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Exposure of free-living jaguars to *Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis neurona* in the Brazilian Pantanal

Exposição de onças-pintadas a *Toxoplasma gondii*, *Neospora caninum* e *Sarcocystis neurona* no Pantanal brasileiro

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Abstract

Toxoplasma gondii, Neospora caninum and *Sarcocystis neurona* are related apicomplexan parasites that cause reproductive and neurological disorders in a wide range of domestic and wild animals. In the present study, the immunofluorescence antibody test (IFAT) was used to investigate the presence of antibodies against *T. gondii, N. caninum* and *S. neurona* in the sera of 11 free-living jaguars (*Panthera onca*) in two protected areas in the Pantanal region of Mato Grosso state, Brazil. Ten jaguars (90.9%) showed seropositivity for *T. gondii*, eight (72.7%) for *S. neurona*, and seven (63.6%) for *N. caninum* antigens. Our findings reveal exposure of jaguars to these related coccidian parasites and circulation of these pathogens in this wild ecosystem. To the best of our knowledge, this is the first serological detection of *N. caninum* and *S. neurona* in free-living jaguars.

Keywords: *Panthera onca*, antibodies, *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, conservation unit.

Resumo

Toxoplasma gondii, Neospora caninum e *Sarcocystis neurona* são coccídios relacionados responsáveis por causar desordens reprodutivas e neurológicas em uma ampla variedade de animais domésticos e selvagens. No presente estudo, a Reação de Imunofluorescência Indireta (RIFI) foi utilizada para investigar a presença de anticorpos contra *T. gondii*, *N. caninum* e *S.neurona* em soros de 11 onças-pintadas de vida livre (*Panthera onca*), provenientes de duas áreas protegidas na região do Pantanal do Estado de Mato Grosso , Brasil. Dez (90,9%), sete (63,6%) e oito (72,7%) onças amostradas foram soropositivas para *T. gondii*, *N. caninum* e *S. neurona*, respectivamente. Os resultados indicam a exposição a esses coccídios relacionados entre as onças-pintadas e a circulação ambiental desses patógenos nesse ecossistema selvagem. Este é o primeiro relato da detecção sorológica de *N. caninum* e *S. neurona* em onças-pintadas de vida livre .

Palavras-chave: *Panthera onca*, anticorpos, *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis neurona*, unidade de conservação.

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The complex dynamic relationship between wildlife, humans and domestic animals at the boundaries of protected areas underscores the importance of adopting proper control measures to prevent the transmission of infectious or parasitic diseases from humans or domestic animals to wildlife. This concern has been growing in recent decades for reasons that include the conservation of threatened species and the increasing recognition of emerging zoonotic diseases (DASZAK et al., 2000). Serologic studies have revealed the occurrence of antibodies against some infectious diseases in wildlife. However, reports about the health status of free-living jaguars (*Panthera onca*), the largest felid in the Americas, are still scanty, notwithstanding their situation in different categories of risk as a result of anthropogenic activities throughout the Brazilian territory (MORATO et al., 2013). Knowledge about health parameters is essential for the construction and implementation of action plans for global wildlife conservation. In Brazil, jaguars can be found in five different biomes, and the Pantanal biome is considered an important area for long-term jaguar conservation.

Toxoplasma gondii, Neospora caninum and *Sarcocystis neurona* are related apicomplexan parasites responsible for causing reproductive and neurological disorders in a wide range of domestic and wild animals (KENNY et al., 2002; DUBEY et al., 2001a, 2003b, 2007). The domestic cat (*Felis catus*) is the definitive host of *T. gondii*, an intracellular protozoan of worldwide distribution, although neotropical felids are also recognized as definitive hosts (JEWELL et al., 1972), emphasizing the importance of wild felines in the epidemiology of the agent in environments where cats do not exist (RENDÓN-FRANCO et al., 2012). The presence of anti-*T. gondii* antibodies has been reported in felids and canids in Brazilian zoos (SILVA et al., 2001; MATTOS et al., 2008; ANDRÉ et al., 2010), including seropositive captive jaguars. On the other hand, studies of exposure to *T. gondii* in free-living jaguars are still scanty (FURTADO, 2010).

Neospora caninum is considered one of the most important causes of abortion in cattle and of neurological disease in dogs. Antibodies against *N. caninum* have been reported in several wild species, such as wild herbivores and canids (DUBEY et al., 1999c; CAÑON-FRANCO et al., 2004; VALADAS et al., 2010; ALMERÍA, 2013), which can establish a plausible sylvatic cycle of the parasite, since coyotes (*Canis latrans*), dingoes (*C. lupus dingo*) and gray wolves (*C. lupus lupus*) are definitive hosts, similarly to domestic dogs (MCALLISTER et al., 1998; GONDIM et al., 2004a; KING et al., 2010; DUBEY et al., 2011). The presence of anti-*N. caninum* antibodies in several free-living and captive wild feline species has also been reported worldwide (CHEADLE et al., 1999; FERROGLIO et al., 2003; SPENCER et al., 2003; SEDLÁK & BÁRTOVÁ, 2006; ANDRÉ et al., 2010), but no clinical cases resulting from this parasite infection have been described in naturally infected felids (ALMERÍA, 2013).

Sarcocystis neurona and *Neospora* spp. are the etiological agents of equine protozoal myeloencephalitis (EPM). *Sarcocystis neurona* is one of the most commonly diagnosed pathogens of neurological disorders in horses in North America (DUBEY et al., 2003a), and a high prevalence of antibodies has been reported in horses in Brazil, Argentina, and Costa Rica (DUBEY et al., 1999a, b; HOANE et al, 2006; DANGOUDOUBIYAM et al., 2011). Currently, the opossum (*Didelphis virginiana*) is the only known definitive host for *S. neurona* in the United States (DUBEY & LINDSAY, 1998), and *Didelphis albiventris* has been implicated as the definitive host for the transmission of *S. neurona* in South America (DUBEY et al., 2001b; HOANE et al., 2006). Serological and parasitological studies have found that cats are natural intermediate hosts of *S. neurona* (STANEK et al., 2003; MENESES, 2012). However, to the best of our knowledge, there are no reports of serological studies or any information of naturally occurring *S. neurona* antibodies in wild felids, including jaguars.

The objective of this study was to evaluate the exposure of free-living jaguars to *T. gondii*, *N. caninum* and *S. neurona* in two protected areas in the Pantanal ecosystem of Mato Grosso state, central-western region of Brazil.

The study sites were the Taiamã Ecological Station (TES) (16° 50' 34.31" S, 57° 35' 03.70" W) and the Pantanal Matogrossense National Park (PNPM) (17° 50'47. 33" S, 57° 24'12.67" W), two Conservation Units in the Pantanal wetlands of the state of Mato Grosso, Brazil, considered one of the world's largest floodplains (Figure 1). The animals were captured intentionally during the dry season because they are impossible to catch when Pantanal is flooded. Captures were authorized by the National System for Biodiversity Research – SISBIO, under permit nos. 30896-1 and 18699-1. All the jaguars were captured using foot snares (BALME et al., 2009), immobilized with a dosage of 10 mg/kg of tiletamine/zolazepam (Zoletil 100®, Virbac SA, Carros-Cedex, France) for blood collection and clinical examination, and fitted with GPS/VHF/satellite radio-collars (Vectronic Aerospace GmbH, Germany and Followit, Stockholm, Sweden). After these procedures, all the animals were released at the same sites where they were captured. Blood samples were collected by femoral venipuncture and stored in Brazil's Biological Samples Bank at the National Research Center for the Conservation of Carnivorous Mammals (CENAP/ ICMBio).

The immunofluorescent antibody test (IFAT) was performed to detect serum antibodies (IgG) against *T. gondii, N. caninum* and *S. neurona*, using the following antigens: *T. gondii* RH strain tachyzoites, as described by Camargo (1964); tachyzoites of *N. caninum* NC-Bahia strain (GONDIM et al., 2001), as described by Dubey et al. (1988); and *S. neurona* SN37R strain (SOFALY et al., 2002), as described by Duarte et al. (2003). The cut-offs adopted were dilutions of 1:16, 1:25, 1:25 for *T. gondii*, *N. caninum* and *S. neurona,* respectively (RIVETTI et al., 2008; ANDRÉ et al., 2010; MENESES, 2012). Serum samples from domestic cats (*Felis catus*) were used as positive and negative controls, and a goat anti-cat immunoglobulin G (anti-IgG) labeled with fluorescein isothiocyanate was used as conjugate. The slides were examined using an epifluorescence microscope. The samples considered positive were subjected to a two-fold serial dilution to obtain the final titer.

Eleven jaguars (7 males/4 females) were captured between July 2010 and November 2012. In general, all the animals were in good overall health, except jaguar #1, which showed low body weight, dermatological signs of systemic disease, and died 45 days after capture. We were unable to determine the cause of death, or to correlate it with any of the infections examined in this study. The age of the animals was estimated based on tooth wear and

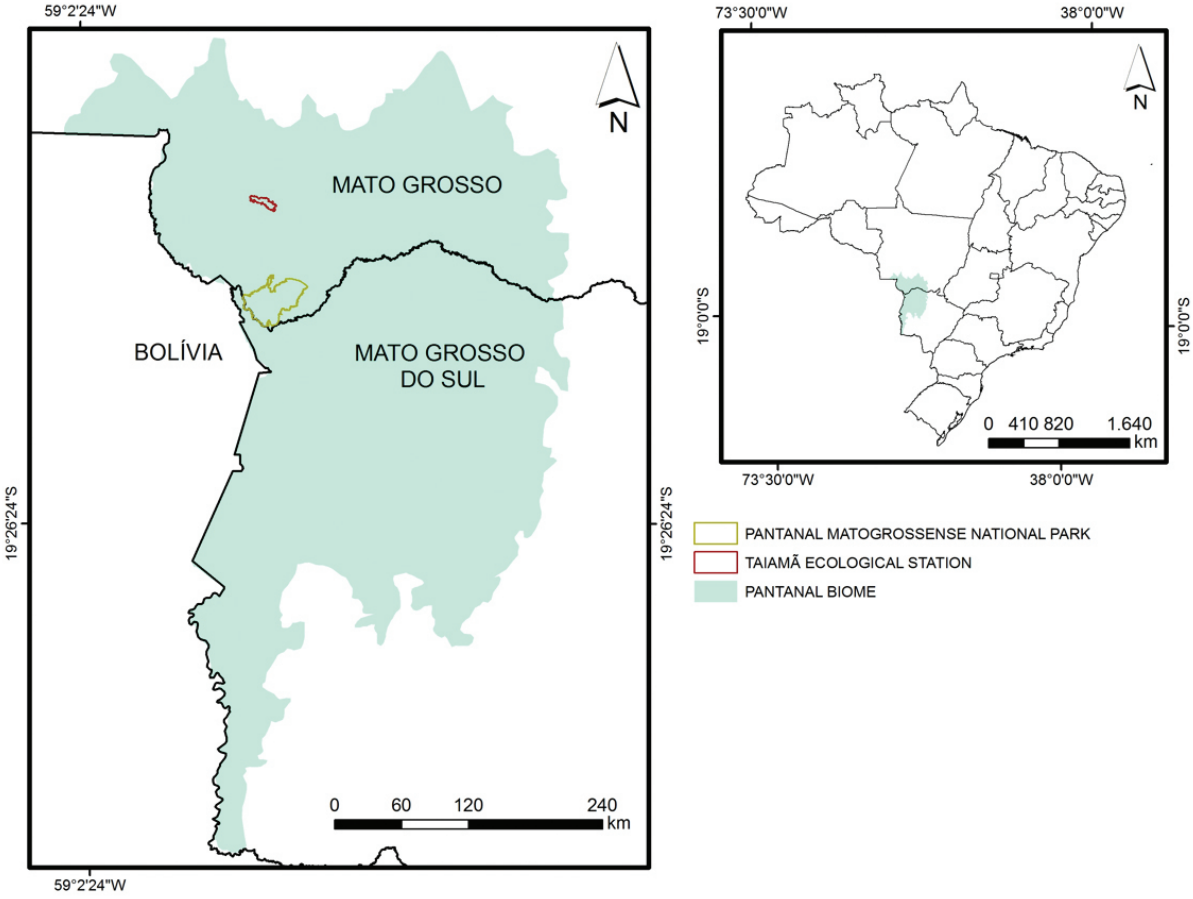

Figure 1. Location of the two Conservation Units in the Brazilian Pantanal where this study was conducted.

color, and all animals were considered adults, with estimated ages ranging from 4 to 10 years.

The results of serological assay are presented in Table 1. Ten (90.9%), eight (72.7%) and seven (63.6%) jaguars were seropositive for *T. gondii*, *S. neurona* and *N. caninum* antigen by IFAT, respectively. Titers of antibodies ranged from the dilutions 1:256 to 1:8,192 for *T. gondii*, from 1:100 to 1:1,600 for *N. caninum,* and from 1:25 to 1:800 for *S. neurona.* Five animals were reactive to all the antigens.

This study showed, for the first time, the occurrence of antibodies against three of the most important coccidia for veterinary medicine in free-living jaguars captured in the Pantanal, an important biome of South America, which is characterized by an extraordinary abundance of wildlife. A high frequency of antibodies against all protozoans was detected by IFAT, indicating the existence of a sylvatic cycle in these areas, most likely as a consequence of frequent exposure to these parasites.

Studies have shown antibodies to or evidence of infection with *T. gondii, N. caninum* and *S. neurona* in wild and domestic animals around the world, most of which are also present in the Pantanal, such as capybara, deer, canids, primates, birds, marsupials, as well as domestic dogs, cats, horses and cattle (DUBEY et al., 1988, 1999a, c; ROSSANO et al., 2002; CAÑON-FRANCO et al., 2003, 2004; GENNARI et al., 2004; LEITE et al., 2008; ALMERÍA, 2013). These domestic species are raised on cattle ranches and in rural communities in the Pantanal region on lands adjoining protected areas. As jaguars are at the top of the food chain, these findings may suggest that parasite infections occur further down the food chain (STUART et al., 2013), and that the role of predator-prey sentinels can be considered. However, further investigations involving this carnivore's ecology and prevalence within different species must be conducted in order to clarify this issue.

The high frequency of jaguars seroreactive against *T. gondii* appears to be common in Brazil. Our findings are similar to those reported by Furtado (2010), who detected seropositivity in all free-living jaguars sampled in Southern Pantanal, also suggesting a widespread sylvatic cycle of toxoplasmosis, although no statistical differences were found between prevalence in protected and disturbed areas.

Jaguar #1 was found dead 45 days after capture (data not shown). This animal was captured in the vicinity of a house (<300 meters) where several domestic dogs and cats lived and where predation had been reported. This jaguar presented anti-*T. gondii* antibody titers of 1:1024, which were higher than antibody titers against *N. caninum* or *S. neurona* (T= 1:100 and 1:25, respectively). However, a conclusive diagnosis could not be reached, because histopathological and immunohistochemical postmortem data were not available due to the level of decomposition of the carcass.

The *T. gondii* titer of 1:8,192 detected in jaguar #11 suggests active infection, despite the absence of clinical signs during its

Table 1. Results of serological assay performed on blood sera of free-living jaguars (*Panthera onca*) from the Pantanal region of Mato Grosso, Brazil.

IFA serological endpoint titers against each antigen			
Jaguar	**Toxoplasma gondii**	**Neospora caninum**	**Sarcocystis neurona**
1	1:1,024	1:100	1:25
2	1:2,048	1:800	1:800
3	1:1,024	1:1,600	1:100
4	N	1:200	1:50
5	1:2,048	N	1:50
6	1:256	N	N
7	1:1,024	1:800	1:800
8	1:1,024	1:100	N
9	1:2,048	N	1:50
10	1:256	N	N
11	1:8,192	1:100	1:50

N= negative; IFA= immunofluorescent antibody test.

period of capture. In this case, other factors correlated with clinical signs of infection, such as virulence and genetic diversity of *T. gondii* and host immune response (PENA et al., 2011; CAÑÓN-FRANCO et al., 2013), must be considered. Clinical toxoplasmosis is rarely observed in domestic and wild felids, but reports of toxoplasmosis in domesticated cheetahs (*Acinonyx acinonyx*), captive lions (*Panthera leo*), Siberian tiger (*Panthera tigris altaica*), and Pallas cat (*Otocolobus manul*) attest to the susceptibility of Felidae species to this agent (DORNY & FRANSEN, 1989; OCHOLI et al., 1989; KENNY et al., 2002; LLOYD & STIDWORTHY, 2007).

This is the first serological detection of antibodies to *N. caninum* in free-living jaguars. The prevalence in this study (63.6%) is higher than that reported for Brazilian wild captive and exotic felids (ANDRÉ et al., 2010) and for wild felids in protected areas in Kenya, East Africa (FERROGLIO et al., 2003). Jaguar #3 had been feeding on horses (data not shown) in a private reserve adjacent to the PMNP and presented the highest antibody titers for *N. caninum* (1:1; 600), in addition to antibody titers for *T. gondii* (1:1,024) and *S. neurona* (100). Therefore, this proximity to human dwellings likely reflects frequent contact with probable domestic or domesticated animal hosts, which may act as sources of infection for all the tested antigens. Other sources of *N. caninum* infection should be considered. Serosurveys conducted in Brazil have revealed the occurrence of antibodies against *N. caninum* in capybaras (*Hydrochaeris hydrochaeris*) (YAI et al., 2008; VALADAS et al., 2010) and in pampas-deer (*Ozotoceros bezoarticus*) (TIEMANN et al., 2005), which are species commonly found in this region of the Pantanal and are known prey of jaguars, making infected animals available through carnivorism in the sylvatic cycle of the parasite. Although felids probably act only as intermediate hosts in neosporosis (ALMERÍA, 2013), the role of this top predator in the life cycle of this parasite and the influence of *N. caninum* infection on the health status of jaguar populations remains undefined. Serological evidence of infection and isolation of *N. caninum* in several wild species, added to the evidence that the parasite can be transmitted between wildlife and domestic animals (GONDIM et al., 2004b), involve new issues related to the transmission cycle of the parasite and to preventive measures in conservation units and their surrounding areas.

To the best of our knowledge, this is also the first report of *S. neurona* antibodies in jaguars. In addition to domestic cats, other wild species such as nine-banded armadillos (*Dasypus novemcinctus*), raccoons *(Procyon lotor),* sea otters (*Enhydra lutris*) , striped skunks (*Mephitis mephitis*) and birds (*Molothrus ater*) have been described as intermediate hosts of *S. neurona* (CHEADLE et al., 2001a, b; DUBEY et al., 2001c, d; MANSFIELD et al., 2008). The frequency of seroreactive jaguars found in this study is higher than that found in cats in the USA and Brazil (ROSSANO et al., 2002; STANEK et al., 2003; MENESES, 2012). Despite the paucity of studies focusing on the identification of natural intermediate hosts in South America, there are Procyonidae and Edentate species in Brazil which can be infected with *S. neurona* and which have been shown to be intermediate hosts for this agent in North America (CHEADLE et al., 2001a; HOANE et al., 2006). These species may play an important role in the natural maintenance of this parasite in this region, but there are no reports of anti-*S. neurona* antibodies in wild felid species, and little information about *S. neurona* infection in cats is available in Brazil. Despite the suggested participation of domestic and feral cats in the epidemiology of MPE in ecological niches shared with marsupials and other intermediate hosts (STANEK et al., 2003), predatory species do not often serve as intermediate hosts for many of the parasitic life cycles (STANEK et al., 2003), and studies are needed to clarify the role of jaguars in the life cycle of this agent.

The lack of reports of clinical signs of *T. gondii*, *N. caninum* and *S. neurona* infection in jaguars in natural environments prevent a clear understanding of the potential impact on this species of conservation concern, despite evidence of the influence of parasitic infections in birth and death rates, even when clinical signs are not obvious (TELFER et al., 2008). A better understanding is needed of environmental contamination by oocysts and of transmission mechanisms in flooded areas such as the Pantanal ecosystem, in view of their public health, economic and biodiversity implications. Whenever possible, diagnostic techniques such as histopathology, immunohistochemistry and molecular analysis associated with serological data would be useful to clarify the real participation of this species in the epidemiology of *T. gondii*, *N. caninum* and *S. neurona*.

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References

Almería S. Neospora caninum and Wildlife. *ISRN Parasitol* 2013; 2013:23. http://dx.doi.org/10.5402/2013/947347

André MR, Adania CH, Teixeira RHF, Silva KF, Jusi MMG, Machado STZ, et al. Antibodies to Toxoplasma gondii and Neospora caninum in captive neotropical and exotic wild canids and felids. *J Parasitol* 2010; 96(5): 1007-1009. http://dx.doi.org/10.1645/GE-2502.1. PMid:20950109

Balme GA, Hunter LTB, Slotow R. Evaluating methods for counting cryptic carnivores. *J Wildl Manage* 2009; 73(3): 433-441. http://dx.doi.org/10.2193/2007-368.

Camargo ME. Improved technique of indirect immunofluorescence for serological diagnosis of toxoplasmosis. *Rev Inst Med Trop Sao Paulo* 1964; 6(3): 117-118. PMid:14177810.

Cañon-Franco WA, Yai LEO, Joppert AM, Souza CE, D'Auria SRN, Dubey JP, et al. Seroprevalence of Toxoplasma gondii antibodies in the rodent capybara (Hidrochoeris hidrochoeris) from Brazil. *J Parasitol* 2003; 89(4): 850. http://dx.doi.org/10.1645/GE-80R. PMid:14533702

Cañón-Franco WA, Yai LEO, Souza SLP, Santos LC, Farias NAR, Ruas J, et al. Detection of antibodies to Neospora caninum in two species of wild canids, Lycalopex gymnocercus and Cerdocyon thous from Brazil. *Vet Parasitol* 2004; 123(3-4): 275-277. http://dx.doi.org/10.1016/j.vetpar.2004.06.004. PMid:15325054

Cañon-Franco WA, Araújo FAP, Gennari SM. Toxoplasma gondii in small neotropical wild felids. *Braz J Vet Res Anim Sci* 2013; 50(1): 50-67.

Cheadle MA, Spencer JA, Blagburn BL. Seroprevalences of Neospora caninum and Toxoplasma gondii in nondomestic felids from southern Africa. *J Zoo Wildl Med* 1999; 30(2): 248-251. PMid:10484140.

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. 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. PMid:11403777

Dangoudoubiyam S, Oliveira JB, Víquez C, Gómez-García A, González O, Romero JJ, et al. Detection of antibodies against Sarcocystis neurona, Neospora spp., and Toxoplasma gondii in horses from Costa Rica. *J Parasitol* 2011; 97(3): 522-524. http://dx.doi.org/10.1645/GE-2722.1. PMid:21506839

Daszak P, Cunningham AA, Hyatt AD. Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* 2000; 287(5452): 443-449. http://dx.doi.org/10.1126/science.287.5452.443. PMid:10642539

Dorny P, Fransen J. Toxoplasmosis in a Siberian tiger (Panthera tigris altaica). *Vet Rec* 1989; 125(26-27): 647. PMid:2617817.

Duarte PC, Daft BM, Conrad PA, Packham AE, Gardner IA. Comparison of a serum indirect fluorescent antibody test with two Western blot tests for the diagnosis of equine protozoal myeloencephalitis. *J Vet Diagn Invest* 2003; 15(1): 8-13. http://dx.doi.org/10.1177/104063870301500103. PMid:12580288

Dubey JP, Lindsay DS. Isolation in immunodeficient mice of Sarcocystis neurona from opossum (Didelphis virginiana) faeces, and its differentiation from Sarcocystis falcatula. *Int J Parasitol* 1998; 28(12): 1823-1828. http://dx.doi.org/10.1016/S0020-7519(98)00166-0. PMid:9925260

Dubey JP, Hattel AL, Lindsay DS, Topper MJ. Neonatal Neospora caninum infection in dogs: isolation of the causative agent and experimental transmission. *J Am Vet Med Assoc* 1988; 193(10): 1259-1263. PMid:3144521.

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, Venturini MC, Venturini L, McKinney J, Pecoraro M. Prevalence of antibodies to Sarcocystis neurona, Toxoplasma gondii and Neospora caninum in horses from Argentina. *Vet Parasitol* 1999b; 86(1): 59-62. http://dx.doi.org/10.1016/S0304-4017(99)00127-2. PMid:10489203

Dubey JP, Hollis K, Romand S, Thulliez P, Kwok OC, Hungerford L, et al. High prevalence of antibodies to Neospora caninum in white-tailed deer (Odocoileus virginianus). *Int J Parasitol* 1999c; 29(10): 1709-1711. http://dx.doi.org/10.1016/S0020-7519(99)00142-3. PMid:10608458

Dubey JP, Lindsay DS, Saville WJA, Reed SM, Granstrom DE, Speer CA. A review of Sarcocystis neurona and equine protozoal myeloencephalitis (EPM). *Vet Parasitol* 2001a; 95(2-4): 89-131. http://dx.doi.org/10.1016/S0304-4017(00)00384-8. PMid:11223193

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* 2001b; 95(2-4): 295-304. http://dx.doi.org/10.1016/S0304-4017(00)00395-2. PMid:11223209

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* 2001c; 100(3-4): 117-129. 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* 2001d; 87(6): 1387-1393. http://dx.doi.org/10.1645/0022-3395(2001)087[1387:SNIISO]2.0.CO;2. PMid:11780826

Dubey JP, Mitchell SM, Morrow JK, Rhyan JC, Stewart LM, Granstrom DE, et al. Prevalence of antibodies to Neospora caninum, Sarcocystis neurona, and Toxoplasma gondii in wild horses from central Wyoming. *J Parasitol* 2003a; 89(4): 716-720. http://dx.doi.org/10.1645/GE-66R. PMid:14533680

Dubey JP, Zarnke R, Thomas NJ, Wong SK, Van Bonn W, Briggs M, et al. Toxoplasma gondii, Neospora caninum, Sarcocystis neurona, and Sarcocystis canis-like infections in marine mammals. *Vet Parasitol* 2003b; 116(4): 275-296. http://dx.doi.org/10.1016/S0304-4017(03)00263-2. PMid:14580799

Dubey JP, Schares G, Ortega-Mora LM. Epidemiology and control of neosporosis and Neospora caninum. *Clin Microbiol Rev* 2007; 20(2): 323-367. http://dx.doi.org/10.1128/CMR.00031-06. PMid:17428888

Dubey JP, Jenkins MC, Rajendran C, Miska K, Ferreira LR, Martins J, et al. Gray wolf (Canis lupus) is a natural definitive host for Neospora caninum. *Vet Parasitol* 2011; 181(2-4): 382-387. http://dx.doi.org/10.1016/j.vetpar.2011.05.018. PMid:21640485

Ferroglio E, Wambwa E, Castiello M, Trisciuoglio A, Prouteau A, Pradere E, et al. Antibodies to Neospora caninum in wild animals from Kenya, East Africa. *Vet Parasitol* 2003; 118(1-2): 43-49. http://dx.doi.org/10.1016/j.vetpar.2003.09.006. PMid:14651874

Furtado MM. *Estudo epidemiológico de patógenos circulantes nas populaçõs de onça-pintada e animais domésticos em áreas preservadas de três biomas brasileiros: Cerrado, Pantanal e Amazônia* [Thesis]. São Paulo: Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo; 2010.

Gennari SM, Canón-Franco WA, Yai LEO, de Souza SL, Santos LC, Farias NAR, et al. Seroprevalence of Toxoplasma gondii antibodies from wild canids from Brazil. *Vet Parasitol* 2004; 121(3-4): 337-340. http://dx.doi.org/10.1016/j.vetpar.2004.02.023. PMid:15135875

Gondim LFP, Pinheiro AM, Santos POM, Jesus EEV, Ribeiro MB, Fernandes HS, et al. Isolation of Neospora caninum from the brain of a naturally infected dog, and production of encysted bradyzoites in gerbils. *Vet Parasitol* 2001; 101(1): 1-7. http://dx.doi.org/10.1016/S0304-4017(01)00493-9. PMid:11587828

Gondim LFP, McAllister MM, Pitt WC, Zemlicka DE. Coyotes (Canis latrans) are definitive hosts of Neospora caninum. *Int J Parasitol* 2004a; 34(2): 159-161. http://dx.doi.org/10.1016/j.ijpara.2004.01.001. PMid:15037103

Gondim LFP, McAllister MM, Mateus-Pinilla NE, Pitt WC, Mech LD, Nelson ME. Transmission of Neospora caninum between wild and domestic animals. *J Parasitol* 2004b; 90(6): 1361-1365. http://dx.doi.org/10.1645/GE-341R. PMid:15715229

Hoane JS, Gennari SM, Dubey JP, Ribeiro MG, Borges AS, Yai LEO, 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

Jewell ML, Frenkel JK, Johnson KM, Reed V, Ruiz A. Development of Toxoplasma oocysts in neotropical felidae. *Am J Trop Med Hyg* 1972; 21(5): 512-517. PMid:5075665.

Kenny DE, Lappin MR, Knightly F, Baler J, Brewer M, Getzy DM. Toxoplasmosis in Pallas' cats (Otocolobus felis manul) at the Denver Zoological Gardens. *J Zoo Wildl Med* 2002; 33(2): 131-138. PMid:12398302.

King JS, Slapeta J, Jenkins DJ, Al-Qassab SE, Ellis JT, Windsor PA. Australian dingoes are definitive hosts of Neospora caninum. *Int J Parasitol* 2010; 40(8): 945-950. http://dx.doi.org/10.1016/j.ijpara.2010.01.008. PMid:20149793

Leite TNB, Maja TA, Ovando TM, Cantadori DT, Schimidt LR, Guércio AC, et al. Ocorrência de infecção por Leishmania spp. e Toxoplasma gondii em macacos-prego (Cebus apella) de Campo Grande, MS. *Rev Bras Parasitol Vet* 2008;17(Suppl 1): 307-310. PMid:20059866.

Lloyd C, Stidworthy MF. Acute disseminated toxoplasmosis in a juvenile cheetah (Acinonyx jubatus). *J Zoo Wildl Med* 2007; 38(3): 475-478. http://dx.doi.org/10.1638/2007-0016.1. PMid:17939359

Mattos BC, Patrício LLF, Plugge NFR, Lange RR, Richartz RRTB, Dittrich RL. [Seroprevalence of antibodies anti-Neospora caninum and anti-Toxoplasma gondii in captive wild canids]. *Rev Bras Parasitol Vet* 2008;17(Suppl 1): 267-272. PMid:20059860.

McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM. Rapid communication: Dogs are definitive hosts of Neospora caninum. *Int J Parasitol* 1998; 28(9): 1473-1478. http://dx.doi.org/10.1016/S0020-7519(98)00138-6. PMid:9770635

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

Meneses IDS. *Ocorrência de anticorpos anti- Sarcocystis neurona em gatos (Felis domesticus) de Salvador, Bahia* [Dissertation]. Bahia: Escola de Medicina Veterinária e Zootecnia, Universidade Federal da Bahia; 2012.

Morato RG, Beisiegel B, Ramalho EE, Campos CB, Boulhosa RLP. Avaliação do risco de extinção da onça pintada (Panthera onca) no Brasil. *Biodiversidade Bras* 2013; 3(1): 122-132.

Ocholi RA, Kalejaiye JO, Okewole PA. Acute disseminated toxoplasmosis in two captive lions (Panthera leo) in Nigeria. *Vet Rec* 1989; 124(19): 515-516. http://dx.doi.org/10.1136/vr.124.19.515. PMid:2756626

Pena HFJ, Marvulo MFV, Horta MC, Silva MA, Silva JCR, Siqueira DB, et al. Isolation and genetic characterisation of Toxoplasma gondii from a red-handed howler monkey (Alouatta belzebul), a jaguarundi (Puma yagouaroundi), and a black-eared opossum (Didelphis aurita) from Brazil. *Vet Parasitol* 2011; 175(3-4): 377-381. http://dx.doi.org/10.1016/j.vetpar.2010.10.015. PMid:21055880

Rendón-Franco E, Caso-Aguilar A, Jiménez-Sánchez NG, Hernandez-Jauregui DMB, Sandoval-Sánchez AL, Zepeda-López HM. Prevalence of anti-Toxoplasma gondii antibody in free-ranging ocelots (Leopardus pardalis) from Tamaulipas, Mexico. *J Wildl Dis* 2012; 48(3): 829-831. http://dx.doi.org/10.7589/0090-3558-48.3.829. PMid:22740559

Rivetti AV Jr, Caxito FA, Resende M, Lobato ZIP. Avaliação sorológica para Toxoplasma gondii pela imunofluorescência indireta e detecção do vírus da imunodeficiência felina pela nested PCR em felinos selvagens. *Arq Bras Med Vet Zootec* 2008; 60(5): 1281-1283. http://dx.doi.org/10.1590/S0102-09352008000500038.

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

Sedlák K, Bártová E. Seroprevalences of antibodies to Neospora caninum and Toxoplasma gondii in zoo animals. *Vet Parasitol* 2006; 136(3-4): 223-231. http://dx.doi.org/10.1016/j.vetpar.2005.11.021. PMid:16387445

Silva JC, Ogassawara S, Marvulo MF, Ferreira-Neto JS, Dubey JP. Toxoplasma gondii antibodies in exotic wild felids from Brazilian zoos. *J Zoo Wildl Med* 2001; 32(3): 349-351. PMid:12785684.

Sofaly CD, Reed SM, Gordon JC, Dubey JP, Ogleebee 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:EIOEPM]2.0.CO;2. PMid:12537112

Spencer JA, Higginbotham MJ, Blagburn BL. Seroprevalence of Neospora caninum and Toxoplasma gondii in captive and free-ranging nondomestic felids in the United States. *J Zoo Wildl Med* 2003; 34(3): 246-249. http://dx.doi.org/10.1638/02-046. PMid:14582785

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 protozoal 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

Stuart P, Zintl A, De Waal T, Mulcahy G, Hawkins C, Lawton C. Investigating the role of wild carnivores in the epidemiology of bovine neosporosis. *Parasitology* 2013; 140(3): 296-302. http://dx.doi.org/10.1017/S0031182012001588. PMid:23068142

Telfer S, Birtles R, Bennett M, Lambin X, Paterson S, Begon M. Parasite interactions in natural populations: insights from longitudinal data. *Parasitology* 2008; 135(07): 767-781. http://dx.doi.org/10.1017/S0031182008000395. PMid:18474121

Tiemann JCH, Souza SLP, Rodrigues AAR, Duarte JMB, Gennari SM. Environmental effect on the occurrence of anti-Neospora caninum antibodies in pampas-deer (Ozotoceros bezoarticus). *Vet Parasitol* 2005; 134(1-2): 73-76. http://dx.doi.org/10.1016/j.vetpar.2005.07.015. PMid:16112811

Valadas S, Gennari SM, Yai LEO, 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

Yai LEO, Ragozo AMA, Cañón-Franco WA, Dubey JP, Gennari SM. Occurrence of Neospora caninum antibodies in capybaras (Hydrochaeris hydrochaeris) from São Paulo State, Brazil. *J Parasitol* 2008; 94(3): 766. http://dx.doi.org/10.1645/GE-1468.1. PMid:18605804