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

Valeria C. Colombo[a], Santiago Nava[b], Leandro R. Antoniazzi[a], Lucas D. Monje[a], Andrea L. Racca[a], Alberto A. Guglielmone[b], Pablo M. Beldomenico[a,*]

[a] *Laboratorio de Ecología de Enfermedades (LEcEn), ICiVet, UNL-CONICET. R.P Kreder 2805, CP 3080 Esperanza, Santa Fe, Argentina*
[b] *Instituto Nacional de Tecnología Agropecuaria (INTA), EEA Rafaela. CC 22, CP 2300 Rafaela, Santa Fe, Argentina*

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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.

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

* Corresponding author. Fax: +54 3496 426304.
E-mail address: pbeldome@fcv.unl.edu.ar (P.M. Beldomenico).

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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 *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 Phytogeo-graphic 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, *Oymycterus 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 oeustrus, 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 humoural 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