Borrelia sp. In Naturally Infected *Didelphis aurita* (Wied, 1826) (Marsupialia: Didelphidae)

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ABSTRACT

Fifty-six opossums (Didelphis aurita) were captured on the campus of Universidade Federal Rural do Rio de Janeiro, Seropédica county, Rio de Janeiro state, in order to investigate the occurrence of Borrelia sp among them in relation with the study of spirochaetemia and its ectoparasites. Blood tests were made through dark field and phase contrast microscopy, as well as the obtainment of blood smears. Smears were stained with Giemsa stain, which did not prove efficacy. There was no relation between results obtained through blood tests (13 opossum positive for Borrelia sp.), and this technique (two positive animals). Parasitaemia studies of 37 animals kept in captivity as well as of several recaptures in which animals once negative proved to be positive days later, showed that haemoscopical studies could be used as an effective diagnosis tool. Ectoparasites from nine animals were classified; with the occurrence of nymphal Amblyomma cajennense and adult Ctenocephalides sp..

Key words: *Borrelia* sp., *Didelphis aurita*, marsupial, *Amblyomma cajennense*, *Ctenocephalides* sp.

INTRODUCTION

Opossums are Metatheria mammals having a close relationship with many zoonotic pathogens. This phenomenon may have resulted from the intense urbanization and decrease of local populations of wild mammals. There is evidence that these animals are reservoirs for *Trypanosoma cruzi* (Herrera & Urdaneta-Morales, 1992), *Leishmania* spp. (Sherlock *et al.*, 1984), *Sarcocystis* spp., *Leptospira* sp. and the alfavírus (Scorza, 1992), as well as for other infectious agents. The adjustment of these marsupials to peri-residence makes possible the introduction, dispersion and maintenance of wild pathogens.

Microorganisms of the genus *Borrelia* Swellengrebel, 1907 are pathogenic bacteria, whose common reservoirs are ticks and rodents. However, such agents infect many other hosts, such as domestic and wild animals as well as humans (Barbour *et al.*, 1986). Borreliosis has been assuming relevance since two past decades, and has been registred in North America, Europe, Asia, Africa and South America (Bennett, 1995).

B. burgdorferi lato sensu is adapted to rodents (Gordus & Theis, 1993), deers (Gill *et al*., 1993), migratory birds (McLean *et al.*, 1993), bears (Kazmierczak *et al.*, 1988), foxes (Doby *et al*., 1991) and opossums (Bonoldi *et al.*, 1996) with the wild cycle involving ixodid ticks as vectors. In Brazil, little is known about borreliosis; however, there are descriptions about Lyme borreliosis in humans (Yoshinari *et al*., 1997), serologic studies of domestic animals (Fonseca *et al*., 1996; Ishikawa *et al*., 1997), as well as records of *Borrelia* sp. in opossums (Bonoldi *et al.*, 1996).

The objective of the present study was to investigate the occurrence of *Borrelia* sp. in naturally infected opossums associated with the study of external parasite.

MATERIALS AND METHODS

Opossums were captured on the campus of Universidade Federal Rural do Rio de Janeiro and the neighbouring municipality of Seropédica, Rio de Janeiro State, from January 1995 to May 1998. Drain-pipe placed vertically (14,5cm of wide x

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70cm long) were used as a trap. Animals were lured by ripe banana. Peri-residence areas where the occurrence of opossums was reported were chosen as the setting of traps. Captured animals were narcotized, investigated for the presence of external parasites, measured and marked with collars. Blood was collected with anticoagulant by means venopuntion; then the opossums were released close to the place of capture. Blood was used for fresh exam, and analysed by dark field and phase contrast microscope (LEITZ LABORLUX S). Blood smears were obtained which were stained with Giemsa stain diluted in sorensen buffer at pH 6.8.

External parasites collected were taken to the laboratory. Engorged nymphs of ticks infesting the opossums were kept in a biological chamber at 28°C and 85 ± 5% relative humidity in order to proceed with moult for identifying them. Later, they were fixed and kept in 70% ethanol and prepared. The arthropods were identified by the Aragão key (1936) for ticks and the Bicho & Ribeiro key (1998) for fleas. Recaptured animals were subjected to the same procedures.

Out of the captured opossums, 37 were kept in captivity in order to accompany the spirochaetemia phase by *Borrelia* sp.. These animals were released whenever no spirochaete were observed in the blood between the second and the third month in captivity. Each each week, animals studied were weighed and the blood collected for fresh exam.

RESULTS

Fifty-six opossums *Didelphis aurita* (Wied, 1826) (Marsupialia: Didelphidae) were captured, out of which 33 were males and 23 were females. Nine (16.07%) showed external parasites. Fleas of the *Ctenocephalides* genus were frequently found and in three opossums nymphs of the *Amblyomma cajennense* tick also were found (Table 1).

Out of 37 opossums kept in captivity, 13 animals were shown to be positive for *Borrelia* sp., although only nine were bearing borrelia at the moment of capture. Weekly observations showed that four animals acquired spirochaetemia on day 30 in captivity. Opossums that were positive at capture or after staying in captivity showed decrease of spirochaetemia, which remained at low level, or absent during the time in which the animals stayed in capitivity.

On fresh exams it was possible to observe spirochaetes in the blood of 13 (23.21%) opossums, eight of which were males and five were females (Table 1). Blood smear only showed *Borrelia* sp. in three (5.35%) animals (Figure 1), which were young, showed high spirochaetemia and did not bear external parasites when they were captured (Table 1).

Out of nine marsupials bearing external parasites, three (33.33%) showed spirochaetes in the blood (Table 1). Four animals were recaptured, of which one was negative at capture and a second one was positive. However, on recaptures on days 15 and 150 they were positive and negative, respectively. None of the captured animals bore clinical symptoms.

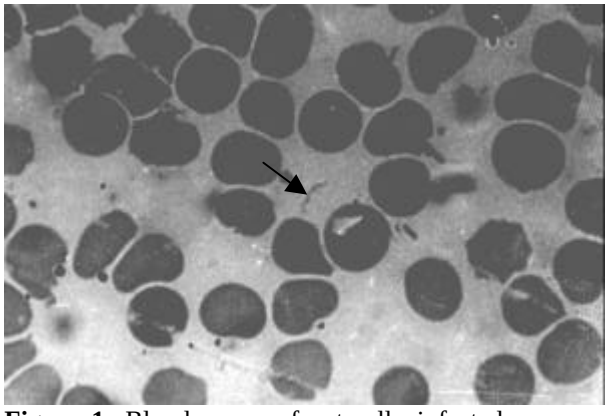

Figure 1- Blood smear of naturally infected opossum (*Didelphis aurita*) by *Borrelia* sp.. Giemsa stain, 1000 x.

Table 1 - Ocurrence of *Borrelia* sp. and external parasites in *Didelphis aurita* in Seropédica Municipality, Rio de Janeiro State

			Borrelia sp.		External parasites			
	Animal n°	Sex	BS	FE	Ticks	Fleas	Date of capture	Date of recapture
	1	M	+	+	–	–	Jan/95	–
	2	F	+	+	–	–	Jan/95	–
	3	F	–	+	–	–	Mar/95	–
	4	M	–	+	–	–	Mar/95	–
	5	F	–	+	–	–	Mar/95	–
	6	M	–	–	–	–	Apr/95	–
	7	F	–	+	–	–	Apr/95	–
	8	F	–	+	–	–	Apr/95	–
	9	M	–	–	–	–	May/95	–
	10	M	–	–	–	–	May/95	–
	11	M	–	+	–	–	Jun/95	Apr/97
	12	F	–	+	–	–	Jul/95	–
	13	F	–	–	–	–	Sep/95	–
	14	M	–	–	–	–	Mar/96	–
	15	M	–	–	–	–	May/96	–
	16	F	–	–	–	–	May/96	May/96, Oct/96
	17	M	–	–	–	–	Jun/96	–
	18	M	–	–	–	+	Jul/96	–
	19	F	–	–	–	–	Aug/96	–
	20	F	–	–	–	–	Aug/96	–
	21	F	–	–	–	–	Aug/96	–
	22	M	–	–	–	+	Aug/96	–
	23	M	–	–	–	–	Aug/96	–
	24	M	–	+	–	+	Sep/96	–
	25	F	–	–	+	+	Sep/96	Oct/96, Jun/97, Sep/97
	26	M	–	–	+	+	Sep/96	Nov/96
	27	M	–	–	+	+	Sep/96	–
	28	F	–	+	–	+	Oct/96	–
	29	F	–	–	–	–	Oct/96	–
	30	F	–	–	–	–	Oct/96	–
	31	M	–	–	–	+	Nov/96	–
	32	F	–	+	–	+	Dec/96	–
	33	F	–	–	–	–	Mar/97	–
	34	M	–	–	–	–	Mar/97	–
	35	M	–	–	–	–	Mar/97	–
	36	M	–	–	–	–	Mar/97	–
	37	F	–	–	–	–	Apr/97	–
	38	F	–	–	–	–	Apr/97	–
	39	F	–	–	–	–	Apr/97	–
	40	F	–	–	–	–	Apr/97	–
	41	F	–	–	–	–	Apr/97	–
	42	M	–	–	–	–	May/97	–
	43	M	–	–	–	–	May/97	–
	44	M	–	–	–	–	Jun/97	–
	45	M	–	–	–	–	Jun/97	–
	46	M	–	–	–	–	Jun/97	–
	47	M	–	–	–	–	Jun/97	–
	48	M	–	–	–	–	Jun/97	–
	49	M	–	–	–	–	Jun/97	–
	50	M	–	–	–	–	Jun/97	–
	51	M	–	–	–	–	Oct/97	–
	52	M	–	–	–	–	Sep/97	–
	53	M	–	–	–	–	Sep/97	–
	54	M	–	–	–	–	Jan/98	–
	55	F	–	–	–	–	Jan/98	–
	56	M	+	+	–	–	Apr/98	–
Total	56	33M; 23F	3	13	3	9	Jan/95 – Apr/98	7

M – male of *Didelphis aurita*; F – female of *D. aurita;* BS – blood smear; FE – fresh exam; F – fleas of the genus *Ctenocephalides*; T – nymph of the *Amblyomma cajennense* tick*;* (-)negative; (+)positive

DISCUSSION

The present results on external parasites affecting opossums are in concordance with the study of Krupp & Quillin (1964), who accounted that fleas were common in these marsupials, whilst ticks were observed only in one animal. Barros & Baggio (1992) also observed ticks in opossums captured in the state of Paraná, Brazil but (n= 8) parasitized by females and nymphs of the *Ixodes* and the *Amblyomma* genus.

The best-known vectors of *Borrelia* are the ticks belonging to the *Ixodes* genus; however, Schulze *et al*. (1984) isolated and identified spirochaetes from *A. americanum* attached to a characteristic lesion of Lyme borreliosis in two humans. This fact prompted him to regard this species as a secondary vector of this borreliosis. In the same manner as *A. americanum*, all the instars of *A. cajennense* infested humans. Although, this tick had preference for equines, it is know as a Brazilian ixodid species of the broader parasitism (Aragão, 1936; Flechtmann, 1985). Due to the little specificity and the association of the ixodids of this genus with borreliosis, this tick species may behave as a potencial vector of many pathogenic agents in the area studied.

The presence of fleas could also be significant in relation of the transmission of *Borrelia* sp.. Presently, this spirochaete was diagnosed in other bloodsucking arthropods, as well as in ticks. Magnarelli & Anderson (1988) reported *B. burgdorferi* on *Stomoxys calcitrans*, tabanids and mosquitoes; Butler & Denmark (1990) suggested the involvement of the argasid tick in the transmission of *Borrelia;* and Doby *et al.* (1991) reported the flea *Spilopsyllus cuniculi* infected by *B. burgdorferi* in foxes in northern of France.

The staining technique used for staining blood smear was not very effective, as there was no correspondence between the results in this method and the ones on the fresh exam. Giemsa staining was quoted by Pêssoa (1963) and Aberer & Duray (1991) as an usual technique in the diagnosis of spirochaetes. However, according to Aberer & Duray (1991) the best methods were the modified Whartin-Starry, Dieterle and Bosma-Steiner silver staining. According to these authors, the indigo staining are good for biological fluids, as the ease preparation and the chemical stability offer advantages for exams of biological fluids for the research of spirochetes.

There was no correspondence between the spirochaetemia observed in 13 animals on fresh exam and on blood smear exams, wherein only three animals were positive. The fact that nine opossums had been positive at the moment of capture and other four showed spirochetes only in captivity can be justified by the adjustment phenomenon as well as to stress. Gordus & Theis (1993) mentioned that in wild animals the spirochaetemia are at low level because borreliae have preference for interstitial areas. Epidemiological studies in Southeast region of Brazil by Fonseca *et al.* (1996) with cattle, showed a high positivity in animals at Itaguaí-Seropédica microregion as well as in the neighboring municipalities of the state of Rio de Janeiro. Serologic studies for canine borreliosis in the state of Rio de Janeiro showed a prevalence of 20% (Soares, 1998). This corroborated the report of *Borrelia* sp. affecting the captured marsupials used in this piece of research. Opossums *D. aurita* can be regarded as reservoirs of *Borrelia* sp..The study of spirochaetemia and the recaptures showed that haemoscopical analysis could be used as a diagnosis tool for *Borrelia* sp..

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RESUMO

Cinquenta e seis gambás (*Didelphis aurita*) foram capturados no *campus* da Universidade Federal Rural do Rio de Janeiro, Seropédica - Rio de Janeiro, com o intuito de se investigar a ocorrência de *Borrelia* sp. entre eles, associada ao estudo da espiroquetemia e de seus ectoparasitos. Amostras de sangue dos animais foram

examinadas à microscopia de campo escuro e contraste de fase, além deste ter se destinado à obtenção de esfregaços sanguíneos, corados pelo método de Giemsa. Não houve relação entre os resultados obtidos através dos exames diretos do sangue (13 gambás positivos para *Borrelia* sp.) e aqueles observados nos esfregaços sanguíneos (dois animais positivos), o que demonstra a baixa eficácia desta técnica. Estudos sobre a parasitemia de 37 animais mantidos em cativeiro, assim como as várias recapturas nas quais animais uma vez negativos mostraram-se positivos dias mais tarde, revelaram que os estudos hemoscópicos podem ser utilizados eficientemente como ferramentas de diagnóstico. Ectoparasitos colhidos de nove animais foram classificados. Houve ocorrência de ninfas *Amblyomma cajennense* e adultos *Ctenocephalides* sp..

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