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INFECÇÃO NATURAL POR *SARCOCYSTIS* SPP. EM GATOS DO SUL DO BRASIL: DESENVOLVIMENTO DE CISTOS MUSCULARES, EPIDEMIOLOGIA E SOROPREVALÊNCIA

Santa Maria, RS, Brasil
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Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Medicina Veterinária, Área de concentração em Patologia e Patologia Clínica Veterinária, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Medicina Veterinária.**

Orientador: Mariana Martins Flores

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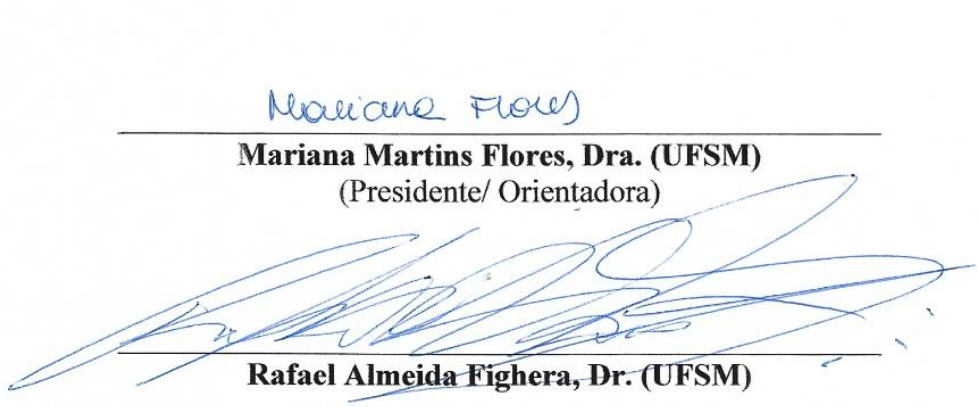

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Gatos são poemas ambulantes. Pisam na Terra como se estivessem no céu e seus olhos atravessam as fronteiras dos mundos invisíveis.

(Roseana Murray)

RESUMO

INFECÇÃO NATURAL POR *SARCOCYSTIS* SPP. EM GATOS DO SUL DO BRASIL: DESENVOLVIMENTO DE CISTOS MUSCULARES, EPIDEMIOLOGIA E SOROPREVALÊNCIA

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Sarcocystis spp. são protozoários pertencentes ao filo Apicomplexa capazes de infectar uma ampla variedade de animais. Dentre as diversas espécies que compõem o gênero *Sarcocystis*, há uma notável variabilidade em relação aos hospedeiros envolvidos, entretanto, a formação de cistos teciduais no hospedeiro intermediário (HI) é uma característica inerente a todas as espécies desse protozoário. Felinos são classicamente reconhecidos como hospedeiros definitivos para determinadas espécies de *Sarcocystis*, porém, estudos recentes demonstram que esses animais podem desempenhar o papel de HI natural para *S. felis* e *S. neurona.* Há relatos de gatos que adoeceram devido à infecção por *Sarcocystis* spp. no sistema nervoso central (SNC). Na grande maioria dos casos, *S. neurona* era a espécie envolvida e os animais apresentaram sinais neurológicos graves. No Brasil, a importância do gato doméstico como HI natural no ciclo de qualquer espécie de *Sarcocystis* permanece desconhecida. Com base nisso, é importante investigar a ocorrência de *Sarcocystis* spp. em tecidos de gatos domésticos brasileiros, a fim de verificar se esses animais podem atuar como HI naturais para alguma espécie de *Sarcocystis* em nosso território. Em vista disso, este trabalho tem o objetivo de avaliar a presença de sarcocistos musculares em gatos submetidos à necropsia com posterior identificação da espécie envolvida e, em paralelo, avaliar a soroprevalência de *Sarcocystis* spp. na população felina do Sul do Brasil. Para verificar a presença de sarcocistos musculares, 100 gatos submetidos à necropsia de rotina tiveram fragmentos de determinados grupos musculares coletados para análise histológica e, realização de reação de polimerase em cadeia (PCR). Dados epidemiológicos (sexo, idade, raça e *status* reprodutivo) e informações associadas ao uso prévio de drogas com potencial imunossupressor, ao *status* de infecção para o vírus da imunodeficiência felina (FIV) e o vírus da leucemia felina (FeLV) e ao acesso à rua foram coletadas do histórico clínico. A causa de morte ou razão para eutanásia foi obtida através do laudo de necropsia. Casos positivos na histologia e PCR foram encaminhados para sequenciamento genético. Adicionalmente, os soros de 497 gatos atendidos rotineiramente no Hospital Veterinário Universitário (HVU) foram submetidos à técnica de reação de imunofluorescência indireta (RIFI), em busca de anticorpos para *Sarcocystis* spp. Dos 100 gatos analisados histologicamente, 5 (5/100; 5%) tiveram sarcocistose muscular e a única espécie envolvida em todos os casos foi confirmada como *S. neurona* pela PCR. Na análise sorológica, 24 gatos (24/497; 4,82%) apresentaram anticorpos anti-*Sarcocystis* spp. Nossos resultados demonstram que há circulação de *Sarcocystis* spp. entre a população felina estudada e confirmam que gatos domésticos são capazes de desempenhar papel de hospedeiro intermediário natural para *S. neurona*. Baseado em nossos dados, amplia-se o conhecimento da infecção natural por *S. neurona* em gatos, e recomenda-se a inclusão de *Sarcocystis* sp. como diagnóstico diferencial em doenças neurológicas de gatos da nossa região.

Palavras-chave: Sarcocistose muscular. Hospedeiro intermediário. Felinos.

ABSTRACT

NATURALLY OCCURRING *SARCOCYSTIS* INFECTION IN CATS FROM SOUTHERN BRAZIL: DEVELOPMENT OF MUSCULAR CYSTS, EPIDEMIOLOGY AND SEROPREVALENCE

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Sarcocystis spp. are protozoa belonging to the phylum Apicomplexa capable of infecting a wide variety of animals. Among the several species from the genus *Sarcocystis*, there is a remarkable variability in relation to the involved hosts, however, the formation of tissue cysts in the intermediate host is an inherent characteristic of all species of this protozoan. Domestic cats are classically recognized as definitive hosts for certain species of Sarcocystis, but recent studies show that these animals play a role as natural intermediate host (IH) for *S. felis* and *S. neurona*. Some cats have developed central nervous system (CNS) infection due to *S. neurona*. In the majority of cases, S. neurona was the species involved and the animals developed severe neurologic signs. In Brazil, the importance of the domestic cat as natural IH for any species of *Sarcocystis* remains unknown. Based on this, it is important to investigate the occurrence of *Sarcocystis* spp. in Brazilian domestic cat tissues, in order to verify if these animals can act as natural IH for some species of Sarcocystis in our territory. Therefore, this study aims to evaluate the presence of muscle sarcocysts in cats submitted to necropsy with subsequent identification of the involved species and to evaluate the seroprevalence of *Sarcocystis* spp. in the feline population of Southern Brazil as well. To verify the presence of muscle sarcocysts, 100 cats submitted to routine necropsy had fragments of certain muscle groups collected for histological analysis and polymerase chain reaction (PCR). Epidemiological data (gender, age, race and reproductive status) and information associated with previous use of drugs with immunosuppressive potential, feline immunodeficiency virus (FIV)/feline leukemia virus (FeLV) infection *status* and outdoor access were collected from the clinical history. The cause of death or reason for euthanasia was obtained through the autopsy report. Positive histology and PCR positive cases were referred for genetic sequencing. Additionally, sera from 497 cats attending to the Hospital Veterinário Universitário (HVU) were submitted to the indirect fluorescence antibody test (IFAT) technique for antibodies anti-*Sarcocystis* spp. detection. Of the 100 cats analyzed, 5 (5/100; 5%) had muscular sarcocystosis and the only species involved in all cases was confirmed as *S. neurona* by PCR. In the serological analysis, 24 cats (24/497; 4.82%) presented anti-*Sarcocystis* spp antibodies. Our results confirm that *Sarcocystis* spp. circulates among the studied feline population and confirm that domestic cats are able to play a role as natural intermediate host for *S. neurona*. Our study aids to what is already known concerning natural *S. neurona* infection in cats. Additionally, *S. neurona* should be considered a differential diagnosis in cases of CNS involvement in cats in our region.

Keywords: Muscular sarcocystosis. Intermediate host. Felines.

SUMÁRIO

1 INTRODUÇÃO

Sarcocystis é um protozoário intracelular obrigatório, pertencente ao filo Apicomplexa e à família Sarcocystidae (MAC et al., 2018). Outros protozoários que compartilham o mesmo filo incluem *Toxoplasma*, *Babesia, Plasmodium, Cryptosporidium parvum* e *Isospora belli (*MAKHIJA, 2012). A primeira espécie de *Sarcocystis* foi descrita pela primeira vez por Miescher em 1843 na musculatura esquelética de um rato capturado em sua casa na Suíça. Provavelmente, o parasita encontrado anos mais tarde no camundongo doméstico (*Mus musculus)* e denominado de *Sarcocystis muris* seja o mesmo descrito por Miescher naquela época. Como o próprio nome sugere, o parasito forma cistos teciduais (sarcocistos) que são encontrados nos músculos e no sistema nervoso central de animais homeotérmicos e pecilotérmicos (DUBEY, 2015a ; GREENE, 2015).

Uma importante característica do parasita é a necessidade de ciclo de dois hospedeiros, realizados em indivíduos diferentes. A fase sexuada ocorre nas células intestinais do hospedeiro definitivo, normalmente um carnívoro predador que libera esporocistos já esporulados em suas fezes. Já, a fase assexuada, ocorre quando um herbívoro ou onívoro ingere esses esporocistos, desenvolvendo sarcocistos em seus tecidos musculares ou nervosos (ELSHEIKHA et al., 2006). Uma vez que os herbívoros ingerem esporocistos infectantes eliminados nas fezes do hospedeiro definitivo, há passagem para o intestino delgado, onde ocorre a liberação dos esporozoítos. Esses esporozoítos migram através do epitélio intestinal e penetram nas células endoteliais de vasos sanguíneos. Nos vasos, ocorre a primeira de quatro reproduções assexuadas (esquizogonia ou merogonia), produzindo numerosos merozoítos após 15 ou 16 dias da ingestão dos esporocistos. Merozoítos de segunda geração são detectados no sangue periférico 27 dias após a infecção inicial (FAYER, 2004). Em seguida, os merozoítos originários desse processo migram para músculos esqueléticos e/ou neurônios, onde formam os sarcocistos (GREENE, 2015). Na musculatura, esses cistos se desenvolvem e amadurecem ao longo de meses, causando mínima ou nenhuma resposta inflamatória por parte do hospedeiro. Quando os músculos são ingeridos por um carnívoro predador, o ciclo se completa (COOPER & VALENTINE, 2016). Sarcocistos são capazes de permanecer nos tecidos musculares durante meses ou anos (FAYER, 2004). O ciclo de vida de *Sarcocystis* spp. difere do de outros coccídeos de animais domésticos, visto que os oocistos esporulam no interior do intestino do hospedeiro definitivo e são excretados nas fezes já na forma infectante (GREENE, 2015).

O sarcocisto muscular torna-se infectante para o hospedeiro definitivo somente quando os bradizoítos são formados, o que ocorre, aproximadamente, dois a três meses após a infecção.

Os esquizontes e os metrócitos, formas intermediárias do parasita, não são infectantes (DUBEY, 1976; GONÇALVES et al., 2016). Após o hospedeiro definitivo ingerir tecidos infectados, os bradizoítos se transformam em gametas masculino e feminino nos enterócitos. Nessas células ocorre gametogonia e esporogonia com formação dos oocistos esporulados, fechando o ciclo entre 7 a 14 dias (GONÇALVES et al., 2016).

Há uma ampla variedade de espécies de *Sarcocystis*, sendo que mais de 90 espécies são descritas em aves, mamíferos e répteis (COOPER & VALENTINE, 2016). Contudo, esses protozoários são espécie-específicos, tanto para hospedeiros intermediários, quanto para definitivos. Por exemplo, esporocistos de *Sarcocystis hominis* infectam bovinos, mas não porcos, enquanto que esporocistos de *S. suihominis* são capazes de infectar porcos, porém, não bovinos. Da mesma forma, cães e coiotes são hospedeiros definitivos de *S. cruzi*, enquanto que em humanos e felinos, o ciclo dessa espécie não se completa (FAYER, 2004).

Humanos também podem ser acometidas por *Sarcocystis* spp. e desenvolver sarcocistose clínica. De fato, seres humanos parecem ser os únicos hospedeiros definitivos que adoecem devido à infecção por *Sarcocystis* spp. (TAPPE et al., 2013). Apenas os bovinos e suínos são atualmente considerados hospedeiros intermediários de espécies zoonóticas de *Sarcocystis*, sendo que a contaminação ocorre ao ingerir carne de gado ou de porco crua ou mal cozida infectada com *S. hominis* e *S. suihominis*, respectivamente (DUBEY, 2015b; TAPPE et al., 2013). Em um estudo brasileiro, sete voluntários ingeriram quibes crus confeccionados a partir de carne bovina e seis tiveram esporocistos de *S. hominis* detectados em suas fezes, enquanto que dois desenvolveram diarreia (PENA et al., 2001). Quando desenvolvem sinais clínicos, normalmente são leves e essencialmente gastrointestinais, com as apresentações mais frequentes de diarreia, vômito, náusea e dores abdominais (DUBEY, 2015b). Como forma de prevenção de sarcocistose intestinal, é necessário congelar ou cozinhar as carnes antes do consumo, com o propósito de inativar os bradizoítos (FAYER, 2004). Sarcocistos presentes em carne suína se tornaram não infectivos quando cozidos a 60ºC durante 20 minutos, a 70ºC durante 15 minutos e a 100ºC durante 5 minutos. O congelamento também se mostrou eficaz em inativar os cistos, mantidos a -4ºC a durante 48 horas e a -20ºC durante 24 horas (SALEQUE et al., 1990).

Há relatos de seres humanos desempenhando papel de hospedeiro intermediário para *Sarcocystis* spp. e, dessa forma, sendo evidenciados sarcocistos em seus tecidos musculares. A denominação *Sarcocystis lindemanni* foi originalmente proposta para todas as sarcocistoses intramusculares em pessoas, porém, não é claramente definida e a evidência de múltiplos cistos morfologicamente distintos, sugere que há presença de diferentes espécies de *Sarcocystis*

envolvidas na infecção humana (FAYER, 2004). A maioria dos casos de sarcocistose em humanos tem sido descrita em regiões de clima tropical ou subtropical, tais como, sudeste da Ásia, China, Índia e Américas do Sul e Central (FAYER, 2004). A Malásia é um país que concentra diversos surtos de sarcocistose muscular em humanos (TAPPE et al., 2013). Um estudo soroepidemiológico realizado na região oeste da Malásia, detectou que 19,7% de 243 pessoas apresentavam anticorpos reagentes para *Sarcocystis* spp., sendo que os títulos mais altos foram encontrados em aborígenes e habitantes da zona rural, provavelmente refletindo os hábitos alimentos e condições sanitárias desses indivíduos (THOMAS & DISSANAIKE, 1978). Quando ocorre sarcocistose muscular, os locais mais comumente afetados são músculo esquelético e cardíaco, mas músculos da laringe, faringe e esôfago superior também podem ser acometidos (GONÇALVES et al., 2016).

Na grande maioria dos casos, a presença de sarcocistos na musculatura de animais é considerada um achado incidental, sem evidência de inflamação local ou doença clínica (COOPER & VALETINE, 2016). Entretanto, de forma menos comum, hospedeiros intermediários podem exibir sinais de doença sistêmica, sobretudo se a infecção ocorrer em outros sítios, que não a musculatura (TAPPE et al., 2013). Já foi observado acometimento do sistema nervoso central (SNC) em gatos de cinco (BISBY et al., 2010), quatro (DUBEY et al., 2003) e três meses (DUBEY et al., 1994). Em todos os casos, os gatos apresentaram sinais neurológicos. Além disso, Sykes et al. (2011) descrevem o caso de dois cães que desenvolveram miosite grave associada à presença de *Sarcocystis* spp. em sua musculatura. Ambos os animais apresentaram sinais sistêmicos (febre, apatia e inapetência), além de atrofia muscular e alterações laboratoriais, como linfopenia, trombocitopenia e aumentos séricos da atividade das enzimas hepáticas alanina aminotransferase e fosfatase alcalina.

Os equinos, classificados como hospedeiros finais de *S. neurona*, desenvolvem uma importante doença neurológica associada a esse protozoário, denominada de mieloencefalite equina por protozoário (MEP) (STANEK et al., 2003). Normalmente, a doença é progressiva e debilitante, e os sinais clínicos variam de agudos a insidiosos, focais ou multifocais, envolvendo encéfalo e/ou medula espinhal (DUBEY et al., 2001).

O ciclo de *Sarcocystis neurona* na natureza ainda não é totalmente elucidado. O gambá-da-Vírginia (*Didelphis virginiana*) é tradicionalmente considerado o hospedeiro definitivo clássico desse protozoário (FENGER et al., 1995). Esse gambá não é encontrado em território brasileiro (CASAGRANDE et al., 2011), no entanto, outros gambás podem igualmente desempenhar o papel de hospedeiro definitivo para *S. neurona*. No Rio Grande do Sul, *Didelphis albiventris*, popularmente conhecido como gambá-de-orelha-branca, é a espécie de

gambá de maior ocorrência, com ampla distribuição no estado (CÁCERES et al., 2013; VIEIRA & IOB, 2003). Esses animais são onívoros e oportunistas, apresentando alta sinantropia e convivendo com os homens no meio rural e nas cidades, onde são cada vez mais frequentes (MULLER et al., 2005). Estudos já demonstraram a presença de esporocistos de *S. neurona* nas fezes de *D. albiventris* (DUBEY et al., 2001), fortalecendo a ideia de que esses animais são, provavelmente, os hospedeiros definitivos desse parasita no Brasil. Informações relacionadas aos hospedeiros intermediários de *S. neurona* são mais vagas, no entanto, mamíferos como tatus (*Dasypus novemcinctus*) (CHEADLE et al., 2001) e guaxinins (*Procyon lotor*) (DUBEY et al., 2001) já foram relatados.

Felídeos já foram descritos como hospedeiros definitivos para 15 espécies de *Sarcocystis*, como *S. hirsuta*, *S. arieticanis*, *S. gigantea*, *S. fayeri* e *S. muris* (BOWMAN & LYNN, 1999). Entretanto, o gato doméstico recentemente tem sido relatado como um possível hospedeiro intermediário de *S. felis* e *S. neurona* (BISBY et al., 2010; COOPER & VALENTINE, 2016). A imunossupressão foi sugerida como fator predisponente na patogênese da sarcocistose muscular de gatos (HILL et al., 1988), entretanto esse fato não foi confirmado em felinos domésticos ou selvagens (BISBY et al., 2010), visto que a presença desses cistos é observada tanto em animais imunodeprimidos quanto saudáveis (GILLIS et al., 2003). Em dois estudos norte-americanos que investigaram a presença de sarcocistos em músculos de gatos domésticos, a única espécie de *Sarcocystis* encontrada foi *S. felis* (ELSHEIKHA et al., 2006; GILLIS et al., 2003). Quanto a *S. neurona*, a infecção experimental (DUBEY et al., 2000) e natural (TURAY et al., 2002) de gatos já foi demonstrada. Apesar da existência desses estudos anteriores, maiores detalhes relacionados a essa forma de infecção permanecem ainda pouco explorados.

Com exceção de alguns sarcocistos (*S. gigantea*, de ovelhas, por exemplo), a maioria das espécies de *Sarcocystis* não é visível macroscopicamente. Raramente, quando os cistos induzem uma resposta inflamatória significativa, podem ocorrer allterações macroscópicas pela inflamação, mas não pelos cistos em si (COOPER & VALETINE, 2016). Em um estudo conduzido por Fiori & Lowndes (1988), os músculos provenientes dos gatos analisados não apresentavam cistos visíveis ou qualquer outra alteração macroscópica.

Histologicamente, os cistos teciduais têm formato ovoide e raramente são acompanhados de reação inflamatória, necrose ou degeneração das fibras musculares. Ao adentrar a fibra muscular, no entanto, o organismo pode incomumente induzir uma discreta infiltração de linfócitos na região afetada. Degeneração e necrose da fibra invadida também podem ocorrer, de forma incomum, no momento da invasão por estes organismos. Além disso,

ocasionalmente se observam eosinófilos, macrófagos e tecido de granulação associados a cistos degenerados e rompidos. Não se sabe se essas alterações representam cistos excessivamente maduros ou se são achados aleatórios, e que não possuem relação com o estágio/idade do cisto (COOPER & VALETINE, 2016). O tamanho dos cistos varia, mas pode passar de 100 µm (FIORI & LOWNDES, 1988). Dependendo de sua espécie e "idade", os sarcocistos podem apresentar paredes finas a espessas. O interior dos cistos é fortemente corado pela hematoxilina, adquirindo um aspecto de "emaranhado de minhocas" que corresponde aos bradizoítos. Esses bradizoítos podem ser facilmente identificados em maior aumento microscópico, e em imersão, pode-se observar o formato de meia – lua ou de banana dessas estruturas (MAKHIJA, 2012). À medida que o cisto amadurece, a sua cápsula se torna mais espessa e proeminente na fibra muscular, sendo mais fácil diferenciá-la do sarcoplasma da fibra afetada. Além disto, se sabe que poros infimamente pequenos (e, portanto, não visíveis na microscopia de luz) comunicam o cisto com o sarcoplasma da célula, permitindo a captura de nutrientes do hospedeiro para a sobrevivência do protozoário. No entanto, aspectos mais detalhados desta relação protozoário-hospedeiro ainda são pouco conhecidos (COOPER & VALENTINE, 2016).

Embora os sarcocistos sejam facilmente reconhecidos por patologistas veterinários, eles apresentam similiaridades morfológicas com outros protozoários do filo Apicomplexa, o que pode exigir técnicas diagnósticas mais avançadas para a confirmação destes protozoários, particularmente quando se trata de trabalhos de pesquisa. Além disso, na histopatologia não é possível diferenciar uma espécie da outra: todos os cistos têm as mesmas características histológicas (BISBY et al., 2010).

Para tanto, diagnósticos moleculares representam uma importante ferramenta para pesquisas epidemiológicas envolvendo sarcocistose. Desde cerca de 1990, os métodos moleculares têm sido cada vez mais utilizados para complementar e apoiar os dados morfológicos e biológicos na identificação de várias espécies de *Sarcocystis*, particularmente aquelas que infectam animais domésticos (GJERDE, 2013). Diversos estudos utilizam a técnica de reação em cadeia da polimerase (PCR) com amplificação do gene 18S rRNA para o diagnóstico de infecções por *Sarcocystis* spp., com ênfase na identificação das espécies utilizando sequenciamento e/ou a técnica de polimorfismo no comprimento do fragmento de restrição (RFLP) (MORÉ et al., 2011). A identificação entre as espécies de *Sarcocystis* pode ajudar a estimar a prevalência, distribuição, fonte de infecção e fatores de risco associado a essas infecções (MORÉ et al., 2013).

Adicionalmente, testes sorológicos têm sido desenvolvidos para diagnóstico de infecções por *Sarcocystis* spp. nos hospedeiros intermediários (DUBEY, 1989). Esses testes

são úteis como exames de triagem, permitindo a detecção de anticorpos, com isso, determinando se há circulação do protozoário em determinada população. Métodos de diagnóstico convencionais, como pesquisa de cistos teciduais, podem não ser aplicáveis em estudos de larga escala envolvendo alto número de indivíduos (SAVINI et al., 1994). De forma geral, o teste de Western Blot (WB) tem sido considerado o padrão ouro para diagnósticos sorológicos, especialmente, quando utilizado em pesquisas, porém, é uma técnica cara e laboriosa. Por outro lado, a reação de imunofluorescência indireta (RIFI) é igualmente precisa e mais rápida, barata e menos dispendiosa quando comparada ao WB (DUARTE et al., 2003).

Como já comentado anteriormente, a importância dos gatos desempenhando a função de hospedeiros intermediários para *Sarcocystis* spp. tem sido investigada apenas a partir dos anos 2000 (GILLIS et al., 2003). Foram realizados dois estudos norte-americanos envolvendo necropsia de gatos domésticos que identificaram a presença de *S. felis* na musculatura desses animais, porém *S. neurona* não foi encontrado (ELSHEIKHA et al., 2006; GILLIS et al., 2003). Um estudo brasileiro observou *S. felis* como única espécie presente em músculos de felinos selvagens mortos em acidentes em rodovias no estado do Rio Grande do Sul (CAÑÓN-FRANCO et al., 2016). Dubey et al. (2000) demonstrou que o gato doméstico era um possível HI experimental para *S. neurona*, por meio da infecção de gatos em laboratórios e posterior demonstração dos sarcocistos musculares nesses animais. Logo em seguida, Turay et al. (2002) procurou cistos de *S. neurona* nos músculos de gatos ferais, encontrando-os em apenas um indivíduo, mas confirmando a possibilidade de gatos agirem como HI naturais. Apesar de já se saber que o gato pode agir como HI de *S. neurona* em condições experimentais e naturais, a importância do gato como HI, a frequência com que isso ocorre, os aspectos epidemiológicos dessa forma de infecção e os músculos mais frequentemente acometidos pelos cistos permanecem pouco explorados. Além disso, até o momento, não há nenhum estudo que tenha pesquisado a ocorrência de sarcocistos musculares em gatos domésticos da região Sul do Brasil. Dessa forma, torna-se importante caracterizar a infecção natural por *Sarcocystis* spp. em gatos domésticos da nossa região, verificando se há a ocorrência desse protozoário e, com isso ampliar o conhecimento de seu ciclo. Adicionalmente, tendo em vista que há relatos de gatos que adoeceram em decorrência da infecção por *Sarcocystis* spp. no sistema nervoso central, determinar a circulação do parasita na nossa população felina pode ser importante no aspecto clínico, uma vez que seria recomendado incluir infecção por *Sarcocystis* spp. como diagnóstico diferencial em gatos que apresentarem sinais neurológicos centrais.

Em vista disso, o objetivo deste estudo foi investigar a infecção natural por *Sarcocystis* spp. em um grupo heterogêneo de gatos domésticos da região central do Rio Grande do Sul

(RS). Os gatos incluídos apresentavam diferentes estilos de vida (*i.e.* possuir ou não acesso à rua), compondo uma amostra populacional heterogênea. Esse estudo visou investigar: (1) a ocorrência dos cistos musculares em um grupo de gatos submetidos à necropsia de rotina, confirmando a espécie envolvida por meio de PCR e sequenciamento genético; (2) a caracterização epidemiológica e anatomopatológica dos gatos positivos para cistos musculares; e (3) a ocorrência de anticorpos anti-*Sarcocystis* spp. em um grupo de gatos atendidos em um Hospital Veterinário Universitário (HVU).

2 MANUSCRITO

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Naturally occurring *Sarcocystis* infection in domestic cats (*Felis catus*) from Southern Brazil: a serologic and histologic study

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Abstract

Despite of classically acting as definitive hosts for different *Sarcocystis* species, domestic cats (*Felis catus*) have uncommonly been involved as intermediate hosts for *S. neurona* and *S. felis*. Few studies have investigated the occurrence of muscular sarcocistosis in domestic cats. Additionally, in Brazil, rare serologic studies have investigated the circulation of *Sarcocystis* among cat populations. This manuscript examined the presence of anti-*Sarcocystis* antibodies and of muscular sarcocysts in two different subsets of cats without clinical *Sarcocystis* infection. The aim of this investigation was to determine whether *Sarcocystis* spp. circulates among cats from Southern Brazil, and how frequently do these animals develop muscular cysts for one or more species of *Sarcocystis.* Morphologic and epidemiologic aspects of muscular sarcocistosis were also analyzedd. A total of 497 serum samples from cats attending a veterinary hospital were investigated for anti-*Sarcocystis* spp. antibodies using indirect immunofluorescence reaction. For the histopathologic study, diaphragm, esophagus, myocardium, tongue, intercostal, subscapular and vastus lateralis muscle samples were collected from 100 cats submitted for necropsy at a Veterinary Pathology Service and evaluated histologically. Cases with tissue sarcocysts were characterized according to the muscle involved, the size and amount of sarcocysts and the presence of inflammation or degeneration. Positive cases were also submitted to Polymerase Chain Reaction and sequencing in order to identify the involved species. Additionally, epidemiologic information was collected from the clinical history of the 100 cats. Of the 497 analyzed serum samples, 24 (4.8%) had detectable anti-*Sarcocystis* antibodies on the indirect immunofluorescence reaction. Histologic sarcocysts were found in five cats (5/100; 5%). These cysts were identified in vastus lateralis, intercostal, subscapular and diaphragm muscles. Four cats (4/5; 80%) had numerous and multifocal cysts affecting various muscles, while one (1/5; 20%) presented a single cyst in the left vastus lateralis muscle. No secondary inflammation was seen associated to the cysts. *S.*

neurona was the only identified species. While outdoor access was consistently reported in the clinical history of all positive cats, only two sarcocyst-positive cats were positive for retrovirosis, and the use of potentially immunosuppressive drugs was not reported in neither of these animals. Our results confirm that *Sarcocystis* spp. do circulate among domestic cats from Southern Brazil. Muscular sarcocistosis due to *S. neurona* was occasionally observed in cats without *Sarcocystis*-associated disease. These findings highlight the possible participation of feral and pet cats from urban areas as natural intermediate hosts for *S. neurona*.

Keywords: sarcocystosis, *S. neurona*, intermediate host, histopathology, polymerase chain reaction, natural infection.

1. Introduction

Domestic cats (*Felis catus*) are natural definitive hosts for several *Sarcocystis* species, including *S. hirsuta, S. arieticanis, S. gigantea, S. fayeri* and *S. muris* (Bowman & Lynn, 1999). However, some studies suggest that these mammals may occasionally act as intermediate hosts for some species of this parasite, developing cysts that mainly affect muscle tissues (Kirkpatrick et al., 1986; Hill et al., 1988; Gillis et al., 2003). Data regarding natural muscular sarcocystosis in domestic cats is scarce, since the few studies aiming to investigate this form of infection have detected a small number of cats with sarcocysts (Turay et al., 2002; Gillis et al., 2003). Furthermore, these studies present divergent findings. While one investigation found only one cat with sarcocystosis caused by *S. neurona* (Turay et al., 2002), a subsequent study aiming to explore the role of domestic cats as intermediate hosts for *S. neurona* has only found *S. felis* (Gillis et al., 2003). The authors from this later study concluded that further work is needed to confirm the cat as a natural intermediate host for *S. neurona.*

Sarcocystis neurona is one of the most important species of *Sarcocystis* in Veterinary Medicine, since it is one of the etiologic agents of equine protozoal myeloencephalitis (Stanek et al., 2003; Cantile & Youssef, 2016). While the Virginia opossum (*Didelphis virginiana*) is the definitive host for *S. neurona* in the United States (Fenger et al., 1995), in Brazil, this role is attributed to *D. albiventris* (Dubey et al., 2001). Cats have been already demonstrated as experimental (Dubey et al., 2000) and natural (Turay et al., 2002) intermediate hosts for *S. neurona*, however, the natural presentation of this form of infection is little described in the literature.

Based on the paucity of studies investigating the natural occurrence of muscular sarcocistosis in domestic cats and on the fact that even in these studies, the infection is considered uncommon, epidemiologic and morphologic data regarding this form of infection, whether by *S. neurona* or *S. felis*, remains scarce. In fact, external circumstances that may

influence the development of muscular cysts in cats are still undetermined. The role of immunosuppression has been mentioned (Hill et al., 1988; Edwards et al., 1988), however, it remains controversial. Characterizing the occurrence of muscular sarcocistosis in cats is important and may help understanding, preventing and controlling *Sarcocyst*-associated clinical diseases in the future. For instance, if *S. neurona* sarcocystsosis is detected in a significant subset of cats from a specific region, it is possible that these animals may be participating of the natural cycle of the parasite, which in turn may contribute to parasite maintenance, facilitating the infection of horses living in the same area. If this was the case, cat populations residing in areas with high incidence of equine protozoal myeloencephalitis should be controlled. Additionally, if feline muscular sarcocistosis is relevant in specific geographic regions, *Sarcocystis* spp. infection should be considered as a possible etiology in cats with muscular or neurologic disease in these areas. This information could facilitate an early diagnosis, enabling the clinician to make rapid therapeutic choices that might rise the chances of response to treatment.

This manuscript examines the presence of anti-*Sarcocystis* antibodies and of muscular sarcocysts, each in one different subset of cats without clinical *Sarcocystis* infection. The aim of this investigation was to determine whether *Sarcocystis* spp. circulates among cats from Southern Brazil, and how frequently do these animals develop muscular cysts for one or more species of *Sarcocystis*, acting as intermediate hosts for these protozoa. Muscular sarcocystosis was studied in more detail in order to characterize the morphologic and epidemiologic aspects of this form of infection.

2. Materials and methods

2.1. Study design

This study was divided in two parts: (1) a serologic study in cats attending to a Brazilian veterinary hospital; and (2) a histopathologic study of muscles from cats submitted for necropsy

at a veterinary pathology service. Additionally, epidemiologic data was collected from clinical histories, when present. All muscles collected were also submitted to Polymerase Chain Reaction (PCR) and those positive for *Sarcocystis* spp. were sequenced for species identification.

2.2. Serologic investigation

Four hundred ninety-seven serum samples from cats attending to the Hospital Veterinário Universitário (HVU) from the Universidade Federal de Santa Maria (UFSM) were included. These cats had been submitted to clinical evaluation and blood work for various reasons between October 2018 and May 2019. The blood samples were centrifugated (3.400 rpm, 4 min), for serum separation before being routinely used, and the leftovers were frozen. All feline serum samples leftovers kept frozen (-20ºC) for future disposal that contained a minimum of 300 µL were included in this study. The serologic study was performed at the Laboratório de Doenças Parasitárias (LADOPAR) of UFSM.

It was performed for detection of anti-*Sarcocystis* spp antibodies using indirect immunofluorescence reaction. For the realization of the IFAT, as slides were sensitized with bradizoos coming from the heart of a bovine naturally with *Sarcocystis neurona*. The bradizoos were diluted in PBS solution (pH 7.2) to a concentration of approximately 1.5 - 2.0 x 10^3 bradizoits per mL and instilled (1 drop per well) into IFRS-specific slides with 10 wells per side. . It was used as negative control of a known negative animal and positive control, a sample known positive for Sarcocystis spp. Excess wells were instilled one drop (4 µL) of feline sera at 1:40 dilution. After this process, the slides were stored in an oven at 37ºC for 30 minutes and washed twice in PBS solution and once in distilled water. With the slides dried, 1 drop (2 µL) of the conjugated commercial antibody (put name of the antibody), feline specific species at 1: 200 dilution was installed. Finally, as slides were incubated, washed and dried as described above. For reading, a glycerine buffer (9 volumes glycerin + 1 volume PBS) was added, covered

with a slide and analyzed under the immunofluorescence microscope. Dimensions were qualitatively classified as positive or negative according to the reaction observed in each well.

2.3. Necropsy investigation

2.3.1. Animals

All cats older than 3 months submitted for routine necropsy at Laboratório de Patologia Veterinária (LPV) of UFSM between March 2018 and September 2019 were included (n=100).

2.3.2. Collection and storage of muscular tissues

Two fragments of approximately 1.0 x 0.5 x 0.5 cm were collected from each of the following sites: diaphragm, esophagus, myocardium, tongue, intercostal muscles, right and left subscapular muscles, and right and left lateral vastus muscle. One fragment from each muscle was placed on 10% buffered formalin for histopathology and the other fragment was kept frozen for PCR.

2.3.3. Histopathology

Formalin-fixed muscular samples were routinely processed and stained with Hematoxilin and Eosin (HE). Histologic slides from the 100 cats were analyzed separately by two investigators. The cases were classified as positive on histopathology when at least one cyst morphologically compatible with *Sarcocystis* spp. was observed in at least one muscle section. When a minimum of 10 cysts was observed in the same histologic muscle section, the case was classified as containing "multiple cysts". Cases were considered negative when no cysts were observed. When present, the cysts were measured using the CellSens® (CellSens – Company, Netherlands) software. Surrounding muscles were searched for inflammatory or degenerative lesions associated with the cysts.

2.3.4. *Epidemiologic data*

Information regarding gender, reproductive status (spayed or intact), breed, age, lifestyle (with or without outdoor access; feral or pet; from urban or rural area), status for feline

immunodeficiency virus (FIV) and/or feline leukemia virus (FeLV) and previous administration of potentially immunosuppressive drugs were collected when present in the clinical history. Information regarding FIV and FeLV status, when present, was based on the snap test (IDEXX) performed during routine clinical examination by the clinician. The following drugs were considered as potentially immunosuppressive: glucocorticoids (such as prednisolone or methylprednisolone acetate), doxorubicin and vincristine. When the cat was positive for muscular sarcocystosis, the cause of death or reason for euthanasia was collected from the necropsy report.

2.3.5. Polymerases chain reaction (PCR)

The frozen muscle fragments of the 100 cats were sent for PCR in the UFSM LADOPAR. DNA extraction was performed from a pool of frozen muscles using the commercial QIAamp DNA Tissue Kit (QIAGEN, United States - USA) according to the manufacturer's instructions. The nested-PCR reaction was performed according to Silva et al. (2009), using multispecies primers for Sarcocystis neurone, Neospora caninum and Toxoplasma gondii. The external primers were used: Tg18s48F 5 '-CCATGCATGTCTAAGTATAAGC-3' and Tg18s359R 5 '-GTTACCCGTCACTGCCAC-3', and the internal primers: Tg18s58F 5 '- CTAAGTATAAGCTTTTATACGGC-3' TCC18G8T8 fragment 290 base pairs of the 18SrDNA gene. Each PCR was performed at a final volume of 25 µl containing 1X Buffer (Promega, USA); 10mM dNTPS (Ludwig Biotec, Brazil); 10 pmol initiator (IDT, USA); 1 U Taq DNA Polymerase (Pomega, USA); Approximately 20 ng of DNA was used in the first reaction, while in the second reaction 2 µL of the amplified product of the first PCR were used. Genomic DNA extracted from a pool of 50 Sarcocystis spp. was used as positive control, and milliQ water as negative control. *T'he* reaction was run in a thermal cycler (T100 ™ Thermalcycler, Bio-Rad, Singapore) with initial denaturation of 94 ° C for 5 minutes; followed by 35 cycles of 94 ° C for 45 sec, 55 ° C for 45 sec and 72 ° C for 45 seconds, and

final extension of 72 ° C for 5 min. The conditions were the same for the first and second reaction. PCR products were analyzed on SYBR® safe DNA gel stain-stained 2% agarose gel (Invitrogen ™, USA) and visualized on ultraviolet light transilluminator.

2.3.6. Genetic sequencing

The amplified DNA product from each sample was subjected to sequencing to identify the species involved. First, products from the second phase of PCR purification were purified using a commercial QIAquick PCR Purification Kit (QUIAGEM, USA) according to the manufacturer's instructions. Then 5 volumes of PB buffer were added to 1 volume of the PCR reaction and homogenized. This mixture was applied to a column located inside a microtube, centrifuged for 1 minute, 17 x 900 g and the supernatant discarded. Then 5 µl Buffer PE was added to the column and centrifuged under the same conditions as described above. After centrifugation, the supernatant was discarded and the centrifugation step was repeated. The column was reallocated in a clean new microtube, added 50 µl Buffer EB and centrifuged for 1 minute, 17 x 900 g. After centrifugation, the column was discarded and concentrated DNA was analyzed using a NanoDrop 1000 spectrophotometer (ThermoScientific, USA). After the purification process, DNA sequencing was performed using 5 pmol of external primers separately, 30 - 60 ng of purified PCR product and MiliiQ water, resulting in a final volume of 6 µl. This content was dehydrated at 60 ºC for 2 hours and then sent to genetic sequencing (ACTGene - Sequencing Service, Brazil). The result obtained was analyzed using StandenPackage software and the generated nucleotide sequences evaluated in the Genebank NCBI database (http://www.ncbi.nlm.nih.gov/BLAST).

2.4. Statistical analyses

A descriptive analysis of data was performed. Scalar variables were presented as median ± standard error and nominal variables as percentage frequencies. The IBM SPSS Version 25 software was used as a computational tool for statistical analysis of data.

3. Results

3.1. Serologic investigation

Of the 497 serum samples analyzed, 24 (4.8%) were positive on the IFAT for anti-*Sarcocystis* spp. antibodies.

3.2. Necropsy investigation

3.2.1. Histologic muscular sarcocysts

Histologic examination of muscle samples revealed sarcocysts in five out of 100 cats (5%). Four of the five sarcocyst-positive cats had multiple sarcocysts affecting at least two different muscles (Fig. 1A). One positive cat had a single sarcocyst affecting a section of lateral vastus from the left pelvic limb (1/5; 20%). Sections of lateral vastus and intercostal muscles were the most frequently affected (3/5; 60%) (Table 1). No cysts were observed in the histologic sections of tongue, esophagus or myocardium.

Histologically, each sarcocyst was inserted within the sarcoplasm of a myocyte, frequently occupying all sarcoplasm. Their length varied from approximately 15 µm to 800 µm and they were approximately 10 to 20 µm wide. They were encapsulated, and when the capsule was visible, it was weakly eosinophilic and was up to 1 µm-thick. In several sarcocysts, the capsule was not clearly differentiated from the muscle sarcoplasm. The interior of the sarcocysts was filled with multiple strongly basophilic bradyzoites (Fig. 1B). No inflammatory reaction or degeneration was seen, even in cases with multiple sarcocysts.

3.2.2. Epidemiologic data

Epidemiologic information from sarcocyst-positive (5/100) and negative (95/100) cats is disposed in Table 2. Two of the sarcocyst-positive cats were feral and three were pet cats with outdoor access (Table 1). Information regarding the habit of hunting and ingesting wild rodents and birds was present in one clinical history. All sarcocysts were considered incidental necropsy findings, not being involved as a cause of death or euthanasia (Table 1).

3.2.3. PCR and sequencing

All muscles histologically affected by sarcocysts were positive for *Sarcocystis* spp. (5/5; 100%) on PCR. Comparative analysis of the sequences available in GenBank NCBI revealed greater identification with *S. neurona* (AH009986.2), with 98-100% of similarity in all cases (5/5; 100%). All muscles histologically free of sarcocysts were negative on PCR.

4. Discussion

This investigation confirms that protozoa from the genus *Sarcocystis* do circulate among cats from Southern Brazil. Muscular sarcocystsosis was uncommon and always associated with *S. neurona*. All cats with muscular sarcocysts were males from urban areas and with outdoor access. Two of these animals were feral, and three were pets. These findings highlight the possible participation of feral and pet cats as natural intermediate hosts for *S. neurona*.

All cases of muscular sarcocystosis were attributed to *S. neurona*. Other studies have mentioned the possibility of domestic cats acting as intermediate hosts for this parasite based on evidence from experimental infection (Dubey et al., 2000), individual case reports (Dubey et al., 2003; Bisby et al., 2010) or prospective research of natural cases (Dubey et al., 2002a; Dubey et al., 2002b; Rossano et al., 2002; Turay et al., 2002; Stanek et al., 2003). However, prospective studies investigating the presence of muscular sarcoysts in cats without *Sarcocystis*-associated disease, whether by *S. neurona* or by other species of *Sarcocystis*, are uncommon and present divergent findings (Turay et al., 2002; Gillis et al., 2003). A small investigation found *S. neurona* sarcocysts in only one out of nine feral cat carcasses (Turay et al., 2002). Subsequently, a larger study aiming to determine whether the domestic cat is a natural host for *S. neurona* ended up only detecting *S. felis* sarcocysts (Gillis et al., 2003). In fact, the authors from this North-American study concluded that “further work is needed to determine the role of the domestic cat in the life cycle of *S. neurona*” (Gillis et al., 2003), making it clear that information on the subject remained scarce at that time. Since then, this subject has been little investigated.

Muscular sarcocysts were found in 10 to 11% of the investigated cats from previous studies (Turay et al., 2002; Gillis et al., 2003), a higher prevalence when compared to the one found in this investigation (5%). This prevalence was possibly affected by the fact that all cats included in this study were from urban areas, and only 9% were considered feral. All the cats investigated by Gillis et al. (2003), however, were primarily free roaming, which enhances the probability of contact with sporocysts. Moreover, none of the cats studied here had a history of living in the countryside or even in farms. Stanek et al. (2003) stated that farm cats had a higher seroprevalence for *S. neurona* when compared to urban cats, justifying that cats living in rural areas are more likely to interact with wildlife and become infected. Another interesting characteristic of the five sarcocyst-positive cats from this study is that all animals were males and had outdoor access. It is well known that male cats have a higher tendency to roam outside when compared to females (Loyd et al., 2013), thus increasing the risk of exposure and infection by sporocysts (Rossano et al., 2002; Gillis et al., 2003).

Of the five cats with muscular sarcocysts in this survey, one was FIV and another one was FeLV-positive on the snap test. No sarcocyst-positive cat had been submitted to any medical treatment before death. The role of immunosuppression in the development of muscular sarcoysts is still controversial. While it has been suggested as a possible risk factor for the development of tissue sarcocysts (Hill et al., 1988; Edwards et al., 1988), some studies show that cats affected by these sarcocysts are frequently in good health conditions and are often negative for retrovirus infections (Gillis et al., 2003). When *S. neurona* sporocysts where experimentally fed to domestic cats with and without cortisone administration, cats from both groups developed muscular sarcocysts, suggesting that other factors other than drug-related immunosuppression may influence sarcocyst development (Dubey et al., 2000). The low rate of sarcocyst-positive cats in this study makes it difficult to draw any association between potentially immunosuppressive conditions and the development of muscular sarcocysts. To

investigate this kind of association, studies including a higher number of infected cats are necessary.

No cat included in this investigation had its cause of death or euthanasia associated with sarcocysts. There are few reports of clinical disease and/or death associated with *Sarcocystis* infection in cats. Most of these cases involved central nervous system infection (Dubey et al., 1994; Dubey et al., 2003; Bisby et al., 2010).

While studies investigating the natural occurrence of muscular sarcocysts in domestic cats are uncommon (Turay et al., 2002; Gillis et al., 2003), serologic investigations are more frequent (Dubey et al., 2002a; Rossano et al., 2002; Turay et al., 2002; Stanek et al., 2003; Cray et al., 2005). Investigating the natural occurrence of muscular sarcocysts is important, since positive animals may act as intermediate hosts. Serologic evidences, although relevant, detect a broader category of animals – including those cats that have been recently exposed to the parasite but have not developed muscular cysts. Therefore, it does not allow the confirmation of cats acting as intermediate hosts. The seroprevalence of cats for *Sarcocystis* spp. in this study was low. This, in part, may be attributed to the fact that all cats from our study were from urban areas and the majority were pets, as discussed earlier. Additionally, it is well known that *sarcocystys*-induced immunity is of short-term duration. The reduced antigenicity of many protozoans causes serum antibody levels to become low to undetectable at times of decreased parasitemia (Tizzard, 2014). Thus, it is probable that only recently exposed cats had a high enough antibody titration to be detected. Since the serologic investigation was not specific for any *Sarcocystis* species, it was not possible to determine whether these cats had *S.neurona*-specific antibodies or if other *Sarcocystis* species were also involved. Two Brazilian studies have investigated the serologic status of domestic cats for *Sarcocysts*. While none of the 502 investigated cats from São Paulo state had detectable serum antibodies against *S. neurona*

(Dubey et al., 2002b), 4% of the tested samples from Bahia state were seropositive (Meneses et al., 2014).

The natural cycle of *S. neurona* is still not completely elucidated. While Virgina opossums (*Didelphis virginiana*) are traditionally considered definitive hosts (Fenger et al., 1995), information regarding natural intermediate hosts are vague. Encephalic and/or muscular cysts have been encountered in different mammals such as armadillos (*Dasypus novemcinctus*), racoons (*Procyon lotor*), skunks (*Mephitis mephitis)* and sea otters (*Enhydra lutris*) (Dubey et al., 2000; Cheadle et al., 2001). As mentioned above, all sarcocyst-positive cats had outdoor access, which theoretically enhances the chances of infection by sporocysts, either by the predation of intestines containing sporocyst-contaminated feces (Dubey et al., 2000), either by the ingestion of contaminated water, food or grass (Dubey et al., 2002a). The Virginia opossum is not part of the Brazilian fauna (Casagrande et al., 2011), however, other opossums can equally act as definitive hosts, such as the White-eared opossum (*Didelphis albiventris*), the most common species in Rio Grande do Sul State (Vieira e Iob, 2003; Cáceres et al., 2013). These mammals are omnivorous and opportunistic, living with men in rural and urban areas, where they are increasingly frequent (Muller et al., 2005). The circulation of opossums in urban areas could facilitate the contact of domestic urban cats with sporocysts, leading to *S. neurona* infection and, in some cases, to the development of muscular sarcocysts. In fact, the infection of domestic cats by ingestion of sporocysts from opossum feces followed by muscular sarcocyst development has been experimentally demonstrated (Dubey et al., 2000). Despite all this, investigating the definitive host and its interaction with domestic cats in natural environment is beyond the scope of this study. Further research is needed to confirm the source and mechanism of infection of cats from Southern Brazil.

Among all histologic muscle sections investigated in this study, sections of lateral vastus and intercostal muscles were the most frequently affected by sarcocysts. Gillis et al. (2003)

found that *S. felis* sarcocysts were more abundant in sections of quadriceps muscles when compared to sections of tongue and diaphragm, however, details regarding the frequency of which each muscle was affected was not reported by these authors. Interestingly, the only sarcocyst encountered by Turay et al. (2003) and later identified as *S. neurona*, was present at the tongue, whereas in our study, all tongue sections were negative for sarcocysts. Four of the five sarcocyst-positive cats in this study had multiple sarcocysts affecting sections of more than one muscle. The detection of cases with multiple cysts is epidemiologically important, whereas the greater the number of muscular sarcocysts the greater the chances of protozoan spread and contamination of the definitive host. It is important to mention, however, that histopathology may not be useful in cases where sarcocysts are too rare to be found (Moré et al., 2011). Polymerase chain reaction, in the other hand, is expected to be more sensitive in the detection of tissue infection by sarcocysts and has been widely used on the detection and characterization of different *Sarcocystis* species (Yang et al., 2002). We emphasize that the molecular investigation applied in this study had the sole propose of confirming the genus and species of the protozoa observed histologically, and not to compare sensibility between diagnostic methods.

5. Conclusions

Our results confirm that *Sarcocystis* spp. do circulate among domestic cats from Southern Brazil. Muscular sarcocistosis due to *S. neurona* was occasionally observed in cats without *Sarcocystis*-associated disease. These findings highlight the possible participation of feral and pet cats from urban areas as natural intermediate hosts for *S. neurona*. The involvement of cats as natural hosts for *S. felis* remains undetermined, since this species was not encountered in this study.

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Conflict of interest

The authors declare no conflict of interest.

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Figure captions

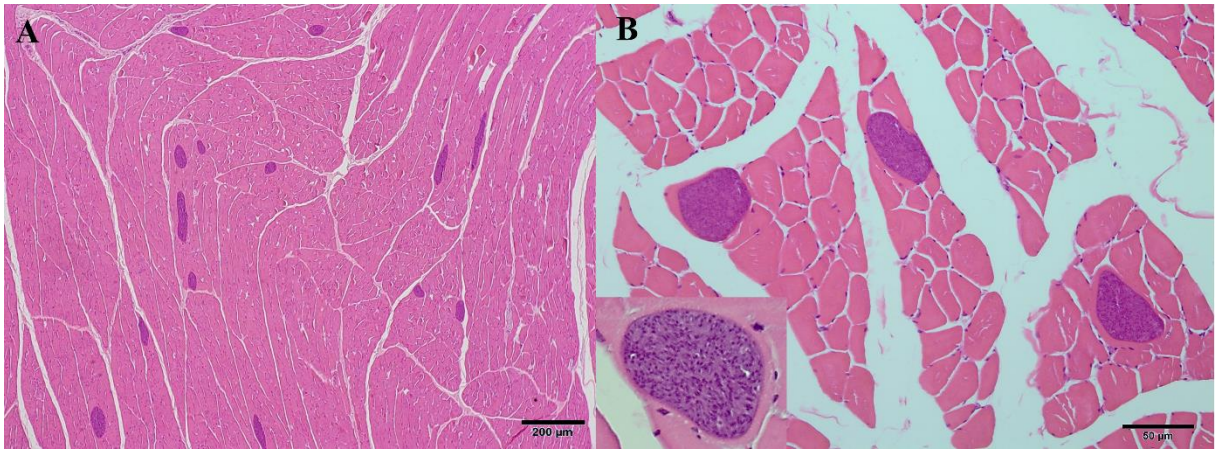

Fig. 1. Sarcocystosis in the skeletal striated muscle of a domestic cat (*Felis catus*) from Southern Brazil. (A) Multiple oval to elongated cysts of varying sizes, are observed within muscle fibers. Hematoxylin and eosin (H&E). (B) A sarcocyst surrounded by a thin eosinophilic capsule and containing numerous basophilic bradyzoites is observed within a muscle fiber. H&E.

Tables

Table 1. Histologic investigation of sarcocysts in muscular tissues from domestic cats: characterization of the sarcocyst-positive group according to clinical, epidemiologic, pathologic information.

Description	Sarcocyst-positive cats				
	Cat 1	Cat 2	Cat 3	Cat 4	Cat 5
Sarcoysts	Multiple	Multiple	Single	Multiple	Multiple
Affected muscle(s)[a]	Intercostal; Diaphragm	Intercostal; Diaphragm	LV (LPL)	SS (RTL; LTL) LV (RPL; LPL)	Intercostal SS (RTL; LTL) LV (RPL; LPL)
Species	*S. neurona*	*S. neurona*	*S. neurona*	*S. neurona*	*S. neurona*
Cause of death[b]	Idiopathic pyothorax	Idiopathic pyothorax	CKD	Chronic pancreatitis	Acute myeloid leukemia
Breed	Mixed breed	Mixed breed	Mixed breed	Mixed breed	Mixed breed
Gender	Male	Male	Male	Male	Male
Age (years)	2	1	5	7	3
Outdoor access	Yes	Yes	Yes	Yes	Yes
Feral or pet	Pet	Feral	Pet	Feral	Pet
FIV / FeLV	Negative	Negative	No informed	FIV +	FeLV +

[a] LV = Lateral vastus; SS = Subscapular; RTL = Right thoracic limb; LTL = Left thoracic limb; RPL = Right pelvic limb; LPL = Left pelvic limb.

[b] CKD = Chronic kidney disease.

Table 2. Histologic investigation of sarcocysts in muscular tissues from domestic cats: characterization of the sarcocyst-positive group and comparison with the total necropsy group.

Data	Total group	Sarcocyst-negative	Sarcocyst-positive
Males			
Intact	35	33	2
Spayed	22	19	3
Females			
Intact	19	19	0
Spayed	24	24	0
Breed			
Purebreed	5	5	0
Mixed breed	95	90	5
Median age at death	5	5	3
Access to the outdoors			
Yes	41	36	5
No	17	17	0
No informed	42	42	0
Feral or pet			
Feral	9	7	2
Pet	91	88	3
Area of origin			
Rural	0	0	0
Urban	100	95	5
FIV/FeLV status			
FeLV positive	15	14	1
FIV positive	3	3	1
FIV and FeLV negative	17	15	2
Not informed	65	64	1
PIS[a] drug administration[b]			
Yes	5	5	0
No	95	95	5
Total	100	95	5

[a]PIS=Potentially immunosuppresive; [b]prednisolone, methylprednisolone acetate, doxorubicin and/or vincristine

3 CONCLUSÃO

Os resultados obtidos neste estudo demonstram que gatos tem potencial de atuarem como hospedeiros intermediários naturais de *S. neurona* em nossa região, embora a sarcocistose muscular tenha sido incomum no nosso trabalho. A atuação desses gatos como hospedeiros intermediários para *S. felis* permanece desconhecida, tendo em vista que nenhum dos casos positivos para sarcocistos musculares foi associado a essa espécie de *Sarcocystis*. Todos os gatos positivos para *S. neurona* eram machos com acesso à rua, e dois deles eram positivos para retroviroses felinas.

O número pequeno de gatos portadores de cistos impossibilitou o estabelecimento de associações entre a infecção por *Sarcocystis* spp. e diferentes aspectos epidemiológicos. Estudos futuros com um maior número de gatos positivos para *Sarcocystis* spp. poderiam permitir a investigação de tais associações.

Os resultados obtidos nesse estudo trazem contribuições acerca do papel do gato doméstico no ciclo natural de *S. neurona*, contribuindo para o entendimento do ciclo do parasita, particularmente na região Sul do Brasil.

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