Exposure of small mammals to ticks and rickettsiae in Atlantic Forest patches in the metropolitan area of Recife, North-eastern Brazil

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SUMMARY

Between December 2007 and March 2009, small mammals were captured in 6 Atlantic Forest patches in Brazil. We assessed tick-host associations and whether they differ among forest strata, sites, seasons, and host age classes or between sexes. Moreover, we assessed the exposure of animals to *Rickettsia* spp. In total, 432 animals were captured and 808 ticks were found on 32·9% of them. Significant differences were found among host species, collection sites, and forest strata; microhabitat preference was a strong risk factor for tick infestation. The highest tick density rates were recorded in forest fragments settled in rural areas; 91·3% of the ticks were collected from animals trapped in these forest fragments. A high prevalence (68·8%) of antibodies to *Rickettsia* spp. was detected among animals. This study suggests that disturbed Atlantic Forest fragments provide an environment for ticks and small mammals, which are highly exposed to rickettsiae. It also indicates that forest patches settled in rural areas are usually associated with higher small mammal diversity as well as with higher tick density rates.

Key words: Atlantic forest, ticks, *Rickettsia*, serology, forest fragments, Recife.

INTRODUCTION

The Atlantic Forest is one of the richest biomes in the world, being home to an outstanding diversity of plants and animals (Galindo-Leal and Câmara, 2003). For instance, out of 658 mammal species found in Brazil, 250 are present in the Atlantic Forest, 55 being exclusively found in this biome (Reis *et al*. 2006). Originally, almost the entire Brazilian coast was covered by Atlantic Forest, which now has been reduced to a series of small fragments (Ranta *et al*. 1998; Ribeiro *et al*. 2009). Moreover, disturbances in such an important natural ecosystem can eventually bring humans into contact with wildlife-associated pathogens (Bradley and Altizer, 2006).

Some fragments of Atlantic Forest in Brazil are embedded within highly urbanized areas (Galindo-Leal and Camara, 2003), bringing wildlife and their associated pathogens into greater contact with domestic animals and humans. For instance, small mammals have been implicated in the transmission cycles of many tick-borne pathogens worldwide, including *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, and *Rickettsia rickettsii* (Bown *et al*. 2008; Zhan *et al*. 2009). Incidentally, tick-borne diseases represent an emerging public health concern in Brazil (Silveira *et al*. 2007; Spolidorio *et al*. 2010). In this scenario, marsupials and small rodents have been regarded as some of the most probable amplifier hosts for *R. rickettsii* among *Amblyomma cajennense* and *Amblyomma aureolatum* ticks, respectively (Labruna, 2009). For example, opossums can serve as a source of *R. rickettsii* to *Am. cajennense* in the laboratory (Horta *et al*. 2009). In addition, *Borrelia*-like spirochetes were isolated from marsupials, rodents and their ticks in South-eastern Brazil, suggesting their participation in the epidemiology of the

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so-called 'Baggio-Yoshinari Syndrome' (Abel *et al*. 2000). These findings highlight the importance of studying the effects of Atlantic Forest fragmentation on small mammal-tick systems to better understand the eco-epidemiology of tick-borne diseases in this country.

Habitat fragmentation in the United States is associated with a reduction of mammal species diversity and elevation of population densities of white-footed mice (*Peromyscus leucopus*), the principal reservoir of *B. burgdorferi* (Bradley and Altizer, 2006). Even if ticks infesting mammals in Brazil have long been studied (Barros-Battesti *et al*. 1998, 2000; Bittencourt and Rocha, 2003; Martins *et al*. 2009), data on habitat fragmentation and its effects on tick-host-pathogen associations in the Atlantic Forest are meagre. We hypothesized that the Atlantic Forest provides an environment for small mammal communities and ticks, and that small mammals are constantly exposed to pathogens, such as *Rickettsia* spp. We assessed small mammal-tick associations in 6 Atlantic Forest patches and whether they differ among forest strata, sites, seasons, and host age classes or between sexes. We also investigated the exposure of small mammals to *Rickettsia* spp. This study suggests that disturbed Atlantic Forest fragments provide an environment for ticks and small mammals, which are highly exposed to *Rickettsia* spp. It also points out that forest patches settled in rural areas are often associated with higher diversity and density rates of both small mammals and ticks. These findings suggest that habitat destruction and urbanization might affect the risk of exposure to ticks and tick-borne pathogens in Atlantic Forest fragments.

MATERIALS AND METHODS

Study area

This study was conducted in 6 Atlantic Forest fragments (Fig. 1) located in the Recife metropolitan region (Pernambuco State, North-eastern Brazil), as follows: Estação Ecológica de Caetés (ESEC Caetés); Parque Estadual de Dois Irmãos (PEDI); Parque Ecológico São José (PEC São José); Campo de Instrução Marechal Newton Cavalcanti (CIMNC); Mata de Aldeia/Chã da Peroba (Aldeia); Estação Ecológica de Tapacurá (ESEC Tapacurá) (Table 1). With over 3·5 million inhabitants, this is the 5th largest metropolitan area of Brazil and scattered Atlantic Forest fragments are irregularly distributed over the region. Although each forest fragment studied here might have its own microclimate, they share the same macroclimatic characteristics. Accordingly, the region has a typical tropical climate, characterized by a wet season (March-August) and dry season (September-February).

Animal trapping and identification

From December 2007 to March 2009, small mammals were captured in the 6 Atlantic Forest patches. Field missions were carried out at 3-month intervals, during 5 consecutive nights (200 traps per night), corresponding to an overall sampling effort of 25 231 traps-nights. In 4 Atlantic Forest fragments (ESEC Caetés, PEDI, PEC São José, CIMNC), grids of 80 capture stations (2 traps per capture station) were set, 25 m apart, covering an area of approximately 3·9 ha. Animals were captured with 2 types of traps: Sherman live trap (23 × 8 × 9 cm) (H.B. Sherman Trap Co., Tallahassee, FL, USA) and handmade wire-mesh (Tomahawk) trap (small (30 × 10 × 10 cm) or large (50 × 17 × 17 cm)) (Gabrisa, Cafelândia, Brazil). Two traps (1 Sherman trap and 1 Tomahawk trap) were set in each capture station, one being placed on the ground and another in the understory attached to tree branches at 1–2 m above the ground. In addition, linear transects (range of length, 400–600 m) were established in pre-existent trails with traps ($n = 40$) positioned 10–15 m apart. Each collection station included 1 trap, the position of which (on the ground or in the understory) was alternated at each station. In 2 Atlantic Forest fragments (ESEC Tapacurá and Aldeia) only linear transects were established, which maintained the same number of collection stations and disposition of traps as per grids. Trap types and positions were alternated to obtain an homogeneous sampling effort over the fragments surveyed.

Traps were baited with pieces of pineapple mixed with a Brazilian peanut candy called *paçoca*. Every morning, all traps were inspected to assess the presence of animals and to check the baits, which were changed whenever needed. Once captured, animals were restrained physically, placed into individual cloth bags and transported to our field facilities. Before tick collection, animals were subjected to anaesthesia with an association of ketamine (30 mg/kg for marsupials, 100 mg/kg for rodents, and 50 mg/kg for lagomorphs) plus xylazine (2 mg/kg for marsupials, 5 mg/kg for rodents, and 5 mg/kg for lagomorphs). Physiological parameters were monitored and after recovery from anaesthesia, animals were released in their original capture site. Before releasing, the animals were either marked with numbered ear tags or tattooed on their inner right thigh, type of marking being decided based on the animal's size.

Small mammals were identified using field guides of Neotropical rainforest mammals (Redford and Eisenberg, 1992; Emmons and Feer, 1997; Bonvicino *et al*. 2008). Voucher specimens were deposited in the Mammal Collection of the Universidade Federal de Pernambuco, Brazil. Trapping and handling of small mammals has been authorized by the Instituto Chico Mendes para Conservação da Biodiversidade (ICMBio/SISBIO, No. 11854-1, 11854-2, 10769-2).

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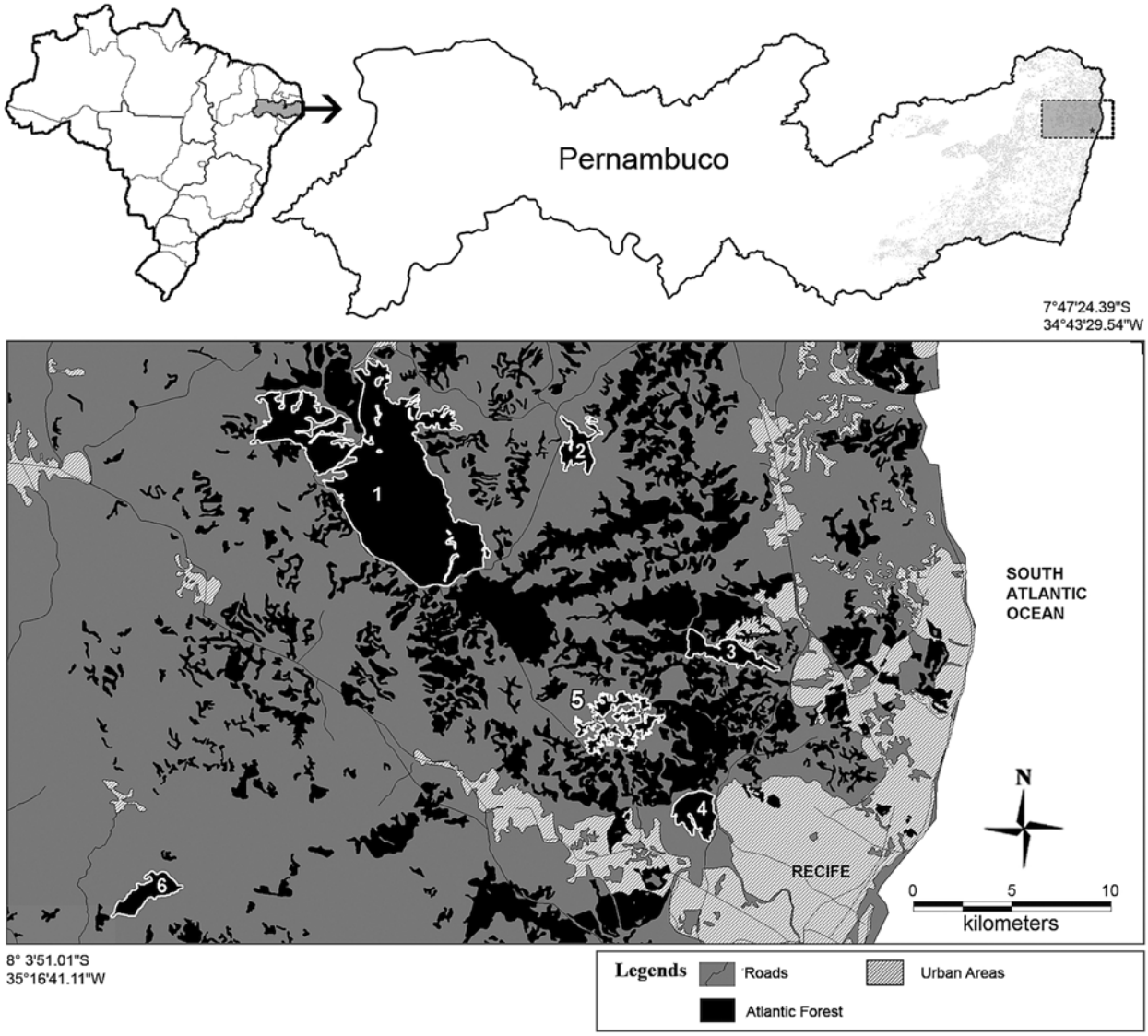

Fig. 1. Location of the 6 Atlantic Forest fragments in Pernambuco (North-eastern Brazil) surveyed during this study, with a view of main landscape features of the surrounding areas. 1, Campo de Instrução Marechal Newton Cavalcanti. 2, Parque Ecológico São José. 3, Estação Ecológica Caetés. 4, Parque Estadual de Dois Irmãos. 5, Mata de Aldeia. 6, Estação Ecológica de Tapacurá.

Tick collection and identification

Ticks were collected from the hosts by hand and placed into individualized labelled plastic bottles. In the laboratory, non-engorged or partially engorged ticks were placed into plastic bottles containing 70% ethanol. Conversely, fully engorged nymphs were kept in their original bottles and maintained in an incubator (27 ± 1 °C, 80% RH, 12-h light/12-h dark photoperiod) to moult into adults to facilitate species-level identification. Additionally, ticks found on researchers involved in the fieldwork were also collected. Ticks were identified using morphological keys for adults (Aragão and Fonseca, 1961; Onofrio *et al*. 2009) and nymphs (Martins *et al*. 2010). Nomenclature of ticks follows a recent checklist of ticks found in Brazil (Dantas-Torres *et al*. 2009) and generic names are abbreviated as proposed elsewhere (Dantas-Torres, 2008). Voucher tick specimens were deposited in the following collections (Accession numbers within parentheses): Instituto Butantan, São Paulo, SP, Brazil (IBSP) (9971, 9973–9975); Coleção Nacional de Carrapatos of the Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, São Paulo, SP, Brazil (CNC) (1328–1339, 1319–1327, 1538–1545, 1549–1552); Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brazil (IOC) (0975–0978).

*Detection of anti-*Rickettsia *spp. antibodies in rodents and marsupials*

Sera from small mammals ($n = 218$) were tested by indirect immunofluorescence assays, as described in detail elsewhere (Horta *et al*. 2007, 2010). In particular, serum samples were tested for antibodies reactive to *R. bellii* (strain Mogi), *R. amblyommii*

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Table 1. Characteristics of the six Atlantic Forest fragments located in North-eastern Brazil

Collection site	Municipality	Coordinates	Area (ha)	Landscape
Estação Ecológica de Caetés (ESEC Caetés)	Paulista	7°55′46·79″ S 34°55′56·74″ W	157	Dense lowland rainforest settled in a suburban area, separated by non-paved roads and private properties
Parque Estadual de Dois Irmãos (PEDI)	Recife	8°0′2·05″ S 34°56′45·98″ W	384·42	Dense lowland rainforest settled in an urban area, bordering zones of native vegetation to the north
Parque Ecológico São José (PEC São José)	Igarassu	7°50′19·03″ S 34°59′57·98″ W	319	Dense lowland rainforest settled in a rural area, bordered by sugarcane plantations, crossed by roads (crop transportation)
Campo de Instrução Marechal Newton Cavalcanti (CIMNC)	Paudalho	7°50′25·13″ S 35°6′7·07″ W	6280	Seasonal semideciduous forest settled in a rural area, crossed by non-paved roads
Mata de Aldeia/Chã da Peroba (Aldeia)	Camaragibe	7°57′32·59″ S 34°59′2·03″ W	488	Dense lowland rainforest settled in a suburban area, interspersed by small properties, orchards, and wasteland
Estação Ecológica de Tapacurá (ESEC Tapacurá)	São Lourenço da Mata	8°2′12·48″ S 35°11′41·12″ W	776	Seasonal semideciduous forest settled in a rural area, encompassed by rural properties and sugarcane plantations

(strain Ac37) and *R. rickettsii* (strain Taiaçu). Slides were incubated with fluorescein isothiocyanate-labelled sheep anti-opossum IgG (CCZ, São Paulo, Brazil) and rabbit anti-guinea pig IgG (Sigma, St Louis, MO, USA) for marsupials and rodents, respectively; sera were considered positive if reactive at a 1:64 dilution.

Ecological parameters

Species richness (S) was recorded for both hosts and ticks for each Atlantic Forest fragment. Prevalence (P), mean intensity (MI), mean abundance (MA), and tick relative density indices (TRDI) were calculated as described previously (Bush *et al*. 1997; Barros-Battesti *et al*. 2000). Tick records ($n=158$) included in the calculation of ecological parameters have been reported by Martins *et al*. (2009).

Statistical analyses

Data comparisons were performed using Kruskal-Wallis, one-way ANOVA, Mann-Whitney U test, Student's *t*-test or Chi-square tests, as appropriate. Before analysis, data were checked for normality using Lilliefors test and, when appropriate, a logarithmic transformation (log 10) was used to eliminate heterogeneity of variances across groups of data. If the heterogeneity persisted, we used a nonparametric test. Differences were considered significant if $P<0·05$. Odds ratio (OR) was used to estimate the relative risk for tick infestation among forest strata, host age classes and sexes. Ninety-five per cent confidence intervals (95% CI) were calculated for each prevalence estimate. Pearson (r) (for parametric data) and Spearman (rs) (for non-parametric data) correlation coefficients were used to determine the level of correlation between some of the ecological parameters recorded. Two host species (represented by only 1 individual) and 1 forest fragment (i.e., PEDI, where only 5 animals were captured) were excluded from the analyses. Analyses were performed using BioEstat 5.0 (Ayres *et al*. 2007). Evenness (E) was calculated using PAST version 1.99 (Hammer *et al*. 2001).

RESULTS

Host species, sex, age classes, and habitat preference

In total, 432 small mammals belonging to 20 species were captured. The number of individuals captured from each host species varied significantly ($\chi^2=183·59$, D.F. = 8, $P<0·0001$, one-sample Chi-square test), 1 rodent species (*T. laurentius*) being the most frequently captured host (Table 2).

Ticks were found on 142 small mammals belonging to 11 species, with overall prevalence of 32·9% (95% CI: 28·4–37·3%), mean intensity of 5·7 and mean abundance of 2·1 (Table 2). The highest mean intensities and abundances of infestation were recorded for 3 marsupial species (*Didelphis albiventris, Didelphis aurita, Monodelphis domestica*) and 1 rodent (*Thrichomys laurentius*) (Fig. 2), which were frequently exposed to multiple tick stages (data not shown). Indeed, the number of infested individuals varied significantly according to host species ($\chi^2=131·93$, D.F. = 8, $P<0·0001$, one-sample Chi-square test).

The sex ratio of small mammals captured was close to unity (198 females/184 males). Males harboured a larger number of ticks than females ($\chi^2=76·12$, D.F. = 1, $P<0·0001$, one-sample Chi-square test), but there was no difference in prevalence of infestation in relation to sex ($\chi^2=3·32$, D.F. = 1, $P=0·07$, two-sample Chi-square test). Most of the captured hosts

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Table 2. Small mammal species collected, number of ticks collected, prevalence, mean intensity and mean abundance

Order	Species	No. captured/ no. infested	No. of ticks	Prevalence (95% CI)	Mean intensity	Mean abundance
Rodentia	*Cerradomys subflavus*	6/4	8	66·7 (22·3–95·7)	2·0	1·3
	Guerlinguetus alphonsei	1/1	7	100·0 (2·5–100·0)	7·0	7·0
	Nectomys rattus	28/3	6	10·7 (2·4–29·2)	2·0	0·3
	Thrichomys laurentius	105/45	342	42·9 (33·2–53·9)	7·6	3·3
Marsupialia	*Didelphis albiventris*	60/40	183	66·7 (53·3–78·3)	4·6	3·1
	Didelphis aurita	26/19	120	73·1 (52·2–88·4)	6·3	4·6
	Marmosa murina	67/9	25	13·4 (6·3–24·0)	2·8	0·4
	Metachirus nudicaudatus	23/8	25	34·8 (16·4–57·3)	3·1	1·1
	Micoureus demerarae	49/5	5	10·2 (3·3–21·8)	1·0	0·1
	Monodelphis domestica	16/7	82	43·8 (19·8–70·1)	11·7	3·4
Lagomorpha	*Sylvilagus brasiliensis*	1/1	5	100·0 (2·5–100·0)	5·0	5·0
	Total	382/142	808	32·9 (28·4–37·3)	5·7	2·1

were adults ($n=292$, 76·44%) ($\chi^2=106·82$, D.F. = 1, $P<0·0001$, one-sample Chi-square test), but no difference was found in relation to host age class ($\chi^2=0·40$, D.F. = 1, $P=0·53$, two-sample Chi-square test). However, crossing sex with age class data, adult males were significantly more infested than adult females ($\chi^2=4·71$, D.F. = 1, $P=0·03$, two-sample Chi-square test). Accordingly, adult males were at a higher risk of being infested by ticks as compared with adult females (OR = 1·70; 95% CI: 1·05–2·75; $P=0·04$).

When grouping small mammals by microhabitat preference, the prevalence was much higher on predominantly terrestrial ($n=265$) (48·0%; 95% CI: 41·9–53·9) than on arboreal ($n=117$) mammals (12·9%; 95% CI: 6·8–18·9) ($\chi^2=42·83$, D.F. = 1, $P<0·0001$, two-sample Chi-square test). Accordingly, the mean intensity and mean abundance was higher among terrestrial (6·1 *vs* 2·9) than on arboreal mammals (2·5 *vs* 0·3). Fitly, terrestrial mammals had a 6-times higher chance of being infested by ticks than arboreal mammals (OR = 6·3; 95% CI: 3·5–11·3, $P<0·0001$).

Nine small mammal species captured were free of ticks (no. of individuals captured is within parentheses): *Akodon cursor* (32); *Calomys expulsus* (1); *Caluromys philander* (3); *Monodelphis americana* (2); *Mus musculus* (1); *Oecomys bahiensis* (1); *Oxymycterus dasythrichus* (1); *Rattus rattus* (8); *Rhipidomys mastacalis* (1). Some of these animals were infested by other ectoparasites, such as fleas and laelapid mites (data not shown).

Tick-small mammal associations

A total of 808 ticks (357 larvae, 416 nymphs, 20 females, 15 males) were collected, ranging from 1 to 68 ticks per host. Three tick genera were identified: *Amblyomma* ($n=737$; 91·2%); *Haemaphysalis* ($n=4$; 0·5%); and *Ixodes* ($n=67$; 8·3%). Four tick species were identified: *Amblyomma fuscum; Amblyomma dubitatum; Haemaphysalis leporispalustris*; and *Ixodes loricatus*. *Amblyomma fuscum* was found on 7 host species (*Cerradomys subflavus, D. albiventris, D. aurita, Metachirus nudicaudatus, M. domestica, Nectomys rattus, T. laurentius*) whereas *Ix. loricatus* was found on 5 marsupial species (*D. aurita, D. albiventris, M. nudicaudatus, Micoureus demerarae, Marmosa murina*). *Amblyomma dubitatum* and *Ha. leporispalustris* were exclusively found on a rodent (*N. rattus*) and on a lagomorph (*Sylvilagus brasiliensis*) species, respectively. Three *D. aurita* and 1 *D. albiventris* were co-infested by *Am. fuscum* (nymphs) and *Ix. loricatus* (adults) whereas 1 *S. brasiliensis* was co-infested by *Amblyomma* sp. (larvae) and *Ha. leporispalustris* (nymphs).

The majority of the ticks collected were *Amblyomma* larvae ($n=353$, 43·7%) and nymphs ($n=384$, 47·5%) that were found co-feeding on small mammals during the entire study period. Although the overall larva/nymph ratio was close to unity, larvae were almost 3 times more abundant during the wet season than in the dry season (mean abundances, 1·4 *vs* 0·5). *Amblyomma* immature ticks were collected from 109 hosts belonging to 9 species: *C. subflavus* (4); *D. albiventris* (28); *D. aurita* (13); *M. nudicaudatus* (7); *Guerlinguetus alphonsei* (1); *M. domestica* (7); *N. rattus* (3); *T. laurentius* (45); and *S. brasiliensis* (1). With the exception of 1 nymph of *Am. dubitatum* collected from a water rat (*N. rattus*), all nymphs were identified as *Am. fuscum*. Accordingly, 25 nymphs kept under laboratory conditions moulted into adults (female/male ratio, 1·5), being all *Am. fuscum*. These 25 nymphs were collected from 10 hosts belonging to 3 species: *D. albiventris* (2); *D. aurita* (3); and *T. laurentius* (5). Of note, no single adult specimen of *Am. fuscum* was found on any of the small mammals captured.

All adult ticks (20 females/15 males) collected were identified as *Ix. loricatus*, which was collected from 23 hosts belonging to 4 species: *D. albiventris* (13); *D. aurita* (8); *M. nudicaudatus* (1); and

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Table 3. Number of small mammals captured/infested, ticks collected, tick relative density (TRD), prevalence, mean intensity, and mean abundance recorded among Atlantic Forest fragments

Forest fragment	No. captured/ no. infested	No. of ticks	TRD	Prevalence (95% CI)	Mean intensity	Mean abundance
ESEC Tapacurá	199/79	546	2764·7	39·7 (32·9–46·9)	6·9	2·7
ESEC Caetés	52/10	24	25·2	19·2 (9·6–32·5)	2·4	0·5
Aldeia	35/21	45	47·0	60·0 (42·1–76·1)	2·1	1·3
PEC São José	51/8	13	18·1	15·7 (7·0–28·6)	1·6	0·3
CIMNC	40/23	179	195·0	57·5 (40·9–73·0)	7·8	4·5
PEDI	5/1	1	0·1	20·0 (0·5–71·6)	1·0	0·2

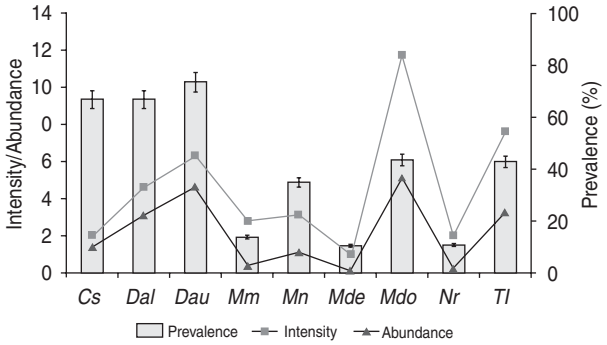

Fig. 2. Prevalence, mean intensity and mean abundance of tick infestation among host species. Cs, *Cerradomys subflavus*. Dal, *Didelphis albiventris*. Dau, *Didelphis aurita*. Mm, *Marmosa murina*. Mn, *Metachirus nudicaudatus*. Mde, *Micoureus demerarae*. Mdo, *Monodelphis domestica*. Nr, *Nectomys rattus*. Tl, *Thrichomys laurentius*. Error bars indicate 95% confidence intervals.

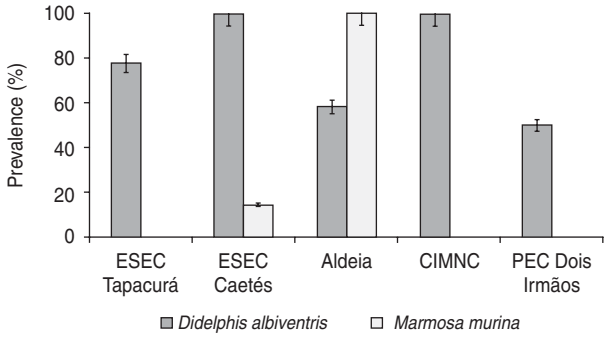

Fig. 3. Prevalence of tick infestation on *Didelphis albiventris* and *Marmosa murina* in different forest fragments. Neither *D. albiventris* nor *M. murina* were found infested in PEC São José, which for this reason is not shown in the graphic. Error bars indicate 95% confidence intervals.

Micoureus demerarae (1). Larvae and nymphs of *Ix. loricatus* were collected from 14 marsupials of 3 species: *Marmosa murina* (9); *M. demerarae* (4); and *D. aurita* (1). Neither adults nor immature stages of *Ix. loricatus* were found on rodents.

Only 4 nymphs of *Haemaphysalis* were collected from a tapeti (*S. brasiliensis*). One nymph maintained in the laboratory moulted into a male of *Ha. leporispalustris*.

Tick infestation among forest fragments, seasons and strata

The overall number of animals captured in each forest fragment varied significantly ($\chi^2 = 256{\cdot}04$, D.F. = 4, $P < 0{\cdot}0001$, one-sample Chi-square test) as well as the number of animals infested ($\chi^2 = 120{\cdot}53$, D.F. = 4, $P < 0{\cdot}0001$, one-sample Chi-square test), the prevalence ($\chi^2 = 44{\cdot}69$, D.F. = 4, $P < 0{\cdot}0001$, one-sample Chi-square test), the number of ticks collected ($\chi^2 = 1255{\cdot}75$, D.F. = 4, $P < 0{\cdot}0001$, one-sample Chi-square test). Tick relative density indices recorded varied as well, being much higher in ESEC Tapacurá (TRDI = 2764·7) and in CIMNC (TRDI = 195·0) (Table 3).

When animals were stratified by species, no differences were found in relation to the number of individuals captured ($H = 4{\cdot}33$, D.F. = 4, $P = 0{\cdot}363$, Kruskal-Wallis) or infested ($H = 2{\cdot}10$, D.F. = 4, $P = 0{\cdot}717$, Kruskal-Wallis). However, 2 small mammal species (*D. albiventris* and *M. murina*) were evenly distributed (present in all the forest fragments), but the prevalence of ticks on them varied widely. Indeed, *M. murina* was captured in all forest fragments, but was found infested by ticks in only 2 forest fragments (Fig. 3). No significant correlations were found between forest fragment land area and any of the ecological parameters (data not shown).

The number of animals captured did not vary between seasons ($t = 0{\cdot}552$, D.F. = 8, $P = 0{\cdot}596$, Student's *t*-test) as well as the number of infested animals ($t = 0{\cdot}292$, D.F. = 8, $P = 0{\cdot}778$, Student's *t*-test). Again, the number of ticks collected did not vary according to season ($t = 0{\cdot}050$, D.F. = 8, $P = 0{\cdot}961$, Student's *t*-test). The prevalence of infestation did not differ significantly by season, except in 1 forest fragment (CIMNC) where the prevalence increased more than 2-fold during the wet season ($\chi^2 = 7{\cdot}82$, D.F. = 1, $P < 0{\cdot}01$, two-sample Chi-square test). Again, only 1 tick species (*Am. fuscum*) was found during the dry season whereas 3 (*Am. fuscum*, *Ha. leporispalustris*, and *Ix. loricatus*) were identified during the wet season in CIMNC.

The number of small mammals captured on the ground in each forest fragment was higher than that captured in the understory ($Z = 2{\cdot}402$, $P = 0{\cdot}016$,

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Table 4. Number of small mammals captured/infested on the ground and in the understory in each Atlantic Forest fragment[a]

Forest fragment	No. captured/no. infested		Fisher exact test	Odds ratio (95% CI)	*P*
	Ground	Understory			
ESEC Tapacurá	161/74	28/2	<0·01	11·1 (2·5–48·2)	<0·01
ESEC Caetés	35/7	11/3	0·68	0·7 (0·1–3·2)	0·93
Aldeia	26/10	9/6	0·25	0·3 (0·1–1·5)	0·28
PEC São José	36/6	19/2	0·70	1·7 (0·3–9·4)	0·83
CIMNC	30/20	8/1	0·01	14·0 (1·5–130·0)	<0·05
Total	288/117	75/14	<0·01[b]	3·0 (1·6–5·6)	<0·01

[a] This table includes only animals ($n=363$) for which information on place of capture (ground or understory) was available.
[b] $\chi^2=12{\cdot}44$, D.F. = 1, $P<0{\cdot}01$, two-sample Chi-square test.

Mann-Whitney U test). Again, the number of infested mammals captured on the ground was higher than that captured in the understory ($t=3{\cdot}330$, D.F. = 8, $P=0{\cdot}010$, Student's *t*-test) (Table 4). Indeed, mammals captured on the ground had a 3 times higher risk of being infested than those captured in the understory (OR = 3·0; 95% CI: 1·6–5·6; $P=0{\cdot}01$). In particular, mammals captured on the ground in ESEC Tapacurá (OR = 11·1; 95% CI: 2·5–48·2; $P<0{\cdot}001$) and CIMNC (OR = 14·0; 95% CI: 1·5–130·6; $P=0{\cdot}02$) had an over 10 times higher risk of being infested by ticks than those in the understory.

Host richness and evenness were similar among forest fragments (data not shown). Accordingly, no correlation was found between host richness and tick species richness ($r=0{\cdot}22$, $R^2=0{\cdot}05$, $P=0{\cdot}68$), forest fragment land area ($r=0{\cdot}22$, $R^2=0{\cdot}05$, $P=0{\cdot}68$), trapping effort ($r=0{\cdot}22$, $R^2=0{\cdot}05$, $P=0{\cdot}68$) or number of small mammals captured ($r=0{\cdot}22$, $R^2=0{\cdot}05$, $P=0{\cdot}68$).

Antibodies to Rickettsia *spp. in rodents and marsupials*

Out of 218 serum samples tested for antibodies to *Rickettsia* spp., 150 (68·8%; 95% CI: 62·7–75) were reactive to one or more antigens. In particular, 83 (68·6%; 95% CI: 60·3–76·9) out of 121 rodents tested positive. Forty-four rodents were positive to a single antigen, being 42 to *R. rickettsii* and 2 to *R. bellii*. Again, 18 rodents were reactive to 2 antigens, being 9 to *R. bellii* and *R. rickettsii*, 8 to *R. amblyommii* and *R. rickettsii*, and 1 to *R. amblyommii* and *R. bellii*. Finally, 21 rodents reacted simultaneously to *R. amblyommii* and *R. bellii* and *R. rickettsii*. In the same way, 67 (69·1%; 95% CI: 59·9–78·3) out of 97 marsupials tested positive. Eleven marsupials were reactive to 1 antigen, being 9 to *R. bellii*, 1 to *R. amblyommii* and 1 to *R. rickettsii*. Again, 21 reacted to 2 antigens, being 20 to *R. bellii* and *R. rickettsii*, and 1 to *R. amblyommii* and *R. bellii*. Importantly, 35 marsupials reacted simultaneously to all 3 antigens.

The seropositivity among tick-infested small mammals (75·3%; 95% CI: 66·3–83·7) and non-infested ones (63·7%; 95% CI: 55·1–72·2) did not differ significantly ($\chi^2=3{\cdot}39$, D.F. = 1, $P=0{\cdot}07$, two-sample Chi-square test).

Human parasitism by ticks

A total of 16 larvae of *Amblyomma* were collected from 3 researchers involved in the fieldwork. In particular, 2 cases of infestation were recorded in the dry season (1 in PEDI and another in CIMNC) and 1 (again in CIMNC) in the wet season.

DISCUSSION

This study suggests that disturbed Atlantic Forest fragments provide an environment for small mammals and ticks and that small mammals living in these areas are highly exposed to rickettsial organisms. This is inferred from the numbers of small mammals and ticks collected in all forest fragments studied, which generally remained constant across seasons. Interestingly, we found that over 90% of the ticks collected came from small mammals captured in 3 Atlantic forest fragments settled in rural areas. In such forest fragments, rodents and *Amblyomma* ticks predominated. On the other hand, marsupials and *Ixodes* ticks were typically found in forest fragments located in urban or suburban areas. These findings suggest that the risk of tick-borne pathogen transmission will vary according to environmental characteristics of the forest fragment, which in turn will directly influence both small mammal communities and tick populations. Because the human parasitism by *Amblyomma* larvae appears to be common in Atlantic Forest fragments (Dantas-Torres *et al.* 2010), people undergoing activities (ecotourism, research activities, forest dwellers) in these areas might be at risk of exposure to ticks and rickettsial organisms. Indeed, 16 *Amblyomma* larvae were collected from 3 researchers involved in the fieldwork of the present study.

We found that small mammals captured on the ground were at a higher risk of being infested by ticks. The tick burden was also heavier on certain host

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species (e.g., *T. laurentius*) and generally on adult males. These findings confirm that microhabitat preference and/or sex-related behaviour increase their risk of tick infestation in small mammals living in Atlantic Forest patches. Male small mammals have, generally, a larger home range than females, particularly during the breeding season (Streilen, 1982), which might increase their chances of being exposed to ticks. This is also true for predominantly terrestrial mammals whose ground activities can increase the risk tick infestation. In this regard, most of the small mammals trapped on the ground were infested by nymphs and larvae of *Am. fuscum*, which until recently was regarded as a rare South American tick species (Barros-Battesti *et al*. 2005). Adults of *Am. fuscum* predominantly parasitize cold-blooded animals, particularly boa snakes (*Boa constrictor*) (Dantas-Torres *et al*. 2008, 2010). It follows that, in nature, *Am. fuscum* undergoes a typical ditropic life cycle, that is, its immatures feed principally on small mammals whereas adults often parasitize reptiles. However, it is worth mentioning that *Am. fuscum* adults have occasionally been found on non-reptile hosts, including humans (Marques *et al*. 2006). This might indicate that *Am. fuscum* has a low host-specificity, as previously hypothesized (Marques *et al*. 2006), or that its host specificity might vary from region to region or even seasonally, according to the host community available. Certainly, besides the ecological aspect, the low host specificity of larvae and nymphs of *Am. fuscum* is also relevant from an epidemiological standpoint.

The overall prevalence of tick infestation and particularly the tick relative density recorded in this study was much higher than that reported in a previous study conducted in a disturbed Atlantic Forest fragment in South-eastern Brazil (Barros-Battesti *et al*. 2000). Certainly, local factors (i.e., microclimatic and physiographic features and host community) might influence the ecology of ticks infesting small mammals in Atlantic Forest fragments. In other words, the same tick species might exhibit different ecological patterns or behaviour in different geographical regions. This information might be relevant to understand the patterns of tick-borne pathogen transmission in different regions, as the local behaviour of a particular tick species with regard to the host community might increase the risk of pathogen transmission to humans. In the same way, the abundance of *Amblyomma* larvae in the studied small mammal communities was almost 3 times higher during the wet season. These data suggest that the risk of human parasitism by *Amblyomma* larvae (Dantas-Torres *et al*. 2010) might be higher during the wet season, when the population of immature ticks reaches its pinnacle. The finding of multiple tick stages co-feeding on small mammals will also stimulate discussions about co-feeding transmission and its importance to the circulation of pathogens in disturbed Atlantic Forest fragments, considering the known importance of this mode of transmission for the perpetuation of Lyme disease spirochetes in nature (Randolph and Gern, 2003).

Ticks identified in this study are widespread in Brazil and have previously been found infected by spotted fever group rickettsiae (Labruna, 2009). However, their role in pathogen transmission to humans is uncertain, mainly because the human parasitism by some of them is rare (e.g., *Ha. leporispalustris*) or unrecorded (e.g., *Ix. loricatus*) (Guglielmone *et al*. 2006). For instance, *Ix. loricatus* has been shown to maintain *R. bellii*, a bacterium of unknown pathogenicity, through transstadial and transovarial passages (Horta *et al*. 2006). Again, a recent study supported the role of *Ha. leporispalustris* in the enzootic maintenance of *R. rickettsii* (Freitas *et al*. 2009). Our findings demonstrate that rodents and marsupials inhabiting Atlantic Forest fragments in North-eastern Brazil are highly exposed to *Rickettsia* spp. While our results do not allow us to confirm whether the small mammals were actually infected by *R. rickettsii*, they suggest the circulation of spotted fever group rickettsiae in the studied area.

Our results suggest that people conducting activities in these areas are exposed to tick bites and, potentially, to tick-borne pathogens. Further research is needed to assess whether fragmented forests within an urban matrix have greater or lower tick infestation levels than more intact forests in a similar biogeographical area. Indeed, it is important to better understand whether small mammals living in Atlantic Forest patches in Brazil are involved in the maintenance of the enzootic cycle of tick-borne pathogens that could represent a threat to small mammals themselves or even to humans.

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REFERENCES

Abel, I. S., Marzagão, G., Yoshinari, N. H. and Schumaker, T. T. (2000). *Borrelia*-like spirochetes recovered from ticks and small mammals collected in the Atlantic Forest Reserve, Cotia county, State of São Paulo, Brazil. *Memórias do Instituto Oswaldo Cruz* **95**, 621–624.

Aragão, H. B. and Fonseca, F. (1961). Notas de ixodologia. VIII. Lista e chave para os representantes da fauna ixodológica brasileira. *Memórias do Instituto Oswaldo Cruz* **59**, 115–129.

Ayres, M., Ayres, M., Jr. and Ayres, D. L. (2007). *BioEstat 5.0: Aplicações Estatísticas nas áreas das Ciências Biológicas e Médicas*. MCT, IDSM, CNPq, Belém.

https://doi.org/10.1017/S0031182011001740 Published online by Cambridge University Press

Barros-Battesti, D. M., Arzua, M., Linardi, P. M., Botelho, J. R. and Sbalqueiro, I. J. (1998). Interrelationship between ectoparasites and wild rodents from Tijucas do Sul, state of Paraná, Brazil. *Memórias do Instituto Oswaldo Cruz* **93**, 719–725.

Barros-Battesti, D. M., Yoshinari, N. H., Bonoldi, V. L. and Castro Gomes, A. (2000). Parasitism by *Ixodes didelphidis* and *I. loricatus* (Acari: Ixodidae) on small wild mammals from an Atlantic Forest in the State of Sao Paulo, Brazil. *Journal of Medical Entomology* **37**, 820–827.

Barros-Battesti, D. M., Onofrio, V. C., Labruna, M. B., Martins, J. R. and Guglielmone, A. A. (2005). Redescription of *Amblyomma fuscum* Neumann, 1907 (Acari: Ixodidae), a rare South America tick confirmed in Brazil. *Systematic Parasitology* **61**, 85–92.

Bittencourt, E. B. and Rocha, C. F. (2003). Host-ectoparasite specificity in a small mammal community in an area of Atlantic Rain Forest (Ilha Grande, State of Rio de Janeiro), Southeastern Brazil. *Memórias do Instituto Oswaldo Cruz* **98**, 793–798.

Bonvicino, C. R., Oliveira, J. A. and D'Andrea, P. S. (2008). *Guia dos Roedores do Brasil comCchaves para Gêneros Baseadas em Caracteres Externos*. Centro Pan-Americano de Febre Aftosa, Rio de Janeiro, Brasil.

Bown, K. J., Lambin, X., Telford, G. R., Ogden, N. H., Telfer, S., Woldehiwet, Z. and Birtles, R. J. (2008). Relative importance of *Ixodes ricinus* and *Ixodes trianguliceps* as vectors for *Anaplasma phagocytophilum* and *Babesia microti* in field vole (*Microtus agrestis*) populations. *Applied and Environmental Microbiology* **74**, 7118–7125.

Bradley, C. and Altizer, S. (2006). Urbanization and the ecology of wildlife diseases. *Trends in Ecology and Evolution* **22**, 95–102.

Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al*. revisited. *The Journal of Parasitology* **83**, 575–583.

Dantas-Torres, F. (2008). Towards the standardization of the abbreviations of genus names of ticks (Acari: Parasitiformes: Ixodida). *Veterinary Parasitology* **154**, 94–97.

Dantas-Torres, F., Ferreira, D. R., Melo, L. M., Lima, P. A., Siqueira, D. B., Rameh-de-Albuquerque, L. C., de Melo, A. V. and Ramos, J. A. (2010). Ticks on captive and free-living wild animals in northeastern Brazil. *Experimental and Applied Acarology* **50**, 181–189.

Dantas-Torres, F., Oliveira-Filho, E. F., Soares, F. A., Souza, B. O., Valença, R. B. and Sá, F. B. (2008). Ticks infesting amphibians and reptiles in Pernambuco, Northeastern Brazil. *Revista Brasileira de Parasitologia Veterinária* **17**, 218–221.

Dantas-Torres, F., Onofrio, V. C. and Barros-Battesti, D. M. (2009). The ticks (Acari: Ixodida: Argasidae, Ixodidae) of Brazil. *Systematic and Applied Acarology* **14**, 30–46.

Emmons, L. H. and Feer, F. (1997). *Neotropical Rainforest Mammals: a Field Guide*. University of Chicago Press, Chicago, IL, USA.

Freitas, L. H., Faccini, J. L. and Labruna, M. B. (2009). Experimental infection of the rabbit tick, *Haemaphysalis leporispalustris*, with the bacterium *Rickettsia rickettsii*, and comparative biology of infected and uninfected tick lineages. *Experimental and Applied Acarology* **47**, 321–345.

Galindo-Leal, C. and Câmara, I. G. (2003). *The Atlantic Forest of South America: Biodiversity Status, Threats, and Outlook*. Island Press, Washington, DC, USA.

Guglielmone, A. A., Beati, L., Barros-Battesti, D. M., Labruna, M. B., Nava, S., Venzal, J. M., Mangold, A. J., Szabó, M. P., Martins, J. R., González-Acuña, D. and Estrada-Peña, A. (2006). Ticks (Ixodidae) on humans in South America. *Experimental and Applied Acarology* **40**, 83–100.

Hammer, Ø., Harper, D. A. T. and Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* **4**, 1–9.

Horta, M. C., Pinter, A., Schumaker, T. T. and Labruna, M. B. (2006). Natural infection, transovarial transmission, and transstadial survival of *Rickettsia bellii* in the tick *Ixodes loricatus* (Acari: Ixodidae) from Brazil. *Annals of the New York Academy of Science* **1078**, 285–290.

Horta, M. C., Labruna, M. B., Pinter, A., Linardi, P. M. and Schumaker, T. T. (2007). *Rickettsia* infection in five areas of the state of São Paulo, Brazil. *Memórias do Instituto Oswaldo Cruz* **102**, 793–801.

Horta, M. C., Moraes-Filho, J., Casagrande, R. A., Saito, T. B., Rosa, S. C., Ogrzewalska, M., Matushima, E. R. and Labruna, M. B. (2009). Experimental infection of opossums *Didelphis aurita* by *Rickettsia rickettsii* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense*. *Vector-Borne and Zoonotic Diseases* **9**, 109–118.

Horta, M. C., Sabatini, G. S., Moraes-Filho, J., Ogrzewalska, M., Canal, R. B., Pacheco, R. C., Martins, T. F., Matushima, E. R. and Labruna, M. B. (2010). Experimental infection of the opossum *Didelphis aurita* by *Rickettsia felis, Rickettsia bellii*, and *Rickettsia parkeri* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense* and *Amblyomma dubitatum*. *Vector-Borne Zoonotic Diseases* **10**, 959–967.

Labruna, M. B. (2009). Ecology of *Rickettsia* in South America. *Annals of the New York Academy of Science* **1166**, 156–166.

Marques, S., Col, R. D., Matos, M. O., Jr., Gonçalves, E. F. B., Pinter, A. and Labruna, M. B. (2006). Parasitismo de *Amblyomma fuscum* (Acari: Ixodidae) em humanos. *Ciência Rural* **36**, 1328–1330.

Martins, T. F., Dantas-Torres, F., Nieri-Bastos, F. A., Marcili, A., de Siqueira, D. B., Aléssio, F. M., Mauffrey, J.-F., DA Silva, J. C. R. and Labruna, M. B. (2009). Host records for the immature stages of *Amblyomma fuscum* (Acari: Ixodidae), a South American tick species. *Entomological News* **120**, 370–374.

Martins, T. F., Onofrio, V. C., Barros-Battesti, D. M. and Labruna, M. B. (2010). Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. *Ticks and Tick-borne Diseases* **1**, 75–99.

Onofrio, V. C., Barros-Battesti, D. M., Labruna, M. B. and Faccini, J. L. (2009). Diagnoses of and illustrated key to the species of *Ixodes* Latreille, 1795 (Acari: Ixodidae) from Brazil. *Systematic Parasitology* **72**, 143–157.

Randolph, S. and Gern, L. (2003). Co-feeding transmission and its contribution to the perpetuation of the Lyme disease spirochete *Borrelia afzelii*. *Emerging Infectious Diseases* **9**, 893–894.

Ranta, P., Blom, T. O. M., Niemela, J., Joensuu, E. and Siitonem, M. (1998). The fragmented Atlantic Rain Forest of Brazil: size, shape, and distribution of forest fragments. *Biodiversity and Conservation* **7**, 385–403.

Redford, K. H. and Eisenberg, J. F. (1992). *Mammals of the Neotropics. The Southern Cone: Chile, Argentina, Uruguay, Paraguay*. University of Chicago Press, Chicago, IL, USA.

Reis, N. R., Peracchi, A. L., Pedro, W. A. and Lima, I. P. (2006). *Mamíferos do Brasil*. Universidade Estadual de Londrina, Londrina., Brazil.

Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J. and Hirota, M. M. (2009). The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. *Biological Conservation* **142**, 1141–1153.

Silveira, I., Pacheco, R. C., Szabó, M. P., Ramos, H. G. and Labruna, M. B. (2007). *Rickettsia parkeri* in Brazil. *Emerging Infectious Diseases* **13**, 1111–1113.

Spolidorio, M. G., Labruna, M. B., Mantovani, E., Brandao, P. E., Richtzenhain, L. J. and Yoshinari, N. H. (2010). Novel spotted fever group rickettsiosis, Brazil. *Emerging Infectious Diseases* **16**, 521–523.

Streilen, K. E. (1982). The ecology of small mammals in the semiarid Brazilian Caatinga. III. Reproductive biology and population ecology. *Annals of Carnegie Museum* **51**, 251–269.

Zhan, L., Cao, W. C., Chu, C. Y., Jiang, B. G., Zhang, F., Liu, W., Dumler, J. S., Wu, X. M., Zuo, S. Q., Zhang, P. H., Huang, H. N., Zhao, Q. M., Jia, N., Yang, H., Richardus, J. H. and Habbema, J. D. (2009). Tick-borne agents in rodents, China, 2004–2006. *Emerging Infectious Diseases* **15**, 1904–1908.