Blood parasites, total plasma protein and packed cell volume of small wild mammals trapped in three mountain ranges of the Atlantic Forest in Southeastern Brazil

Silva, MAML.[a]*, Ronconi, A.*[a], *Cordeiro, N.*[b], *Bossi, DEP.*[b], *Bergallo, HG.*[c], *Costa, MCC.*[a], *Balieiro, JCC.*[a] *and Varzim, FLSB.*[a]

[a]Faculdade de Medicina Veterinária do Centro Universitário da Fundação de Ensino Octávio Bastos, Av. Dr. Octávio Bastos, s/n, Campus 2, CEP 13874-159, São João da Boa Vista, SP, Brazil

[b]Departamento de Parasitologia - Instituto de Biologia, Universidade Estadual de Campinas, CP 6109, CEP 13083-970, Campinas, SP, Brazil

[c]Departamento de Ecologia, Instituto de Biologia, Universidade do Estado do Rio de Janeiro, Rua São Francisco Xavier, 524, CEP 20559-900, Rio de Janeiro, RJ, Brazil

*e-mail: psxerife@uol.com.br, lab1hovet@feob.br

Abstract

A study of blood parasites in small wild non-flying mammals was undertaken in three areas of the Atlantic Forest in Southeastern Brazil: Serra de Itatiaia, RJ, Serra da Bocaina, SP and Serra da Fartura, SP, from June 1999 to May 2001. A total of 450 animals (15 species) were captured in traps and it was observed in 15.5% of the blood smears the presence of *Haemobartonella* sp. and *Babesia* sp. in red blood cells. There was no statistically significant difference between parasited and non-parasited specimens regarding total plasma protein, packed cell volume and body weight, which strongly suggests that these specimens might be parasite reservoirs.

Keywords: blood parasites, wild mammals, Atlantic Forest, plasma protein, packed cell volume.

Hemoparasitismo, proteína total plasmática e volume globular em pequenos mamíferos silvestres em três áreas serranas da Mata Atlântica no Sudeste do Brasil

Resumo

A presença de hemoparasitos em pequenos mamíferos silvestres não voadores foi pesquisada em animais de três áreas serranas do Sudeste brasileiro, pertencentes ao complexo da Serra do Mar e da Serra da Mantiqueira, nos Estados de São Paulo, Rio de Janeiro e Minas Gerais. Foram capturados 420 animais de 15 espécies, durante dois anos, dos quais, 15,5% apresentaram *Haemobartonella* sp. e *Babesia* sp., observadas em lâmina de esfregaço sangüíneo no interior de suas hemácias. Os níveis de proteína total plasmática e de volume globular não apresentaram diferença estatisticamente significativa entre os indivíduos parasitados e não parasitados, assim como o peso corporal, o que sugere fortemente que esses animais possam ser reservatórios desses parasitos.

Palavras-chave: hemoparasitos, mamíferos silvestres, Mata Atlântica, proteínas plasmáticas, volume globular.

1. Introduction

Domestic animals, both of economic importance and pet animals, as well as humans, are susceptible to diseases caused by blood parasites. Several blood cells parasites, such as *Babesia* sp, *Hemobartonella* sp and others, that can cause death of domestic and wild animals and, rarely, of humans, are transmitted by biological vectors such as ticks or fleas, which are ectoparasites of a wide variety of animals, and are also reservoirs of blood parasites (Daszak, Cunningham and Hyatt, 2000 and 2001; Bossi et al., 2002; Moraes, Bossi and Linhares, 2003;

Rios et al., 2003; Quintão-Silva and Ribeiro, 2003; Barreira et al., 2004).

There are several studies on the clinical, pathologic and hematologic alterations caused by hemoparasites in domestic animals, its diagnosis and transmission (Madruga et al., 2002; Mendonça et al., 2003). However, few studies have been done on hosts and reservoirs in Brazilian native species (Dall´olio et al., 2001; Gazeta et al., 2004).

This work focused on the presence of blood parasites in blood smears of small wild mammals from tropical

Braz. J. Biol., 67(3): 531-535, 2007

531

mountain ranges, regarding the packed cell volume (PCV), the total plasma protein (TPP) and the body weight, determining a possible epidemiological link of important blood parasitoses in domestic and wild animals and men.

2. Materials and Methods

Three different areas were chosen in different mountain ranges of Southeastern Brazil: Area I- Itatiaia National Park (Itatiaia, RJ – 22° 26.187' S and 44° 37.511' W) between São Paulo and Rio de Janeiro states; Area II- Serra da Bocaina National Park (São José do Barreiro, SP – 22° 44.125' S and 44° 37.007' W) between Rio de Janeiro and Minas Gerais states and Area III- Serra da Fartura (São João da Boa Vista, SP – 21° 53.621' S and 46° 45.188' W) between São Paulo and Minas Gerais states. Area I belongs to the Sea Mountain Range (Serra do Mar) and Areas II and III, to Mantiqueira Mountain Range (Serra da Mantiqueira). They all belong to the morphoclimatic domain of the mountainous regions of the humid tropics (Ab'Saber, 1970).

Small mammal trapping was conducted in an irregular grid covering 5 ha, with 14 transects varying from 200 to 240 m, spaced 20-m from each other and 1000 to 1500 m above sea level. Sherman® live traps (100) baited with banana or manioc with peanut butter were set on the forest floor for four consecutive nights. Each trap site was recorded, and trapped animals were taken to a nearby station laboratory. Handling of small mammals was similar to the procedure used by Gettinger (1992). The animals were placed in plastic bags with cotton balls soaked with ethyl ether until they became unconscious, after which they were marked using an ear-code (Monteiro-Filho, 1987), weighed and sexed. The blood was collected by an incision in the tail made with a lancet and the animals were subsequently released at the same spot as the trap site. A voucher collection of skins and skulls of males and females of all collected host species were deposited at the Department of Ecology, State University of Rio de Janeiro. Mammal identification was based on morphological characters and cariotype, using material from the sacrificed specimens. Each trapping was carried out for four consecutive nights, totaling eight collections in each location, and the total trapping effort was 3200 traps/night/site.

The hemoparasite infection rates were determined by microscopic examination of Giemsa-stained blood smears. In this work, the blood parasite species were not determined.

Packed cell volumes (PCV) were determined when the collected amount of blood was enough to fill a heparinized microhematocrit capillary tube. In those cases after the reading, the capillary was broken just above the white globules and the plasma transferred to the refractometer and total plasma protein (TPP) was determined by refractometry, as recommended by Feldman et al. (2000).

Data on species, sex, weight, blood parasite frequency and trap site were recorded and stored for each host.

They were analyzed by the GLM procedure of the SAS (1987) software.

3. Results

A total of 374 rodents and 46 marsupials belonging to 15 species were collected. The rodents were represented by nine species: *Akodon montensis* (Thomas), *Akodon serrensis* (Thomas), *Delomys dorsalis* (Henzel), *Delomys sublineatus* (Thomas), *Nectomys squamypes* (Brants), *Oligorysomys nigripes* (Olfers), *Oxymycterus* sp (Watherhouse), *Thaptomys nigrita* (Lichtenstein), and *Trynomis dimediadus* (Günter). The six collected marsupial species were: *Didelphis aurita* (Wied-Neuwied), *Gracilynanus* sp (Burmeister), *Marmosops incanus* (Lund), *Monodelphis scallops* (Thomas), *Philander frenata* (Olfers), *Thylamis velutinus* (Wagner).

The frequency of each collected species whose red blood cells had parasites are presented in Table 1. A total of 65 specimens (15%) belonging to 9 species (6 rodents and 3 marsupials) were infected with *Hemobartonella* sp and/or *Babesia* sp. Only one specimen of *Akodon montensis* showed *Theileria* sp in its lymphocytes.

The prevalence of parasitism was greater in rodents and for *Hemobartonella* sp. The five most abundantly collected rodent species, were: *O. nigripes* (23.5%) followed by *D. sublineatus* (19%), *A. montensis* (18%), *A. serrensis* (11.8%) and *D. dorsalis* (8%). For the marsupials, the three most abundantly collected species were *M. incanus* (28.6%), *P. frenata* (12.5%) and *T. velutinus* (10%). However *Babesia* sp was found only in the rodents *A. montensis, D. sublineatus* and *N. squamypes.*

The distribution of parasitism in the three studied areas and the body weight averages is presented in Table 2. The greater prevalence occurred in Serra da Bocaina (Area II), with 47.7% of hemoparasitized specimens. There was no significant difference in body weight ($p < 0.01$) among the studied species and hosts.

Packed cell volume (PCV) and the total plasma protein (TPP) for the specimens infected and non-infected by blood parasites are presented in Table 3. There was no significant difference ($p < 0.01$) among parasitized and non-parasitized specimens and among the study areas. Only 102 specimens from six species provided enough blood to fill the microhematocrit capillary tube in all specimens, and were included in the study: *A. montensis, A. serrensis, D. dorsalis, D. sublineatus, O. nigripes* and *T. dimediadus.*

4. Discussion

Direct microscopic identification of parasites in stained blood smears is confirmatory of their presence, but it is usually difficult to find the organisms in the parasitized animals. Therefore, despite the high specificity, microscopic examinations of blood smears are of low sensitivity for the detection of parasites in host animals. Serological methods, such as complement fixation tests and the indirect fluorescent antibody test are

532

Braz. J. Biol., 67(3): 531-535, 2007

Table 1. Species, total number of slides, number of positive slides, percentage of each blood parasite regarding its host and percentage of males and females.

Specie	Total number of slides	Number of positive Slides	Blood parasites (%)			Males (%)	Females (%)
			Haemobartonella sp	*Babesia sp*	*Theileria sp*		
Akodon montensis	172	31	90.6	6.3	3.1	38.7	33.3
Akodon serrensis	76	09	100	-	-	66.7	33.3
Delomys dorsalis	25	02	100	-	-	100	-
Delomys sublineatus	42	08	75	25	-	87.5	12.5
Nectomys squamypes	03	02	66.7	33.3	-	-	100
Oligorysomys nigripes	34	08	100	-	-	75	25
Oxymiterus sp	04	-	-	-	-	50	50
Thaptomys nigrita	11	-	-	-	-	63.6	36.4
Trynomys dimediatus	07	-	-	-	-	71.4	28.6
Gracilynanus sp	06	-	-	-	-	33.3	66.7
Marmosoptes incanus	07	02	100	-	-	100	-
Monodelphis scalops	03	-	-	-	-	100	-
Phylander frenata	16	02	100	-	-	100	-
Thylamys velutinatus	10	01	-	-	-	100	-
Didelphys aurita	04	-	-	-	-	50	50
Total	420	65	-	-	-	-	-

Table 2. Species, number of positive slides, number of hosts in each mountain range and weight (g) averages ± standard deviation (s).

Specie	Positive slides	Area I Itatiaia	Area II Bocaina	Area III Fartura	Weight g ± s	Host Weight g ± s
Akodon montensis	31	-	19	12	0.367 ± 0.078	0.350 ± 0.057
Akodon serrensis	9	4	5	-	0.370 ± 0.129	0.373 ± 0.012
Delomys dorsalis	2	-	2	-	0.503 ± 0.088	0.505 ± 0.008
Delomys sublineatus	8	-	5	3	0.550 ± 0.079	0.560 ± 0.097
Nectomys squamypes	2	2	-	-	0.396[a]	0.394[a]
Oligorysomys nigripes	8	-	-	8	0.390 ± 0.107	0.385 ± 0.089
Oxymiterus sp	-	-	-	-	0.280[a]	-
Thaptomys nigrita	-	-	-	-	0.430 ± 0.098	-
Trynomys dimediadus	-	-	-	-	0.384 ± 0.089	-
Gracilyanus sp	-	-	-	-	0.436 ± 0.102	-
Marmosoptes incanus	2	2	-	-	0.457 ± 0.077	0.445 ± 0.103
Monodelphis scalops	-	-	-	-	0.423[a]	-
Phylander frenata	2	-	-	2	0.518 ± 0.075	0.521 ± 0.072
Thylamys velutinatus	1	-	-	1	0.397 ± 0.082	0.387[a]
Didelphis aurita	-	-	-	-	0.355[a]	-
Total	65	8	31	26	-	-

[a]Standard deviation was not calculated due to the small number of captured animals

commonly used for detecting hemoparasite infections. However these tests can cause errors, because they are generally restricted to antibody detection, so a cross reactivity can occur (Baldani et al, 2004a). The protector immunity is not related with antibody levels, because the antibodies are produced against parasite-antigen and host-antigen, resulting in inflammatory reaction (Bittar et al., 2004). The enzyme-linked immunosorbent assay (ELISA) and several other techniques including in vitro cultures, DNA probes, polymerase chain reaction

Braz. J. Biol., 67(3): 531-535, 2007

533

Table 3. Species, number of each analyzed specimen (n), Packed Cell Volume (PCV - %) averages ± sd (s) and Total Plasma Protein (TPP - g.dL⁻¹) averages ± sd (s) in each area and host.

Specie	n	Area I-Itatiaia		Area II-Bocaina		Area III-Fartura		Host	
		PCV	TPP	PCV	TPP	PCV	TPP	PCV	TPP
Akodon montensis	47	47.50 ± 6.8	6.30 ± 0.9	43.70 ± 5.5	7.00 ± 1.5	47.70 ± 3.76	6.90 ± 1.6	45.8 ± 5.2	7.2 ± 1.2
Akodon serrensis	28	42.50 ± 6.5	7.00 ± 0.8	55.00 ± 5.8	6.90 ± 0.8	-	-	48.0 ± 4.3	6.0 ± 0.9
Delomys dorsalis	07	45.70 ± 2.7	6.40 ± 1.1	46.50 ± 2.9	7.50 ± 0.9	-	-	46.3 [a]	6.8 [a]
Delomys sublineatus	13	42.70 ± 4.5	7.70 ± 0.7	-	-	48.50 ± 4.4	7.90 ± 0.6	44.3 ± 4.9	6.8 [a]
Oligorisomys sp.	03	-	-	-	-	47.50 [a]	7.10	50.0 [a]	7.0 [a]
Trinomys dimediatus	04	-	-	56.00[a]	6.50	-	-	-	-
Total	102								

[a]Standard deviation was not calculated due to the small number of captured animals.

(PCR), nested polymerase chain reaction and western blot analysis, have been used for the diagnosis for blood parasites. However, except for serology, these methods are laborious, expensive and time-consuming (Baldani et al., 2004b). The presence of *Haemobartonella* sp and *Babesia* sp in the erythrocyte of rodents and marsupials from three mountain ranges in the Atlantic Forest in southeastern Brazil, showed that they are potentially hosts, so they can lead to an infection, transmitted by fleas and ticks, to domestic and wild animals, and men (Burato et al., 2004).

This work showed that the majority of hosts in all three areas had *Haemobartonella* sp (Table 1). A study of the associations between small mammals and fleas was undertaken in the same three areas where the present work was carried out. A total of 185 specimens belonging to 17 species (11 rodents and 6 marsupials) were parasitized by fleas. The rodent *A. montensis* was the host with the largest number of flea species collected (Moraes et al., 2003).

Haemobartonella muris can be found in the erythrocytes of mice, rats and hamsters. The clinical manifestations of this disease were observed when the animals presented immune suppression (Pereira, 2002).

Haemobartonella and *Eperythrozoon* species infecting dogs and cats have been reclassified as mycoplasmal parasites and are now known as *Mycoplasma haemofelis* (Ohio or large form of *H. felis*), *M. haemominutum* (California or small form of *H. felis*), and *H. haemocanis* (*H. canis*) (Souza and Almosny, 2002; Messick, 2003).

Babesiosis is a tick-borne disease of domestic and wild animals and, rarely, of man (Madruga et al., 2002; Mendonça et al., 2003; Rios, 2003). From the blood smear examinations, 4 animals infected with *Babesia* sp (3 *Akodon serrensis* and 1 *Delomys sublineatus*, collected in Serra da Bocaina- Area II) were found, and in those specimens only nymphs of ixodid ticks were collected. In the Atlantic Rainforest (part of it) where our studies were undertaken, the invasion of domestic animals (cat-

tle and equinus) is normal. Ixodid ticks can be infecting both hosts (Dall'Olio et al, 2001).

The occurrence of babesiosis from blood smear identification, was studied in 44 small rodents of six species captured in Silva Jardim and Nova Iguaçu municipalities, in the state of Rio de Janeiro, Brazil. The prevalence of infection was 27,3% in two species: *Oligoryzomys nigripes* and *Rattus norvergicus*. The other captured species were: *Nectomys squamipes*, *Oryzomys angoya*, *Holochilus brasiliensis* and *Akodon cursor* (Gazeta et al., 2004).

No information on the species studied in this work has been found in the literature, regarding the patterns of packed cell volume and total plasma protein. Nevertheless, some papers have proposed the standardization of those values for rodents used as lab animals. The packed cell volume (PCV) and total plasma protein (TTP) showed similar values to those of lab animals, such as hamsters, rats and mice (Moore, 2000ab).

Determination of packed cell volume is important to determine whether the captured specimens with blood parasites had decreased PCV, indicating anemia. High total plasma protein can indicate dehydration, and low, malnutrition, liver or kidney insufficiency. In this study, the specimens that had blood parasites were not anemic and neither had protein alterations, suggesting that the specimens are reservoirs of those parasites, which could be transmitted by ectoparasites such as fleas and ticks, to other mammals, including humans.

References

AB'SABER, AN., 1970. Províncias geológicas e domínios morfoclimáticos no Brasil. *Boletim do CEPEGE*, vol. 3, no. 1, p. 85-123.

534

Braz. J. Biol., 67(3): 531-535, 2007

BALDANI[a], CD., FERRAZ, ART., NETO, AQ. and MACHADO, RZ., 2004. Estudo comparativo de técnicas diretas no diagnóstico de *Babesia equi* em eqüinos naturalmente infectados e submetidos a treinamento físico de intensidade progressiva. *Rev Bras Parasitol Vet*, vol. 13, suplemento no. 1, p. 231.

BALDANI[b], CD., MACHADO, RZ., BOTTEON, PTL., TAKAKURA, FS. and MASSARD, CL., 2004. An enzyme-linked immunosorbent assay for the detection of IgG antibodies against *Babesia equi* in horses. *Cienc Rural*, vol. 34, no. 5, p. 1525-1529.

BARREIRA, JD., DORIA ROSSI, MI., SILVA, GVO., PIRES, FA. and MASSARD, CL., 2004. Avaliação clinico-parasitológica de *Meriones unguilatus* frente à infecção experimental com amostras modificadas de *Babesia bovis* e *B.bigemina*. *Rev Bras Parasitol Vet*, vol. 13, suplemento no. 1, p. 230.

BITTAR, JFF., RIBEIRO, MFB., MARCIANO, APV., SALCEDO, JHP., MARTINS-FILHO, OA. 2004. Perfil fenotípico de linfócitos periféricos de bovinos de raças européias. *Arq Bras Med Vet Zootec*, vol. 56, p. 107-110.

BOSSI, DEP., LINHARES, AX. and BERGALLO, HG., 2002. Parasitic arthropods of some wild rodents from Juréia-Itatins Ecological Station, state of São Paulo, Brazil. *Mem Inst Oswaldo Cruz*, vol. 97, no. 7, p. 959-963.

BURATO, AC., BELO, NO., SILVEIRA, JAG., MOREIRA, SM., BASTOS, CV. and PASSOS, LMF., 2004. Hemobartonelose felina: aspectos epidemiológicos, clínicos e hematológicos. *Rev Bras Parasitol Vet*, vol. 13, suplemento no. 1, p. 241.

DALL´OLIO, AJ., RONCONI, A., BOSSI, DEP. and SILVA, MAML. September, 2001. Ixodid ticks vectors of babesiosis to rodents of brazilian rain Forest. *Third International Congress of Vector Ecology*. Barcelona, Spain, 16/21 de Setembro, p. 18.

DASZAK, P.; CUNNINGHAM, AA. and HYATT, AD., 2000. Emerging infectious diseases of wildlife-threats to biodiversity and human health. *Science*, vol. 287, no. 5452, p. 443-449.

-, 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop*, vol. 78, no. 2, p. 103-116.

FELDMAN, BF., ZINCKL, JG. and JAIN, NC., 2000. *Schalm's Veterinary Hematology*. 5th ed., Lippincott Williams & Wilkins, Philadelphia. p. 1111-1119.

GAZETA, GS., CARVALHO, RW., AVELAR, RF., AMORIM, M. and ABOUD-DUTRA, AE., 2004. Ocorrência de *Babesia* sp em pequenos roedores no Brasil. *Arq Bras Med Vet Zootecn*, vol. 56, no. 6, p. 741-744.

GETTINGER, D., 1992. Host specificit of Laelaps (Acari: Laelapidae) in Central Brazil. *J Med Entomol*, vol. 29, no. 1, p. 71-77.

MADRUGA, CR., LEAL, CRB. and FERREIRA, AMT. et al., 2002. Análise genética e antigênica de isolados de *Babesia bigemina* das cinco regiões fisiográficas do Brasil. *Pesq Vet Bras*, vol. 22, no. 4, p. 153-160.

MENDONÇA, CL., VIEIRA, D. and KOHAYAGAWA, A., 2003. Avaliação clínica e hematológica em bezerros Nelore infectados experimentalmente com isolados de *Babesia bigemina* das regiões Sudeste, Nordeste e Norte do Brasil. *Pesq Vet Bras*, vol. 23, no. 2, p. 52-60.

MESSICK, JB., 2003. New perspectives about *Hemotrophic mycoplasma* (formerly, *Haemobartonella* and *Eperythrozoon* species) infections in dogs and cats. *Vet Clin North Am Small Anim Practice*, vol. 33, no. 1, p. 1453-1465.

MONTEIRO FILHO, ELA., 1987. *Biologia Reprodutiva e Espaço Domiciliar de Didelphis albiventris em uma Área Perturbada na Região de Campinas, Estado de São Paulo*, MSc Thesis, State University of Campinas, Brazil.

MOORE, DM., 2000. Hematology of Mongolian gerbil e Syrian (golden) Hamster. In, Feldman, BF., Zinckl, JG. and Jain, NC. *Schalm's Veterinary Hematology*. 5th ed. Lippincott Williams & Wilkins, Philadelphia. p. 1111-1119.

-. Hematology of rat and mouse. In, FELDMAN, BF., ZINCKL, JG., and JAIN, NC. *Schalm's Veterinary Hematology*. 5th ed. Lippincott Williams & Wilkins, Philadelphia. p. 1210-1224.

MORAES, LB., BOSSI, DEP. and LINHARES, AX., 2003. Siphonaptera parasites of wild rodents and marsupials trapped in three mountain ranges of the Atlantic Forest in Southeastern Brazil. *Mem Inst Oswaldo Cruz*, vol. 98, no. 8, p. 1071-1076.

PEREIRA, AM., 2002. Principais doenças dos camundongos, ratos e hamsters. In, Andrade, A. et al. *Animais de Laboratório – criação e experimentação*. Editora Fiocruz, Rio de Janeiro. p. 105-137.

QUINTÃO-SILVA, MG. and RIBEIRO, MFB., 2003. Infection rate of *Babesia* spp sporokinetes in engorged *Boophilus microplus* from na area of enzootic stability in the state of Minas Gerais, Brazil. *Mem Inst Oswaldo Cruz*, vol. 98, no. 8, p. 999-1002.

RIOS, L., ALVAREZ, G. and BLAIR, S., 2003. Serological and parasitological study and report of first case human babesiosis in Colombia. *Rev Soc Bras Med Trop*, vol. 36, no. 4, p. 493-498.

SAS Institute, Inc., 1987. *S.A.S. Users Guide: Statistic*. Vesion 6th ed. Caru, n.c., E.U.A.

SOUZA, AM. and ALMOSNY, NRP., 2002. Hemobartonelose em pequenos animais domésticos e como zoonose. In, Almosny, NRP. *Hemoparasitoses em pequenos animais domésticos e como zoonose*. L.F.Livros, Rio de Janeiro. p. 89-109.