Occurrence of *Sarcocystis* spp. in opossums (*Didelphis aurita* and *Didelphis albiventris*) in regions of the State of São Paulo, Brazil

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Recebido para publicação: 30/07/2007
Aprovado para publicação: 25/09/2008

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Abstract

The objective of this study was to determine the occurrence of *Sarcocystis* spp. in *Didelphis albiventris* and *D. aurita* in three regions of the state of São Paulo. Ninety-eight dead *Didelphis* were employed in this study, among which 66 were *D. aurita* and 32 *D. albiventris*. Twenty-eight living *D. aurita* and five D. *albiventris* were also analyzed. Flotation-centrifugation in sucrose solution was used in the isolation of *Sarcocystis* spp. of the small intestine and feces. *Sarcocystis* spp. was found in the small intestines of 9.1% of the *D. aurita* (6/66); in four of them, the feces were also positives. There was no statistically significant difference between males and females (P= 0,522), or among samples that came from different regions of the state of São Paulo (P= 0,627), regarding the occurrence of *Sarcocystis* spp. However, there was a significant difference of positive samples harvested from captive compared to free-ranging animals (P = 0.009), and between adults and offspring (P= 0,004). Adults were more affected by the parasite than the offspring, and only free-ranging animals were positives. From the samples collected from 28 living *D. aurita*, *Sarcocystis* spp. was found in 7.1% (2/28) of them. A total of 32 *D. albiventris* were studied, none of which had positive tests for *Sarcocystis* spp. in samples of intestine of feces, and five animals live were also negative. We conclude that the occurrence of *Sarcocystis* spp. in *D. aurita* and *D. albiventris* in these three regions of the state of São Paulo is low in the conditions assessed in this study.

Key words:
Opossum.
Sarcocystis.
São Paulo.
Brazil.

Introduction

There are four opossum species in South America: *Didelphis aurita*, *D. albiventris*, *D. marsupialis* and *D. imperfecta*.[1] In the state of São Paulo, Brazil, the only species found are *D. aurita* and *D. albiventris*.[2]

Sarcocystis is an Apicomplexan protozoan parasite that affects mammals, birds and reptiles. *Sarcocystis* species have two hosts in their life cycle, a definitive host, usually a predator, and an intermediate host (heteroxenic cycle).[3]

Didelphis virginiana (North American opossum) is considered a definitive host of three pathogenic *Sarcocystis* species: *S. falcatula*[4], *S. neurona*[5], and *S. speeri*[6]. The first report on the occurrence of *S. speeri* in Brazil was in 2000[7], when sporocysts were obtained from a *D. marsupialis* in São Paulo.

In 2001 *S. neurona* was isolated of *D. albiventris* from São Paulo for the first time from the intestines of Brazilian opossums[8]. The first report on the occurrence of *S. falcatula* in opossums from the state of São Paulo was in 2000, when sporocysts were obtained from opossums (*D. albiventris*) of Jaboticabal (SP)[9]. In 2001, *S. falcatula* was isolated again from Brazilian *D. albiventris* and was isolated for the first time from D. *marsupialis*.

Sarcocystis neurona, *S. falcatula* and *S. speeri* sporocysts are morphologically similar, but they can be distinguished by means of their pathogenicity and infectivity to immunodeficient mice and birds.

There are few studies involving *Sarcocystis* spp. in Brazil. Opossums are synanthropic wild animals, and frequently found in our environment. Therefore, the objective of the present study was to determine the occurrence of *Sarcocystis* spp. in *D. albiventris* and *D. aurita* in three regions of the state of São Paulo, Brazil. These regions were central area of the state (Grande São Paulo), country side (municipality of Sorocaba) and shore of the state (Baixada Santista).

Material and Method

From March 2005 to July 2006, 98 dead *Didelphis* were studied, among which 66 were *D. aurita* and 32 *D. albiventris*. Twenty eight living *D. aurita*, and five D. *albiventris* were also analyzed. These animals were supplied by Quinzinho de Barros Municipal Zoo (Sorocaba - SP), São Bernardo do Campo Zoo (São Bernardo – SP), Veterinary Section of Park and Green Areas Departament (São Paulo – SP), Tietê Ecological Park (São Paulo – SP) and the Orchid Breeding Facility in Santos (Santos – SP). Animals were classified according to their species, gender, and age, following the guidelines determined by Hunsaker[11]. They were also classified based on the season when they were captured, region of the state of São Paulo where they captures, and whether they were captive or free-ranging prior to the study.

Small intestines were scraped[3] and feces were collected from the cloaca in the *post-mortem* examination. Only fecal samples were collected from the animals that were alive. Flotation-centrifugation in sucrose solution[12] was the technique used in the isolation of sporocysts of *Sarcocystis* spp. from the small intestines and feces.

For this procedure, 11 ml of a sucrose solution (sp. gr. 1.203) were used, adding 1g of the homogenized contents of small intestines and cloaca feces, which were processed separately. These were sieved through gauze and transferred to a 15 ml tube, which was centrifuged at 400*g* for 10 min. After that, one drop collected from the surface of the centrifuged solution was transferred to a slide by means of an adapted platinum loop, covered with a 1-cm^2 coverslip and observed under a compound microscope (400χ). Morphologically, the length and width of sporocysts of *Sarcocysts* spp. was approximately 11 x 7 ìm. Internally, all sporocysts contained four sporozoites and residual bodies.

The test of two proportions (MINITAB® release 14.1 / MINITAB® Inc.) was used in the statistical analysis of the results. Level of significance was set at $P \leq 0.05$ with a 95% confidence interval.

Results

- *Occurrence of* Sarcocystis *spp. in* Didelphis aurita

From all studied samples *Sarcocystis* spp. were found in the small intestines of 9.1% *D. aurita* (6/66); in four of 66, *Sarcocystis* spp. was also observed in the fecal samples collected from the cloaca. Thirty six samples (54.5%) came from males and 30 (45.5%) from females. Positive samples were 6.7% (2/30) in females and 11.1% in males (4/36). As for age, 44 (66.7%) of the individuals were offspring in the marsupial pouch or still dependent on the mother / mother's milk to live. The other 22 (33.3%) individuals were classified in an only group formed by subadults, adults and old animals. There were

no positive samples in the offspring group. Adults showed 27.3% (6/22) positive samples. Factors associated with the presence of *Sarcocystis* spp. in *D. aurita* are shown in table 1.

There was no statistical difference between positive males and females with P = 0.522. No statistical differences were found between positive samples that came from different regions of the state of São Paulo either, with P = 0.627. However, there was a significant statistical difference in relation to the positive captive and free-ranging animals, with P = 0.009, only free-ranging animals were affected. Additionally, a difference was observed between positive adults (subadults, adults and old animals) and offspring, with P = 0.004 (Table 1). Adults were more affected by the parasite than the offspring. From the six animals infected, four presented sporocysts in their large intestines and/or feces, and two other did not present sporocysts either in the large intestines or in feces.

From the samples collected from 28 living *D. aurita* (two captive and 26 free-ranging animals), 39.3% were from females (11/28) and 60.7% (17/28) from males; 53.6% (15/28) were from offspring and 46.4% (13/28) from adults. As for the season, 14.3% (4/28) of the samples were collected in the spring, 3.6% (1/28) in the summer, 7.2% (2/28) in the autumn and 75.0% (21/28) in the winter. In relation to the origin, 60.7% (17/28) of the animals were from Grande São Paulo; 39.3% (11/28) were from country site and shore of the state. *Sarcocystis* spp. was found in 7.1% (2/28) of the feces samples collected from this group of animals. These positive samples came from adult, free ranging males of Grande São Paulo, one collected in the autumn and one in the winter.

- *Occurrence of* Sarcocystis *spp. in* Didelphis albiventris

A total of 32 D. albiventris were studied: 37.5% (12/32) were females and 62.5% (20/32) were males; 53.1% (17/32)

Table 1 - Factors associated with the occurrence of S*arcocystis* spp. of the fecal samples in *Didelphis aurita* of regions of the State of São Paulo and the statistical difference between these factors - São Paulo - 2007

FACTOR		No. (% animals)	No. (% positive)
Age	Offspring	44 (66.7%)	0 (0.0%)[A]
	Adult	22 (33.3%)	6 (27.3%)[A]
Sex	Male	36 (54.5%)	4 (11.1%)*
	Female	30 (45.5%)	2 (6.7%)*
Season	Winter	13 (19.7%)	1 (7.7%)
	Autumn	10 (15.2%)	1 (10.0%)
	Spring	41 (62.1%)	4 (9.8%)
	Summer	2 (3.0%)	0 (0.0%)
Origin	Grande São Paulo	38 (57.6%)	4 (10.5%)*
	Country site and shore	28 (42.4%)	2 (7.1%)*
Nature	Captive	12 (18.2%)	0 (0.0%)[B]
	Free-ranging	54 (81.8%)	6 (9.3%)[B]

[A,B] there was statistical difference; [A] P = 0.004; [B] P = 0.009; * there was no statistical difference

were offspring and 46.8% (15/32) were adults; 93.8% (30/32) came from the city of Sorocaba; 96.9% (31/32) were free-ranging animals; 3.1% (1/32) animals were captive and came from the city of São Paulo. From these samples, 90.6% (29/32) were collected in the spring, 6.2% (2/32) in the winter and 3.1% (1/32) in the autumn. All samples were negative for Sarcocystis spp. Feces were also collected from five live free-ranging D. albiventris offspring from the city of Sorocaba, from which 60.0% were females (3/5) and 40.0% (2/5) were males. All these feces samples were also negative.

Discussion and Conclusions

This is the first study of occurrence of *Sarcocystis* spp. in *Didelphis aurita* and *D. albiventris* from the state of São Paulo. This occurrence is an important information in the determination of adequate control and prevention strategies for different animals affected by the parasite, minimizing economic losses in horse breeding as well as the impact caused by the death of birds.

Infection rate in *D. aurita* from March 2005 to July 2006 in the state of São Paulo was 9.1% (6/66). In the United States, prevalence in *D. virginiana* was reported to be 18.0% (37/206) in Michigan[13] and 33.3% (24/72) in Mississippi[14]. The lower occurrence of *Sarcocystis* spp. infection observed in the present study can be due to the fact that 66.6% (40/66) of the individuals sampled were offspring in the marsupial pouch or still dependent on their mothers / mother's milk to survive. Older animals would have potentially more contacts in which transmission can have occurred, resulting in a higher risk of infection[13]. As for *D. albiventris*, no positive animals were observed, maybe due to the small number of individuals studied. Only 32 animals of this species were analyzed, and from these, 46.8% (15/32) were offspring. *Sarcocystis* prevalence can also have been underestimated due to the low sensitivity of the method used, mainly for mild infections.

The presence of sporocysts was investigated in relation to factors such as age, origin, and source. Other conditions, such as season, were also analyzed. *Sarcocystis* spp. was not detected in offspring because infection can occur later on the lives of these animals. The results observed here showed that adults were significantly more affected than offspring. It can be assumed that how older the animals, more chances for them to be exposed to infection, as it was also reported.[13,14,15,16]

No statistical differences were observed in the infection rates of males and females. This was also reported by others studies[13,14,16], although Elsheikha, Murphy and Mansfield[15] observed that females were more affected than males, this finding could not be explained by these authors.

As for season, it was not possible to submit the data collected to statistical analysis, because the study was not long enough and the number of samples was irregularly distributed throughout the seasons. Positive animals were found in all seasons, except in the summer. However, this cannot rule out the occurrence of positive animals during this season, once the number of samples cannot have been sufficient for the detection of affected animals.

No difference was detected in the positive results obtained for animals that came from different regions of the state of São Paulo. Animals that came from Grande São Paulo and other regions of the state (seaside and countryside) were equally affected.

In relation to the source, it was observed that prevalence was significantly higher in free-ranging animals than in those in captivity. This can be due to the relative control of the feed supplied to captive animals. Free-ranging animals can feed more frequently on infected preys, being more exposed to the risk of infection. Another important factor is that 81.8% (54/66) of the subjects were free-ranging animals, and the remaining 18.2% (12/66) captive ones were mostly offspring, what can have interfered in the results.

The presence of sporocysts in the

feces was also analyzed in order to assess if the animals were shedding the parasite at the moment of examination. The test indicated the presence of sporocysts in the feces of four from the six *D. aurita* positive samples, that is, these animals can potentially have transmitted the parasite. In the 28 living *D. aurita* from which only feces samples were analyzed, there were 7.1% (2/28) positive results, showing that these animals were shedding the parasite into the environment. However, without the analysis of the small intestine, it was not possible to assess if these sporocysts were definitely infecting the opossum or only passing through it.

We conclude that the occurrence of *Sarcocystis* spp. in *D. aurita* and *D. albiventris* in these three regions of the state of São Paulo is low in the conditions assessed in this study.

Acknowledgements

We would like to thank CNPq for the financial support. We would also like to thank Professor PhD. Ricardo Augusto Dias, from Department of Preventive Veterinary Medicine and Animal Health, from School of Veterinary Medicine, University of São Paulo for statistical analysis support.

Ocorrência de *Sarcocystis* spp. em gambás (*Didelphis aurita* e *Didelphis albiventris*) em regiões do Estado de São Paulo, Brasil

Resumo

O objetivo deste estudo foi de determinar a ocorrência de *Sarcocystis* spp. em *D. albiventris* e *D. aurita* em três regiões do Estado de São Paulo. Para tal, utilizou-se noventa e oito *Didelphis* mortos, sendo 66 *D. aurita* e 32 *D. albiventris,* e também 28 *D. aurita* e cinco *D. albiventris* vivos. O método de centrífugo-flutuação em solução de sacarose foi empregado para isolamento dos oocistos/esporocistos de *Sarcocystis* spp. do intestino delgado e das fezes. Encontrou-se *Sarcocystis* spp. no intestino delgado de 9,1% dos *D. aurita* (6/66), sendo que quatro destes também houve positividade nas fezes. Não houve diferença estatística significativa entre machos e fêmeas positivos (P= 0,522), e entre os positivos de diferentes origens do Estado de São Paulo (P= 0,627), quanto a ocorrência de *Sarcocystis* spp. Entretanto, houve diferença estatística significativa entre animais de vida livre e de cativeiro (P = 0.009), sendo que somente os de vida livre foram positivos. Entre adultos e filhotes positivos também houve diferença (P= 0,004), sendo os adultos mais parasitados que os filhotes. Das amostras provenientes dos 28 *D. aurita* vivos, encontrou-se *Sarcocystis* spp. em 7.1% (2/28) deles. Dos 32 *D. albiventris*, todos foram negativos para *Sarcocystis* spp. nas amostras de intestino delgado e fezes. Os cincos *D. albiventris* vivos também foram negativos. Sendo assim, pode-se observar que a ocorrência de *Sarcocystis* spp. em *D. aurita* e *D. albiventris* nestas três regiões do Estado de São Paulo é baixa para estas condições analisadas.

Palavras-chaves:
Gambá.
Sarcocystis.
São Paulo.
Brasil.

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