age of a rodent is highly correlated with its size (Zullinger et al., 1984). As in observational studies (like this one), age cannot be easily estimated. We used 'body length' as a factor indicating both indivisible variables: size and age. Although host age is considered a key factor for host-parasite interactions (Soliman et al., 2001; Hudson et al., 2002; Brunner and Ostfeld, 2008; Cardon et al., 2011), previous studies assessing associations between age and tick burdens in wild rodents failed to find an association (Beldomenico et al., 2005; Hawlena et al., 2006). However, we found a strong negative correlation between body length and NN loads, but again conditional on the parasitism by other ectoparasite. Burdens of NN increased with age/size, but this association was stronger the greater the burden of *I. loricatus*. This, again, might reveal an interaction between ectoparasites, in this case taking place predominantly at small sizes/young ages.

Host body condition was found associated with NN counts, but, once again, this depended on other ectoparasite. The larger the *I. loricatus* burden, the greater the negative correlation between body condition and NN. Larger burdens of parasites can cause the body condition to decline by extracting resources from the host

(Whiteman and Parker, 2004) or due to energy invested in the immune response (Sheldon and Verhulst, 1996), especially when other parasite is acting synergistically to cause physiological deterioration (Marzal et al., 2008). On the other hand, a host with poor body condition might have dampened resistance to parasites (Nelson et al., 1975; Simon et al., 2003; Krasnov et al., 2005). Longitudinal or experimental approaches are needed to confirm the cause-effect direction of this association, as well as to evaluate the possibility that they are engaged in a circular synergistic process where both are cause and effect (Beldomenico and Begon, 2010).

To assess whether the immune system of the host was associated with *A. triste* burdens, we used generic indicators of cellular (WBC) and humoral (NAb) immunity. Only NAb were positively correlated with LL burdens. NAb provide unspecific humoral immunity independent of antigenic stimulation and are considered to be stable over time (Ochsenbein and Zinkernagel, 2000). This confers them potential to be good indicators of immune competence in wild animals (Racca et al., 2014), but it has not been established as yet whether they co-vary with specific antibodies, or there are trade-offs between innate and specific immunity, or what is the behaviour of NAb under particular physiological conditions. For example, while antibody production is known to be suppressed by chronic stress (Padgett and Glaser, 2003), it has been shown in capybaras that prolonged food restriction increases NAb levels (Eberhardt et al., 2013). Therefore, the elucidation of the meaning of this association requires further investigation of the biology of NAb.

Relating to environmental factors, we found that burdens of *A. triste* on *A. azarae* were strongly associated with the environmental variables studied. The seasonality of *A. triste* was previously studied in Uruguay (Venzal et al., 2008) and in the same locality where the current study was performed (Nava et al., 2011), and in general terms, our results are in agreement with the seasonal pattern described by Venzal et al. (2008) and Nava et al. (2011). In addition, including season in our analysis allowed us to control for the potential confounding and effect modification that may result if that variable is ignored.

Regarding host abundance, with many hosts collecting questing ticks, the burden on each is expected to decrease (Sorci et al., 1997; Stanko et al., 2002; Brunner and Ostfeld, 2008). At the same time, because adult stages of *A. triste* use different hosts than LL and NN, there is opportunity for intricate interactions to occur. A paucity of suitable hosts for immature stages would render few questing adults to infest larger mammals. Conversely, lack of suitable hosts for adult stages would result in low infestation by immature ticks in their rodent hosts, independently on their abundance. In turn, more hosts for adult stages would represent greater chance for increasing the tick reproductive output, and consequently there will be more questing larvae seeking for rodent hosts. Studies of systems with a tick that has a similar dissociation in host preference, *Ixodes scapularis*, showed that the burden of LL and NN on rodent hosts depend on the density of the preferred host for adult stages, *Odocoileus virginianus* (Stafford, 1993; Kilpatrick et al., 2014). In the Parana Delta region, the hosts recorded for adult stages of *A. triste* were *B. dichotomus*, *H. hydrochaeris*, cattle, horses and dogs (Nava et al., 2011). Of those domestic animals, cattle are frequently parasitized by *A. triste* adults in the study area (PM Beldomenico, unpublished data). Engorged females feeding on cattle successfully lay eggs (Nava et al., 2011). Our findings show that the presence of cattle was positively correlated with *A. triste* burdens, both for LL and NN. For larvae, the effect of cattle depended on the abundance of rodents. When rodent trappability was low, larval *A. triste* burdens were much higher in sites with cattle, but this difference disappeared and tended to reverse as rodent captures increased. The interaction between cattle and rodent abundance might be explained by a dilution of existing questing immature stages as their host

populations grow, but the burdens where cattle was absent were independent of rodent density, perhaps indicating low availability of suitable hosts for adult stages. In the study area, *B. dichotomus* and *H. hydrochaeris* are among the few large wild mammals species known to be suitable hosts for *A. triste* (Nava et al., 2011). Despite there is no available data neither on the densities of *B. dichotomus* and *H. hydrochaeris*, nor information about their tick burdens during the development of the present study, we can speculate that these hosts may play an important role in the ecology of *A. triste*. However, from anecdotal evidence and direct observation, cows are at higher densities than deer and capybaras in the study area; so, the effect of these domestic animals on *A. triste* burdens could be stronger. More studies integrating data from marsh deer, capybara, cattle, rodents and questing ticks are needed to understand the whole scenario.

The other anthropogenic factor evaluated in this study was also significantly associated with the mean abundance of *A. triste* on *A. azarae*. Larval burdens were higher in *A. azarae* captured in *Populus* spp. plantations than in those from natural vegetation, but, as consistently present in our analyses, an interaction with other ectoparasite was found. Individuals with null or low *I. loricatus* counts had generally low larval *A. triste* burdens, independently of the type of vegetation where they were captured. However, among individuals parasitized by higher burdens of *I. loricatus*, rodents from implanted forests had much larger larval *A. triste* burdens than those captured in natural grasslands. The vegetation structure is known to affect tick abundance (L'Hostis et al., 1995; Gray, 1998; Boyard et al., 2008; Kiffner et al., 2011). Studies in Europe found that the abundance of all stages of *Ixodes ricinus* depended on tree species composition and shrub cover (Tack et al., 2012), and that shrub clearing caused the *I. ricinus* abundance to decrease (Tack et al., 2013). These effects can be attributed to habitat modifications that are disadvantageous to the ticks (Tack et al., 2013), or also result from differential land use by the hosts (Tack et al., 2012). Rodent density was higher in natural settings when compared to implanted forests (*A. azarae* captured were 298 and 91, respectively) but, in the analysis, 'rodent abundance' was taken into account so the size of the effect reported was estimated at equal rodent abundance levels. However, the fact that larval *A. triste* burdens in *A. azarae* parasitized by *I. loricatus* were greater in implanted forests remains unexplained.

Within the host, parasites may interact in diverse ways (Lello et al., 2004; Pedersen and Fenton 2007; Lafferty, 2010; Lutermann et al., 2015). There may be inter-specific antagonistic interactions as a result of competition (exploitation or interference) and cross-effective immune response (Mideo, 2009; Ulrich and Schmid-Hempel, 2012), or synergistic interactions where infestation by one parasite is facilitated by another one, either by a general impoverishment of the host's defenses (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). In the present study, fleas were positively correlated with larval *A. triste* burdens when lice were absent or present at low intensities, and a similar pattern was observed for lice, but only in females. However, greater burdens of either ectoparasite made the association of the other with larval *A. triste* burdens increasingly negative. *I. loricatus* was positively associated with nymphal and larval *A. triste* burdens, but the size of this effect depended on vegetation type, season, body condition and body size. The fact that these associations were so strong, and that they often interacted with other significant factors, merits the construction of explanatory hypotheses. Indeed, true interactions between co-infesting parasites should not be ruled out as a plausible explanation to our findings. Nonetheless, although our analyses controlled for some potential risk factors, including indicators of physiological condi-

tion and immunocompetence, other relevant ones were ignored (for example, host behavior). Foraging activity, for example, is positively correlated with parasite encounter (Fourie and Kok, 1992). Future studies should further explore associations among ectoparasite communities and the mechanisms underlying them.

The findings of greater significance and implications of our study are two. Firstly, anthropogenic factors such as silvopastoral practices contribute to increasing the population size of *A. triste*, consequently affecting the eco-epidemiology of *R. parkeri*. Secondly, interactions between ectoparasites may be more important for parasite dynamics than has so far been appreciated. Furthermore, our findings indicate that patterns of parasitism related to host or environmental factors could be influenced and distorted by the remaining, and generally ignored, parasite community; thus contributing to explain some of the conflicts present in the literature regarding ectoparasite ecology.

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