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Molecular detection of *Sarcocystis* spp. in the breast muscle of captive birds from the southern region of Brazil

Detecção molecular de *Sarcocystis* spp. no músculo peitoral de aves de cativeiro da região sul do Brasil

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Highlights

Molecular detection of *Sarcocystis* spp. in birds.
Detection of *Sarcocystis* spp. in samples of avian breast muscle.
Different bird species acting as intermediate hosts for *Sarcocystis* spp.

Abstract

Studies on diseases of wild birds are essential in the context of public health, as these animals act as sentinels, allowing information regarding a determined geographic area. In addition, birds are food protein sources for animals, and therefore play an important role in the life cycle of the protozoan *Sarcocystis* spp. This study aimed to identify the *Sarcocystis* spp. in breast muscle samples of naturally infected captive birds. The breast muscle of 89 birds were sampled, and the DNA amplified by PCR targeting the 18S ribosomal RNA gene to detect *Sarcocystis* spp. PCR products were sequenced and 5.61% (5/89) samples showed 100% similarity with *Sarcocystis* spp. (one *Cyanoliseus patagonus*, one *Psittacula krameri*, two *Pyrrhura frontalis*, and one *Ramphastos dicolorus*). The large number of naturally infected species analyzed by molecular methods allowed the detection of *Sarcocystis* spp. in different bird species, corroborating the epidemiology of *Sarcocystis* spp.

Key words: *Sarcocystis*. PCR. Captive birds. Naturally infected.

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Resumo

Estudos sobre doenças de aves silvestres são essenciais no contexto da saúde pública, pois esses animais atuam como sentinelas, permitindo obter informações sobre uma determinada área geográfica. Além disso, as aves são fontes de proteína alimentar para os animais e, portanto, desempenham um papel importante no ciclo de vida do *Sarcocystis*. Este estudo teve como objetivo identificar *Sarcocystis* spp. nos músculos do peito de aves de cativeiro naturalmente infectadas. Os músculos do peito de 89 aves foram coletados, e o DNA amplificado pela PCR do gene RNA ribossômico 18S para detecção de *Sarcocystis* spp. Os produtos da PCR foram sequenciados e 5,61% (5/89) amostras apresentaram 100% de similaridade com o *Sarcocystis* spp. (um *Cyanoliseus patagonus*, um *Psittacula krameri*, dois *Pyrrhura frontalis* e um *Ramphastos dicolorus*). O grande número de espécies naturalmente infectadas analisadas por métodos moleculares permitiu a detecção de *Sarcocystis* spp. em diferentes espécies de aves, corroborando a epidemiologia de *Sarcocystis* spp.

Palavras-chave: *Sarcocystis*. PCR. Aves em cativeiro. Naturalmente infectadas.

Introduction

The genus *Sarcocystis* was first described in Switzerland by Miescher (1843), who observed lesions with an appearance of "milky white threads" in the musculature of rats. In 1882, based on the appearance and location of the muscular cyst, Lankester coined the name *Sarcocystis* (greek sarkos (flesh) and kystis (bladder)). *Sarcocystis* is an intracellular obligatory protozoan characterized by a life cycle in two hosts. In intermediate hosts usually omnivores asexual multiplication of the parasite occurs with the formation of cysts in the musculature. In definitive hosts usually carnivores the sexual phase occurs in the small intestine with the formation of oocysts/sporocysts (Dubey et al., 2016).

The macroscopic identification of the *Sarcocysts* is difficult. In this way, the microscopic analysis or molecular tools are required for *Sarcocystis* detection. Different techniques for sequencing the ribosomal RNA gene and mtDNA contributed to describe a series of new species, and many investigations of wild bird populations, indicating that a relatively high host specificity exists in several cases. Recent studies have described *Sarcocystis* spp. that affect birds, i.e., *S. albifronsi, S. anasi* (Kutkienė et al., 2012), *S. calchasi* (Parmentier et al., 2019), *S. columbae* (Olias et al., 2010), *S. falcatula* (Konradt et al., 2017; Llano et al., 2022; Rêgo et al., 2021), *S. halieti, S. lari* (Gjerde et al., 2018), *S. lindsayi* (Dubey et al., 2001a), *S. ramphastosi* (Dubey et al., 2008), and *S. rileyi* (Dubey et al., 2010). The definitive hosts of *S. falcatula* are *Didelphis albiventris* (Dubey et al., 2000), *D. aurita* (Gallo et al., 2018) and *D. marsupialis* (Dubey et al., 2001b).

The presence of *Sarcocystis* DNA in the breast muscle of birds may constitute evidence that these birds serve as intermediate hosts of *Sarcocystis.* This study aimed to detect *Sarcocystis* spp. in the breast muscles of naturally infected captive birds in the city of Santa Maria, Rio Grande do Sul, Brazil.

Materials and Methods

Carcasses of captive birds in the city of Santa Maria, Rio Grande do Sul, Brazil (29° 41′ 29′′ S and 53° 48′ 3′′ W), were subjected to necropsy at the Avian Pathologies Diagnosis Central Laboratory (LCDPA) of the Federal University of Santa Maria (UFSM). In this study, a total of 89 captive birds classified into 35 species, belonging to 19 families were studied (Table 1). Breast muscle samples were collected from 89 birds (1g, approximately), macerated using a scalpel blade under a petri dish and 20mg collected for stored at -20 °C until DNA extraction.

Polymerase chain reaction (PCR) was conducted at the Laboratory of Parasitic Diseases (LADOPAR) of the UFSM. DNA was extracted from breast muscle samples using a commercial kit (Wizard® Genomic DNA Purification Kit-Promega), according to the manufacturer's instructions with one modification, i.e., the lysis step was performed overnight at 55 °C in accordance with the protocol proposed by Moré et al. (2011). After extraction, DNA concentration was measured at the NanoDrop 1000 (Thermo Scientific, USA), to the absorbance at 260 nm, with a range of 20-50 ng/μL. The DNA samples were stored at -20 °C until PCR.

PCR amplification of the 18S ribosomal RNA gene was performed to detect members of the genus *Sarcocystis*, using the following primers: 2L (5′-GGATAAACCGTGGTAATTCTATG-3′) and 3H (5′-GGCAAATGCTTTCGCAGTAG-3′) sequence primers 5′ and 3′amplifying a 900 bp fragment (Yang et al., 2001). Each PCR was carried out in a total volume of 25 μL, comprising 2.5 μL of 10x buffer (Promega, USA), 1 mM dNTPs (Ludwig Biotec, Brazil), 1 μM of each primer (Sigma-Aldrich, Brazil), 1 U Taq DNA polymerase (Promega, USA), 1.5 mM MgCl2, and 50 ng template DNA. DNA extracted from a pool of 50 Sarcocystis cysts was used as a positive control (unidentified species) and Milli-Q water was used as a negative control. PCR was performed on a T100 thermal cycler (BioRad, USA), as follow: 2 min at 95 °C for the initial hot denaturation step, followed by 40 cycles of 40 s at 94 °C, 50 s at 56 °C, and 60 s at 72 °C, and a final extension step of 6 min at 72 °C. The PCR products were visualized using UV illumination after electrophoresis on a 1% agarose gel stained with Gel Red Nucleic Acid Stain (Biotium, USA).

Table 1
List of birds tested according to their classification, family, scientific name and common name*

Family	Scientific name	Common name	Nº tested birds
Accipitridae	*Rupornis magnirostris*	Roadside Hawk	5
Ardeidae	*Tigrisoma lineatum*	Rufescent Tiger-Heron	1
Cacatuidae	*Nymphicus hollandicus*	Cockatiel	1
Cardinalidae	*Cyanoloxia brissonii*	Ultramarine Grosbeak	1
Columbidae	*Zenaida auriculata*	Eared Dove	5
Corvidae	*Cyanocorax caeruleus*	Azure Jay	1
Cracidae	*Crax fasciolata*	Bare-faced Curassow	1
Falconidae	*Falco sparverius*	American Kestrel	1
Fringillidae	*Euphonia cyanocephala*	Golden-rumped Euphonia	1
	Paroaria coronata	Red-crested Cardinal	7
	Serinus canaria	Domestic canary	1
Jacanidae	*Jacana jacana*	Wattled Jacana	1
Motacillidae	*Anthus correndera*	Correndera Pipit	1
Phasianidae	*Pavo cristatus*	Blue Peafowl	2
Psittacidae	*Amazona aestiva*	Amazon Parrot	11
	Amazona pretrei	Red-spectacled Parrot	4
	Amazona vinacea	Vinaceous-breasted Parrot	1
	Ara ararauna	Blue-and-yellow Macaw	5
	Cyanoliseus patagonus	Burrowing Parrot	2
	Forpus xanthopterygius	Blue-winged Parrotlet	1
	Myiopsitta monachus	Monk Parakeet	7
	Pionites leucogaster	White-bellied Parrot	3
	Pionus maximiliani	Scaly-headed Parrot	1
	Poicephalus senegalus	Senegal Parrot	1
	Pyrrhura frontalis	Maroon-bellied Parakeet	7
	Triclaria malachitacea	Blue-bellied Parrot	1
Psittaculidae	*Psittacula krameri*	Rose-ringed Parakeet	2
Rallidae	*Gallinula galeata*	Common Gallinule	1
Ramphastidae	*Pteroglossus castanotis*	Chestnut-eared Aracari	4
	Ramphastos dicolorus	Red-breasted Toucan	2
Strigidae	*Athene cunicularia*	Burrowing Owl	3
	Megascops choliba	Tropical Screech-Owl	1
Thraupidae	*Saltator aurantiirostris*	Golden-billed Saltator	1
	Stephanophorus diadematus	Diademed Tanager	1
Threskiornithidae	*Theristicus caudatus*	Buff-necked Ibis	1
19	**35**	**35**	**89**

Positive PCR products were purified using a QIAquick® PCR Purification Kit (Qiagen, Germany) according to the manufacturer's instructions. The final purified DNA was analyzed using a NanoDrop 1000 spectrophotometer (Thermo Scientific, USA), for concentration determination. After PCR purification, sequencing reactions were assembled using 5 pmol primers (separately), 30-60 ng purified PCR product, and MilliQ water in a final volume of 6 µL. This reaction mixture was dehydrated at 60 °C for 2 h and then sequenced (ACTGENE - Sequencing Service, Brazil). The obtained results were analyzed using StandenPackage and the generated nucleotide sequences were evaluated using Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/ BLAST).

The collection of biological material from captive birds was authorized by the Chico Mendes Institute for Biodiversity Conservation under Biodiversity Authorization and Information System (registration number 76022-1). An approval from the Ethics Committee, i.e., Comissão de Ética na Utilização de Animais was not required as this study involved only dead animals.

Results and discussions

Five birds (5.61%) resulted positive to the PCR for *Sarcocystis* spp. These birds belonged to four species of three families (two Psittacidae, one Psittaculidae, and one Ramphastidae) (Figure 1); one *Cyanoliseus patagonus*, one *Psittacula krameri*, two *Pyrrhura frontalis* and one *Ramphastos dicolorus.* The amplified sequences exhibited 100% similarity with *Sarcocystis* spp. Sequences deposited in GenBank (accession nos. OQ154263, OQ154264, OQ154266, OQ154265 and OQ154271, respectively). From these isolates, a phylogenetic tree was constructed (Figure 2).

The infected birds did not present macroscopic cysts of sarcocystosis and died of various causes, with a higher occurrence of endoparasites. This demonstrates that possibly in natural infections, when birds are exposed to the infective forms of the parasite, they develop subclinical infections, and consequently, the findings in these birds are often incidental. Taking into consideration the feeding habits of the captive birds with the presence of *Sarcocystis* spp., it was possible to identify two forms of feeding, and of the five infected birds, three (60%) were granivorous and two (40%) were omnivorous.

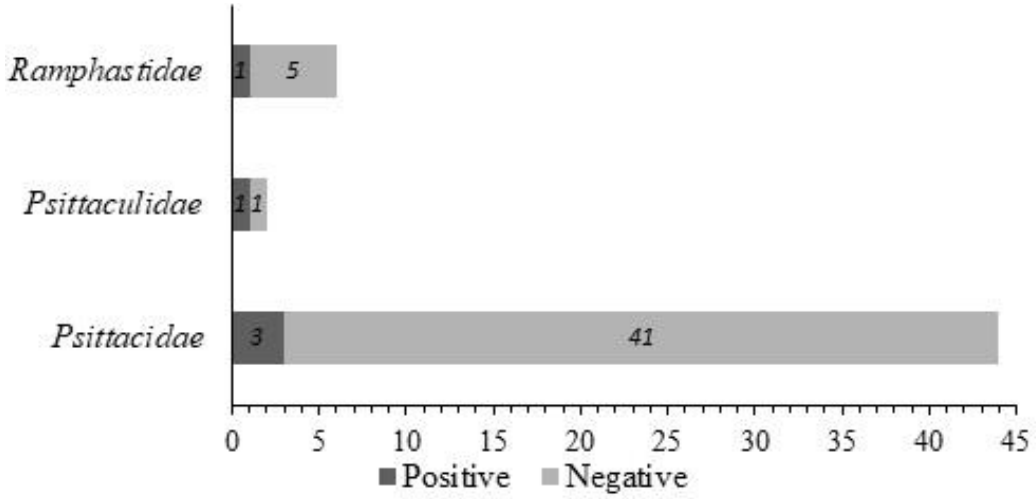

Figure 1. Number of birds positive for *Sarcocystis* spp. (Family-based analysis).

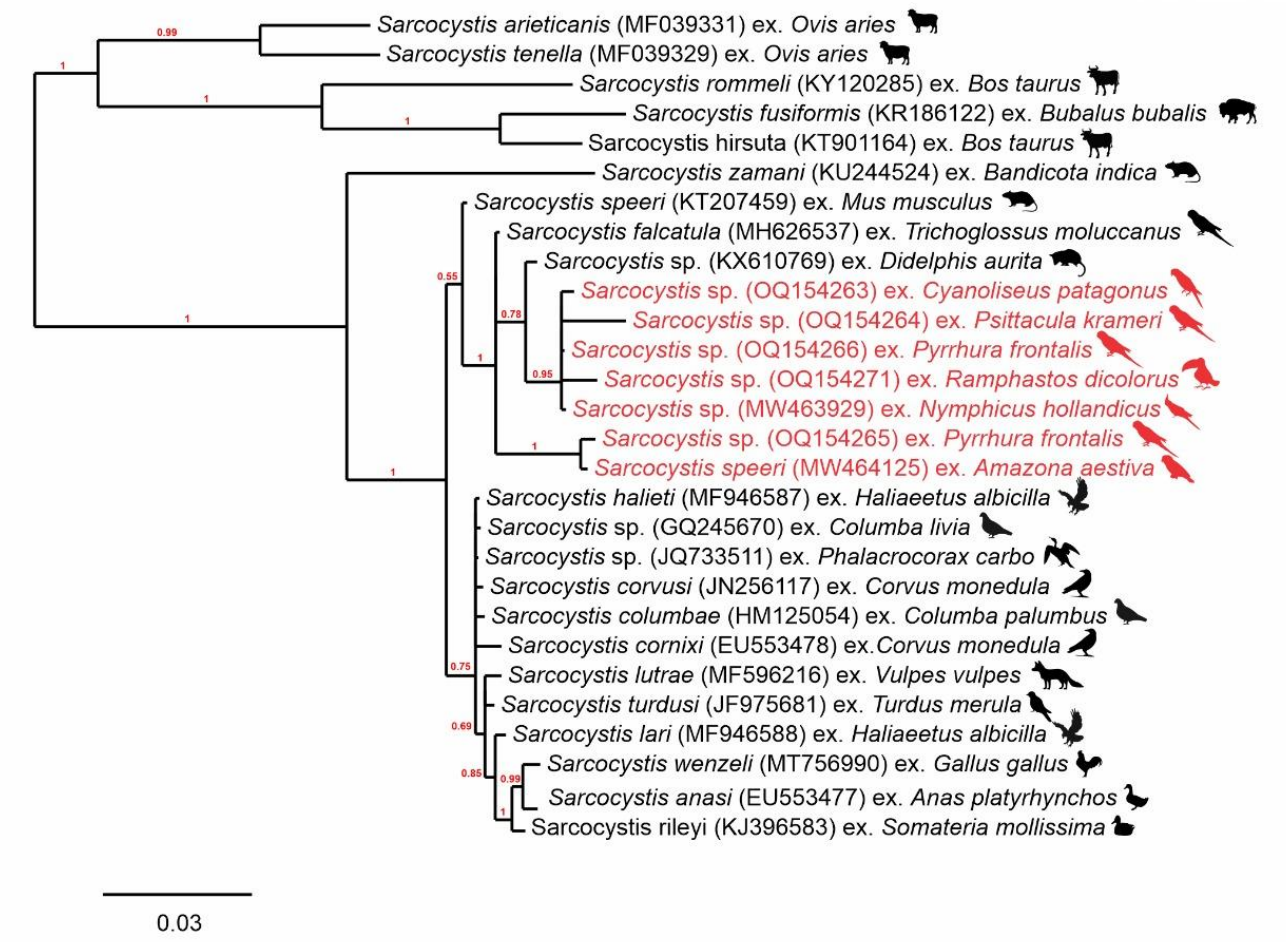

Figure 2. Phylogenetic analysis of 18S rRNA subunit obtained from various isolates of *Sarcocystis* spp. The phylogenetic tree was constructed using the bayesian inference method implemented in the MrBayes program v3.2.6. The phylogenetic tree were performed with TreeDyn v198.3.

The birds *C. patagonus* (Psittacidae), *P. frontalis* (Psittacidae), and *R. dicolorus* (Ramphastidae) are unique to South America (New World). *P. krameri* is a bird naturally distributed in Asia and Africa (Old World). *S. falcatula* infection was first described in Brazil in a zoo in Belo Horizonte, where parakeets (*P.5rameria*) died suddenly without manifesting any clinical signs (Ecco et al., 2008). An outbreak in a zoo in Foz do Iguaçú, Brazil led to the death of 38 parrots, most of which were from the Old World (Godoy et al., 2009). Omnivorous, carnivorous, and scavenger birds have the habit of consuming carcasses; such feeding habits facilitate the ingestion of cysts and/or parasite oocysts, thereby providing a route for the birds to be infected (Dubey et al., 2010). Dubey et al. (2016) demonstrated that in intermediate hosts, which are generally omnivorous, the formation of cysts in the musculature occurs via asexual multiplication of the parasite.

In South America, *S. falcatula*-like species was first isolated from sporocysts found in the intestine of opossum (*D. marsupialis*) from the city of São Paulo, Brazil (Dubey et al., 2000). *S. lindsayi* was proposed as a new species isolated from an opossum (*D. albiventris*) in Jaboticabal, São Paulo, Brazil (Dubey et al., 2001b). The experimental intermediate host for both *Sarcocystis* species is *Melopsittacus undulatus*; however, its natural intermediate host remains unknown (Dubey et al., 2000, 2001b).

Rêgo et al. (2021) evaluated samples of wild free-ranging birds, naturally infected and rescued in the state of Minas Gerais, Brazil, presenting 5/44 (11.4%) samples positive for *Sarcocystis* spp. using the 18S ribosomal gene. However, Llano et al. (2022) performed nested-PCR, with ITS1 gene primers, showing 28/400 birds infected by *Sarcocystis falcatula, Sarcocystis halieti* and *Sarcocystis* spp. unknown or not described. In our study, PCR was performed using the 18S RNA gene, which may be the reason for not identifying the species.

In North America, birds of the orders Psittaciformes, Passeriformes, and Columbiformes canaries (*Serinus canarius*), mandarin (*Poephila guttata*), budgerigars (*M. undulatus*), domestic pigeon (*Columba livia*), chicken (*Gallus gallus*), and guinea fowl (*Numida meleagris*) have been reported as the intermediate hosts of *S. falcatula* (Box & Smith, 1982).

As the sporocysts of *S. neurona*, *S. falcatula*, and *S. speeri* are similar, these species can be distinguished based on their pathogenicity and infectivity in immunodeficient birds and mice in experimental infections. *S. speeri* and *S. neurona* are not infectious to *M. undulatus*, whereas they are infectious to interferon gamma-knockout (KO) mice. In contrast, *S. falcatula* and *S. lindsayi* species are infectious to *M. undulatus*, while they are not infectious to KO mice (Dubey et al., 2016). In Brazil, *S. falcatula* infection has been described as necrotizing meningoencephalitis in a tapirucu (*Phimosus infuscatus*), and the bird manifested wing paralysis and mild issues with motor coordination (Konradt et al., 2017).

In Costa Rica, researchers have reported the presence of macroscopic sarcocysts of *S. ramphastosi* in a toucan (*Ramphastos sulfuratus*) (Dubey et al., 2004). Eleven individual sarcocysts were subjected to DNA extraction and PCR amplification of

the 18S rRNA gene; the results demonstrated a close phylogenetic relationship with *S. falcatula* and *S. neurona* (Dubey et al., 2008). Gjerde et al. (2018) reported a secondary finding of numerous sporulated *Sarcocystis* oocysts in the intestinal mucosa of a white-tailed sea eagle (*Haliaeetus albicilla*) in Western Norway. In their study, DNA was extracted from 10 mucosal scrapings containing oocysts, and was subjected to PCR amplification and sequencing of four DNA regions, i.e., the 18S and 28S rRNA genes, ITS1 region, and cox1 gene; the results revealed the presence of *S. halieti* n. sp. and *S. lari.* Necropsy revealed that the cause of death was inflammation and occlusion of bile ducts.

In most studies aimed at identifying *Sarcocystis* species, the 18S rRNA gene has been used because the sequence of this region is highly conserved (Dubey et al., 2015). Prakas et al. (2014) suggested that the 18S rRNA gene was not sufficiently variable to identify *Sarcocystis* species that use birds as intermediate hosts. Recent studies on the molecular characterization of *Sarcocystis* species that infect birds have used the first transcript space (ITS1) along with the 28S rRNA gene (Bamac et al., 2020; Prakas et al., 2014). In addition, for the phylogenetic classification of species, the associations between loci 18S rRNA gene, 28S rRNA gene, and ITS1 have been used (Dubey et al., 2015; Gjerde et al., 2018; Parmentier et al., 2019). These data indicate the need for further studies aimed at designing a methodology with good sensitivity and specificity to characterize *Sarcocystis* species that use birds as intermediate hosts.

In this study, birds with *Sarcocystis* spp. in their breast muscle showed no macroscopic lesions. A study in geese (*Anser* sp.) from public parks and captivity in Curitiba, Brazil, verified through indirect immunofluorescence assay (IFA) that 28% (42/149) of the birds had antibodies against *Sarcocystis* spp., with 90% of the birds showing no clinical signs; nonetheless, only four birds with positive serology manifested nonspecific clinical signs (diarrhea, cloaca prolapse, and difficult breathing) (Konell et al., 2019). The frequency of reports of natural infection in New World birds by *Sarcocystis* species is low because of co-evolution with the parasite (Dubey et al., 2001a).

Conclusions

The observed results indicates the role of the captive birds as intermediate hosts of *Sarcocystis* spp. and the importance of these bird species to the epidemiology chain of this parasite.

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Declarations

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Conflicts of interest/Competing interests

The authors declare that there are no conflicts of interest.

Authors' contributions

All authors have read and agree to the fnal draft of the manuscript. Conceptualization and methodology, Marta Elena Machado Alves, Fagner D'ambroso Fernandes, Patrícia Bräunig, Helton Fernandes dos Santos, Luis Antonio Sangioni, Fernanda Silveira Flores Vogel; investigation, Marta Elena Machado Alves, Fagner D'ambroso Fernandes, Patrícia Bräunig, Helton Fernandes dos Santos, Luis Antonio Sangioni, Fernanda Silveira Flores Vogel; writing original draft preparation, Marta Elena Machado Alves, Fagner D'ambroso Fernandes, Patrícia Bräunig, Helton Fernandes dos Santos, Luis Antonio Sangioni, Fernanda Silveira Flores Vogel; writing review and editing, Luis Antonio Sangioni, Fernanda Silveira Flores Vogel; supervision, Luis Antonio Sangioni, Fernanda Silveira Flores Vogel.

References

Bamac, O. E., Rogers, K. H., Arranz-Solís, D., Saeij, J. P., Lewis, S., Duerr, R., Skoglund, J., Peronne, L., & Mete, A. (2020). *International Journal for Parasitology: Parasites and Wildlife, 12*, 185-191. doi: 10.1016/j.ijppaw.2020.06.005

Box, E. D., & Smith, J. H. (1982). The intermediate host spectrum in a Sarcocystis species of birds. *Journal of Parasitology, 68*, 668-673. doi: 10.2307/3280926

Dubey, J. P., Calero-Bernal, R., Rosenthal, B. M., Speer, C. A., & Fayer, R. (2016). *Sarcocystosis of animals and humans* (2nd ed.). CRC Press.

Dubey, J. P., Lane, E., & Van Wilpe, E. (2004). *Sarcocystis ramphastosi* sp. nov. and *Sarcocystis sulfuratusi* sp. nov. (Apicomplexa, Sarcocystidae) from the keel-billed toucan (*Ramphastos sulfuratus*). *Acta Parasitologica, 49*(2), 93-101.

Dubey, J. P., Lindsay, D. S., Rezende, P. C. B., & Costa, A. J. (2000). Characterization of an unidentified *Sarcocystis falcatula*-like parasite from the South American opossum, *Didelphis albiventris* from Brazil. *Journal of Eukaryotic Microbiology, 47*(6), 538-544. doi: 10.1111/j.1550-7408.2000.tb00087.x

Dubey, J. P., Lindsay, D. S., Rosenthal, B. M., Kerber, C. E., Kasa, N., Pena, H. F., Kwok, O. C., Shen, S. K., & Gennari, S. M. (2001b). Isolates of *Sarcocystis falcatula*-like Organisms from South American Opossums *Didelphis marsupialis* and *Didelphis albiventris* from São Paulo, Brazil. *Journal of Parasitology, 87*(6), 1449-1453. doi: 10.1645/0022-3395(2001)087[1449:IOSFLO]2.0.CO;2

Dubey, J. P., Morales, J. A., Leandro, D., & Rosenthal, B. M. (2008). Molecular phylogeny implicates new world opossums (Didelphidae) as the definitive hosts of *Sarcocystis ramphastosi*, a parasite of the keel-billed toucan (*Ramphastos sulfuratus*). *Acta Protozoologica, 47*(1), 55-61.

Dubey, J. P., Rosenthal, B. M., & Felix, T. A. (2010). Morphologic and molecular characterization of the Sarcocysts of *Sarcocystis rileyi* (Apicomplexa: Sarcocystidae) from the mallard duck (*Anas platyrhynchos*). *Journal of Parasitology, 96*(4), 765-770. doi: 10.1645/GE-2413.1

Dubey, J. P., Rosenthal, B. M., & Speer, C. (2001a). Sarcocystis lindsayi n. sp. (Protozoa: Sarcocystidae) from the South American opossum, *Didelphis albiventris* from Brazil. *Journal of Eukaryotic Microbiology, 48*(5), 595-603. doi: 10.1111/j.1550-7408.2001.tb00196.x

Dubey, J. P., Verma, S. K., Dunams, D., Calero-Bernal, R., & Rosenthal, B. M. (2015). Molecular characterization and development of *Sarcocystis speeri* sarcocysts in gamma interferon gene knockout mice. *Parasitology, 142*(13), 1555-1562. doi: 10.1017/S0031182015001109

Ecco, R., Luppi, M. M., Malta, M. C. C., Araújo, M. R., Guedes, R. M. C., & Shivaprasad, H. L. (2008). An outbreak of Sarcocystosis in Psittacines and a pigeon in a zoological collection in Brazil. *Avian Diseases, 52*(4), 706-710. doi: 10.1637/8303-040408-Case.1

Gallo, S. S. M., Lindsay, D. S., Ederli, N. B., Matteoli, F. P., Venancio, T. M., & Oliveira, F. C. R. de. (2018). Identification of opossums *Didelphis aurita* (Wied-Neuweid, 1826) as a definitive host of *Sarcocystis falcatula*-like sporocysts. *Parasitology Research, 117*, 213-223. doi: 10.1007/s00436-017-5695-4

Gjerde, B., Vikøren, T., & Hamnes, I. S. (2018). Molecular identification of *Sarcocystis halieti* n. sp., *Sarcocystis lari* and *Sarcocystis truncata* in the intestine of a white-tailed sea eagle (*Haliaeetus albicilla*) in Norway. *International Journal of Parasitology: Parasites Wildlife, 7*(1), 1-11. doi: 10.1016/j.ijppaw.2017.12.001

Godoy, S. N., Paula, C. D. de, Cubas, Z. S., Matushima, E. R., & Catão-Dias, J. L. (2009). Occurrence of *Sarcocystis falcatula* in captive psittacine birds in Brazil. *Journal of Avian Medicine and Surgery, 23*(1), 18-23. doi: 10.1647/2008-006R.1

Konell, A. L., Sato, A. P., Stival, M., Malaguini, N. P., Anjos, A. dos, Ferreira, R. F., & Locatelli-Dittrich, R. (2019). Serosurvey of *Toxoplasma gondii*, *Sarcocystis* sp. and *Neospora caninum* in geese (*Anser* sp.) from urban parks and captivity. Brazilian *Journal of Veterinary Parasitology, 28*(2), 221-228. doi: 10. 1590/S1984-29612019042

Konradt, G., Bianchi, M. V., Leite, R. V., Fº., Silva, B. Z. da, Soares, R. M., Pavarini, S. P., & Driemeier, D. (2017). Necrotizing meningoencephalitis caused by *Sarcocystis falcatula* in bare-faced ibis (*Phimosus infuscatus*). *Parasitology Research, 116*, 809-812. doi: 10.1007/s00436-016-5341-6

Kutkienė, L., Prakas, P., Sruoga, A., & Butkauskas, D. (2012). Description of *Sarcocystis anasi* sp. nov. and *Sarcocystis albifronsi* sp. nov. in birds of the order Anseriformes. *Parasitology Research, 110*, 1043-1046. doi: 10.1007/s00436-011-2588-9

Llano, H. A. B., Polato, H. Z., Keid, L. B., Oliveira, T. M. F. S., Zarwarg, T., Oliveira, A. S., Sanches, T. C., Joppert, A. M., Gondim, L. F. P., & Soares, R. M. (2022). Molecular screening for Sarcocystidae in muscles of wild birds from Brazil suggests a plethora of intermediate hosts for *Sarcocystis falcatula. International Journal for Parasitolology: Parasites Wildlife, 17*, 230-238. doi: 10.1016/j.ijppaw.2022.03.002

Miescher, E. (1843). Uber eigenthumliche schlãuche in den muskeln einer hausmaus. Berl. Verh. Naturforsch. Ges. Basel., Augsburg, 5: 198-202, 1843 apud Levine, N. D. (1977) Nomenclature and of Sarcocystis in the ox and sheep and of fecal coccidia of the dog and cat. *Journal of Parasitology, 63*(1), 38-51.

Moré, G., Abrahamovich, P., Jurado, S., Bacigalupe, D., Marin, J. C., Rambeaud, M., Venturini, L., & Venturini, M. C. (2011). Prevalence of *Sarcocystis* spp. in Argentinean cattle. *Veterinary Parasitology, 177*(1-2), 162-165. doi: 10.1016/j.vetpar.2010.11.036

Olias, P., Olias, L., Lierz, M., Mehlhorn, H., & Gruber, A. D. (2010). *Sarcocystis calchasi* is distinct to *Sarcocystis columbae* sp. nov. from the wood pigeon (*Columba palumbus*) and *Sarcocystis* sp. from the sparrow hawk (*Accipiter nisus*). *Veterinary Parasitology, 171*(1-2), 7-14. doi: 10.1016/j.vetpar.2010.03.021

Parmentier, S. L., Maier-Sam, K., Failing, K., Gruber, A. D., & Lierz, M. (2019). High prevalence of *Sarcocystis calchasi* in racing pigeon flocks in Germany. *PloS One, 14*(4), e0215241-x. doi: 10.1371/journal.pone.0215241

Prakas, P., Oksanen, A., Butkauskas, D., Sruoga, A., Kutkienė, L., Švažas, S., & Butkauskas, D. (2014). Identification and intraspecific genetic diversity of *Sarcocystis rileyi* from ducks, *Anas* spp., in Lithuania and Finland. *Journal for Parasitology, 100*(5), 657-661. doi: 10.1645/13-395.1

Rêgo, W. M. F., Costa, J. G. L., Baraviera, R. C. A., Pinto, L. V., Bessa, G. L., Lopes, R. E. N., Silveira J. A. G. da, & Almeida Vitor, R. W. (2021). Sarcocystidae in wild birds of southeastern Brazil. *Revista Brasileira de Parasitologia Veterinária, 30*(1). doi: 10.1590/S1984-296120201094

Yang, Z., Zuo, Y., Yao, Y., Chen, X., Yang, G., & Zhang, Y. (2001). Analysis of the 18S rRNA genes of Sarcocystis species suggests that the morphologically similar organisms from cattle and water buffalo should be considered the same species. *Molecular and Biochemical Parasitology, 115*, 283-288. doi: 10.1016/s0166-6851(01)00283-3