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The parasitism of immature stages of *Ixodes loricatus* (Acari: Ixodidae) on wild rodents in Argentina

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Abstract. *Ixodes loricatus* has long been considered with strict-total specificity to New World Marsupials. However, frequent findings of its immature stages on rodents suggest that these vertebrates play an important role in the tick's life cycle. Aspects dealing with the ecology of Sigmodontinae rodents infestation by *I. loricatus* are unknown. To contribute to the knowledge of the ecology of this tick species, environmental factors, as well as host species, sex and age, were evaluated to find associations of immature *I. loricatus* infestation of the most abundant wild rodent species from riparian locations of Buenos Aires province (Argentina). A total of 290 hosts belonging to the subfamily Sigmodontinae were captured and examined for ticks at six locations. The data analysis showed that there was a preference of *I. loricatus* towards *Akodon azarae*, but only for those hosts captured in lands not prone to flooding. The results suggest that the host preference of *I. loricatus* is mainly related to factors such as habitat type and host density.

Introduction

Ixodes loricatus Neumann, 1899 is a three-host hard tick species (Acari: Ixodidae) widely spread in the New World, from southern Mexico to Argentina (Guglielmone et al. 2003). Although this species is considered with strict-total specificity to New World Marsupials (Didelphimorphia) (Hoogstraal and Aeschlimann 1982), several studies reported the presence of larvae and nymphs of *I. loricatus* infesting wild rodents (Muridae, Sigmodontinae) in Argentina (Lareschi 1996; Liljeström and Lareschi 2002; Beldomenico et al. 2003; Lareschi et al. 2003; Nava et al. 2003), Uruguay (Venzal et al. 2003) and Brazil (Cooley and Kohls 1945; Barros-Battesti and Knysak 1999). These findings suggest that rodents may be more than accidental hosts for immature stages of this tick species.

In the past 10 years, an important number of studies have quantified the relationship between arthropod ectoparasites and Sigmodontinae rodents in Argentina, particularly in Buenos Aires Province (Lareschi 1996, 2000; Liljesthröm and Lareschi 2002; Lareschi et al. 2003; Nava et al. 2003), but little

has been published about environmental and host factors associated with Sigmodontinae infestation by *I. loricatus*.

In this same region, nymphs and adults of *I. loricatus* were found on the marsupials *Didelphis albiventris* Lund, 1840 and *Lutreolina crassicaudata* (Desmarest 1804) (Mauri and Navone 1988), and in field observations by the authors, larvae and nymphs of *I. loricatus* appear to preferentially infest rodents of the genus *Akodon*, but more elaborated information is needed to confirm it.

To contribute to the knowledge of the ecology of this tick species, environmental factors, as well as host species, sex and age, were evaluated to find associations of immature *I. loricatus* infestation of the most abundant wild rodent species from riparian locations of Buenos Aires province (Argentina).

Materials and methods

Study area

The study area comprised six locations of Buenos Aires province: Ramallo (33°32′S; 59°52′W) on the coast of Paraná river. Reserva Natural Hudson (34°45′S; 58°06′W), Reserva Selva Marginal de Punta Lara (34°47′S; 58°1′W), Palo Blanco (34°53′S; 57°50′W), Balneario Bagliardi (34°54′S; 57°48′W), and La Balandra (34°56′S; 57°42′W), along the coast of La Plata river. The whole area is situated in the Biogeographic province La Pampa (Morrone 2001), and is home of the greatest biodiversity found in Buenos Aires, given that the rivers act as biological corridors that enable the spread of plants and animals from northern provinces (Barrios and Moschione 1993). The climate is temperate humid-mesothermal, with an undefined rainy season and scarce incidence of freezing conditions (Dascanio et al. 1994). In Punta Lara, La Balandra and Hudson, the landscape is a mixture of graminoid swamps and forested wetlands (Dascanio et al. 1994), whereas Palo Blanco and Balneario Bagliardi are frequently flooded areas characterized by scrublands. In Ramallo there are xeromorphic woods with trees and shrubs, and along the coast there is riparian marshland where rushes dominate. Towards the east, grazing lands are predominant.

Rodent capture and tick collection

The rodents were captured in April, July and December 1995 and 1996 in Punta Lara, Palo Blanco, Balneario Bagliardi and La Balandra, in April and September 1995 in Hudson, and monthly from January 2000 through March 2001 in Ramallo. Captures were carried out with $7.5 \times 15 \times 8$ cm Kuns-Massoia live trap cages, in accordance with regulations and policies of

the Dirección de Administración y Difusión Conservacionista del Ministerio de Asuntos Agrarios, Buenos Aires. In Ramallo, a rectangular grid with 100 trap stations (10 × 10) 10 m apart was used, whereas in the other locations, 80 trap stations (8 × 10) 3 m apart from one other were used. Traps were baited with oiled bread and left activated for one night. The rodents were sacrificed by inhalation of a lethal dose of sulfuric ether and frozen in individual plastic bags. A voucher collection was added to the Collection of Mammals of the Museo de La Plata, Buenos Aires, Argentina (MLP).

The skin of the hosts was examined with a magnifying lens in order to recover all the ticks, which were preserved in 70% ethanol. All tick specimens were classified as larvae and nymph of *I. loricatus* following Cooley and Kohls (1945) who provided description and figures of the nymph, and Marques et al. (2004) and were compared with known laboratory material provided by José Manuel Venzal (Departamento de Parasitología, Facultad de Veterinaria, Montevideo, Uruguay).

Statistical analysis

The association of ecological factors with tick infestation on rodents were analyzed in localities where infestation by *I. loricatus* was identified. After the tick species were determined, the infestation was measured as a dichotomous variable (infested/not infested) or as counts (total number of larvae, total number of nymphs, total number of ticks).

The data accompanying each captured rodent included the date, locality, type of environment, species, sex, age, and presence of other ectoparasites. Each of these variables were converted into categories as follows: date was recorded as the 4-value variable season; locality remained the same with 4 values; type of environment was dichotomized into lowlands (prone to flooding) and highlands; sex remained male or female; age was assessed as juvenile and adult; species was split into three dichotomous variables, each of which were one of the three most abundantly captured species vs. the rest; and other ectoparasites were measured as presence or absence of ectoparasites other than ticks.

The three most abundant rodent species were confronted with environment to evaluate preference of habitat by Pearson χ^2 tests. Infestation was confronted with each of the examined variables using contingency tables and the association was assessed using Pearson χ^2 tests, or Fisher's exact test when appropriate. For dichotomous variables, the magnitude of the association was measured using odds ratio (OR) with 95% confidence intervals (95% CI). To identify confounding or effect modification phenomena, stratification by each of the remaining variables was made in every analysis, and reported whenever identified.

Results

A total of 290 hosts belonging to the following species were captured and examined for ticks: Rodentia: Muridae, Sigmodontinae: *Scapteromys aquaticus* Thomas; *Oxymycterus rufus* (Fischer); *Akodon azarae* (Fischer); *Oligoryzomys flavescens* (Waterhouse); *Holochilus brasiliensis* (Desmarest); *Oligoryzomys delticola* Thomas; and *Deltamys kempi* Thomas. The number of rodents captured by locality is shown in Table 1. Rodents captured in Palo Blanco and Balneario Bagliardi were not infested by ticks; therefore, they were left out of further analyses. In the other locations, 66% of the 205 captured rodents were male and most of the animals captured (75%) were adults. These proportions did not vary significantly for each value of location, species and environment. However, age did vary with season ($p = 0.043$), being the proportion of juveniles highest in autumn (33.7%). The three most abundantly captured host species (*S. aquaticus*, *O. rufus* and *A. azarae*) were predominantly captured in different type of environment ($p < 0.001$), being *A. azarae* mostly found in highlands (70.7%), and *S. aquaticus* and *O. rufus* mainly in lowlands (80.5 and 62.2%, respectively).

The only tick species found was *I. loricatus*. From 23 specimens of infested hosts (11.1%), 51 specimens of *I. loricatus* (35 larvae and 16 nymphs) were collected. The maximum number of larvae and nymphs found on a host were 10 and 4, respectively; but infested hosts had a mean number of 1.48 larvae and 0.55 nymphs. Given the low proportion of animals infested, the predominance of low tick burdens in those infested (70% of the infested had $\leq$ 2 ticks total), and the fact that the presence of larvae and nymphs were positively correlated (Spearman correlation coefficient r = 0.204, $p < 0.001$), further analyses were conducted treating the response as the dichotomous variable infested/not infested. The number of inmature ticks by rodent species and locality are shown in Table 2.

The infestation was not different for adults and juveniles ($p = 0.363$) and did not vary by sex ($p = 0.393$). The infestation was not significantly associated ($p > 0.05$) with other ectoparasites, either when confronted all together

Table 1. Number of wild rodent species captured in Argentine riparian localities.

Locality	Host Species							Total
	S. aquaticus	*O. rufus*	*A. azarae*	*O. flavesc.*	*H. brasil.*	*O. delticola*	*D. kempi*	
Ramallo	22	0	17	8	8	0	0	55
Hudson	8	20	7	5	0	7	0	47
Punta Lara	5	9	7	6	0	0	0	27
Palo Blanco	36	0	0	4	0	0	0	40
Balneario Bagliardi	38	1	2	2	0	0	2	45
La Balandra	42	16	10	8	0	0	0	76
Total	151	46	43	33	8	7	2	290

Table 2. Number of nymphs (NN) and larvae (LL) of *Ixodes loricatus* collected on wild rodent species in Argentine riparian localities.

Locality	Host Species														Total	
	S. aquaticus		*O. rufus*		*A. azarae*		*O. flavescens*		*H. brasiliensis*		*O. delticola*		*D. kempi*			
	NN	LL	NN	LL	NN	LL	NN	LL	NN	LL	NN	LL	NN	LL	NN	LL
Ramallo	0	0	0	0	6	16	1	0	0	0	0	0	0	0	7	16
Hudson	0	0	0	0	1	8	0	1	0	0	1	0	0	0	2	9
Punta Lara	0	2	0	0	3	7	0	0	0	0	0	0	0	0	3	9
Palo Blanco	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Balneario Bagliardi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
La Balandra	1	0	3	0	0	1	0	0	0	0	0	0	0	0	4	1
Total	1	2	3	0	10	32	1	1	0	0	1	0	0	0	16	35

or separately fleas, lice and mites. *Ixodes loricatus* infestation did not vary by season ($p > 0.05$), not even when considering larvae and nymphs separately. When analyzing type of environment, the infestation in rodents captured in highlands was 22.2%, whereas that observed in lowlands was 4.0% [$p < 0.001$, OR = 6.89 (95% CI: 2.45–19.61)]. Although not significantly ($p = 0.054$), the proportion of infested appeared to vary by location, being highest in Punta Lara (21.4%) and lowest in La Balandra (5.2%). However, there was an effect of modification that was evident when stratifying by species. When accounting only for *A. azarae* the prevalence of infestation was not homogeneous across locality ($p < 0.001$) being as high as 85.7% in Hudson and as low as 10.0% in La Balandra. Conversely, for the rest of the species this difference was not found ($p = 0.99$). This association was absent when further stratifying by environment, ($p = 0.60$ and 0.90 for highlands and lowlands, respectively).

Regarding species, *S. aquaticus* was found to be less infested than other rodents [$p = 0.01$, OR = 0.22 (95% CI: 0.06–0.78)], association that disappeared when stratifying by locality. The specimens of *O. rufus* were also found less infested when compared with the rest [$p = 0.03$, OR = 0.15 (95% CI: 0.02–1.10)]. When *A. azarae* was confronted with the rest of the rodents the highest association ($p = 10 \times 2^{-9}$) was found; the odds of being infested were 14.5 times higher for *A. azarae* than for the remaining rodent species [OR = 14.537 (95% CI: 5.438–38.858)]. Interestingly, this association varied greatly when stratifying by locality and environment, as shown in Table 3. When excluding *A. azarae*, the infestation remained low (2.5–5.0%) for every locality and type of environment.

Discussion

In this work, *I. loricatus* was the only tick species obtained from the rodents examined. This result is in accordance with the literature, since to date the great

Table 3. Level of infestation of *Akodon azarae* vs. other rodent species sampled, sorted by locality and environment type.

Locality or type of environment	Percentage of rodents captured in highlands	Fisher's exact test *p*-value	Odds ratio (95% confidence intervals)[a]
Punta Lara	93%	**0.001**	50.00 (3.74–668.35)
La Balandra	0%	0.434	2.67 (0.25–28.8)
Hudson	62%	**0.00002**	114.00 (8.9–1460.30)
Ramallo	47%	**0.028**	11.39 (1.16–111.37)
Highlands		**0.000004**	17.50 (4.43–69.18)
Lowlands		0.339	2.50 (0.26–24.37)

[a]The odds ratio indicates how many times the odds of *A. azarae* being infested are greater than the odds of other rodent species being infested.

majority of the ticks collected on Sigmodontinae rodents in Buenos Aires province were *I. loricatus* (op cit).

The tick-specific richness in the present study was low in comparison with other similar study that reported 3 species of *Ixodes* on 203 Sigmodontinae rodents captured in northwestern Argentina, where the climate and the vegetation have subtropical conditions (Beldomenico et al. 2004). This richness difference agrees with biodiversity studies on several taxonomic groups (Morrone 2001), where a decreasing gradient in biodiversity is shown from the biogeographical province of Chaco, where the study by Beldomenico et al. (2004) was conducted, towards La Pampa, where the present study was conducted. However, in the study carried out in northwestern Argentina, the proportion of rodents infested by *I. loricatus* was 2.5%, lower in contrast to the proportion found in the present study (11.1%).

In Brazil, host–parasite studies of *I. loricatus* and *Ixodes didelphidis* Fonseca & Aragão, 1951 (this last species synonymyzed with *I. loricatus* by Labruna et al. (2002)) on small rodents showed seasonal variation (Barros-Battesti et al. 2000). Lareschi (2000), on the basis of monthly samples at Reserva Selva Marginal de Punta Lara in 1990 and 1991, observed that infestations were more frequent in spring. In the present study, conclusions regarding seasonality cannot be stated, because sample size did not allow for individual analyses by year or locality. Further studies with larger sample sizes should explore the seasonal variation for each tick stage.

Juvenile rodents predominated in autumn, as expected for Sigmodontinae, and the type of environment preferred by the most abundant host species was in accordance with a previous study (Sánchez López 1998). The homogeneity of infestation between juvenile and adults differed from the results obtained in Northwestern Argentina for *I. pararicinus*, where adults were more parasitized (Beldomenico et al. 2004).

The correlation between larvae and nymphs coincides with the results of Randolph et al. (1999), who found that those hosts feeding large numbers of *Ixodes ricinus* Linnaeus 1758 larvae were simultaneously feeding the greatest numbers of nymphs. The infestation similarity between males and females coincides with the findings of Beldomenico et al. (2004), but contrasts with the results obtained by Hughes and Randolph (2001), who showed experimental evidence that testosterone depresses the innate and acquired resistance to *I. ricinus* in rodents. Nevertheless, Lareschi (2000) found a marked preference of *I. loricatus* towards male hosts.

When accounting only for *A. azarae* the infestation was not homogeneous across localities being highest in Hudson (where highlands are dominant) and lowest in La Balandra (where lowlands prevail). Further, in those localities frequently flooded (Palo Blanco and Balneario Bagliardi), no ticks were collected, and only two *A. azarae* individuals were captured. For the rest of the host species this difference was not found. This fact could be explained by the difference in the type of environments.

There is a preference of *I. loricatus* towards *A. azarae*. Considering that *I. loricatus* infestation was found associated with highlands, and that while *S. aquaticus* is associated with aquatic microhabitats and *O. rufus* is commonly found there, *A. azarae* is mostly associated with grasslands (Bonaventura et al. 1991), the habitat use could be expected to explain this host preference. Lowlands are periodically flooded, and therefore they would not be a propitious habitat for *I. loricatus* to develop its biological cycle. However, for rodent species other than *A. azarae* the infestation remained equally low both in highlands and lowlands. Moreover, when just lowlands were considered, the *I. loricatus* preference for *A. azarae* disappeared and remained consistently low as for other sigmodontin species. A fact that might explain this unequal association is that in highlands both *A. azarae* and *I. loricatus* densities were high. In a study involving *Ixodes scapularis* Say 1821 larvae and two rodent species, Shaw et al. (2003) found that microhabitat segregation plays an important role in the tick–host encounter rate. In highlands, the low density of species like *S. aquaticus* and *O. rufus* would diminish the tick–host encounter probability, whereas in lowlands, where *S. aquaticus* and *O. rufus* are abundant, the encounter rate would be low because *I. loricatus* is scarce. According to Kollars (1996), host importance may change in different regions. In our sample, *A. azarae* appeared as the main host for *I. loricatus* immature stages, while in a study conducted in a different region, southwestern Itapevi (São Paulo, Brazil), the most frequently infested host was *Oligoryzomys nigripes* (Olfers), even though *Akodon cursor* (Winge) was the most abundantly captured host (Barros-Battesti et al. 2000). This suggests that the host preference of *I. loricatus* is not related to a particular genus, and mainly relies on factors such as habitat type and host density.

Another factor that could have impact on *I. loricatus* abundance in the environment is the presence of marsupials, which are known to be the preferred hosts for the adults of this tick (Guglielmone et al. 2003). In the study area, two marsupials species are found, *D. albiventris* and *L. crassicaudata*. These species have sympatric distribution and similar habits (Mares and Braun 2000; Massoia et al. 2000; Parera 2002), being *D. albiventris* captured in both high and lowlands, while *L. crassicaudata* prefers flooded microhabitats, since it has never been captured away from a water course (Cajal 1981; Massoia et al. 2000; Parera 2002). However, the infestation parameters and the ecological associations of *I. loricatus* parasitizing marsupials are unknown in Argentina, and further studies would be necessary to explore in detail the incidence of this factor on the abundance and the host preference of this tick species.

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