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Epidemiological aspects of the occurrence of anti-*Sarcocystis spp.* and anti-*Toxoplasma gondii* antibodies in dogs in the Northern region of Brazil*

Aspectos epidemiológicos da ocorrência de anticorpos anti-*Sarcocystis* spp. e anti-*Toxoplasma gondii* em cães da região norte do Brasil

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Abstract

We conducted a seroepidemiological study on the occurrence of anti-*Sarcocystis* spp. and anti-*Toxoplasma gondii* antibodies in dogs from family farming properties in the municipality of Ji-Paraná, Rondônia. Blood samples were collected from apparently healthy dogs between September 2012 and November 2013. In total, 181 blood serum samples were analyzed using an indirect immunofluorescence assay, among which 57 (31.49%) and 20 (11.04%) were positive for anti-*T. gondii* and anti-*Sarcocystis* spp., respectively. Statistical analyses showed that the type of food fed to the dogs was associated with the occurrence of anti-*Sarcocystis* spp. antibodies. In contrast, age and access to bovine carcasses were the risk factors for anti-*T. gondii*. The high occurrence of seropositive dogs for *Sarcocystis* spp. and *T. gondii* evidences the wide distribution of these agents in the studied area, possibly due to human and animal exposure to these protozoan species. In addition, anti-*T. gondii* antibodies were directly proportional to dog age. The increase in the number of positive animals with age was statistically significant. Furthermore, high antibody titers (up to 800) against *Sarcocystis* spp. in dogs suggest the possibility of recent exposure, in addition to environmental contamination by oocysts/sporocysts eliminated by the feces of these animals.

Keywords: Amazon, immunofluorescence, sarcosporidiosis, toxoplasmosis, zoonoses.

Resumo

Conduzimos um estudo soroepidemiológico sobre a ocorrência de anticorpos anti- *Sarcocystis* spp. e anti-*Toxoplasma gondii* em cães de propriedades de agricultura familiar no município de Ji-Paraná, Rondônia. Amostras de sangue foram coletadas de cães aparentemente saudáveis, entre setembro de 2012 e novembro de 2013. Ao todo, foram analisados 181 soros sanguíneos por meio do ensaio de imunofluorescência indireta, sendo positivas 57 (31,49%) e 20 (11,04%) amostras para anticorpos anti-*T. gondii* e anti-*Sarcocystis* spp., respectivamente. As análises estatísticas demonstraram que o tipo de alimentação fornecida aos cães esteve associado **à ocorrência de anticorpos** anti-*Sarcocystis* spp. Em contraste a idade e o acesso à carcaça bovina foram fatores de risco para a presença de anticorpos anti-*T. gondii*. A alta ocorrência de cães soropositivos para *Sarcocystis* spp. e *T. gondii* evidencia a ampla distribuição desses agentes na área estudada, possivelmente devido à exposição humana e animal a essas espécies de protozoários. Além disso, o resultado dos anticorpos anti-*T. gondii* relacionados a idade do cão mostraram diferença estatística, com aumento significativo no número de animais positivos com a idade. Além disso, altos títulos de anticorpos (até 800) contra *Sarcocystis* spp. em cães sugerem a possibilidade de exposição recente, além da contaminação ambiental por oocistos/esporocistos eliminados pelas fezes desses animais.

Palavras-chave: Amazônia, imunofluorescência, sarcosporidiose, toxoplasmose, zoonoses.

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Introduction

Sarcocystis spp. is a common coccidian recognized in the musculature of herbivores (intermediate hosts) and carnivores (definitive hosts), such as domestic dogs (*Canis familiaris*). The *Sarcocystis* life cycle (Dubey et al., 1989; Chhabra and Samantaray, 2013) includes developing gamonts and oocysts in the lamina propria of the small intestine in definitive hosts. Although carnivores are asymptomatic in most cases, they may spread diseases in livestock and equines depending on the coccidian species involved (Chhabra and Samantaray, 2013). Furthermore, species of *Sarcocystis* have been identified in humans as definitive hosts. In addition, recognized zoonotic *Sarcocystis* species have also infected human tissues (Fayer et al., 2015).

Toxoplasma gondii is a widespread zoonotic protozoan that infects most, if not all, bird and mammal species, including domestic dogs (Dubey, 2010). Felids are definitive hosts, and all non-feline animals are intermediate hosts. *Toxoplasma gondii* can affect the central nervous system, muscles, and viscera of these animals (Dubey, 2010; Calero-Bernal and Gennari, 2019). Dogs rarely suffer from toxoplasmosis infection (Calero-Bernal and Gennari, 2019), and have been used as sentinel animals for *T. gondii* infection due to their close contact with humans and cats (Salb et al., 2008).

The present study aimed to perform a seroepidemiological study on the presence of anti-*Sarcocystis* spp. and *anti-T. gondii* antibodies in dogs living in dairy cattle production systems, and to evaluate the risk factors associated with seropositivity in rural areas.

Materials and Methods

This study sampled dogs living in the dairy cattle production systems of family farms in the municipality of Ji-Paraná, Rondônia, in the Western Brazilian Amazon region. The dogs were sampled primarily for another study on the epidemiology of neosporosis (Vilas Boas et al., 2015) between September 2012 and November 2013. Blood samples were collected from all dogs (n=181) from 61 of the 63 farms. A person responsible for each farm answered a questionnaire addressing epidemiological factors related to handling animals on their corresponding farm.

The levels of anti-*Sarcocystis* spp. and anti-*T. gondii* antibodies were measured by an indirect immunofluorescence assay (IFA) using the following antigens: *T. gondii* RH strain tachyzoites and merozoites of the SN37R strain of *Sarcocystis neurona*, according to methods previously described by Camargo (1964) and Duarte et al. (2003), respectively. The cut-offs adopted were 1:16 for *T. gondii* **(**Rivetti-Júnior et al., 2008) and 1:25 for *S. neurona* (Oliveira et al., 2020). Positive and negative control samples were included on each slide, and rabbit polyclonal anti-dog immunoglobulin G (anti-IgG) whole molecule FITC at 1:100 dilution was used as the conjugate. Samples considered positive were subjected to two-fold serial dilution to obtain the final titer. Only cultured merozoites of *S. neurona* were available for serology as an antigen. Because of possible cross-reactivity that occurs by IFA between *Sarcocystis* species, we assumed that dogs seropositive for *S. neurona* antigen were seropositive for *Sarcocystis* spp.

Statistical analyses were performed using EPIINFO 7.0. The associations between seropositive dogs (presence of anti-*Sarcocystis* spp. and/or anti-*T. gondii* antibodies) and the variables were analyzed using chi-square (χ^2) and Fisher exact tests, with a significance of 5%. The covariate variables analyzed were the presence of dogs; dogs tested seropositive for anti-*Sarcocystis* spp. and/or anti-*T. gondii* on farm; occurrence of abortion; wild canids nearby; dogs with access to cattle food; dogs with access to water for cattle; the presence of felids; the presence of Equidae; dogs with access to the remains of dead cattle carcasses; dogs fed with heat-treated food or foodstuffs scrapped (with uncooked or undercooked meat); cattle grazing with dogs present; destination of the dead animals; the destination of fetuses, when observed; dogs that got stuck or loose; farming system (extensive or intensive); recently acquired dogs; farrowing of dogs on the property; and age and sex of dogs. Statistical analyses were performed by comparing these covariates and farms with dogs seropositive for *Sarcocystis* spp. and *T. gondii* using generalized linear models (GLM).

The Bioethical Committee for Animal Research of the Federal University of Mato Grosso approved the present study (protocol no. 23108.015662/12-5).

Results

Anti-*Sarcocystis* spp. antibodies (titers ≥ 25) were detected in 11.04% (20/181) of the serum samples, with titers ranging from 25 to 800. In contrast, anti-*T. gondii* antibodies (titers ≥ 16) were found in 31.49% (57/181) of the sampled dogs, with titers ranging from 16 to 2,048 (Table 1).

Table 1: Positive results of indirect immunofluorescence assay (IFA) endpoint titers were obtained from 181 dogs from 61 farms in the municipality of Ji-Paraná, Rondônia, Brazil, between September 2012 and December 2013, using *Sarcocystis neurona* and *Toxoplasma gondii* as antigens.

Sarcocystis neurona		***Toxoplasma gondii***	
Titer	**Positive sample (%)**	**Titer**	**Positive sample (%)**
25	1 (5)	16	2 (3.51)
50	2 (10)	32	8 (14.03)
100	5 (25)	64	14 (24.56)
200	6 (30)	128	17 (29.82)
400	5 (25)	256	6 (10.53)
800	1 (5)	512	5 (8.77)
		1024	2 (3.51)
		2048	3 (5.27)
Total	**20 (100)**	**Total**	**57 (100)**

Univariate correlations between the examined variables and the presence of seropositive dogs showed that the type of food provided to the dogs was associated with the occurrence of anti-*Sarcocystis* antibodies on the studied farms (p=0.021).

Statistical analysis using the Chi-Squared test for linear tendency showed that the presence of anti-*T. gondii* antibodies among the samples of the dogs was significantly associated with the dog age, resulting in χ^2=5.990 and p=0.014. Considering the results

by age group, the odds ratio analysis (OR) obtained was as follows: 0–12 months OR=1.000; 12–24 months OR=0.96; 24–36 months OR=1.56; 36–48 months OR=3.44), and > 48 months OR=2.342. The GLM analyses showed that the presence of anti-*Sarcocystis* spp. and anti-*T. gondii* antibodies in dogs was associated with the type of food provided to dogs (fed raw or undercooked meat; F-test<0.001) and with dogs that had access to bovine carcasses (F-test=0.003), respectively.

Discussion

The information available on serological studies of the exposure to *Sarcocystis* spp. in canine populations is scarce; thus, to our knowledge, only two studies have described the occurrence of anti-*S. neurona* antibodies in Brazilian dogs (Kock et al., 2019; Oliveira et al., 2020), showing lower seropositivity in sampled dogs from Paraná (7%) and Bahia (3.53%), respectively.

Oliveira et al. (2020) observed that serologic cross-reactivity did not occur by IFA using *S. neurona* and *Sarcocystis cruzi* as antigens. The domestic dog is a definitive host for at least twenty-three valid species of *Sarcocystis* (Dubey et al., 2016; Tuska-Szalay et al., 2021; Wu et al., 2022). Because no other *Sarcocystis* species were available for the last report, we assumed that dogs seropositive to *S. neurona* antigen were seropositive to *Sarcocystis* spp. Moreover, dogs are also intermediates or aberrant hosts to other *Sarcocystis* species, such as *S. caninum*, *S. neurona*, and *S. svanai* (Dubey et al., 2015; Dubey et al., 2016). Therefore, the reactivity of blood serum to *S. neurona* antigen might result from the exposure to *S. neurona* or other related species.

Sarcocystis has an obligatory prey-predator 2-host life cycle (intermediate-definitive), with the definitive host becoming infected by ingesting muscular or neural tissue containing mature *Sarcocystis* (Dubey et al., 2016). Therefore, in the present study, the transmission of *Sarcocystis* species could be related to the ingestion of undercooked meat, as we observed occurrences of anti-*Sarcocystis* antibodies in the farms studied associated with the type of food provided to dogs. *Sarcocystis cruzi*, the most common *Sarcocystis* in cattle worldwide, is transmissible via dogs (Dubey et al., 2016). The serum samples available for the present study were collected from dogs living in dairy cattle production systems in rural areas of the western Brazilian Amazon region.

Seropositivity in serum samples suggests the exposure of the farm dogs and, possibly, livestock to *Sarcocystis* through food and water contaminated with sporocysts/oocysts released in the feces of definitive hosts, such as domestic dogs. In addition, it is notable that in one serum sample, we observed reactivity with a high titer (800) of antibodies anti-*S. neurona*, which might result from exposure to this latter species. Although this statement must be proven, *S. neurona* is related to systemic illness in many animals, including dogs infected with myositis and encephalitis (Vashisht et al., 2005; Dubey et al., 2006; Cooley et al., 2007). Furthermore, horses are the etiological agents of equine protozoal myeloencephalitis (Dubey et al., 1991).

Although the *Sarcocystis* species that infect dogs and humans are different, in a study conducted by Agholi et al. (2016), the authors have demonstrated the presence of *S. cruzi* oocysts in immunodeficiency patients in Iran. Thus, highlighting the zoonotic potential and the risk of exposure to these agents in an environment shared with humans, domestic dogs, and livestock.

Serological studies have indicated that anti-*T. gondii* antibodies are found in canine sera worldwide (Dubey et al., 2020). Several serological investigations of *T. gondii* in dogs have reported seroprevalences in Brazil, with titers ranging from 7.0% to 82.2% (Dubey et al., 2020; Ziemniczak et al., 2021) using a 1:16 dilution in IFA as the cut-off. In addition, seroprevalence in dogs from rural areas was higher than that in city dogs and generally increased with age, indicating postnatal infection (Dubey et al., 2020; Sevá et al., 2020). We observed high levels (57%) of anti-*T. gondii* antibodies in serum samples from dogs in the rural area of the Ji-Paraná. The high seroprevalence of anti-*T. gondii* antibodies have already been described in other studies in Rondônia, Western Brazilian Amazon (Canon-Franco et al., 2004; Ziemniczak et al., 2021), thus confirming human and livestock exposure to the protozoa. High seroprevalence in dogs may reflect the magnitude of parasite contamination in the environment (Salb et al., 2008), as the ingestion of food or water contaminated with oocysts excreted by infected cats is a major mode of transmission of *T. gondii* (Dubey et al., 2020). In addition, this high prevalence may be explained by the hot and humid climate of the Amazon region, which may contribute to maintaining the viability of excreted oocysts in the environment (Ziemniczak et al., 2021). Presence of anti-*T. gondii* antibodies and dog age revealed statistically difference by the qui-square test, with a significant increase in the number of positive animals with age. The group of dogs at 36 to 48 months of age was 3.44 times more likely to test positive for anti-*T. gondii* antibodies. The magnitude of the titer is not associated with clinical signs, and this occurs because seroprevalence increases with age because of the chance of *T. gondii* exposure during the lifetime rather than disease susceptibility (Dubey et al., 2009).

Notably, dogs with access to bovine carcasses had an increased probability of the presence of anti-*T. gondii* antibodies, suggesting the possible involvement of cattle in parasite transmission. Among livestock, bovines seem to be more resistant to infection with *T. gondii* than other animals; however, cysts of *T. gondii* may remain viable in their tissues until the slaughtering age and may be a source of infection (Dubey, 2010). Maia et al. (2021) reported a high prevalence of anti-*T. gondii* antibodies in cows destined for human consumption in Midwestern Brazil, with a positive association between the presence of anti-*T. gondii* antibodies and farms in which slaughtered animals are normally used for their own consumption. Additionally, eating raw beef has been reported as a risk factor for *T. gondii* infection in Brazil (Eduardo et al., 2007).

Conclusions

This study investigated the distribution and frequency of seropositivity of the anti-*Sarcocystis* spp. and anti-*T. gondii* antibodies in farm dogs in the municipality of Ji-Paraná, Rondônia, Western Brazilian Amazon. Further studies are required to assess the impact of the seropositivity of dogs to *Sarcocystis* spp. and *T. gondii* on livestock and human health in the region and to establish the requirement of additional control measures related to the type of food provided to dogs.

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