A review of *Sarcocystis* spp. shed by opossums (*Didelphis* spp.) in Brazil

Revisão sobre Sarcocystis spp. excretados por gambás (Didelphis spp.) no Brasil

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Abstract

South American opossums are the definitive hosts of Sarcocystis neurona, Sarcocystis falcatula, Sarcocystis speeri and Sarcocystis lindsayi. The sporocysts of these species of Sarcocystis are morphologically similar and methods like infectivity and pathogenicity for intermediate hosts (immunodeficient mice and psittacine birds) and molecular tools are used for identification. Opossums are synanthropic wild animals, and widely distributed in Brazilian territory. Previous studies have shown high environmental contamination with S. neurona sporocysts in several Brazilian regions. This paper reviews information on Sarcocystis spp. shed by various opossum species and its occurrence in Brazil.

Keywords: *Sarcocystis* spp.. Brazil. *Didelphis* spp.. *Sarcocystis neurona. Sarcocystis falcatula*.

Resumo

Os gambás Sul-americanos são os hospedeiros definitivos de *Sarcocystis falcatula, Sarcocystis neurona, Sarcocystis speeri* e *Sarcocystis lindsayi*. Estas espécies de *Sarcocystis* são morfologicamente similares, mas podem ser distinguidas por sua patogenicidade e infectividade em hospedeiros intermediários (aves e camundongos imunodeficientes) e técnicas moleculares. Os gambás são animais silvestres e sinantrópicos e amplamente distribuídos no território nacional. Estudos anteriores demonstraram uma alta contaminação ambiental com esporocistos de *S. neurona* em diversas regiões brasileiras. Este artigo revisa informações sobre *Sarcocystis* spp. excretados por gambás e sua ocorrência no Brasil.

Palavras-chave: *Sarcocystis* spp.. Brasil. *Didelphis* spp.. *Sarcocystis neurona. Sarcocystis falcatula*.

INTRODUCTION AND HISTORY

The genus *Sarcocystis* belongs to the protozoan phylum Apicomplexa. Members of this phylum cause significant human and animal disease including malaria, coccidiosis, and toxoplasmosis. Members of the genus *Sarcocystis* are closely related to *Toxoplasma gondii* and other tissue cyst-forming-parasites. They have evolved to have an obligatory heteroxenous life cycle consisting of a definitive host that is a predator or omnivore and an intermediate host that is an omnivore or herbivore. Miescher first reported *Sarcocystis* in 1843 as "milky white threads" in the skeletal muscle of a mouse in Switzerland. In 1882, Lankester introduced the genus name *Sarcocystis* (in Greek, *Sarkos* = flesh, *kystis* = bladder) (DUBEY et al., 2015a) based on the appearance and location of the characteristic muscle cyst stage now known as a sarcocyst. It was not until 1972 that the mandatory two-host life cycle was described in experimentally infected animals and the identity of the *Sarcocystis* oocyst was determined. It now became apparent that coccidia named "*Isospora bigemina*" observed in dog and cat feces were actually *Sarcocystis* species (LINDSAY et al., 1997).

The definitive host becomes infected after ingesting raw muscle (occasionally neural tissue) that has mature

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sarcocysts containing asexual stages known as bradyzoites. Bradyzoites are released from the sarcocyst by digestion in the stomach and intestine. Bradyzoites penetrate the mucosa of the small intestine and transform into male (micro) and female (macro) gamonts in the lamina propria. After fertilization, a thin oocyst wall develops around the zygote and the unsporulated oocyst is formed. Sporulation of the oocysts occurs in the lamina propria, resulting in two sporocysts each containing four sporozoites. Because the oocyst wall is thin, it often ruptures while exiting the lamina propria releasing sporocysts into the intestinal lumen that are passed in feces. The prepatent period for most *Sarcocystis* species is between seven to 14 days, while the patent period is a month or longer (DUBEY et al., 2015a).

The intermediate host becomes infected by ingesting sporocysts from the environment while grassing or in contaminated food or water. Sporozoites excyst from sporocysts in the small intestine and migrate to endothelial cells in vessels or other cells, where they undergo asexual reproduction by schizogony and produce merozoites. The number of generations of schizogony and the type of host cell infected can vary with each species of *Sarcocystis*. Merozoites liberated from the terminal generation of schizogony penetrate striated muscle (occasionally neural) cells and initiate sarcocyst formation. Merozoites transform into metrocytes that divide by endodyogeny and form bradyzoites. As the sarcocyst matures, it is filled with bradyzoites, which are infectious for the definitive host (DUBEY et al., 2001c, 2015a).

The number and distribution of sarcocysts may vary. This is influenced by several factors, such as the number of sporocysts ingested, the species of *Sarcocystis*, the species of the intermediate host, and its immune status. The majority of sarcocysts develop in skeletal muscle; however they can occasionally be found in smooth muscle (*S. mucosa*) and the central nervous system (DUBEY et al., 1989).

There are about 180 valid named *Sarcocystis* species; however, the complete life cycles are known only for 26 of them (DUBEY et al., 2015a). Molecular methods are gaining importance in naming new species but morphological descriptions of sarcocysts and animal feeding studies are also important in validating a new species.

SARCOCYSTIS SPP.

South American opossums are the definitive hosts of *Sarcocystis neurona*, *Sarcocystis falcatula*, *Sarcocystis speeri* (DUBEY; LINDSAY, 1998; DUBEY; LINDSAY, 1999) and *Sarcocystis lindsayi* (DUBEY et al., 2001d).

Bioassay is used to aid in species identification of opossum transmitted *Sarcocystis* because it is not possible to identify the species using morphology of sporocysts. Bioassay separates the sample into avian infective (*S. falcatula*, *S. lindsayi*, *S. falcatula* Arg) and mammalian infective (*S. neurona*, *S. speeri*). All avian infective species form sarcocysts in budgerigars while *S. speeri* will form sarcocysts in immunodeficient mice and *S. neurona* will cause encephalitis but not make sarcocysts (MARSH et al., 1997; DUBEY; LINDSAY, 1998).

Sarcocystis neurona

Sarcocystis neurona is the main cause of equine protozoal myeloencephalitis (EPM). The clinical syndrome associated with EPM was first described in the 70s. In 1991, the parasite was first isolated from neural tissue of infected horses, described as a *Sarcocystis* species, and named by Dubey et al. (1991). *S. neurona* was first isolated in Brazil in 2001 from the intestines of two opossums (*D. albiventris*) from the state of São Paulo (DUBEY et al., 2001a).

The North American opossum (*D. virginiana*) was proposed as the definitive host of the parasite in 1995, based on comparisons between cultivated merozoites and sporocysts from the intestine of opossums through the analysis of their SSURNA (FENGER et al., 1995).

In 1997, Fenger et al. (1997) induced clinical EPM in *S. neurona* negative horses fed with sporocysts from opossums. The life cycle was concluded when laboratory-raised opossums fed with muscle tissue of experimentally infected cats (*Felis catus*) shed sporocysts of *S. neurona* (DUBEY et al., 2000b).

The North American opossum (*Didelphis virginiana*) and the South American opossum (*D. albiventris*) are *S. neurona* known definitive hosts. Several other animal species are its intermediate or aberrant hosts. Laboratory-raised opossums excreted sporocysts after feeding naturally infected skunk, armadillo, raccoon and sea otter muscles (CHEADLE et al., 2001, 2002; DUBEY et al., 2001e; 2001f; TANHAUSER et al., 2001).

Horses are considered aberrant hosts for *S. neurona* since mature sarcocysts are not found in tissue of horses. In 2005, a case of symptomatic EPM was reported in a four-month-old foal from Michigan, USA. Mature schizonts were found in its nervous system and the tongue. *S. neurona* was confirmed by genetic, immunologic and morphologic analysis, thus suggesting that occasionally the horse might be an intermediate host for the parasite (MULLANEY et al., 2005).

EPM affects horses of all breeds, sex and ages. However, it is more common among animals of the ages one to six years and the breeds: Thoroughbred (FAYER et al., 1990), American Trotter (ROONEY et al., 1970) and American Quarter Horse (FAYER et al., 1990). The predominant form of EPM is a progressive asymmetrical ataxia that may be accompanied by focal muscle atrophy (ROONEY et al., 1970).

Clinical signs of EPM can vary from acute to insidious onset of focal or multifocal signs of neurologic disease involving the brain, brainstem, spinal cord or any combination of these areas of the CNS. Affected horses normally display a gradual progression of the clinical signs. In some cases, however, the gradual onset might be replaced by the sudden worsening of the severity of the clinical disease, resulting in decubitus. The clinical signs vary due to the random location of the parasite in the white and gray matter (DUBEY et al., 2001c).

Overall, infected horses remain alert and responsive with vital signs within normality; some may present depression and weight loss. Neurological examination normally reveals asymmetrical ataxia, weakness and spasticity of the four limbs. Areas of hyporeflexia, hypoalgesia or complete sensory loss are frequently present (MACKAY et al., 2000; DUBEY et al., 2001c).

The frequency of horses with *S. neurona* antibodies in the United States has ranged from 6.5% to 89.2% by Western Blot and from 26% to 27.6% by indirect fluorescent antibody test (IFAT). In Argentina, the frequency of horses presenting *S. neurona* antibodies has ranged from 26.1% to 35.5% by Western Blot. In Brazil, it has ranged from 33.7% to 69.6% by Enzyme Linked Immuno Sorbent Assay (ELISA). The frequency of horses with *S. neurona* antibodies by ELISA in Costa Rica was 42.4% and 48.5% in Mexico (DUBEY et al., 2015a).

Sarcocystis falcatula

Stiles first reported and named *Sarcocystis falcatula* from North America in 1893; however its life cycle and intermediate hosts were described years later (BOX; SMITH, 1982; BOX et al., 1984). *S. falcatula* was first isolated in Brazil in 2000 from opossums (*D. albiventris*) from Jaboticabal, São Paulo (DUBEY et al., 2000a).

Sarcocystis falcatula can cause severe disease in several members of different orders of birds, namely Passeriformes, Psittaciformes, Columbiformes, Strigiformes and Falconiformes. Clinical disease is commonly observed in outbreaks of acute pulmonary sarcocystosis in zoos (SMITH et al., 1990; HILLYER et al., 1991; CLUBB; FRENKEL, 1992; PAGE et al., 1992; WÜNSCHMANN et al., 2009, 2010).

Birds become infected by the ingestion of sporocysts in food or water contaminated with opossum feces. They also can be infected by ingesting paratenic hosts, such as flies and cockroaches that are carrying sporocysts inside or on their bodies (CLUBB;

FRENKEL, 1992). The sporozoites are released in the small intestine of the bird, where they migrate to the blood stream in order to invade different tissues.

Schizonts undergo two asexual generations in the endothelium. The first reproductive phase occurs in endothelial cells of the arterioles and the second in endothelial cells of capillaries and venules in most organs of the host. The lungs are the primary organs affected, but pathologic changes may also be observed in the liver, spleen, kidneys, intestines, and skeletal muscles (SMITH et al., 1987).

Studies with budgerigars (*Melopsittacus undulatus*) fed sporocysts of *S. falcatula* have shown that the growth of schizonts causes hypertrophy of endothelial cells in pulmonary capillaries, veins and venules that leads to narrowing of the lumen resulting in obstruction of blood in the affected areas of the lung. When the merozoites are released, they can invade other endothelial cells, producing more schizonts and merozoites. The last generation of merozoites migrate to skeletal muscle and produce sarcocysts (SMITH et al., 1987).

The prevalence of mortality resulting from *S. falcatula* varies among different species of birds. The disease in Old World psittacine birds is generally hyperacute and leads to high mortality as a result from severe pulmonary lesions. Species that survive the pulmonary phase of the disease are those that co-evolved with the presence of opossums in the environment (BOX; SMITH, 1982; HILLYER et al., 1991; PAGE et al., 1992).

Pulmonary sarcocystosis is an acute disease and many animals do not show clinical signs before sudden death. Other birds may demonstrate weakness, dyspnea, neurologic abnormalities, or partial or total anorexia. Macroscopic lesions can include pulmonary edema, congestion, and hemorrhage; splenomegaly; and hepatomegaly. Microscopic findings can include schizonts within the endothelium of pulmonary capillaries and pneumonia. Schizogony may result in chronic active hepatitis as well as interstitial myocarditis, myositis, splenitis, nephritis, and

encephalitis (CLUBB et al., 1986; NEIIJU et al., 1989; HILLYER et al., 1991; PAGE et al., 1992).

S. falcatula has been grown in cell cultures inoculated with merozoites from infected lung tissue and from sporozoites obtained from opossum feces. *S. falcatula* merozoites developed to mature schizonts in bovine turbinate cell culture by three to four days postinoculation (PI) and *S. neurona* merozoites developed to mature schizonts by three days PI, being slightly different than that observed for *S. falcatula* (LINDSAY et al., 1999).

Sarcocystis speeri

The prevalence of *S. speeri* in opossums has not been examined in many studies. *S. speeri* sarcocysts in muscle from interferon gamma gene knockout (KO) mice fed sporocysts from a South American opossum (*D. albiventris*) produced patent infections in North American opossums (*D. virginiana*) (DUBEY et al., 2000c).

The natural intermediate host for *S. speeri* is presently not known. It is transmissible to KO mice; schizonts and merozoites are found in many organs, including liver, brain and uterus of KO mice. The last generation of merozoites produces sarcocysts in skeletal muscle. Asexual stages have been grown in cell cultures seeded with merozoites from the liver of a KO mouse infected with an isolate from *D. albiventris* from Argentina. Merozoites from cell culture are not infective to KO mice (DUBEY et al., 2000d).

Sarcocystis lindsayi

Sarcocystis lindsayi has been found in the feces of both *Didelphis albiventris* and *Didelphis aurita* (DUBEY et al., 2001d; STABENOW et al., 2012). The natural intermediate host for *S. lindsayi* is unknown; budgerigars (*Melopsittacus undulatus*) were found experimentally to be an intermediate host. Budgerigars fed *S. lindsayi* sporocysts from opossum feces died of acute sarcocystosis, similar to infection with *S. falcatula* and sarcocysts were found in birds that

survived acute infection. Schizonts and merozoites have been grown in cell culture providing a source of DNA for molecular studies.

MOLECULAR STUDIES

The North American opossum (*D. virginiana*) and the three species of South American opossums (*D. albiventris, D. marsupialis, D. aurita*) are hosts for at least four species of *Sarcocystis, S. neurona, S. falcatula* and *S. lindsayi* and *S. speeri.*

Sarcocystis lindsayi can be differentiated from *S. falcatula* and *S. neurona* through genetic variation in the nuclear large subunit ribosomal RNA gene, the internal transcribed spacer (ITS-1), and each of two other genetic loci (DUBEY et al., 2001d). Recently, *S. speeri* was molecularly characterized with three nuclear (18S rRNA, 28S rRNA and ITS1), and two mitochondrial loci (Cox1 and Cytb) and then the mitochondrial loci of *S. speeri* isolate were sequenced and compared with homologues in other species of *Sarcocystis*. The results showed that *S. speeri* is very closely related to other species of *Sarcocystis* that employ opossums as definitive hosts (DUBEY et al., 2015c).

The very close similarity in the 18S ribosomal DNA of *S. falcatula* and *S. neurona* led to the suggestion that these taxa may correspond to a single species (DAME et al., 1995). However, subsequent analyses of more variable genetic loci provided support for their differentiation (MARSH et al., 1999; TANHAUSER et al., 1999).

The first report of molecular differentiation between *S. neurona* and *S. falcatula* was obtained from molecular markers from RAPD (rapid amplified polymorphic DNA) and the ITS (TANHAUSER et al., 1999). Restriction endonuclease digestion of a genetic locus, termed 33/54, was proposed to differentiate *S. neurona* from *S. falcatula*: a DraI site was suggested to be present in *S. neurona* but absent in *S. falcatula* and a HinfI site present in *S. falcatula* but not in *S. neurona* (TANHAUSER et al., 1999). Sporocysts isolated from

the feces of seven out of nine opossums were categorized as either *S. neurona* or *S. falcatula* based on this genetic characterization (TANHAUSER et al., 1999). However, two isolates from naturally infected opossums (1085, 1086) possessed both DraI and HinfI sites, indicative of either mixed infections or a lineage with a novel genotype.

In 2001b, Dubey et al. used the 33/54 molecular marker described by Tanhauser et al. (1999) to analyze isolates of *Sarcocystis* spp. from feces of opossums and found a mixed profile between *S. falcatula* and *S. neurona*. However, the isolates (n = 9) were pathogenic to budgerigars (*Melopsittacus undulatus*) and were identified as *S. falcatula*-like organisms. Four isolates were identical to *S. falcatula* from a *D. albiventris* from Argentina but distinct from the *S. falcatula* strain Cornell 1 (LINDSAY et al., 1999) and the *S. falcatula*-like isolate from a *D. albiventris* from Jaboticabal, Brazil (isolate B100 = *S. lindsayi*). By the 33/54 marker, the isolate B100 has a profile identical to *S. neurona*, even though it is a strain from a budgerigar orally inoculated with sporocysts from a *D. albiventris*. The lack of infectivity of B100 for KO mice is conclusive it is not *S. neurona*.

Molecular studies with the 25/396 marker revealed that sporocysts of *S. neurona* from *D. albiventris* from Brazil are evolutionary related to each other but distinct from sporocysts of *S. neurona* from North America (ROSENTHAL et al., 2001; ELSHEIKHA et al., 2005).

In a previous study, 34 isolates of *Sarcocystis* spp. from different regions (25 isolates of *S. neurona* from North America, two isolates of *S. neurona* from South America, two isolates of *S. falcatula* from North America and 5 isolates of *S. falcatula* from South America) were characterized with polymorphic microsatellite markers. The multi-locus analysis revealed three genetically distinct groups: *S. neurona* from North America, *S. falcatula* from South America and *S. neurona* from South America along with *S. falcatula* of North America (ASMUNDSSON et al., 2006). The results obtained support the distinction

between North American isolates, which comprehend one genetic lineage, and South American isolates. However, some doubts were raised regarding the classification of the South American isolates of *S. neurona*, because two isolates of *S. neurona* from South America appeared to possess a common ancestor with two isolates of *S. falcatula* (ASMUNDSSON et al., 2006).

A great variability in surface antigens genes (SAGs) present on sporozoites in sporocysts of *Sarcocystis* spp. was found in a previous study in opossums from the south region of Brazil (MONTEIRO et al., 2013). A high number of alleles were found for each gene (*SAG2*, *SAG3* and *SAG4*) and three distinct lineages were defined: two lineages genetically related to *S. falcatula* and one lineage genetically similar to *S. neurona* (*neurona*-like). The authors proposed the use of more conserved markers for a better understanding of the phylogenetic relations between *Sarcocystis* spp. isolates shed by marsupials of the genus *Didelphis*.

DIDELPHIS SPP.

South American marsupials belong to the order Didelphimorphia Gill, 1872, which comprises most of the living American marsupials. Four families are recognized within this order (HERSHKOVITZ, 1992): Marmosidae, Caluromyidae, Glironiidae and Didelphidae. The family Didelphidae currently has 19 genera with 95 recognized species (GARDNER, 2007). Only four *Didelphis* species are found in South America.

Didelphis is derived from the Greek word *di* (two) and *delphys* (womb) in reference to the reproductive tract of females that have branched uteri or single uteri (amphidelphic). Opossums are extremely adaptable to different environments and are considered evolutionary successful (GARDNER, 2007).

The species within the *Didelphis* genus are omnivorous and are found in several habitats, such as forests, areas near woods and streams. They are essentially terrestrial but can climb onto obstacles

rather easily. They are nomads, with lonely habits making it difficult to determine a territory since they can roam long distances and remain in a particular area for relatively short periods, which facilitates the dissemination of pathogens. Known predators include birds of prey and all carnivorous (FINNIE, 1986).

The *Didelphis* genus is divided into two super-groups: *D. marsupialis* (black-eared opossum) group with two allopathic species, *D. marsupialis* Linnaeus, 1758 and *D. aurita* Wied-Neuwied, 1826; *D. albiventris* (white-eared opossum) group with three species *D. albiventris* Lund, 1840, *D. pernigra* J.A. Allen, 1900 and *D. imperfecta* Mondolfi and Pérez-Hernández 1984. Several species and sub-species were named based on minor variations, however, insufficient for a formal recognition. The only other living species recognized in this genus is *D. virginiana* Kerr, 1792, found from Canada to Costa Rica (GARDNER, 2007).

Regarding their geographical distribution, records of sympatry are rare and uncommon (CERQUEIRA, 1985; GARDNER, 2007). However, the geographical overlap seems to occur in areas disturbed by humans (GARDNER, 2007).

The distinction between *D. aurita* and *D. albiventris* is usually made by their morphological characteristics, such as ear color and teeth patterns. Recently molecular studies were conducted with mitochondrial genes confirming sympatry between *D. albiventris* and *D. aurita* in urban fragments of the Atlantic Forest. Cervantes et al. (2010) also reported sympatry between *D. marsupialis* and *D. virginiana* in Mexico.

Geographical distribution

Didelphis albiventris occurs from northeastern and central Brazil (Caatinga and Cerrado habitats, enclaves, and transition zones) into central and southern Paraguay, east in the state of Rio Grande do Sul, Brazil, and south into Uruguay and Argentina as far south as Buenos Aires province in the east and the Monte Desert in the west. *D. albiventris* also is widely distributed in eastern Bolivia (CERQUEIRA, 1985), inhabiting open and deciduous forest type from

northeastern Brazil to mid-Argentina, including areas of low and irregular rainfall such as the Caatinga and Monte Desert habitats (CERQUEIRA, 1985). It is replaced in the wetter Atlantic and Araucaria forests by *D. aurita*, in the Amazonian forests by *D. marsupialis*, and on the slopes of the Andes by *D. pernigra* (GARDNER, 2007).

Didelphis aurita is found in eastern Brazil in the Tropical Atlantic and Araucaria Forest domains, and southward within these habitats to southeastern Paraguay and northeastern Argentina (Misiones). Populations of black-eared opossums still occur in the remnants of the Atlantic Forest of Alagoas and Pernambuco, but are rare (CERQUEIRA; LEMOS, 2000; BROWN, 2004).

Didelphis marsupialis occurs in Trinidad and Tobago, the Guianas, and the greater Amazon basin, including the wet forest habitats of the eastern Andean slopes of Venezuela, Colombia, Ecuador, Peru, and Bolivia. Drier regions, such as Caatinga, Cerrados, and Chaco, limit its range to the east and south. Elsewhere, it is found in Central America and Mexico (GARDNER, 2007).

Didelphis imperfecta is known from isolated populations from south of the Orinoco in Venezuela and adjacent Brazil, as well as in Surinam and French Guiana (GARDNER, 2007).

Didelphis pernigra is found in the forested slopes of the Andes from northwestern Venezuela and Colombia through Ecuador and Peru into Bolivia, and possibly as far as the Andes of northern Argentina (GARDNER, 2007).

EPIDEMIOLOGY IN BRAZIL

The first report of EPM in Brazil was performed in 1986 in southern Brazil when the parasite was observed in the spinal cord of a horse with neurological signs (BARROS et al., 1986). In 1992, *S. neurona* was reported in two horses with ataxia based on *immunohistochemistry* of tissues (MASRI et al., 1992). In 1999, a study with 101 horses revealed 36% of seropositivity for *S. neurona* in horses from São Paulo, Rio de Janeiro and Rio Grande do Sul, Brazil (DUBEY et al., 1999). However, it was only in 2001 that the parasite was first isolated from the intestines of an opossum (*D. albiventris*) from São Paulo, Brazil (DUBEY et al., 2001a). Since then, several studies of occurrence of *Sarcocystis* spp. from Brazil have been reported.

Equids

Antibodies against SnSAG4 were demonstrated in 669 (69.6%) from 961 horses tested from ten different states in Brazil (São Paulo, Minas Gerais, Paraná, Santa Catarina, Rio Grande do Sul, Bahia, Rondônia, Mato Grosso, Mato Grosso do Sul and Goiás), indicating a high environmental contamination with *S. neurona* sporocysts (HOANE et al., 2006). From 1998-2006, 61 horses were evaluated in the UFPel Veterinarian Hospital in Rio Grande do Sul, Brazil. A total of 23 horses were diagnosed with clinical EPM, 18 (78%) horses were positive for *S. neurona* by Western blot (LINS et al., 2012). Also from Rio Grande do Sul, Brazil, a study with 181 mares and their newborns revealed antibodies against *S. neurona* in 33.7% of mares and 6.6% of pre-colostral foals by ELISA (PIVOTO et al., 2014).

A recent study reported the results of serological survey of antibodies against *S. neurona* in sera of 333 donkeys from the northeastern region of Brazil (Alagoas, Paraíba, Pernanbuco, Piauí and Rio Grande do Norte). Antibodies to *S. neurona* were found in ten donkeys (3%) of the samples tested by IFAT and in 69 donkeys (21%) by the direct agglutination test (SAT) (GENNARI et al., 2016). The SAT and IFAT results for *S. neurona* showed a poor concordance in this study, which might be due to the lack of a comprehensive study of sensitivity and specificity of the SAT in equids (DUBEY et al., 2001a, 2015b) and lack of knowledge

about cross-reactivity in both tests with other protozoa that can infect donkeys (GENNARI et al., 2016).

Felids

Domestic cats are one of the confirmed natural intermediate hosts of the parasite and recently the first report of antibodies against *S. neurona* in Brazilian cats was demonstrated in Bahia, Brazil, where 272 feline serum samples were analyzed by IFAT. Positivity was detected in 4.0% (11/272) of the tested samples (MENESES et al., 2014). The same method was also used to investigate the presence of antibodies against *S. neurona* in the sera of 11 free-living jaguars (*Panthera onca*) in two protected areas in the Pantanal region of Mato Grosso state, Brazil. Eight (72.7%) jaguars showed seropositivity for *S. neurona* (ONUMA et al., 2014).

Birds

The first documentation of *Sarcocystis* infection in psittacines and a pigeon from Brazil was performed in Belo Horizonte Zoo in 2008. *Sarcocystis* spp. was confirmed by immunohistochemistry in different species of captive psittacines and in a Luzon bleeding-heart pigeon (*Gallicolumba luzonica*) in the zoological collection. The majority of the birds was found dead and had exhibited no previous clinical signs. Grossly, pulmonary congestion and edema were the most common findings (ECCO et al., 2008).

In 2009, a total of 47 psittacines birds housed in a bird park in Foz do Iguaçu, Paraná, Brazil, died within a 15-month period during an outbreak of infection by *S. falcatula*. Sarcocystosis was considered the cause of death of 38 of the 47 examined psittacine birds. Sixteen species of psittacine birds, including both Old and New World species, were infected with *Sarcocystis* species. The Old World species were the most affected birds and developed the acute pulmonary form of the disease (GODOY et al., 2009).

However, confirmation of *S. falcatula* infection is

needed since *S. calchasi* was diagnosed to cause neurological signs in pigeons and psittacine birds (OLIAS et al., 2010, 2014).

Opossums

From 2005-2006, the occurrence of *Sarcocystis* spp. in three regions of the state of São Paulo was investigated. The study employed 66 *D. aurita* and 32 *D. albiventris* and flotation-centrifugation in sucrose solution was used in the isolation of *Sarcocystis* spp. of the small intestine and feces. Six samples from *D. aurita* were positive for *Sarcocystis* spp. (9.1%) and none of the *D. albiventris* were positive for *Sarcocystis* spp. The lower occurrence of *Sarcocystis* spp. infection could be due to the age and number of the individuals sampled, and low sensitivity of the method used (CASAGRANDE et al., 2009).

The intestinal scraping technique was performed in 19 opossums (*D. albiventris*) from Rio Grande do Sul, Brazil. All samples were positive for *Sarcocystis* spp., but only one proved to be *S. neurona* (LINS et al., 2011).

Sarcocystis lindsayi was first isolated in Brazil in 2001. It was obtained from the lungs and muscles of budgerigars (*Melopsittacus undulatus*) fed sporocysts from a naturally infected opossum (*Didelphis albiventris*) from Jaboticabal, São Paulo (DUBEY et al., 2001d). In 2012, *S. lindsayi* was isolated from the lungs and muscles of budgerigars fed sporocysts from a naturally infected opossum (*Didelphis aurita*) from Seropédica, Rio de Janeiro, establishing *D. aurita* as a new definitive host for this parasite (STABENOW et al., 2012).

Other hosts

In a study with capybaras (*Hydrochoerus hydrochaeris*) from São Paulo, Brazil, only two (3%) of the 63 capybara samples, examined by IFAT, had antibodies to *S. neurona*, demonstrating a low frequency of occurrence of the parasite in these animals (VALADAS et al., 2010).

FINAL CONSIDERATIONS

Opossums are synanthropic wild animals, frequently found in urban areas due to the growing fragmentation of forest remnants on the outskirts of cities. The advances in agriculture and livestock farming by human intervention in ecosystems have led to a closer contact with wild animals, which could facilitate the dissemination of infectious agents and parasites. A high prevalence of *Sarcocystis* infection and exposure has been demonstrated in birds and horses in Brazil.

Genotypic studies on *Sarcocystis* spp. have indicated differences between populations from North America and South America. Moreover, a recent study has shown high genetic diversity among *Sarcocystis* spp. samples shed by opossums in Brazil (MONTEIRO et al., 2013).

Because of the limited amount of information within national literature, additional studies examining opossum transmitted *Sarcocystis* spp. in Brazil are needed. This will help enhance our knowledge of the population genetic structure of *Sarcocystis* spp. in these hosts. Studies examining the interactions of opossums with and the roles of the various intermediate hosts for *Sarcocystis* will bring knowledge about the population biology and the relationships occurring in the transmission of these important pathogens.

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