



Detection of anti-*Sarcocystis* spp. antibodies in domestic cats, in southern Brazil

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Abstract

Parasites of the genus *Sarcocystis* can infect several species of animals and cause multiple diseases such as equine protozoal myeloencephalitis. Felines are considered hosts of this protozoa; therefore, the present study aimed to detect anti-*Sarcocystis* spp.–specific antibodies in domestic cats that were under clinical evaluation, using the indirect immunofluorescence antibody test. Anti-*Sarcocystis*-specific immunoglobulin Gs were detected in 24 out of 497 (4.82%) cat serum samples. These findings support the fact that natural *Sarcocystis* infections do occur in cats. Furthermore, it highlights the importance of domestic cats as both intermediate and definitive hosts in the *Sarcocystis* life cycle, maintaining the parasite and serving as a source of infection for various other animals. To the best of our knowledge, this is the first study to identify antibodies against the genus *Sarcocystis* in cats from a region in southern Brazil.

Keywords Domestic cats · Intermediate host · Genus *Sarcocystis* · Indirect immunofluorescence · Cross-reaction

Introduction

Sarcocystis spp. are intracellular protozoan parasites (Apicomplexa: Sarcocystidae) with an obligatory prey-predator, two-host life cycle. There are two types of hosts involved: the intermediate host (IH), which is infected through the consumption of food or water contaminated with sporocysts,

and the definitive host (DH), which is infected by ingesting tissues containing sarcocysts (Dubey et al. 2015).

Sarcocystis spp. are widespread parasites that infect mammals, birds, and reptiles. Several species of *Sarcocystis* have evolved to be able to infect multiple hosts (Shams et al. 2022). Among these, the most prevalent are *S. cruzi* and *S. hominis*, which infect cattle, and *S. gigantea* and *S. tenella*, which infect sheep (Shams et al. 2022). Other species, such as *S. neurona*, infect horses and cause equine protozoal

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myeloencephalitis (EPM) (Dubey et al. 2000), while *S. falcatula* is known to infect birds (Gondim et al. 2019).

Felines can act as either the IH or DH for *Sarcocystis* spp.: they are known DHs of various *Sarcocystis* species, such as *S. gigantea*, *S. medusiformis*, *S. hirsuta*, *S. porcifelis*, *S. moulei*, *S. bovifelis* (Dubey et al. 2020; Gjerde et al. 2016a, b; Heydorn et al. 1975), *S. sinensis* (Gjerde et al. 2016a), and *S. bovis* (Gjerde et al. 2016b), and can also act as the IH of *S. felis* (Dubey et al. 1992) and *S. neurona* (Cheadle et al. 2001; Turay et al. 2002). Domestic cats (*Felis domesticus*) have the ability to act as either the IH or DH for *S. sinensis* (Gjerde et al. 2016a) and have also been described as an IH of *S. neurona* which is the causative agent of EPM in horses (Dubey et al. 2015). However, studies on the role of domestic cats in *Sarcocystis* epidemiology and *Sarcocystis* spp. detection and characterization, particularly in Brazil, are scarce.

Therefore, the objective of this study was to detect the presence of anti-*Sarcocystis* spp. antibodies in serum samples from domestic cats.

Materials and methods

Serum samples

In total, 497 blood samples were collected from cats that received veterinary assistance at the Hospital Veterinário Universitário (HVU) of Universidade Federal de Santa Maria (UFSM) between October 2018 and May 2019. The blood samples were drawn from the cats during clinical evaluation. After blood count analysis, the samples were centrifuged at 3400 rpm for 4 min, and the serum was collected and stored at $-20\text{ }^{\circ}\text{C}$. The serum samples were analyzed for the presence of antibodies against *Sarcocystis* spp. at the Laboratório de Doenças Parasitárias (LADOPAR) in UFSM.

Indirect immunofluorescence antibody test

Detection of IgGs against anti-*Sarcocystis* spp. in the cat serum samples was performed using indirect immunofluorescence antibody test (IFAT). Slides containing fixed *Sarcocystis* bradyzoites, which were obtained from bovine hearts, were used to perform IFAT. The bradyzoites were diluted in phosphate-buffered solution (PBS, pH 7.4) at a final concentration of $1.5\text{--}2.0 \times 10^3$ bradyzoites/mL. Approximately 20 μL of this solution was added to each well on specific IFAT slides, dried at room temperature, and fixed with methanol.

Sera were diluted at 1:50 in PBS (pH 7.4) (Hsu et al. 2010), placed in wells on the slide, and incubated at $37\text{ }^{\circ}\text{C}$ for 50 min in a humid chamber. The slides were washed

with PBS, followed by an incubation at $37\text{ }^{\circ}\text{C}$ for 50 min in a humid chamber with anti-cat IgG antibody conjugated with fluorescein (Sigma-Aldrich Inc., St. Louis, MO, USA) diluted at 1:200 in PBS. The slides were washed again, mounted with mounting fluid (glycerol/PBS, 50:50) and a cover slip, and then examined under a fluorescent microscope at $400\times$ magnification. The samples were considered positive at a 1:50 dilution. *Sarcocystis* spp.–positive (naturally infected) and *Sarcocystis*–negative cat sera were used as the positive and negative controls, respectively.

Results and discussions

Anti-*Sarcocystis* spp. antibodies were detected in 24 cat serum samples with an antibody detection frequency of 4.82% (24/497). Few studies have investigated the serostatus of Brazilian domestic cats with regard to the genus *Sarcocystis* (Dubey et al. 2002; Meneses et al. 2014; Koch et al. 2019). This is the first study to detect anti-*Sarcocystis* spp. antibodies in domestic cats in southern Brazil. However, several studies have reported the detection of antibodies against *S. neurona* in cats (Meneses et al. 2014), and in some studies, the detection frequency was similar to that in the present study. Furthermore, the detection frequencies of antibodies against *S. neurona* detected by Meneses (2014) and Koch et al. (2019) were 4% and 5%, respectively, similar to the results obtained in our study.

The IFAT test contained bradyzoites from an undetermined species of *Sarcocystis* that infect cattle. However, because cross-reactions among *Sarcocystis* species occur (Ferreira et al. 2020), these using bovine-derived bradyzoite can still be used for the detection of anti-*Sarcocystis* antibodies in cat serum samples. Because of these cross-reactions that allow the use of undetermined species of *Sarcocystis* serological detection, the results obtained are related to anti-*Sarcocystis* spp. antibodies at the genus level.

Sarcocystis infection in cats is usually not associated with clinical signs. However, the detection of anti-*Sarcocystis* spp. IgGs in cat serum confirms that natural infection by *Sarcocystis* does occur in domestic cats and is capable of a specific and quantifiable immune response. Therefore, domestic cats represent an important host involved in the *Sarcocystis* life cycle. This information is especially valuable, as cats are present in both urban and rural areas, where they circulate indoors and outdoors, while coming into close contact with humans and other animals, such as horses. In this way, cats can act as a widespread source of *Sarcocystis* infection, both as an IH and DH, causing disease in various other animals, such as EPM in horses.

Conclusion

Anti-*Sarcocystis* spp. antibodies were detected in serum samples of domestic cats from a region in southern Brazil. To the best of our knowledge, this is the first study to detect antibodies against the genus *Sarcocystis* in Brazilian cats. In addition, our results confirm that natural infection by *Sarcocystis* does occur in domestic cats, and strongly support the participation of cats as hosts in the *Sarcocystis* life cycle.

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Data availability Not applicable.

Declarations

Competing interests The authors declare no competing interests.

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Conflict of interest The authors declare no competing interests.

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