



## Factors associated with hard tick (Acari: Ixodidae) parasitism in medium-sized mammals in the Atlantic Rainforest region of Argentina

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### ABSTRACT

Associations with environmental and host parameters were assessed to describe tick parasitism patterns in two medium-sized mammals of the Atlantic rainforest region of Argentina. Ticks found on 93 specimens of *Nasua nasua* and 26 specimens of *Didelphis aurita* captured at six sites in the Iguazú National Park were collected. Generalized linear models were constructed to explain the presence and abundance of ticks and the most appropriate ones were selected after stepwise simplification. The season, site and host body mass variables were important to explain the abundance of *Amblyomma coelebs* nymphs, while site was important to describe larval abundance of this species. Season was the most important variable for larvae and nymphs of *Haemaphysalis juxtakochi*, while *Amblyomma brasiliense* nymphs were associated with host age. *Amblyomma ovale* larvae were related to host age, and adult ticks with host sex. The results indicate that these medium-sized mammals are important to support the immature tick stages and that both host and environmental factors may be associated with parasite loads.

### 1. Introduction

Hard ticks are arthropods capable of transmitting a great variety of pathogenic microorganisms, including protozoa, viruses, bacteria and fungi of medical and veterinary importance (Sonenshine and Roe, 2014). Interactions between ticks and their hosts are important to understand the cycles of pathogen transmission (Estrada-Peña et al., 2013).

The medium-sized mammals *Nasua nasua* (Carnivora: Procyonidae) and *Didelphis aurita* (Didelphimorphia: Didelphidae) have opportunistic food habits and can reach high population densities in areas with anthropic disturbance (Cáceres and Monteiro-Filho, 2001; Alves-Costa et al., 2004). Both species are usual hosts of different tick species in the Neotropical region (Labruna et al., 2005; Guglielmono et al., 2011; Nava et al., 2017) and they can act as reservoirs or vertebrate amplifiers of tick-borne pathogens (Herrera et al., 2008; Horta et al., 2009).

Macroparasites are subject to the external environment and the within-host environment (Hugot, 2006). The importance of

environmental characteristics in tick adaptation to host is well known (Klompfen et al., 1996; Cumming, 2002), but individual attributes of the hosts like body mass, body size, age and sex are also relevant factors determining the heterogeneous distribution of parasites within their host populations (Pacala and Dobson, 1988; Goodwin et al., 2001; Krasnov et al., 2004, 2005; Brunner and Ostfeld, 2008; Patterson et al., 2008; Esser et al., 2016). Because of the wide variety of factors that may influence the presence and burden of ticks on their hosts, it is important to analyze those factors together. Herein, the influence of environmental and host variables on the presence and abundance of ticks associated to *N. nasua* and *D. aurita* in the Atlantic rainforest region of Argentina was evaluated.

### 2. Materials and methods

#### 2.1. Study area

The Iguazú National Park (INP), located in northern Misiones

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Province, Argentina, contains one of the largest and most biodiverse Atlantic Forest remnants (Plací and Di Bitetti, 2005). The INP belongs to the Parana Forest Biogeographic Province, which comprises southeastern Brazil, northeastern Argentina and eastern Paraguay, and it is included within the Parana sub-region of the Neotropical region (Morrone, 2006). The INP receives more than one million tourists per year, and its borders are directly continued with agricultural landscapes.

## 2.2. Tick collection from mammals

Six trap sessions of *N. nasua* and *D. aurita* specimens were carried out in the INP during the years 2015 to 2017, at sites with variable tourism impact (ethical approval by the Advisory Committee on Ethics and Safety (CAES) of the Faculty of Veterinary Sciences of the National University of the Litoral: 194/14). Fourteen Tomahawk-style traps (60 × 30 × 30 cm) were baited with orange and banana slices and canned tuna to capture *D. aurita* specimens, and they were placed in two areas of the INP called CIES (25°40'42"S, 54°26'57"W) and Apepú (25°33'53"S, 54°17'44"W). These same traps baited with banana slices were used to intercept *N. nasua* groups at five different INP sites: Estación Cataratas (25°41'2"S, 54°26'55"W), Dos Hermanas (25°41'1S, 54°26'43"W), CIES, Garganta (25°42'4"S, 54°26'40"W) and Viejo Hotel (25°40'55"S, 54°26'47"W). Estación Cataratas, Dos Hermanas and Viejo Hotel are considered intensive public use zones by the National Parks Administration (Administración de Parques Nacionales (APN), 2017) where vehicles and tourists movement is permanent, while CIES is considered a special use zone immersed in a restricted zone and Apepú is an intangible zone. Garganta is considered an area of intensive use but is immersed in a vast restricted area, more than 2 km east of the large areas of public use (Administración de Parques Nacionales (APN), 2017). Therefore, Estación Cataratas, Dos Hermanas and Viejo Hotel were considered high tourist impact sites, while Garganta and Apepú were considered low tourist impact sites. The location of the sites can be observed in the maps presented by APN (2017). None of these sites is less than 10 km from the nearest city (Puerto Iguazú city, northeastern Misiones province).

The captured *N. nasua* were sedated by an intramuscular dose of 10–15 mg/kg of 5% ketamine with 1–2 mg/kg of 2% xylazine, while doses of 20–25 mg/kg of 5% ketamine with 10 mg/kg of 2% xylazine were used to sedate *D. aurita* individuals. They were weighed, measured and exhaustively examined in search of ticks, which were determined following Barros-Battesti et al. (2006), Barbieri et al. (2008), Martins et al. (2014), Nava et al. (2017) and by comparison with known laboratory reared material deposited in the Tick Collection of Instituto Nacional de Tecnología Agropecuaria, Estación Experimental Agropecuaria Rafaela (INTA Rafaela), Argentina. Mean, prevalence and confidence intervals by bootstrap of 2000 replications were calculated using the statistical software QP3.0 (Reiczigel and Rózsa, 2005) for each tick species and parasitic stage.

## 2.3. Statistic analysis

Generalized linear models (GLM) were constructed to analyze the data, using the MASS package (Venables and Ripley, 2002) on R 3.4.1 platform (R Core Team, 2017). For the models construction, two groups of variables were considered: I) Variables associated to host: sex, age (specimens 3–12 months old were considered juvenile/specimens more than 12 months old were considered adults) and body mass (kg.) for *N. nasua* and sex and body mass for *D. aurita* (all captured specimens were adults); II) Variables associated with external factors: season (winter/spring/summer/autumn) and capture site determined by the anthropic disturbance degree (high disturbance: Estación Cataratas/Dos Hermanas/Viejo Hotel; low disturbance: CIES/Garganta/Apepú) (Table 1). Ages of *N. nasua* and *D. aurita* were estimated taking into account the reproductive season, and in *D. aurita* the dental class was assessed based

on Schweigmann et al. (1999). Possible associations between explanatory variables were tested in order to evaluate their inclusion in the statistical analysis.

The dispersion parameters of all global models were calculated and the Variance Inflation Factors (VIF) of the variables of all initial models were evaluated with the car package (Fox and Weisberg, 2011) to confirm their permanence in the models before starting the analysis. The selection of the models was made by a stepwise simplification of terms using the Akaike Information Criterion adjusted for small samples (AICc), using the MuMIn package for R (Barton, 2016). The variables that did not cause a reduction of at least two units of the model's AICc were eliminated (Burnham and Anderson, 2002).

Finally, Tukey contrasts analyses were performed to generate multiple comparisons between the different levels of the categorical variables that were significant in the final models.

## 3. Results

A total of 493 ticks belonging to the species *Amblyomma brasiliense* (n: 15), *Amblyomma coelebs* (n: 424), *Amblyomma ovale* (n: 29) and *Haemaphysalis juxtakochi* (n: 25) were collected from 93 specimens of *N. nasua*. In turn, a total of 552 ticks belonging to the species *A. brasiliense* (n: 3), *A. coelebs* (n: 524), *A. ovale* (n: 22) and *H. juxtakochi* (n: 3) were collected from 26 specimens of *D. aurita* (Table 1). *Amblyomma coelebs* represented 90.7% of all collected ticks. The mean and prevalence of the different stages of all tick species found on *N. nasua* and *D. aurita* and their respective confidence intervals are detailed in Table 2.

A GLM with negative binomial response and logarithmic link function was generated to explain the abundance of *A. coelebs* nymphs collected on *N. nasua*. Because the prevalence of *A. brasiliense* nymphs, *A. coelebs* larvae, *A. ovale* larvae and adults, and *H. juxtakochi* larvae and nymphs on *N. nasua* was lower than 30% throughout the samplings, GLMs with binomial response were used to analyze variables associated with tick presence (number of hosts with at least one individual of a tick species and stage), but not with tick abundance (number of individuals of a tick species and stage on each individual host). Larvae and adults of *A. brasiliense* and nymphs of *A. ovale* were found infesting only one individual host, therefore models were not run for them. Regarding *D. aurita*, negative binomial GLMs were made to explain the abundance of *A. coelebs* larvae and nymphs. The prevalence of the remaining tick species on *D. aurita* was lower than 10%, which complicates the construction of statistical models.

The high VIF values in the binomial models on presence of *A. brasiliense* nymphs, *A. ovale* larvae and *H. juxtakochi* larvae and nymphs on *N. nasua*, forced the elimination of a term before beginning the analyses, since that term showed high collinearity with other variables within the model. In the case of *A. ovale* larvae and *A. brasiliense* nymphs, the season variable was eliminated (VIF = 15.39, VIF = 14.77 respectively), while in the *H. juxtakochi* larvae and nymphs models, the subtracted variable was site (VIF = 7.46, VIF = 8.37, VIF = 6.81 respectively). The most highly correlated variables were season and site.

The models explaining the loads of *A. coelebs* nymphs on *N. nasua* and *D. aurita* were finally constructed with three significant explicative variables: season, site and body mass (Table 3). Nymphs on *N. nasua* were significantly less abundant in spring than in autumn ( $p < 0.001$ ), summer ( $p < 0.001$ ) and winter ( $p < 0.001$ ), and were significantly more abundant on specimens captured in Garganta than in Dos Hermanas ( $p < 0.001$ ), and also more abundant in CIES than in Dos Hermanas ( $p = 0.004$ ). Larger body mass was associated with higher burdens of *A. coelebs* nymphs ( $p = 0.024$ ) on *N. nasua*. In the case of *A. coelebs* infestation on *D. aurita*, the abundance of nymphs was significantly higher in winter than in autumn, spring and summer ( $p < 0.001$  in all cases), and it was significantly higher on animals captured in CIES than in Apepú ( $p = 0.002$ ), and higher loads were associated with higher body mass of *D. aurita* ( $p = 0.011$ ) (Fig. 1).

The presence of *A. coelebs* larvae on *N. nasua* was associated with

**Table 1**

Tick specimens collected on *Nasua nasua* and *Didelphis aurita* in different sites of the Iguazú National Park, Argentina. Age, sex and body mass of each host examined per season is also indicated. J: juvenile; A: adult; f: females; m: males.

Host species	Season	Site	Age	Sex	Body mass (kg)	<i>Amblyomma brasiliense</i>			<i>Amblyomma coelebs</i>		<i>Amblyomma ovale</i>				<i>Haemaphysalis juxtakochi</i>					
						Larvae	Nymphs	Male	Larvae	Nymphs	Larvae	Nymphs	Female	Male	Larvae	Nymphs				
<i>Nasua nasua</i>	Summer	CIES	J	f	0.6		1			1	7						1			
			A	f	5.4		1			39	17						18	1		
			A	f	4					58	25					1	1			
	Autumn	Estación Cataratas	A	m	5.5						6					1		2		
			A	f	4		1			4	2									
			A	f	4.5		2			5	5				1					
			J	f	1.6						1									
			J	f	2.5						1		2							
			J	f	1.6						5									
			J	f	2						4									
			J	f	2						6								2	
			J	f	1.9						8									
			A	f	4.5						1					2			1	
			A	f	4.3				1			2								
			A	m	5.5							14								
			Winter	Estación Cataratas	A	m	5						4							
					A	f	3.3			1			3							
	A	m			3.7						2									
	A	f			3.4						2									
	A	f			3.7						4									
	A	f			4.2				1		3								1	
	A	m			4.6				1		9					1	6		1	
	A	f			4						4					1	1			
	J	f			2						3									
	J	m			2.2						2									
	A	m			5.5						1	13							2	
	A	f			3.3														1	
	A	m			4							1								
	A	m			3.4															
	A	f	3.4							16							2			
	A	f	3.4							1										
	A	m	4.2				1			6							1			
	A	m	4.4							2										
	A	m	5.3				2			8					1	4		1		
	Spring	Estación Cataratas	A	m	5					2						1				
			J	f	2.8								1							
			A	f	3.8															
			A	m	3.7							1								
			A	f	3.6															
			A	f	2.8															
A			f	4																
A			f	4									2					1		
CIES		A	m	5.5				1			5				3	1				
		A	m	6						2	5				1	1				
		J	m	2.4						3								1		
		J	f	2.6						1	4							1		
		J	m	2.8						2	1				1			1		
		J	f	2.5						15	2							2		
		J	m	2.3						1	1						1			
		J	m	2.7						5	2							1		
J	f	2.6						3	1				1	2						
J	m	2.3						5				1				1				
J	m	2.5						4												
J	f	2.6						4	1											
J	f	2.5						12	1											

(continued on next page)

Table 1 (continued)

Host species	Season	Site	Age	Sex	Body mass (kg)	<i>Amblyomma brasiliense</i>			<i>Amblyomma coelebs</i>		<i>Amblyomma ovale</i>				<i>Haemaphysalis juxtakochi</i>				
						Larvae	Nymphs	Male	Larvae	Nymphs	Larvae	Nymphs	Female	Male	Larvae	Nymphs			
<i>Nasua nasua</i>	Winter	Estación Cataratas	A	m	5.7					1									
			A	f	4.6		1			4									
			J	f	2					3									
			J	m	2.1					4									
			J	f	1.9					1									
			J	f	1.5						2								
			J	f	1.6						4								1
			J	f	1.9						1	1	1						
			A	m	4.9				1		2								
			A	m	4.8														
			J	m	2						1	3							1
			J	m	2														
			A	f	4.2							4							
			A	f	4							5							
	A	f	4.9							4									
	A	f	4.5							1									
	A	f	3.4						4	7									
	A	m	6.2							1									
	A	f	3.6						1	5									
	Autumn	Dos Hermanas	A	f	5					1									
			J	m	2.2						1								
			J	m	2.4							1							
			A	f	4.3														
			Estación Cataratas	A	m	5.9						5							
				A	m	5.5						3							
			A	f	4.1														2
			J	f	1.9							1							1
			A	f	4.1							3							
A			f	4.1							2								
A			f	3.2					1		2								
A			m	6.5															
A			f	3.3							3								
A			f	4.3							2								
Garganta	A	m	3.9				1		2	15							2		
	A	f	3.9				2		2	11							1		
A	m	5.5							1										
A	f	3.9							3	24									
A	f	3.6					1		1	39					1				
A	f	4								2						1			
<i>Didelphis aurita</i>	Summer	CIES	A	m	1.2				14	4									
	Autumn	CIES	A	m	0.9				3	1									
A			f	0.6					2	3									
Apepú		A	m	0.4					2	16									
		A	f	0.8						2							1		
Winter	CIES	A	f	1					6	76									
		A	m	0.8				1		2	69								
A	m	0.9							2	38									
A	f	0.8							2	62									
A	f	1					1			13									
A	f	0.8								12									
A	f	0.6							2	17			1						
A	f	0.6								14									
Spring	CIES	A	m	0.4					9	2			17						
		A	f	1						2									
A	f	1.3							21	3		4							
A	m	1							1	8									
A	f	0.4								22									
A	m	1							4	5									
Summer	Apepú	A	m	0.3					3	2									
		A	m	1.2						115	18								
A	m	0.8								3									
A	f	0.6					1			1									
A	f	1.1							4	17									
Spring	Apepú	A	f	0.8							11								
		A	m	1							25								

the site where the hosts were captured. Significant differences were found in sites of greater and lower tourism impact: a significantly lower prevalence was found on hosts captured in Estación Cataratas (a site with higher anthropic disturbance) than on hosts captured in CIES

( $p < 0.001$ ) and Garganta ( $p = 0.0218$ ). To analyze the abundance of *A. coelebs* larvae on *D. aurita*, the extreme value of 115 larvae found on a single host was not taken into account because it did not allow running the global model. After that, the abundance of larvae of *A. coelebs*

**Table 2**

Prevalence, mean and confidence interval limits (CL) of ticks collected on *Nasua nasua* and *Didelphis aurita*. In parenthesis, the number of captured individuals and collected ticks.

Host species (n)	Tick species	Tick stage (n)	Prevalence	Prevalence CL (95%)	Mean	Mean abundance BCa CL (95%)
<i>Nasua nasua</i> (93)	<i>Amblyomma brasiliense</i>	Larvae (1)	0.01	0.0006 - 0.0573	0.01	0.00 - 0.03
		Nymphs (18)	0.16	0.0964 - 0.2516	0.19	0.11 - 0.30
		Adults (1)	0.01	0.0006 - 0.0573	0.01	0.00 - 0.03
	<i>Amblyomma coelebs</i>	Larvae(191)	0.29	0.2030 - 0.3920	2.05	1.01 - 4.35
		Nymphs (397)	0.83	0.7375 - 0.8940	4.27	3.25 - 5.78
	<i>Amblyomma ovale</i>	Larvae (7)	0.05	0.0215 - 0.1219	0.08	0.02 - 0.15
		Nymphs (1)	0.01	0.0006 - 0.0573	0.01	0.00 - 0.03
		Adults (39)	0.19	0.1220 - 0.2893	0.42	0.24 - 0.71
	<i>Haemaphysalis juxtakochi</i>	Larvae (28)	0.11	0.0574 - 0.187	0.3	0.09 - 1.06
		Nymphs (19)	0.16	0.0964 - 0.2516	0.2	0.12 - 0.32
<i>Didelphis aurita</i> (26)	<i>Amblyomma brasiliense</i>	Larvae (1)	0.04	0.0020 - 0.1881	0.04	0.00 - 0.12
		Nymphs (2)	0.08	0.0139 - 0.2460	0.08	0.00 - 0.19
	<i>Amblyomma coelebs</i>	Larvae (215)	0.69	0.4945 - 0.8458	8.27	3.19 - 23.23
		Nymphs (442)	1	0.8715 - 1.0000	17	10.50 - 26.69
	<i>Amblyomma ovale</i>	Larvae (21)	0.08	0.0139 - 0.2460	0.81	0.00 - 2.92
		Nymphs (1)	0.04	0.0020 - 0.1881	0.04	0.00 - 0.12
	<i>Haemaphysalis juxtakochi</i>	Larvae (1)	0.04	0.0020 - 0.1881	0.04	0.00 - 0.12
		Nymphs (2)	0.04	0.0020 - 0.1881	0.08	0.00 - 0.23

on *D. aurita* was associated to site, being significantly more abundant in CIES than in Apepú (p = 0.021), as well as *A. coelebs* nymphs.

The presence of *H. juxtakochi* larvae and nymphs on *N. nasua* was associated with the season, showing lower prevalence in autumn than in spring (p = 0.042) and summer (p = 0.034) in the case of larvae, and significantly higher prevalence in autumn in the case of nymphs (p = 0.007). The presence of *A. brasiliense* nymphs on *N. nasua* was associated with age of host. Prevalence was higher on adult hosts (p = 0.036).

The presence of *A. ovale* larvae was associated with the age of *N. nasua* specimens, showing higher prevalence in juvenile specimens (p = 0.059), whereas the presence of adult ticks was associated with host sex, and their prevalence was significantly higher in males than in females (p = 0.035).

The ΔAICc of the final models with the lowest AICc, resulting from the last term elimination, are shown in Table 3.

**4. Discussion**

This study allowed evaluating the significance of variables related to host and environment to explain the presence and abundance of different tick species on two ubiquitous medium-sized mammals in the Atlantic Forest region of Argentina, namely *D. aurita* and *N. nasua*. The models showed that the magnitude of the influence of both types of variables differs among tick species and parasitic stages.

Regarding host capture site, higher parasitic burdens of *A. coelebs* was associated with hosts circulating in the CIES and Garganta area, in

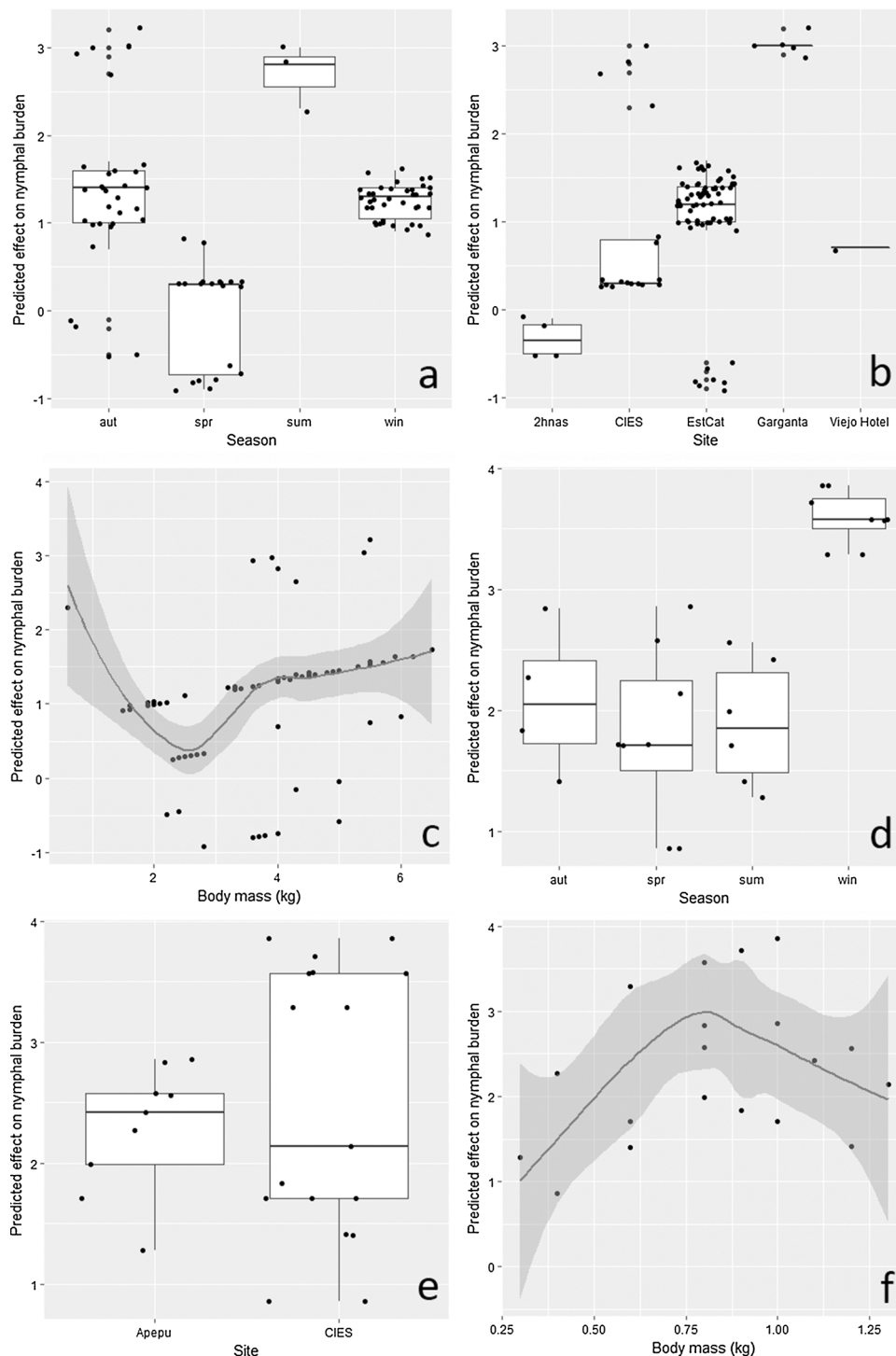
contrast to hosts circulating in Estación Cataratas and Dos Hermanas. The first two sites are those furthest away from the area of greatest tourist presence of the PNI, while the last two are in the epicenter of this area, where people and vehicles circulate daily and where the restaurants for tourists are located. *Nasua nasua* and *D. aurita* have an omnivorous diet and usually feed on rubbish of anthropic origin, as it happens in the tourism area of the PNI (Cáceres and Monteiro-Filho, 2001; Alves-Costa et al., 2004). According to Costa et al. (2009), when there is no food shortage, *N. nasua* groups do not travel long distances, and according to Cáceres and Monteiro-Filho (2001), *D. aurita* has a very small home range of up to 3 ha. This would imply that animals circulating in the INP tourism epicenter where there is copious food supply from human wastes and from fruit trees, would not move too far to other areas. A smaller home-range could imply a lower host-parasite encounter rate and lead to lower tick burdens on these hosts. In addition, the presence of large mammals such as *Panthera onca*, *Puma concolor*, *Tapirus terrestris* and *Mazama americana*, principal hosts of the adult stages of the tick species analyzed in this work (Nava et al., 2017), is less frequent in these areas, which could lead to lower tick abundance in the environment. It is possible to infer, then, that the parasite loads of species such as *A. coelebs* would be lower on animals that circulate in areas where the tourist movement is intense. That is, in areas of high tourism impact, specimens of *N. nasua* and *D. aurita* would have everything in favor: less pressure of large predators, easy access to food of human origin and lower parasitic burdens. Although confounding may occur because not all sites were sampled in the same seasons, comparisons between sites in the same season confirmed the results thrown

**Table 3**

Explanatory variables associated with *Amblyomma coelebs* abundance and *Amblyomma brasiliense*, *Amblyomma ovale* and *Haemaphysalis juxtakochi* presence on *Nasua nasua* and *Didelphis aurita*, resulting from stepwise simplification of Generalized Linear Models.

Host species	Tick species	Tick Stage	Explanatory variables					Δ AICc <sup>a</sup>	Model Family
			Season	Site	Sex	Age	Body mass		
<i>Nasua nasua</i>	<i>Amblyomma coelebs</i>	Larvae		x				37.337	Binomial
		Nymphs	x	x			x	2.409	Negative binomial
	<i>Amblyomma brasiliense</i>	Nymphs				x		5.467	Binomial
		<i>Amblyomma ovale</i>	Larvae				x	2.55	Binomial
	<i>Haemaphysalis juxtakochi</i>	Adults					x	2.519	Binomial
		Larvae	x					18.457	Binomial
<i>Didelphis aurita</i>	<i>Amblyomma coelebs</i>	Nymphs	x					6.364	Binomial
		Larvae		x				1.741	Negative binomial
		Nymphs	x	x			x	1.8	Negative binomial

<sup>a</sup> Δ AICc is the model AICc increase when a single term is eliminated.



**Fig. 1.** Effect of a. season, b. site and c. body mass on *Amblyomma coelebs* nymph abundance on *Nasua nasua*, and effect of d. season, e. site and f. body mass on *Amblyomma coelebs* nymph abundance on *Didelphis aurita*, as predicted by generalized linear models. 2hnas: Dos Hermanas; EstCat: Estación Cataratas.

by the models.

The season variable was important to explain the abundance of *A. coelebs* nymphs and the presence of *H. juxtakochi* larvae and nymphs on *N. nasua*. There are previous studies where season was the variable with greatest ability to predict tick burden (Goodwin et al., 2001; Brunner and Ostfeld, 2008). In this sense, seasonal fluctuation in the abundance of the different parasitic stages of the tick species considered in the current analysis, is usual in areas belonging to the Atlantic Rainforest (Szabó et al., 2009). In a study performed by Lamattina et al. (2018) in northern Misiones province, Argentina, *A. coelebs* and *H. juxtakochi*

nymphs had abundance peaks in winter, while *H. juxtakochi* larvae peaked in summer. These results are in line with information obtained in the present study, except for *H. juxtakochi* nymphs. Nevertheless, this could reflect the heterogeneity of the wild hosts examined by Lamattina et al. (2018), and the fact that few hosts were examined during autumn in that study.

Besides hosts, the abundance and presence of different tick stages depend on environmental factors such as relative humidity, temperature and saturation deficit (Estrada-Peña, 2008), which fluctuate seasonally, therefore it is expected to find seasonal patterns in ticks

collected on their hosts.

A larger host body mass was associated with higher abundance of *A. coelebs* nymphs in both *N. nasua* and *D. aurita*. Body mass is thought to affect parasite burdens by influence of the amount of resource available for parasites (Patterson et al., 2008). The results obtained in the *A. coelebs* nymphs analysis goes in this direction, and they are consistent with a study performed by Harrison et al. (2010), who discovered a positive effect of body mass on *Ixodes ricinus* larval burdens on the small rodent *Apodemus sylvaticus* in northern Ireland.

*Nasua nasua* is a usual host for adults of *A. ovale* (Magalhaes-Matos et al., 2017). The prevalence of *A. ovale* adults was higher on *N. nasua* males than in females. This could be explained by the fact that *N. nasua* are organized in groups formed by adult females, juvenile individuals and/or pups and some males, whereas adult males are usually solitary (Beisiegel, 2001). *Nasua nasua* that live in groups typically show grooming behaviors, during which ectoparasites are removed, a situation that does not happen in solitary males. Furthermore, the ectoparasites removal seems to be dependent on the size of the ticks, since only *A. ovale* adults presented this difference between host genders. This hypothesis is consistent with a study performed by Gompper (2004) on ticks infesting *Nasua narica* in Panama.

In some cases, the  $\Delta AICc$  of the simplified models was lesser than two units. For example, in the case of *A. coelebs* nymphs loads on *D. aurita*, keeping the body mass variable in the model resulted in a  $\Delta AICc$  of 1.8, while taking out this variable provoked the  $\Delta AICc$  rising to 2.671. The difference is very small and it should be evaluated whether it is correct to remove body mass from the model when it has a significant effect ( $p = 0.011$ ). Possibly, the two units consensus of  $\Delta AICc$  to maintain or remove variables should be more flexible in cases like this, where the variable in question also influences significantly the abundance of *A. coelebs* on another host model (*N. nasua*). The same happened with *A. coelebs* larvae. The  $\Delta AICc$  for the site was 1.741 in *D. aurita*, but this variable proved to be important to explain the presence of larvae on *N. nasua*, therefore it should be considered to keep it in the model.

Some variables that have decreased in more than two units the AICc of the model, such as age for the presence of *A. ovale* larvae, had a p-value higher than 0.05. However, these variables were important in the models to keep their low AICc, so it should be considered to accept a significance level of 0.09 for studies like this, in which significant variables are found to explain the presence of ectoparasite species with highly aggregated and even sometimes zero-inflated distributions.

Finally, the importance of these medium-sized mammals to support the immature stages of ticks found in the Atlantic Rainforest region of Argentina was highlighted. Depending on the stage and tick species of interest, both environmental (i.e. anthropogenic disturbance) and host variables could modulate the parasite loads. Given the high level of interactions between these two medium-sized mammals and humans in protected areas and in sites of human settlements neighboring Atlantic Forest protected areas in Argentina, these hosts could play an important role in the enzootic cycles of tick-borne pathogens. This fact highlights the relevance of assessing the variables which have impact on the presence and abundance of ticks on wild hosts.

## Conflicts of interest

The authors declare to have no conflicts of interest.

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