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Antibodies to *Toxoplasma gondii* and *Neospora caninum* in Captive Neotropical and Exotic Wild Canids and Felids

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ABSTRACT: This study was designed to detect antibodies to *Toxoplasma gondii* and *Neospora caninum* in wild captive carnivores maintained in Brazilian zoos. Blood samples were collected from 142 Brazilian wild felids and 19 exotic felids in zoos, and 3 European wolves (*Canis lupus*) and 94 Brazilian wild canids maintained in captivity in Brazilian zoos of São Paulo, Mato Grosso states and Federal District. One hundred and two (63.4%) and 70 (50.3%) of the 161 wild felids tested were seropositive for *T. gondii* and *N. caninum* by indirect immunofluorescent assay test (IFAT), respectively. Among sampled wild canids, 49 (50.5%) and 40 (41.2%) animals were seropositive for *T. gondii* and *N. caninum* antigens by IFAT, respectively. To our knowledge, this is the first serological detection of antibodies to *N. caninum* in Brazilian wild captive felids and bush dogs (*Speothos venaticus* (Lund)).

Toxoplasma gondii and *Neospora caninum* are 2 closely related apicomplexan parasites with a worldwide distribution. Felines and canids are definitive hosts for *T. gondii* (Dubey and Beattie, 1988) and *N. caninum* (McAllister et al., 1998; Gondim et al., 2004; King et al., 2010), respectively.

Felids are important in the epidemiology of *T. gondii* infection because they are the only hosts that can excrete environmentally resistant oocysts in nature (Dubey, 2009). Because the duration of oocyst shedding is relatively short, serosurveys are better indicators of *T. gondii* infection in felids (Dubey and Thulliez, 1989). Oocyst shedding has been demonstrated in pumas (*Puma concolor*) (Miller et al., 1972; Aramini et al., 1998), bobcats (*Lynx rufus*) (Miller et al., 1972; Marchiondo et al., 1976), ocelots (*Felis pardalis*), and panthers (*Panthera onca*) (Patton et al., 1986).

Canids are definitive hosts of *N. caninum* (Dubey et al., 2007). Evidence indicating a sylvatic transmission cycle between wild canids and beef cattle in Texas has been reported previously (Barling et al., 2000). Oocyst shedding has been shown to occur in coyotes (*Canis latrans*) (Gondim et al., 2004), red foxes (*Vulpes vulpes*) (Wapenaar et al., 2006), and Australian dingos (*Canis lupus dingo*) (King et al., 2010).

The present study aimed to detect antibodies to *T. gondii* and *N. caninum* in Brazilian and exotic wild carnivores maintained in captivity in Brazilian zoos.

Serum samples were collected from 142 Brazilian wild captive felids and 19 exotic captive felids, plus 3 European wolves (*Canis lupus*) and 94 Brazilian wild canids maintained in captivity in Brazilian zoos of São Paulo, Mato Grosso states and Federal District (Table I). All samples were collected under IBAMA license numbers S02027.002943/2005 and 15901-1. Animals were immobilized with a mixture of ketamine (10 mg/kg) and xylazine (1 mg/kg).

The presence of anti-*T. gondii* and *Neospora caninum* antibodies in the serum of each animal was detected by indirect immunofluorescent assay test (IFAT). Tachyzoites of the *N. caninum* NC-1 strain were used as an antigen (Dubey et al., 1988). *Toxoplasma gondii* RH strain tachyzoites were used as an antigen as described by Domingues et al. (1998). Antigen slides were removed from storage and allowed to thaw at room temperature for 30 min. Ten microliters of 2-fold dilutions of sera of 1:25 (cut-off for *N. caninum*) and 1:40 (cut-off for *T. gondii*) were placed in wells on antigen slides. Known positive and negative canine and feline sera were used as controls. Slides were incubated at 37 C in a moist chamber for 45 min, washed 3 times in phosphate-buffered saline (pH 7.2) for 5 min, and air-dried at room temperature. Immunoglobulin G (IgG) anti-cat conjugate (dilution of 1:32; Sigma, St. Louis, Missouri) for wild feline samples and IgG anti-dog conjugate (dilution of 1:32; Sigma) for wild canids samples were diluted according to the manufacturer and then added to each well. These slides were incubated again, washed, dried, and overlaid with buffered glycerin (pH 8.7), covered with glass coverslips, and examined using a fluorescence microscope.

One hundred and two (63.4%) and 70 (50.3%) of the 161 wild felids tested were seropositive for *T. gondii* and *N. caninum* antigen by IFAT, respectively (Table I). Titers of antibodies ranged from 1:40 to 1:10,240 for *T. gondii* and from 1:25 to 1:400 for *N. caninum*. Forty-eight wild felids showed antibodies for both parasites. Fifty-four and 22 felids were seropositive only for *T. gondii* and *N. caninum*, respectively. Thirty-six animals were seronegative for both parasites.

Among sampled wild canids, 49 (50.5%) and 40 (41.2%) animals were seropositive for *T. gondii* and *N. caninum* antigen by IFAT, respectively (Table I). Titers of antibodies ranged from 1:40 to 1:10,240 for *T. gondii* and from 1:25 to 1:6,400 for *N. caninum*. Thirty wild canids had antibodies for both parasites. Nineteen and 10 canids were seropositive only for *T. gondii* and *N. caninum*, respectively. Thirty-eight animals were seronegative for both parasites.

The seroprevalence of *T. gondii* found in the present study in captive felids (63.4%) is similar to that found in exotic captive wild felids from Brazilian zoos (Silva et al., 2001) but higher than that found in Neotropical felids in Brazilian zoos (Silva et al., 2007).

The seroprevalence to *T. gondii* (50.5%) among sampled wild canids was higher than that found in free-ranging Brazilian wild canids from Rio Grande do Sul, São Paulo, and Paraíba and Paraná states (Genari et al., 2004), and captive Brazilian foxes from some São Paulo zoos (Catenacci et al., 2010). However, the seroprevalence found herein was lower than that found in captive maned wolves (*Chrysocyon brachyurus*) from southeastern and midwestern Brazil (Vitaliano et al., 2004). The present work is the first serological detection of antibodies to *T. gondii* in Brazilian bush dog (*Speothos venaticus* (Lund)), an endangered wild canid species, with a few individuals maintained in Brazilian zoos.

The present study represents the first serological detection of *N. caninum* in Brazilian wild captive and exotic felids, with a seroprevalence of 50.3%. Unfortunately, there are very few reports of antibody detection for this protozoan among wild felids. A very low seroprevalence (0.6%) was reported among captive and free-ranging feral cats in the United States (Spencer et al., 2003) and Spain (6.8%) (Millán et al., 2009).

The seroprevalence to *N. caninum* (41.2%) among wild canids was higher than that reported for Brazilian free-ranging wild canids (Canón-Franco et al., 2004), and captive Brazilian maned wolves (Vitaliano et al., 2004). It was suggested previously that the detection of specific antibodies in wild canids can be a good indicator of the presence of *N. caninum* in the environment (Hamilton et al., 2005). Further studies are needed to determine whether *N. caninum* has an intestinal phase in Brazilian wild canids resulting in oocyst shedding.

The high seroprevalence to these parasites could reflect an error in the institutional management, facilitating the contact with the infective agent by ingesting oocysts from the environment, food, or water, as well as bradyzoites from tissue cysts of intermediate hosts with chronic infection (Tenter et al., 2000). Zoo animals are susceptible to infection by *T. gondii* because domestic cats are frequently present in these places, along with

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TABLE I. Number, species, and localization of sampled wild captive carnivores.

Species	Common name	No. of seropositives to *T. gondii* (%)	No. of seropositives to *N. caninum* (%)	Total no. of sampled animals
Leopardus pardalis	Ocelot	28 (66.7)	30 (71.4)	42
Leopardus tigrinus	Little-spotted-cat	22 (62.8)	11 (31.4)	35
Leopardus wiedii	Margay	4 (100)	0	4
Oncifelis colocolo	Pampas cat	1 (33.3)	3 (100)	3
Panthera onca	Jaguar	11 (84.6)	8 (61.5)	13
Puma concolor	Puma	14 (77.8)	5 (27.8)	18
Puma yagouaroundi	Jaguarundi	10 (40)	5 (20)	25
Panthera tigris	Tiger	4 (66.7)	4 (66.7)	6
Panthera pardus	Leopard	1 (100)	0	1
Caracal caracal	Caracal	0	1 (100)	1
Leptailurus serval	Serval	1 (100)	1 (100)	1
Genetta genetta	Genetta	1 (100)	0	1
Panthera leo	Lion	5 (55.5)	1 (11.1)	9
Prionailurus viverrinus	Fishing cat	0	1 (100)	1
Speothos venaticus	Bush dog	17 (63)	16 (59.2)	27
Cerdocyon thous	Crab-eating fox	14 (35.9)	13 (33.3)	39
Cerdocyon brachyurus	Maned-wolf	11 (52.4)	5 (23.8)	21
Pseudalopex vetulus	Hoary fox	5 (71.4)	4 (57.1)	7
Canis lupus	European wolf	2 (66.7)	2 (66.7)	3
Total		151	110	257

synanthropic animals, which constitute potential prey for carnivores (Zarnke et al., 2000). In addition, chickens and beef are used as a source of meat for captive wild animals. The seroprevalence to *T. gondii* among chickens in Brazil ranges from 39 to 66% (da Silva et al., 2003; Dubey, Graham et al., 2003; Dubey, Navarro et al., 2003; Dubey et al., 2006, 2007; de Oliveira et al., 2009) and from 1 (Pita Gondim et al., 1999) to 71% (Santos et al., 2009) among cattle. Alternatively, the seroprevalence to *N. caninum* among cattle ranges from 14.3 (Guimarães et al., 2004) to 91.2% (Guedes et al., 2008) in Brazil. Biosecurity measures at the Brazilian zoos were not probed in the present study; therefore, direct contact with other wildlife species (birds, small rodents) is unknown. Furthermore, feeding captive felids with carcasses of accidentally killed (via automobiles) or animals dying for other reasons is an acceptable and very common practice in Brazil (Silva et al., 2007).

To our knowledge, this is the first serological detection of antibodies to *N. caninum* in Brazilian wild captive felids and bush dogs.

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