Parasitology Research (2021) 120:751–754
https://doi.org/10.1007/s00436-020-06979-8

PROTOZOOLOGY - SHORT COMMUNICATION

Lesions caused by *Sarcocystis* spp., parasites of opossums (*Didelphis aurita*), in acute and chronic infections in birds (*Melopsittacus undulatus*)

Laura da Silva Ney[1] · Samira Salim Mello Gallo[1] · Nicole Brand Ederli[2] · Francisco Carlos Rodrigues de Oliveira[1]

Received: 5 May 2020 / Accepted: 15 November 2020 / Published online: 6 January 2021

Abstract

Protozoa of the genus *Sarcocystis* are obligatory heterogenous parasites with both definitive and intermediate hosts. Opossums (*Didelphis aurita*) can shed multiple species of *Sarcocystis* with birds as the intermediate host. The pathologies of *Sarcocystis* species in birds have not been thoroughly elucidated. Therefore, the aim of the present study to determine the main lesions that can occur in acute and chronic infections in intermediate hosts, when they ingest infective sporocysts that are shed in the opossum's feces, using budgerigars as a model. To this end, 12 budgerigars, *Melopsittacus undulatus*, were divided into two groups that received an inoculum with 60 and 120 sporocysts. Birds that died or were euthanized were necropsied, and the lung, tongue, liver, brain, heart, and skeletal striated muscles were collected and fixed in 10% formalin for histopathological analysis. The infectivity varied according to the sample and infective dose. Acute histopathological lesions were characterized by evidence of slightly degenerated hepatocyte cords that permeated the region of the blood vessel and hepatic sinusoids. Pulmonary tissue lesions were also observed in the parabronchial region with the presence of inflammatory infiltrates associated with areas of edema and atelectasis. In chronic infections, few mature cysts were observed in the chest, and many mature cysts in the thigh and tongue muscles. Thus, it was possible to conclude that lesions are highly characteristic in acute infection and, in chronic infections, cysts were present but without major lesions. In this case, the preferred organs of parasitism were the thigh and the tongue.

Keywords Sarcosporidiosis · Opossums · Budgerigars · *Sarcocystis*

Introduction

Protozoans of the genus *Sarcocystis* are found worldwide and in a wide variety of hosts, such as reptiles, birds, and mammals, including humans. A unique feature of the genus is that it has a heterogenous life cycle with two different host types: a definitive host and an intermediate host (Odening 1998). The definitive hosts become infected by ingesting an asexual cyst stage (sarcocyst) encysted in the tissues (muscles) of the intermediate host. A sexual cycle that results in the formation of sporulated oocysts occurs only in the definitive host, and it is restricted to the intestinal lamina propria (Dubey et al. 2015). Few studies have characterized histopathological findings related to the pathogenicity of *Sarcocystis* spp., parasites of the opossum, *Didelphis aurita*, in birds in Brazil (Stabenow et al. 2012; Gallo et al. 2018), thereby justifying research to determine the pathological aspects of parasitism of this protozoan in birds using the budgerigar, *Melopsittacus undulatus*, as an intermediate host model.

Section Editor: David S. Lindsay

✉ Samira Salim Mello Gallo
samiragallo@yahoo.com.br

[1] Laboratório de Sanidade Animal, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000, Campos dos Goytacazes, Rio de Janeiro 28013-602, Brazil

[2] Instituto do Noroeste Fluminense de Educação Superior, Universidade Federal Fluminense, Avenida João Jasbick, Santo Antônio de Pádua, Rio de Janeiro 28470 000, Brazil

Materials and methods

Sporocysts were obtained from five opossums, *D. aurita*, previously captured in the municipality of Campos dos

Goytacazes-RJ according to SISBIO authorization No. 46839-2 and were counted according to the methodology described by Oliveira et al. (2001). The inocula were standardized into solutions containing 60 and 120 sporocysts and stored in a 2.5-mL Eppendorf tube, and a volume of 1 mL was achieved by adding 0.9% saline solution to each tube. Twelve budgerigars, *M. undulatus*, were divided into two groups (60 and 120 sporocysts) that received the inoculum with the standardized number of sporocysts, and two birds were used as controls (Table 1). The birds were euthanized in accordance with Resolution No. 714 of June 20, 2002, Federal Council of Veterinary Medicine, in accordance with State Law No. 3900/02, with the approval of the Ethics Committee for Animal Use of the Universidade Estadual do Norte Fluminense (UENF) under number 290. The inoculum was deposited directly in the birds' swallow using a gavage probe, and each group was maintained separately in cages suitable for the breeding of the species. Birds that died or were euthanized were necropsied and eviscerated, and samples of the lung, tongue, liver, brain, heart, and skeletal striated muscles were collected. Samples were fixed in 10% neutral buffered formalin and processed for a histological examination after staining with hematoxylin and eosin (H&E) according to Prophet et al. (1994). The slides were analyzed under an AxioVision Plus® microscope (Carl Zeiss MicroImaging GmbH, Germany) equipped with a CANON PowerShot A640 digital camera for image capture.

Table 1 Mortality of budgerigars *Melopsittacus undulatus* inoculated with *Sarcocystis* spp. sporocysts recovered from the intestinal mucosa of opossums *Didelphis aurita* from Campos dos Goytacazes, RJ, Brazil

Inoculum/ sample	Death or euthanasia after inoculation							
	Weeks				Months			Total
	1°	2°	3°	4°	2°	3°	4°	
60 sporocysts								
102	-	-	-	-	(42)	-	-	1
111	-	-	-	-	-	-	(125)	1
112	-	-	(16)	-	-	-	-	1
132	-	-	-	-	-	(108)	-	1
174	-	-	(19)*	-	-	-	-	1
Control	-	-	-	-	-	-	(125)	1
Partial total	-	-	2	-	1	1	2	6
120 sporocysts								
102	-	-	(19)	-	-	-	-	1
111	-	-	-	-	-	-	(125)	1
112	-	-	-	-	-	-	(125)**	1
132	-	-	-	-	-	(108)**	-	1
174	-	-	(19)*	-	-	-	-	1
Control	-	-	-	-	-	-	(125)	1
Partial total	-	-	2	-	-	1	3	6
Grand total	-	-	4	-	1	2	5	12

Numbers in parentheses are the days of death or euthanasia after inoculation

*Birds in which meronts were found in the lung

**Birds in which cysts were found in the musculature

Results and discussion

Sarcocysts and meronts were found in the tissues of budgerigars inoculated with sporocysts of *Sarcocystis* spp. from opossums. Inoculum infectivity varied according to the sample and infective dose (Table 1). In agreement with Powell et al. (1986), varying degrees of pathogenicity of sporocyst samples obtained from the intestinal scrapings of opossums were observed. The pathogenicity of samples varied according to the number of sporocysts. Because some birds died 12–19 days after inoculation (DAI) which made impossible to investigate the presence of cysts over longer periods of infection. In most cases, no clinical symptoms are observed in inoculated budgerigars, but some were inactive, remaining in the bottom of the cage with possible loss of flight function, similar to the results of Dubey et al. (1999). In the present study, some birds died of respiratory failure due to acute pulmonary sarcosporidiosis with large infiltration in the air capillaries and interstitial edema and multifocal granulomatous inflammatory interstitial infiltrate in the alveolar and parabronchial regions. Previous studies in experimentally infected birds reported signs of apathy, dyspnea, and partial or total anorexia (Clubb and Frenkel 1992).

Corroborating with the results of Smith et al. (1987a) and Neill et al. (1989), histopathological lesions in the lung and liver were observed in one of the budgerigars that was inoculated with 120 sporocysts from sample 174 and died at 19 DAI. These lesions were characterized by evidence of slightly degenerated hepatocyte cords that permeated the region of the blood vessel and hepatic sinusoids with inflammatory infiltrate represented mainly by multiple heterophils, macrophages, and some lymphocytes. Pulmonary tissue lesions were also observed in the parabronchial region with the presence of inflammatory infiltrates associated with areas of edema and atelectasis. The same lesions were observed in a budgerigar that was inoculated with 60 sporocysts from sample 112 and died at 16 DAI. Meronts were observed in the capillary endothelium of the lungs in this bird and in budgerigars inoculated with 60 and 120 sporocysts from sample 174 (Fig. 1a, b) that died at 19 DAI. According to Smith et al. (1987a, 1987b), schizonts go through two asexual generations in the endothelium; the first occurs in the endothelial cells of the arterioles, and the second occurs in the endothelial cells of the capillaries and venules. The lungs are the main organ affected, corroborating the results found in the present study,

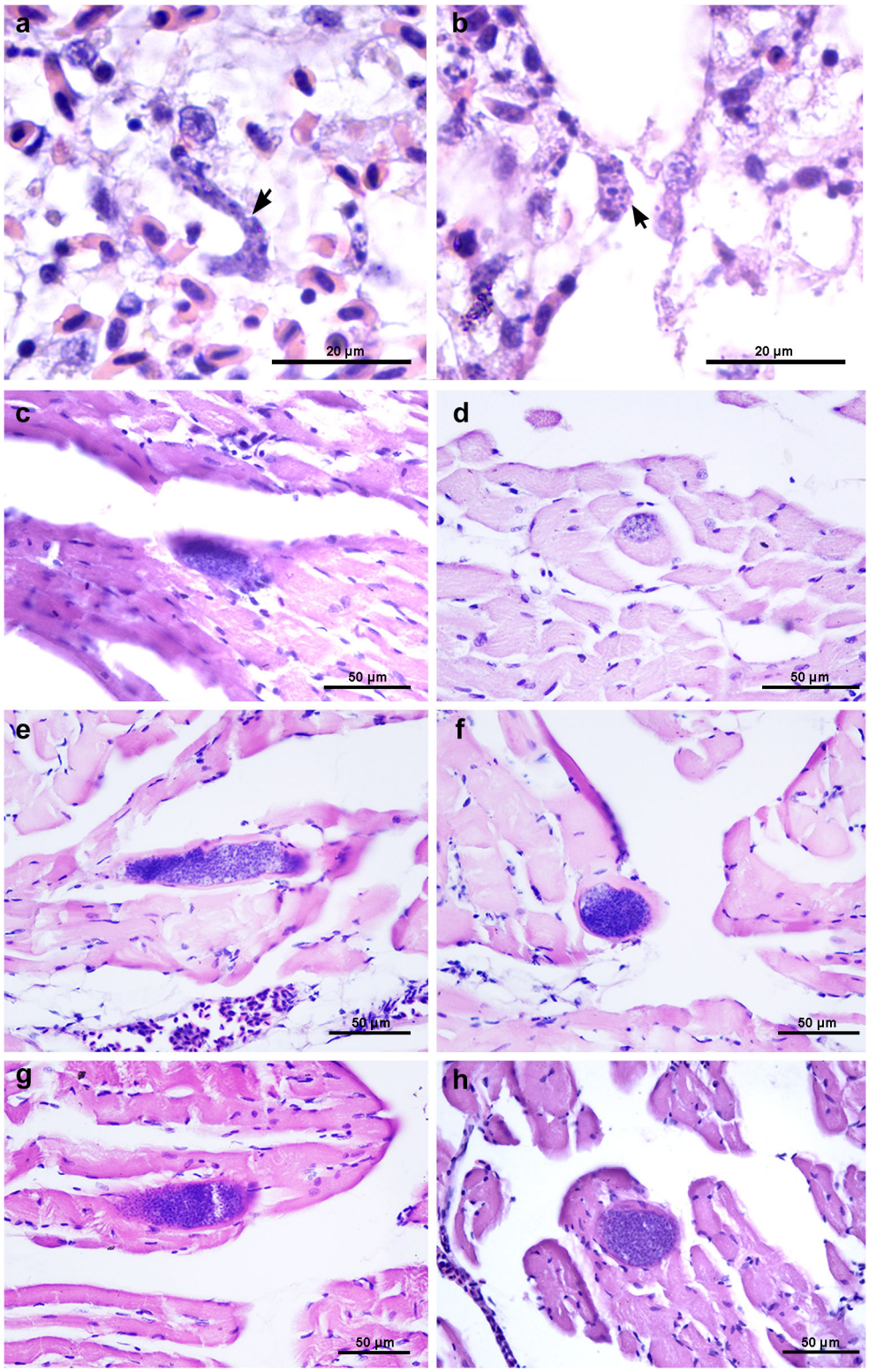

Fig. 1 Micrograph of lung and muscle tissues of budgerigars (*Melopsittacus undulatus*) experimentally inoculated with *Sarcocystis* spp. sporocysts from opossum (*Didelphis aurita*) intestinal scrapings. In **a** and **b**, arrows indicate meronts in the vascular endothelium of venules of birds that received 120 and 60 sporocysts, respectively, and died at 19 DAI. In birds that died at 108 DAI, H&E staining revealed the following: **c** a mature cross-sectional cyst in the chest musculature; **d** an immature cyst in a vertical section in the chest musculature; **e** and **f** mature cysts in the transverse and vertical sections, respectively, of the thigh musculature; and **g** and **h** mature cysts in the cross-sectional and vertical sections of the tongue musculature

in which noteworthy meronts were found in the lungs (Smith et al. 1987a, 1987b; Neill et al. 1989; Stabenow et al. 2012). Smith et al. (1987a) and Neill et al. (1989) noted that *S. falcatula* used the lungs as an important organ for meront proliferation in experimentally infected budgerigars. Regarding the liver, although meronts were not found in our study, active and chronic hepatitis was observed in the histology results, likely caused by parasitism in the second phase of asexual reproduction, which occurs in capillary and venous endothelial cells in most host organs, as stated in several

papers (Neill et al. 1989; Clubb and Frenkel 1992 Granulomatous hepatitis, characterized by multifocal inflammatory infiltrate with a predominance of macrophages, rare lymphocytes, and plasma cells associated with areas of hydropic degeneration, was the most important finding in the liver. These changes observed in liver tissue are likely caused by parasitism (Neill et al. 1989).

Cysts were observed in two budgerigars that were inoculated with 120 sporocysts (Table 1). In the bird that was inoculated with sample 112 and was euthanized at 125 DAI (Table 1), only two mature cysts were observed in the thigh musculature; in the other budgerigar inoculated with sample 132 and that died 108 DAI (Table 1), few mature (Fig. 1c) and immature (Fig. 1d) cysts were observed in the chest musculature. In this same bird, a large quantity of mature cysts were observed in the thigh musculature (Fig. 1e, f), and many mature cysts were observed in the tongue musculature (Fig. 1g, h). The observed sarcocysts were found in the tongue, thigh, and chest muscles in descending order, corroborating the results of Stabenow et al. (2012). These authors associated the parasite predilection of these tissues with opossum attacks on poultry according to popular reports that opossums prefer ingestion of the legs and head, increasing the likelihood of parasite perpetuation. The decreased presence of the parasite in the pectoral and cardiac muscles of birds may also be explained by Neill et al. (1989), who verified cyst degeneration in these tissues; however, in the tongue and thigh muscles, the cysts were maintained and matured.

Conclusion

Lesion characteristics of infection by *Sarcocystis* spp. in birds are observed only in acute infection and generally with the death of the host. In chronic infections with or without bird death, lesions due to parasitism are not observed, and cysts may or may not be present in the host's muscle tissues regardless of the infecting dose.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

Clubb SL, Frenkel JK (1992) *Sarcocystis falcatula* of opossums: transmission by cockroaches with fatal pulmonary disease in psittacine birds. J Parasitol 78:116–124

Dubey JP, Venturini L, Venturini C, Basso W, Unzaga J (1999) Isolation of *Sarcocystis falcatula* from the South American opossum (*Didelphis albiventris*) from Argentina. Vet Parasitol 86:239–244

Dubey JP, Calero-Bernal R, Rosenthal BM, Speer CA, Fayer R (2015) Sarcocystosis of animals and humans. CRC Press, Boca Raton

Gallo SSM, Lindsay DS, Ederli NB, Matteoli FP, Venancio TM, Oliveira FCR (2018) Identification of opossums *Didelphis aurita* (Wied-Neuweid, 1826) as a definitive host of *Sarcocystis falcatula-like* sporocysts. Parasitol Res 117:213–223

Neill PJG, Smith JH, Box ED (1989) Pathogenesis of *Sarcocystis falcatula* (Apicomplexa: Sarcocystidae) in the budgerigar (*Melopsittacus undulatus*). IV. Ultraestructure of developing, mature and degenerating sarcocysts. J Protozool 36:430–437

Odening K (1998) The present state of species systematics in *Sarcocystis* Lankester, 1882 (Protista, Sporozoa, Coccidie). Syst Parasitol 41: 209–233

Oliveira FCR, Albuquerque GR, Lopes CWG, Massad FV, Munhoz AD (2001) Hipnozoítas de *Cystoisospora ohioensis* (Dubey, 1975) Frenkel, 1977 (Apicomplexa:Cystoisosporinae) recuperados de órgãos de camundongos através da digestão péptica. Rev Bras Parasitol Vet 10:29–35

Powell EC, Pezeshkpour G, Dubey JP, Fayer R (1986) Types of myofibers parasitized in experimentally induced infections with *Sarcocystis cruzi* and *Sarcocysits capracanis*. Am J Vet Res 47: 514–517

Prophet EB, Mills B, Arrington JB, Sobín LH (1994) Laboratory methods. In Histotechnology, D. C. Washington, Armed Forces Institute of Pathology

Smith JH, Meier JL, Neill PJG, Box ED (1987a) Pathogenesis of *Sarcocystis falcatula* in the budgerigar. I. Early pulmonary schizogony. Lab Investig 56:60–71

Smith JH, Meier JL, Neill PJG, Box ED (1987b) Pathogenesis of *Sarcocystis falcatula* in the budgerigar. II. Pulmonary pathology. Lab Investig 56:72–84

Stabenow CS, Ederli NB, Lopes CWG, Oliveira FCR (2012) *Didelphis aurita* (Marsupialia: Didelphidae): a new host for *Sarcocystis lindsayi* (Apicomplexa: Sarcocystidae). J Parasitol 98:1262–1265