

# Frequency of antibodies against *Sarcocystis neurona* and *Neospora caninum* in domestic cats in the state of Bahia, Brazil

Frequência de anticorpos contra *Sarcocystis neurona* e *Neospora caninum* em gatos domésticos do Estado da Bahia, Brasil

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Received February 20, 2014

Accepted July 29, 2014

## Abstract

*Sarcocystis neurona* is the major agent of equine protozoal myeloencephalitis. It infects several mammalian species in the Americas, where the definitive hosts, marsupials of the genus *Didelphis* (*D. virginiana* and *D. albiventris*) are found. Domestic cats are one of the confirmed intermediate hosts of the parasite; however, antibodies against *S. neurona* had never before been demonstrated in Brazilian cats. The aim of this study was to determine whether cats in Bahia, Brazil, are exposed to the parasite. A total of 272 feline serum samples (134 from feral and 138 from house cats) were subjected to an indirect fluorescent antibody test using cultured merozoites of *S. neurona* as antigen. Positivity was detected in 4.0% (11/272) of the tested samples, with titers ranging from 25 to 800. The feline sera were also tested for antibodies against the protozoan *Neospora caninum*, with an observed antibody frequency of 2.9%. To the author's knowledge, this is the first study to report antibodies against *S. neurona* in Brazilian cats. We conclude that cats are exposed to the parasite in the region of this study. Further investigations are needed to confirm the role of cats in the transmission cycle of *S. neurona* in Brazil.

**Keywords:** *Sarcocystis neurona*, *Neospora caninum*, feline, epidemiology.

## Resumo

*Sarcocystis neurona* é o principal agente da mieloencefalite protozoária equina. Esse parasito infecta várias espécies de mamíferos nas Américas, onde são encontrados os hospedeiros definitivos, os marsupiais do gênero *Didelphis* (*D. virginiana* and *D. albiventris*). O gato doméstico é um dos hospedeiros intermediários do parasito. Contudo, anticorpos contra *S. neurona* ainda não tinham sido demonstrados em gatos brasileiros. O objetivo deste trabalho foi determinar se gatos da Bahia, Brasil, são expostos ao parasito. Amostras séricas de 272 felinos (134 de gatos errantes e 138 de gatos domiciliados) foram testadas pelo teste de imunofluorescência indireta, utilizando-se como antígeno, merozoítos produzidos em cultura celular. Entre as amostras testadas, 4,0% (11/272) foram positivas com títulos entre 25 e 800. Os soros dos felinos foram também testados para anticorpos contra o protozoário *Neospora caninum*, cuja frequência de anticorpos foi de 2,9%. Esse é o primeiro relato de anticorpos contra *S. neurona* em gatos brasileiros. Conclui-se que os gatos da região estudada são expostos a *S. neurona*. Estudos futuros são necessários, a fim de se confirmar o papel dos gatos no ciclo de transmissão de *S. neurona* no Brasil.

**Palavras-chave:** *Sarcocystis neurona*, *Neospora caninum*, felino, epidemiologia.

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## Introduction

*Sarcocystis neurona* is a tissue cyst-forming protozoan parasite and the main agent of equine protozoal myeloencephalitis (EPM), a serious disease that affects horses in several countries in the Americas, including Brazil (DUBEY et al., 1991; MASRI et al., 1992).

Marsupials of the genus *Didelphis* are definitive hosts (DH) for *S. neurona*; *D. virginiana* is the DH in North America (FENGER et al., 1995), and *D. albiventris* has been identified as a DH in South America (DUBEY et al., 2001a). Domestic cats (*Felis catus*) were the first intermediate hosts of *S. neurona* identified experimentally (DUBEY et al., 2000). In addition to infecting cats, the parasite may also cause disease in this animal species (DUBEY et al., 2003). Myeloencephalitis caused by *S. neurona* was reported in a cat on the third day after surgery for routine castration and onychectomy; the diagnosis was based on morphology and immunostaining of the parasite, albeit not confirmed by molecular analysis (DUBEY et al., 2003).

Infection by *S. neurona* has been confirmed in other mammalian and avian species, such as armadillos (*Dasypus novemcinctus*) (CHEADLE et al., 2001a), raccoons (*Procyon lotor*) (DUBEY et al., 2001b), sea otters (*Enhydra lutris*) (DUBEY et al., 2001c), skunks (*Mephitis mephitis*) (CHEADLE et al., 2001b) and brown-headed cow birds (*Molothrus ater*) (MANSFIELD et al., 2008).

Exposure of cats to *S. neurona* has been reported in North America (ROSSANO et al., 2002; TURAY et al., 2002; STANEK et al., 2003; HSU et al., 2010). Only one seroepidemiological study of *S. neurona* in cats has been conducted in Brazil; however, no positive reactions were observed among the tested animals (DUBEY et al., 2002). The aim of this study was to determine the frequency of antibodies against *S. neurona* in outdoor and indoor cats in the city of Salvador, state of Bahia, Brazil.

## Materials and Methods

### Animals

Blood samples were drawn from 272 cats in the city of Salvador, Bahia, between 2010 and 2011. The sample size was calculated for an infinite population, using an expected prevalence of 50%, accuracy of 6% and a confidence interval of 95%. The animals were divided in two groups: 134 cats were outdoor animals rescued from the streets and kept in a shelter, while 138 animals were indoor cats living with their owners, without outdoor access (Table 1). Blood samples were collected during routine clinical examination of the cats, and placed in tubes without anticoagulant. The sera were separated and stored at  $-20^{\circ}\text{C}$  until tested.

### Parasite culture and serology

*Sarcocystis neurona* merozoites of the strain SN37R (SOFALY et al., 2002) were employed to screen the positive and negative samples. For titration, the SN6 (DUBEY et al.,

**Table 1.** Frequency of antibodies against *Sarcocystis neurona* and *Neospora caninum* tested by IFAT in sera of domestic cats from Salvador, Bahia, Brazil.

Animals (n)	Seropositive animals (%)	
	<i>S. neurona</i>	<i>N. caninum</i>
Indoors (138)	3 (2.17)	3 (2.17)
Outdoors (134)	8 (5.97)	5 (3.73)
Total (272)	11 (4.04)	8 (2.94)

1999b) and SN138 (LINDSAY et al., 2004) strains were also used, in addition to SN37R. The merozoites were maintained in CV-1 cell monolayers cultured with RPMI containing 100 units/mL of penicillin, 100  $\mu\text{g}/\text{mL}$  of streptomycin, 0.25  $\mu\text{g}/\text{mL}$  of amphotericin B, and 5% of bovine calf serum, at  $37^{\circ}\text{C}$  in a 5%  $\text{CO}_2$  atmosphere. The culture flasks were scraped when approximately 80% of the cell monolayers were infected. The cells thus removed were centrifuged at 1200xg for 10 min, washed with sterile PBS pH 7.2, passed through a 26G needle, and filtered through a 5 $\mu\text{m}$  syringe filter.

Purified merozoites were diluted in PBS (500-1000 merozoites/ $\mu\text{l}$ ) and distributed in 12-well Teflon-coated slides. The slides were dried at  $37^{\circ}\text{C}$  for 30 min, fixed in methanol for 5 min, and stored at  $-20^{\circ}\text{C}$  for no longer than 2 months, until analysis.

*Neospora caninum* (NC-Bahia strain) tachyzoites were cultured in the same way as the *S. neurona* merozoites. Vero cells were used instead of CV-1 cells, and the medium was supplemented with 5% of horse serum. The antigen purification and slide preparation methods for *N. caninum* were the same as those described above.

The IFATs for *S. neurona* and *N. caninum* were performed using serum dilutions of 1:25 and 1:50, respectively. A fluorescein isothiocyanate-labeled goat anti-cat IgG (Bethyl, Montgomery, TX, USA) was used as a secondary antibody. Positive sera were diluted 2-fold until an endpoint was reached. Negative and positive control sera for both parasites were included on each slide; these controls consisted of sera from the same tested cats that had been screened previously by IFAT.

### Statistical analysis

The means of the two groups of animals (outdoor and indoor) were compared by Fisher's exact test with a 95% confidence interval.  $P < 0.05$  was considered significant.

## Results

Antibodies against *S. neurona* were detected in 4.0% (11/272) of the feline sera, and *N. caninum* antibodies in 2.9% (8/272) of the cats (Table 1). The antibody titers for *S. neurona* were 25 (1/11), 50 (1/11), 200 (1/11), 400 (2/11), and 800 (6/11). All the cats seropositive for *N. caninum* had titers of 50.

Seropositivity for *S. neurona* or *N. caninum* showed no significant difference between indoor and outdoor cats ( $P = 0.072$ ). Among the 11 cats seropositive for *S. neurona*, four were also positive for *N. caninum*.

## Discussion

In this study, antibodies against *S. neurona* were detected by IFAT in domestic cats in the state of Bahia, Brazil. High antibody titers to the parasite (up to 800) were found, which is suggestive of true exposure to *S. neurona*; however, true infection by this parasite was not confirmed in these cats. To the best of our knowledge, this is the first report of *S. neurona* antibodies in Brazilian cats.

At the time this study was concluded, only one serological survey for *S. neurona* had been performed in Brazilian cats (DUBEY et al., 2002), but no seropositive animals were found. The above cited authors attributed the negative results to the fact that the tested sera came from urban cats, which probably had no contact with the parasite's sporocysts.

In the current study, the difference in seropositivity for *S. neurona* between indoor and outdoor animals was not significant ( $P = 0.072$ ); however, the number of outdoor cats seropositive for the parasite was 2.7 times higher than the number of indoor animals. Cats are intermediate hosts of *S. neurona* and may become infected after ingesting *S. neurona* sporocysts shed by opossums (DUBEY et al., 2000). Opossums also shed sporocysts of other *Sarcocystis* spp., including *S. falcatula*, which is closely related to *S. neurona* (MARSH et al., 1999; TANHAUSER et al., 1999). Nevertheless, the ingestion of *S. falcatula* sporocysts by cats does not seem to induce a serological cross-reaction to *S. neurona*. Cats infected experimentally with *S. neurona* sporocysts developed high antibody titers to the parasite, whereas a cat inoculated with *S. falcatula* sporocysts did not cross react against *S. neurona* (DUBEY et al., 2002).

Antibodies against *S. neurona* have been detected in North American cats, with a prevalence of exposure of up to 10% (GILLIS et al., 2003; HSU et al., 2010; ROSSANO et al., 2002; TURAY et al., 2002). In one study, serum samples from farm cats where EPM had been diagnosed previously were compared with sera from spay/neuter clinic samples (STANEK et al., 2003). Antibodies against *S. neurona* were found in 40% of the farm cats, while 10% of the samples from the spay/neuter clinic were seropositive for the parasite, suggesting that farm cats may be more commonly exposed to *S. neurona*.

In the present survey, the feline serum samples were also tested by IFAT for the closely related parasite *N. caninum*, and 2.94% (8/272) of the cats were seropositive, albeit with low antibody titers. In previous serological studies involving Brazilian cats, variable rates of exposure were reported (BRESCIANI et al., 2007; DUBEY et al., 2002), although natural *N. caninum* infections in cats have never been confirmed.

Among the *S. neurona* positive samples, four were reactive for *N. caninum*. In the same study mentioned above (DUBEY et al., 2002), 60 of 502 cats were positive for *N. caninum* antibodies, but none of the cats were seropositive to *S. neurona*. These results indicate that antibodies against *N. caninum* do not cross react with *S. neurona* antigen.

In Brazil, studies on *S. neurona* have been performed on other animal species, including horses (DUBEY et al., 1999a; HOANE et al., 2006), opossums (DUBEY et al., 2001a) and capybaras (*Hydrochoerus hydrochaeris*) (VALADAS et al., 2010),

but no serological investigation of any confirmed intermediate host of the parasite has been reported, other than cats. Further studies are needed to confirm the role of cats as true intermediate hosts of *S. neurona*. These studies may be performed by testing whether tissues from naturally exposed (seropositive) Brazilian cats are able to induce infection in Brazilian opossums (*D. albiventris*) or in susceptible rodent models, such as interferon gamma knockout mice (DUBEY et al., 1999b).

## Conclusions

The high titers of antibodies against *S. neurona* observed in feline sera possibly indicate exposure of the cats to the parasite in Bahia, Brazil. The low antibody titers observed for *N. caninum* do not confirm true exposure of the cats to this parasite.

## Acknowledgements

This work was partially supported by FAPESB (Bahia Research Foundation). I.D.S. Meneses was the recipient of a fellowship granted by CAPES (Brazil's Federal Agency for the Support and Improvement of Higher Education), while L.F.P. Gondim was the recipient of a fellowship granted by CNPq (Brazil's National Council for Scientific and Technological Development).

## References

- Bresciani KD, Gennari SM, Serrano AC, Rodrigues AA, Ueno T, Franco LG, et al. Antibodies to *Neospora caninum* and *Toxoplasma gondii* in domestic cats from Brazil. *Parasitol Res* 2007; 100(2): 281-285. <http://dx.doi.org/10.1007/s00436-006-0262-4>. PMID:16941188
- Cheadle MA, Tanhauser SM, Dame JB, Sellon DC, Hines M, Ginn PE, et al. The nine-banded armadillo (*Dasypus novemcinctus*) is an intermediate host for *Sarcocystis neurona*. *Int J Parasitol* 2001a; 31(4): 330-335. [http://dx.doi.org/10.1016/S0020-7519\(01\)00177-1](http://dx.doi.org/10.1016/S0020-7519(01)00177-1). PMID:11306111
- Cheadle MA, Yowell CA, Sellon DC, Hines M, Ginn PE, Marsh AE, et al. The striped skunk (*Mephitis mephitis*) is an intermediate host for *Sarcocystis neurona*. *Int J Parasitol* 2001b; 31(8): 843-849. [http://dx.doi.org/10.1016/S0020-7519\(01\)00231-4](http://dx.doi.org/10.1016/S0020-7519(01)00231-4). PMID:11403777
- Dubey JP, Benson J, Larson MA. Clinical *Sarcocystis neurona* encephalomyelitis in a domestic cat following routine surgery. *Vet Parasitol* 2003; 112(4): 261-267. [http://dx.doi.org/10.1016/S0304-4017\(03\)00019-0](http://dx.doi.org/10.1016/S0304-4017(03)00019-0). PMID:12623205
- Dubey JP, Davis SW, Speer CA, Bowman DD, de Lahunta A, Granstrom DE, et al. *Sarcocystis neurona* n. sp. (Protozoa: Apicomplexa), the etiologic agent of equine protozoal myeloencephalitis. *J Parasitol* 1991; 77(2): 212-218. <http://dx.doi.org/10.2307/3283084>. PMID:1901359
- Dubey JP, Kerber CE, Granstrom DE. Serologic prevalence of *Sarcocystis neurona*, *Toxoplasma gondii*, and *Neospora caninum* in horses in Brazil. *J Am Vet Med Assoc* 1999a; 215(7): 970-972. PMID:10511862.
- Dubey JP, Lindsay DS, Hill D, Romand S, Thulliez P, Kwok OC, et al. Prevalence of antibodies to *Neospora caninum* and *Sarcocystis neurona* in sera of domestic cats from Brazil. *J Parasitol* 2002; 88(6): 1251-1252.

- [http://dx.doi.org/10.1645/0022-3395\(2002\)088\[1251:POATNC\]2.0.CO;2](http://dx.doi.org/10.1645/0022-3395(2002)088[1251:POATNC]2.0.CO;2). PMID:12537122
- Dubey JP, Lindsay DS, Kerber CE, Kasai N, Pena HF, Gennari SM, et al. First isolation of *Sarcocystis neurona* from the South American opossum, *Didelphis albiventris*, from Brazil. *Vet Parasitol* 2001a; 95(2-4): 295-304. [http://dx.doi.org/10.1016/S0304-4017\(00\)00395-2](http://dx.doi.org/10.1016/S0304-4017(00)00395-2). PMID:11223209
- Dubey JP, Mattson DE, Speer CA, Baker RJ, Mulrooney DM, Tornquist SJ, et al. Characterization of a *Sarcocystis neurona* isolate (SN6) from a naturally infected horse from Oregon. *J Eukaryot Microbiol* 1999b; 46(5): 500-506. <http://dx.doi.org/10.1111/j.1550-7408.1999.tb06067.x>. PMID:10519218
- Dubey JP, Saville WJ, Lindsay DS, Stich RW, Stanek JF, Speert CA, et al. Completion of the life cycle of *Sarcocystis neurona*. *J Parasitol* 2000; 86(6): 1276-1280. [http://dx.doi.org/10.1645/0022-3395\(2000\)086\[1276:CO TLCO\]2.0.CO;2](http://dx.doi.org/10.1645/0022-3395(2000)086[1276:CO TLCO]2.0.CO;2). PMID:11191904
- Dubey JP, Saville WJ, Stanek JF, Lindsay DS, Rosenthal BM, Oglesbee MJ, et al. *Sarcocystis neurona* infections in raccoons (*Procyon lotor*): evidence for natural infection with sarcocysts, transmission of infection to opossums (*Didelphis virginiana*), and experimental induction of neurologic disease in raccoons. *Vet Parasitol* 2001b; 100(3-4): 117-129. [http://dx.doi.org/10.1016/S0304-4017\(01\)00500-3](http://dx.doi.org/10.1016/S0304-4017(01)00500-3). PMID:11698157
- Dubey JR, Rosypal AC, Rosenthal BM, Thomas NJ, Lindsay DS, Stanek JF, et al. *Sarcocystis neurona* infections in sea otter (*Enhydra lutris*): evidence for natural infections with sarcocysts and transmission of infection to opossums (*Didelphis virginiana*). *J Parasitol* 2001c; 87(6): 1387-1393. [http://dx.doi.org/10.1645/0022-3395\(2001\)087\[1387:SN IISO\]2.0.CO;2](http://dx.doi.org/10.1645/0022-3395(2001)087[1387:SN IISO]2.0.CO;2). PMID:11780826
- Fenger CK, Granstrom DE, Langemeier JL, Stamper S, Donahue JM, Patterson JS, et al. Identification of opossums (*Didelphis virginiana*) as the putative definitive host of *Sarcocystis neurona*. *J Parasitol* 1995; 81(6): 916-919. <http://dx.doi.org/10.2307/3284040>. PMID:8544064
- Gillis KD, MacKay RJ, Yowell CA, Levy JK, Greiner EC, Dame JB, et al. Naturally occurring *Sarcocystis* infection in domestic cats (*Felis catus*). *Int J Parasitol* 2003; 33(8): 877-883. [http://dx.doi.org/10.1016/S0020-7519\(03\)00090-0](http://dx.doi.org/10.1016/S0020-7519(03)00090-0). PMID:12865087
- Hoane JS, Gennari SM, Dubey JP, Ribeiro MG, Borges AS, Yai LE, et al. Prevalence of *Sarcocystis neurona* and *Neospora* spp. infection in horses from Brazil based on presence of serum antibodies to parasite surface antigen. *Vet Parasitol* 2006; 136(2): 155-159. <http://dx.doi.org/10.1016/j.vetpar.2005.10.023>. PMID:16310955
- Hsu V, Grant DC, Dubey JP, Zajac AM, Lindsay DS. Prevalence of antibodies to *Sarcocystis neurona* in cats from Virginia and Pennsylvania. *J Parasitol* 2010; 96(4): 800-801. <http://dx.doi.org/10.1645/GE-2449.1>. PMID:20476809
- Lindsay DS, Mitchell SM, Vianna MC, Dubey JP. *Sarcocystis neurona* (Protozoa: Apicomplexa): description of oocysts, sporocysts, sporozoites, excystation, and early development. *J Parasitol* 2004; 90(3): 461-465. <http://dx.doi.org/10.1645/GE-230R>. PMID:15272465
- Mansfield LS, Mehler S, Nelson K, Elsheikha HM, Murphy AJ, Knust B, et al. Brown-headed cowbirds (*Molothrus ater*) harbor *Sarcocystis neurona* and act as intermediate hosts. *Vet Parasitol* 2008; 153(1-2): 24-43. <http://dx.doi.org/10.1016/j.vetpar.2007.12.016>. PMID:18342449
- Marsh AE, Barr BC, Tell L, Bowman DD, Conrad PA, Ketcherside C, et al. Comparison of the internal transcribed spacer, ITS-1, from *Sarcocystis falcatula* isolates and *Sarcocystis neurona*. *J Parasitol* 1999; 85(4): 750-757. <http://dx.doi.org/10.2307/3285758>. PMID:10461964
- Masri MD, Alda JL, Dubey JP. *Sarcocystis neurona*-associated ataxia in horses in Brazil. *Vet Parasitol* 1992; 44(3-4): 311-314. [http://dx.doi.org/10.1016/0304-4017\(92\)90128-V](http://dx.doi.org/10.1016/0304-4017(92)90128-V). PMID:1466140
- Rossano MG, Murphy AJ, Vrable RA, Vanzo NE, Lewis SK, Sheline KD, et al. Cross-sectional study of serum antibodies against *Sarcocystis neurona* in cats tested for antibodies against *Toxoplasma gondii*. *J Am Vet Med Assoc* 2002; 221(4): 511-514. <http://dx.doi.org/10.2460/javma.2002.221.511>. PMID:12184700
- Sofaly CD, Reed SM, Gordon JC, Dubey JP, Oglesbee MJ, Njoku CJ, et al. Experimental induction of equine protozoan myeloencephalitis (EPM) in the horse: effect of *Sarcocystis neurona* sporocyst inoculation dose on the development of clinical neurologic disease. *J Parasitol* 2002; 88(6): 1164-1170. [http://dx.doi.org/10.1645/0022-3395\(2002\)088\[1164:EOEPM\]2.0.CO;2](http://dx.doi.org/10.1645/0022-3395(2002)088[1164:EOEPM]2.0.CO;2). PMID:12537112
- Stanek JF, Stich RW, Dubey JP, Reed SM, Njoku CJ, Lindsay DS, et al. Epidemiology of *Sarcocystis neurona* infections in domestic cats (*Felis domesticus*) and its association with equine protozoan myeloencephalitis (EPM) case farms and feral cats from a mobile spay and neuter clinic. *Vet Parasitol* 2003; 117(4): 239-249. <http://dx.doi.org/10.1016/j.vetpar.2003.09.002>. PMID:14637026
- Tanhauser SM, Yowell CA, Cutler TJ, Greiner EC, MacKay RJ, Dame JB. Multiple DNA markers differentiate *Sarcocystis neurona* and *Sarcocystis falcatula*. *J Parasitol* 1999; 85(2): 221-228. <http://dx.doi.org/10.2307/3285623>. PMID:10219299
- Turay HO, Barr BC, Caldwell A, Branson KR, Cockrell MK, Marsh AE. *Sarcocystis neurona* reacting antibodies in Missouri feral domestic cats (*Felis domesticus*) and their role as an intermediate host. *Parasitol Res* 2002; 88(1): 38-43. PMID:11826870.
- Valadas S, Gennari SM, Yai LE, Rosypal AC, Lindsay DS. Prevalence of antibodies to *Trypanosoma cruzi*, *Leishmania infantum*, *Encephalitozoon cuniculi*, *Sarcocystis neurona*, and *Neospora caninum* in Capybara, *Hydrochoerus hydrochaeris*, from São Paulo State, Brazil. *J Parasitol* 2010; 96(3): 521-524. <http://dx.doi.org/10.1645/GE-2368.1>. PMID:20020808