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All about neosporosis in Brazil

Tudo sobre neosporose no Brasil

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Abstract

Neospora caninum is protozoan parasite with domestic and wild dogs, coyotes and grey wolves as the definitive hosts and many warm-blooded animals as intermediate hosts. It was cultivated and named in 1988. Neosporosis is a major disease of cattle and has no public health significance. Since 1990's *N. caninum* has emerged as a major cause of abortion in cattle worldwide, including in Brazil. *N. caninum* also causes clinical infections in several other animal species. Considerable progress has been made in understanding the biology of *N. caninum* and there are more than 200 papers on this subject from Brazil. However, most of the reports on neosporosis from Brazil are serological surveys. Overall, little is known of clinical neosporosis in Brazil, particularly cattle. The few reports pertain to sporadic cases of abortion with no information on epidemics or storms of abortion. The objective of the present review is to summarize all reports from Brazil and suggest topic for further research, including prevalence of *N. caninum* oocysts in soil or in canine feces, and determining if there are additional definitive hosts, other than the domestic dog. There is need for a national survey in cattle using defined parameters. Future researches should focus on molecular characterization of *N. caninum* strains, possibility of vaccine production and relationship between wildlife and livestock epidemiology.

Keywords: *Neospora caninum*, neosporosis, domestic animals, wild animals, Brazil.

Resumo

Neospora caninum é um protozoário parasita que possui os canídeos domésticos e selvagens, coiotes e lobos cinzentos como hospedeiros definitivos e vários animais de sangue quente como hospedeiros intermediários. Foi cultivado e nomeado em 1988. A neosporose é uma das principais doenças em bovinos e não tem significância em saúde pública. Desde 1990, *N. caninum* tem emergido como uma das principais causas de aborto em bovinos em todo o mundo, inclusive no Brasil. *N. caninum* também causa infecções clínicas em várias outras espécies animais. Consideráveis avanços foram feitos na compreensão da biologia desse parasita e há mais de 200 trabalhos sobre o assunto no Brasil. No entanto, a maioria dos relatos de neosporose do Brasil são relacionados a sorologia. Em geral, pouco se sabe sobre a neosporose clínica no Brasil, particularmente em bovinos. Os poucos relatos referem-se a casos esporádicos de aborto sem informações sobre epidemias ou surtos de aborto. O objetivo da presente revisão é resumir todos os relatos sobre *N. caninum* no Brasil e sugerir tópicos para pesquisas futuras, incluindo a prevalência de oocistos de *N. caninum* no solo ou em fezes caninas e determinar se há hospedeiros definitivos adicionais, exceto o cão doméstico no país. Uma pesquisa nacional em bovinos usando parâmetros definidos seria de grande importância. Pesquisas futuras deveriam ser focadas na caracterização de cepas de *N. caninum*, possibilidade de produção de vacinas e a relação epidemiológica entre a vida selvagem e o gado.

Palavras-chave: *Neospora caninum*, neosporose, animais domésticos, animais selvagens, Brasil.

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Introduction

Neosporosis is relatively a newly recognized disease. In 1988, the etiologic agent of neosporosis was cultivated and named, *Neospora caninum*. It is ancestrally and morphologically related to *Toxoplasma gondii*. Since 1990's *N. caninum* has emerged as a major cause of abortion in cattle worldwide, including in Brazil. *N. caninum* also causes clinical infections in several other animal species. Considerable progress has been made in understanding the biology of *N. caninum*. A recent book on neosporosis (DUBEY et al., 2017) listed more than 2100 citations and most reports (>200) from Brazil. Many of the reports from Brazil are scattered in local journals. The objective of the present review is to summarize reports from Brazil and suggest topic for further research. To minimize citations, only references from Brazil are listed in bibliography.

Basic Biology

As stated earlier, *N. caninum* and *T. gondii* are morphologically similar but biologically different coccidians. Both parasites have a wide host range but unlike *T. gondii*, *N. caninum* is not considered zoonotic. Canids (domestic and wild canids dogs, coyote, and wolf) are the definitive hosts of *N. caninum* whereas felids (domestic and wild Felidae) are definitive hosts for *T. gondii*. Neosporosis is a major disease of cattle whereas cattle are considered resistant to *T. gondii.* Transplacental transmission is a major route of propagation of *N. caninum* in cattle while, although medically important, is not the major route of transmission for *T. gondii*. There is only one species of *Toxoplasma*, *T. gondii*, but the genus *Neospora* has two species, *N. caninum* and *N. hughesi*; only horses are reported as an intermediate host for *N. hughesi.*

History of Neosporosis in Brazil

The *in vitro* cultivation of *N. caninum* in 1988 in USA made it possible to develop diagnostic tests for neosporosis (DUBEY et al., 2017). However, unlike *T. gondii*, it is difficult to culture *N. caninum* (see later discussion) and only one isolate (NC-1) was available initially at the USDA laboratory in Beltsville, Maryland, USA. Thus, commercial tests were not developed for the diagnosis of neosporosis for several years. Therefore, no research was performed on this subject in Brazil until mid 1990's.

In BA, Gondim et al. (1999b, 2001) first recognized clinical neosporosis in an aborted bovine fetus and first isolated viable *N. caninum* (NC-Bahia) from the brain of a naturally-infected adult dog presenting incoordination and hind-limb paresis. Corbellini et al. (2002) first recognized neosporosis as an important cause of bovine abortion. They documented lesions consistent with protozoal infection in 22 (47.8%) of 46 fetuses submitted from 12 farms in RS. From those, 18 specimens of fetuses with encephalitis reacted to *N. caninum* antisera.

Brazilan Contributions to the General Biology of *N. caninum*

Viable N. caninum isolates and genetic diversity

Unlike *T. gondii*, little is known of genetic diversity of *N. caninum* mainly because there are only few viable isolates available worldwide. One reason for this is the difficulty to isolate viable *N. caninum* from animal tissues, especially from latently infected animals. Table 1 summarizes all viable isolates of *N. caninum* from animals in Brazil. The greatest success of isolating *Neospora* from asymptomatic animals has been achieved by feeding brain tissue from naturally-infected animals to dogs and then examining dog feces for oocyst excretion. By doing so, *N. caninum* was isolated from buffaloes, sheep and cattle.

In addition, to provide these *Neospora* isolates for biological and genetic studies to researchers in other countries (DUBEY et al., 2017), García-Melo in association with researchers from Spain did the first microsatellite typing data for Nc-Goiás isolated from clinically healthy cattle. Although the isolate had most of the alleles already identified, unique alleles were described for this strain at the MS5 and MS10 loci, using 12 microsatellite markers (GARCÍA-MELO et al., 2009). The recent isolate from brain tissue from a naturally-infected cattle (OLIVEIRA et al., 2017) was also found to have unique microsatellite alleles as MS5, MS6a, MS8 and MS10. A dog fed brain tissue from a naturally-infected cattle excreted *N. caninum* oocysts for 14 days starting seven days post infection (dpi), with an average number of 102 oocysts/g of feces. DNA isolated from cell culture derived tachyzoites was characterized using microsatellites. No new alleles were found and comparison showed closest relation to multilocus genotyping with strains from Spain and Argentina. Comparison with Brazilian strains (NC-Bahia; NC-Goiás) revealed variation in three and four of the nine markers used.

Additionally, the Brazilian NC-Bahia strain isolated from the dog was included among the *N. caninum* strains characterized by researchers outside Brazil (AL-QASSAB et al., 2009, 2010; REGIDOR-CERRILLO et al., 2013).

Transmission

Tachyzoites (in groups), bradyzoites (in tissue cysts) and sporozoites (in oocysts) are the three infectious stages of *N. caninum.* Tachyzoites and bradyzoites occur in tissues of intermediate hosts whereas oocysts are excreted in feces of certain canids. Unlike *T. gondii*, relatively few oocysts are excreted by canids after ingesting infected tissues. However, oocysts were excreted with a high frequency by two-three-month old dogs fed brains from six naturally infected water buffaloes (RODRIGUES et al., 2004). An other study showed that oocyst excretion is more efficient by feeding brains than other tissues to dogs (CAVALCANTE et al., 2011). The authors fed 17 two-three month old dogs with different tissues from four cattle naturally infected with *N. caninum*. Each group of dogs received: masseter (n=5), heart (n=5), brain

Table 1. Isolation of *N. caninum* from naturally infected animals from Brazil.

Host	Source	Bioassay		Isolate designation	Genetic data	Remarks	Reference
		Animals	Cell culture				
Cattle	Clinical, blind, neurological calf, 3-months old	SW mice,	Vero	BCN/PR3	No	Tissue cysts were found in the brains of SW mice that were immunocompetent	Locatelli-Dittrich et al. (2003)
	Aborted fetus, 7 months of gestation	Mice, gerbils	Vero	BCN/PR1	Yes	Initial isolation in immunosuppressed mice. Tachyzoites infective to immunocompetent mice and gerbils	Locatelli-Dittrich et al. (2004)
	Asymptomatic, 4 months old	KO mice	MARC-145	Nc-Goiás 1b	Yes	Not pathogenic to BALB/c mice	García-Melo et al. (2009)
	Adult slaughtered in abattoir	Dog, SW mice, gerbils	Vero	NC-SP1	Yes	Isolated from dog bioassay	Oliveira et al. (2017)
Dog	Collie, 7 years old, clinical	Gerbil	Vero (COS-1)	NC-Bahia	Yes	Isolate pathogenic to gerbils	Gondim et al. (2001, 2004)
Sheep	Two 4 months old, clinically normal	Gerbil, SW and vesper mice, dogs	Vero	Not stated	No	Mice remained healthy. Necropsied 60 dpi. Tissue cysts in brains of both gerbils.	Pena et al. (2007)
Water buffalo	6 seropositive from abattoir	Dogs, gerbil, KO mice	CV1	NCBrBuf-1 NCBrBuf-2 NCBrBuf-3 NCBrBuf-4 NCBrBuf-5	No	Both gerbils and KO remained asymptomatic	Rodrigues et al. (2004)

KO=Gamma interferon gene knockout; SW=Swiss Webster.

(n=4), liver (n=3), and a group remaining as control (n=3), no infected. None of the control dogs excreted oocysts and three dogs that received brain, two that received masseter, two that received heart and one that received liver excreted *N. caninum*-like oocysts, from day seven to day 17 after ingestion of tissues. All dogs that received brain and liver excreted only *N. caninum* oocysts. The results were confirmed by polymerase chain reaction (PCR) using Nc5 and ITS-1 amplification and indicated that a variety of visceral, neural, and muscular tissues are infected naturally with *N. caninum*.

How dogs become infected with *N. caninum* in nature is not fully understood. Fecal transmission of *N. caninum* in dogs appears to be less important than carnivorism. Until the study by Bandini et al. (2011) it was unknown if the dogs could be infected via ingestion of oocysts. They fed four dogs with 1,000, 5,000 or 10,000 *N. caninum* oocysts; none of the four dogs excreted *N. caninum*–like oocysts in their feces during the observation period of 30 days. However, the two dogs fed with 10,000 oocysts seroconverted and the two dogs fed with 1,000 or 5,000 oocysts did not. Neither parasite DNA nor parasite stages were demonstrable in tissues of the seropositive dogs euthanized six months after feeding oocysts. These findings suggest that fecal transmission may not be an important mode of transmission of the parasite for the definitive host but results need confirmation.

Tachyzoites are important in transplacental transmission of *N. caninum* infection. Cavalcante et al. (2012) confirmed transplacental transmission of *N. caninum* in dogs. Six bitches in two groups were inoculated with a very high dose (10^8 tachyzoites). In group I, three bitches were inoculated during the third week of gestation, and in group II, three bitches were inoculated at the sixth week of gestation. The bitches were allowed to whelp naturally. Dams and their pups were tested by immunohistochemistry (IHC), serology, and PCR. In group I, six of the ten pups died within 48 hour of birth. In group II, seven of the 13 pups died between five and ten days of birth. *N. caninum* DNA was detected by nested PCR in two pups (hearts of both and liver of one) from group I, and one pup in central nervous system (CNS) and lymph node from group II. The dams and the pups that survived were clinically normal. *N. caninum* was not demonstrable in tissues of any of pups and their dams.

Studies in Brazil showed that *N. caninum* can be transmitted transplacentally in water buffaloes (CHRYSSAFIDIS et al., 2014, 2015). The authors conducted an important experiment in six buffaloes and seven cows inoculated intravenously with *N. caninum* tachyzoites at 70 day of gestation. Three buffaloes and five cows were inoculated with a Brazilian *N. caninum* strain (NC-Bahia); only one cow aborted but all fetuses became infected. The other two cows and three buffaloes were inoculated with the NC-1 strain; all fetuses died by 35 dpi as determined by ultrasound and *N. caninum* DNA was detected in fetal tissues.

Chryssafidis et al. (2011) also showed, for the first time, that *N. caninum* can be transmitted transplacentally in naturally infected buffaloes; they found *N. caninum* DNA in one brain of the nine fetuses from buffaloes slaughtered in an abattoir, aiming Nc5 and ITS-1 DNA regions. Although, viable or intact parasite has not been demonstrated in naturally infected fetuses from buffaloes, this is the first indication of transplacental transmission in this host.

In addition to bradyzoites and oocysts, tachyzoites can also be infectious orally. Four to five day old gerbils were successfully infected by oral inoculation of tachyzoites (FERREIRA et al., 2016). All 17 gerbils died of neosporosis between eight and 17 dpi (one died on each of days 8, 9, 15, 16, and 17, four died on day 12, and five died on day 15 (personal communication to JPD on October 24, 2016) with 4 x 10^5 NC-1 tachyzoites. *N. caninum* DNA was found in heart, lung, spleen, kidney, liver and CNS of gerbils.

Environmental resistance of oocysts

Oocyst is the environmentally resistant stage. Researchers in Brazil found that *N. caninum* oocysts were rendered noninfectious by heating to 100 °C for one minute, and 10% sodium hypochlorite for one hour but not at 60 °C for one minute, and by other commonly used disinfectants (ALVES et al., 2011). Aqueous 2% sulfuric acid has been commonly used to store *N. caninum* oocysts at 4 °C; how long oocysts remain viable under these conditions is not known. *N. caninum* oocysts remained viable for 108 days but not for 46 months when stored in 2% sulfuric acid at 4 °C (UZÊDA et al., 2007a).

Diagnostic tests

Recently, Fehlberg et al. (2017) reported on successful development of a high resolution melting PCR method to distinguish *Neospora, Sarcocystis* and *Toxoplasma* using a single pair of primers targeting 18S rDNA.

Epidemiology

More serological studies for *N. caninum* infection in animals have been conducted in Brazil than rest of the world.

The results, however, are not comparable because of different serological assays used different cut-offs employed, different antigens used. For example, in the indirect fluorescent antibody test (IFAT) and the *Neospora* agglutination test (NAT) whole tachyzoites are used detecting antibodies to surface proteins whereas in the enzyme-linked immunoabsorbent assay (ELISA) different antigens are used, some of them using crude tachyzoite extract while others used soluble antigens (Table 2a). The standardization of serological tests was based on studies in other countries and a full discussion of these is beyond the scope of this review—this subject was recently reviewed (DUBEY et al., 2017). One of the problems with serological testing is the availability of standardized sera from experimentally infected animals. Immunoblotting (IB) was employed in some studies where no such sera were available (Table 2b).

Little has been done in Brazil to characterize *Neospora* recombinant antigens for the serological diagnosis or vaccine development, except the report of Bezerra et al. (2017) who characterized one surface protein, by cloning the sequence and named it as NcSRS67, which has no orthologue with *Toxoplasma gondii*, only with *Hammondia hammondi*.

In Tables 3-12 serological reports in different hosts are summarized. We listed all reports that we found.

Salient features are commented for some of these surveys.

Dogs: Numerous surveys from different regions of Brazil are summarized in Table 3. As stated earlier results of these types of surveys are not strictly comparable. However, 45 of 49 surveys used IFAT as a diagnostic technique, and most of them employed the cut-off value of 1:50, facilitating comparisons of occurrence values. In three surveys, IFAT and ELISA were compared for serological diagnosis of *N. caninum* in dog sera and IFAT was superior to indirect ELISA used (SILVA et al., 2007; HIGA et al., 2000; RAIMUNDO et al., 2015).

In some studies, risk factors were evaluated (Table 3). The age of dogs was a statistically significant factor in nine reports; older dogs were more likely to be seropositive (OLIVEIRA et al., 2004; FERNANDES et al., 2004; AZEVEDO et al., 2005; ANDREOTTI et al., 2006; CUNHA et al., 2008; MINERVINO et al., 2012; NOGUEIRA et al., 2013; BALTHAZAR et al., 2013; RAIMUNDO et al., 2015). Gender and breed were not associated with presence of antibodies. In two surveys mixed breed dogs constituted a risk factor for the infection (BRUHN et al., 2012; RAIMUNDO et al., 2015). The diet in some studies was related with access to street and prevalence was higher in dogs that had outdoor access than in pets with little or no outdoor scavenging (GENNARI et al., 2002; CAÑÓN-FRANCO et al., 2003; BENETTI et al., 2008; SICUPIRA et al., 2012). However, in some studies such an association was not found (MINEO et al., 2004; JESUS et al., 2006; PLUGGE et al., 2011). Vertical transmission was also studied (TAQUES et al., 2016), with 41 stillborn puppies from 23 bitches. By PCR and IFAT, five (21.7%) bitches were positive and 22 (53.6%) stillborn were positive by PCR, utilizing ITS-1 DNA region/locus, being 17 from positive bitches and five from negative ones. Although the prevalence of positive stillborn was higher from positive bitches, no conclusions were made.

Epidemiologically, contact between cattle and dogs has been identified as a possible risk factor that deserves attention (Table 3). Dogs from peri-urban or rural areas had more chance to be infected by *N. caninum* (IFAT 1:50) than urban dogs in the surveys from MG (FERNANDES et al., 2004), PR (PLUGGE et al., 2008), RS (CUNHA et al., 2008), and BA (SICUPIRA et al., 2012), and this risk factor is normally associated with proximity to cattle; access and ingestion of fetal membranes, carcasses, and prey.

Leishmania infantum chagasi is an important parasite of dogs in Brazil; and immunosuppression caused by *Leishmania* spp. may enhance the susceptibility of dogs to *N. caninum* infection (CRINGOLI et al., 2002). Serological surveys correlating *N. caninum* and *Leishmania* spp., had been conducted (GENNARI et al., 2006; ANDREOTTI et al., 2006; GUIMARÃES et al., 2009; GRECA et al., 2010; VALADAS et al., 2010a; LOPES et al., 2011; PAULAN et al., 2013; SEABRA et al., 2015; CONSTANTINO et al., 2016). In dogs from endemic cities of visceral leishmaniosis such as Araçatuba, SP (GENNARI et al., 2006), Campo Grande, MS (ANDREOTTI et al., 2006), and Teresina, PI (LOPES et al., 2011), positive association was found (Table 3).

Cattle: Seroprevalence of *N. caninum* varied with the type of cattle (beef, dairy), different regions, within region and with the type of serological tests used (Table 4).

Table 2a. ELISA techniques used to confirm presence of *N. caninum* antibodies.

Sera source	Antigen	ELISA type*	Results in table	Reference
Dogs	Antigen incorporated into iscoms	A, C, I	3	Mineo et al. (2004)
	NC-1 strain maintained in bovine monocytes cells; soluble antigen	B, C		Silva et al. (2007)
	NC-1 tachyzoites strain in Vero cells	A, C		Raimundo et al. (2015)
Cattle	NC-1 tachyzoites strain in Vero cells, lysed in SDS buffer	J	4	Chahan et al. (2003)
	Commercial	E		Andreotti et al. (2004)
	Commercial	F		Paz et al. (2007)
	Commercial	E		Melo et al. (2001)
	Commercial	E		Melo et al. (2004)
	Antigen incorporated into iscoms	I		Mineo et al. (2006)
	Commercial	E		Locatelli-Dittrich et al. (2001)
	Commercial	E		Locatelli-Dittrich et al. (2008)
	Commercial	E		Marques et al. (2011)
	Commercial	E		Nascimento et al. (2014)
	NC-1 strain in Vero cells; soluble antigen	B, C		Ramos et al. (2016)
	Commercial	E		Munhoz et al. (2006, 2009)
	Commercial	D		Vogel et al. (2006)
	Commercial	E		Sartor et al. (2003)
	Commercial	M		Sartor et al. (2005)
Buffalo	Commercial	E	5	Viana et al. (2009)
	Commercial	D		Vogel et al. (2006)
	Commercial kit	G, H		Silva et al. (2014)
Sheep	NC-1 strain in Vero cells	A, L	6	Andreotti et al. (2009)
	NC-1 strain in Vero cells, soluble antigen	A, C		Rossi et al. (2011)
	Commercial	D		Vogel et al. (2006)
Captive deer	Commercial	E	11	Zimpel et al. (2015)
Horse	NC-1 strain in HeLa cells	A, C	12	Pivoto et al. (2014)
	NC-1 strain in bovine turbinate cell monolayers	C, K		Hoane et al. (2006)

*A = **WT** = Whole tachyzoite extract; **B** = **SA** = soluble antigen; **C** = **IH** = In house; **D** = **CHEKIT** = CHEKIT *Neospora,* indirect ELISA, detergent lysate of tachyzoites, IDEXX Laboratories, The Netherlands; **E** = **IDEXX** = IDEXX HerdChek *Neospora caninum* antibody, indirect ELISA, sonicate lysate of tachyzoites, IDEXX Laboratories, USA; **F** = **VMRD** = *Neospora caninum* cELISA competitive ELISA GP65 surface antigen of tachyzoites VMRD, USA; **G** = **Horse IgG** = Horse IgG(T) ELISA Quantitation Set; Bethyl Laboratories; **H** = **Horse IgM** = Horse IgM ELISA, Kamiya Biomedical Company, Seattle; **I** = **ISCOM** = Detergent extracted tachyzoite antigen incorporated in immune stimulating complex particles; **J** = **NcSAG1** = Recombinant surface antigen; **K** = **NhSAG1** = Recombinant NhSAG1; **L** = **rNcSRS2** = Recombinant antigen protein; **M** = **NS/ND** = not stated/ not done.

Table 2b. Immunoblotting technique used to confirm presence of *N. caninum* antibodies.

Sera source	Antigen*	Sera tested previously	Results in Table	Reference
Dog	NC-1 tachyzoites lysed in SDDS-PAGE buffer	IFAT, 1:25; indirect ELISA	3	Silva et al. (2007)
Cattle	NC-1 tachyzoites lysed in SDDS-PAGE buffer	NcSAG1 ELISA, 60/66 sera positive for ELISA	4	Chahan et al. (2003)
Sheep	NC-1 tachyzoites lysed in 4% sodium dodecyl sulfate buffer	IFAT, 1:50; discordant results	6	Rossi et al. (2011)
Cat	Detergent extracted whole NC-1 tachyzoites	NAT, 1:80	9	Dubey et al. (2002)
Donkey	Whole NC-1 tachyzoites	Sera positive by IFAT, 1:100	12	Galvão et al. (2015)
Human	NC-1 tachyzoites lysed in SDDS-PAGE buffer	Sera with discordant results by IFAT and ELISA	-	Lobato et al. (2006)

*Produced in non reducing conditions.

Table 3. Serological studies of *N. caninum* in dogs from Brazil.

State	Type	No. tested	No. positive	% Positive	Test	Cut-off	Remarks	Reference
Alagoas	Urban	128	5	3.8	IFAT	1:50	Age, sex, breed, area, habitat	Sousa et al. (2012)
	Rural	99	5	4.8	IFAT	1:50		
Amazon region (MT and TO)	Indian communities	325	32	9.8	IFAT	1:50	**Age,** sex, *T. gondii*, others	Minervino et al. (2012)
Bahia	Stray	250	28	11.2	IFAT	1:50	Age, sex, others	Jesus et al. (2006)
	Owned	165	22	13.3	IFAT	1:50		
Bahia	Urban	156	4	2.6	IFAT	1:50	**Area, contact with other dogs, feeding habits,** others	Sicupira et al. (2012)
	Rural	41	6	14.6	IFAT	1:50	**Area, contact with other dogs, feeding habits,** others	
	Peri-urban	214	28	13.1	IFAT	1:50	**Area, contact with other dogs, feeding habits,** others	
Espírito Santo	Rural	187	22	11.7	IFAT	1:50	Sex, *T. gondii*	Acosta et al. (2016)
Goiás	Zoonosis Center	72	26	36.1	IFAT	1:50	Sex, origin	Boaventura et al. (2008)
	Hospital (owned)	125	39	31.2	IFAT	1:50	Sex, origin	
Maranhão	Stray	100	45	45.0	IFAT	1:50	Sex	Teixeira et al. (2006)
Mato Grosso	Clinics	60	27	45.0	IFAT	1:50	Age, sex, diet, **access to streets**	Benetti et al. (2008)
Mato Grosso	Rural	37	25	67.6	IFAT	1:50		Benetti et al. (2009)
Mato Grosso do Sul	Pet	245	65	26.5	IFAT	1:50	**Age**, sex	Oliveira et al. (2004)
Mato Grosso do Sul	Rural	40	12	30.0	IFAT	1:100		Andreotti et. al. (2004)
Mato Grosso do Sul	Urban	345	93	27.2	IFAT	1:50	**Age**, sex, ***Leishmania***	Andreotti et al. (2006)
Minas Gerais	Clinical	163	11	6.7	IFAT	1:25	*T. gondii*	Mineo et al. (2001)
Minas Gerais	Urban	300	32	10.7	IFAT	1:50	**Age**, breed, sex, **area**	Fernandes et al. (2004)
	Peri urban	58	11	18.9	IFAT	1:50		
	Rural	92	20	21.7	IFAT	1:50		
Minas Gerais	Clinic	275	22	7.9	ELISA	A,C	Age, breed, sex, habitat, *T. gondii*, others	Mineo et al. (2004)
	Stray	94	12	12.8	ELISA	I		
Minas Gerais	Clinic, stray	300	32	10.7	IFAT	1:50	Age, breed, sex, *T. gondii*	Silva et al. (2007)
	Clinic, stray	300	105	35.0	ELISA	B,C		
Minas Gerais	Clinics	228	7	3.1	IFAT	1:50	Age, breed, sex *T. gondii, Leishmania, Babesia canis*	Guimarães et al. (2009)
Minas Gerais	Rural	240	36	15.0	IFAT	1:50	Age, **breed**, **origin,** sex, others	Bruhn et al. (2012)
Minas Gerais	Urban	182	15	8.2	IFAT	1:50	**Age**, diet, **hunting,** area	Nogueira et al. (2013)
	Rural	421	58	13.7	IFAT	1:50	**Bovine abortion,** area	
Pará	Rural	72	8	11.1	IFAT	1:50	Sex, area *T. gondii, Leishmania*	Valadas et al. (2010b)
	Urban-stray	57	8	14.0	IFAT	1:50		
Paraíba	Urban Domestic	286	24	8.4	IFAT	1:50	Age, sex, breed, others, **habitat,** *T. gondii*	Azevedo et al. (2005)
Paraná	Dairy farms	134	29	21.6	IFAT	1:50	**Age,** sex, breed	Souza et al. (2002)
Paraná	Neurological	98	0	0.0	IFAT	1:50	*T. gondii*	Giraldi et al. (2002)
Paraná	Sheep farms	24	7	29.1	IFAT	1:50	Age, sex, breed, *T. gondii*	Romanelli et al. (2007)
Paraná	Urban	181	23	12.7	IFAT	1:50	**Area,** habitat	Plugge et al. (2008)
	Peri urban	178	28	15.7	IFAT	1:50		
	Rural	197	50	25.3	IFAT	1:50		
Paraná	Rural	129	32	25.0	IFAT	1:50	Dogs and cattle	Locatelli-Dittrich et al. (2008)

Bold=statistically significant risk factor, Area = urban, peri urban, rural; Habitat=stray, domiciled.

Table 3. Continued...

State	Type	No. tested	No. positive	% Positive	Test	Cut-off	Remarks	Reference
Paraná	Owned	127	14	11.0	IFAT	1:50	*T. gondii*, neurologic signs	Plugge et al. (2011)
	Stray	20	3	15.0	IFAT	1:50		
Paraná	Stray	26	3	11.5	IFAT	1:25	*Leishmania spp, T. gondii, Trypanosoma cruzi*	Constantino et al. (2016)
Pernambuco								
Paulista	Domiciled	289	75	26.0	IFAT	1:50	*T. gondii*, origin	Figueredo et al. (2008)
Amaraji	Domiciled	168	44	26.2	IFAT	1:50		
Garanhuns	Domiciled	168	58	34.5	IFAT	1:50		
Pernambuco	Rural villages	56	0	0	IFAT	1:50	*T.gondii* antibodies surveyed	Arraes-Santos et al. (2016)
Piauí		530	17	3.2	IFAT	1:50	Age, sex, breed, *T. gondii*, ***Leishmania***	Lopes et al. (2011)
Piauí	Rural villages	71	5	7.0	IFAT	1:50	*T.gondii* antibodies surveyed	Arraes-Santos et al. (2016)
Rio de Janeiro	Urban - Clinic	402	34	8.4	IFAT	1:50	**Age,** neurologycal signs, others	Balthazar et al. (2013)
Rio Grande do Sul	Rural	230	47	20.4	IFAT	1:50	**Age**, sex, **area, type of farm, carcasses and abortions not removed**, others	Cunha et al. (2008)
	Urban	109	6	5.5	IFAT	1:50		
Rondônia	Domiciled (Street access)	157	13	8.3	IFAT	1:50	Age, sex, diet, street access	Cañón-Franco et al. (2003)
Rondônia	Farms	174	22	12.6	IFAT	1:50	**Age**, diet, abortion, stillbirth, others	Aguiar et al. (2006)
São Paulo	Hospital	203	44	21.6	IFAT	1:50	*T. gondii*, neurological signs	Higa et al. (2000)
São Paulo	Rural and urban	295	25	8.4	IFAT	1:50	Age, sex, *T. gondii*, neurologic signs	Varandas et al. (2001)
São Paulo	Domiciled	500	49	10.0	NAT	1:25	Age, sex, breed, **habitat**	Gennari et al. (2002)
	Street	611	151	25.0	NAT	1:25		
São Paulo	Urban	204	36	17.6	IFAT	1:50	Age, sex, *T. gondii*, ***Leishmania* spp.**,	Gennari et al. (2006)
São Paulo	Urban	108	17	15.7	IFAT	1:50	Age, sex, diet, others, *T. gondii*	Bresciani et al. (2007b)
São Paulo	Urban	865	223	25.8	IFAT	1:50	Age, sex, area	Moraes et al. (2008)
	Rural	65	11	16.9	IFAT	1:50		
	Peri urban	33	11	33.3	IFAT	1:50		
São Paulo	Rural (stray)	100	14	14.0	IFAT	1:25	*Leishmania* spp.	Greca et al. (2010)
São Paulo	Sheep farms	42	2	4.8	IFAT	1:25	Type of raising (chained or free), access to raw meat or offal	Machado et al. (2011)
São Paulo	Neurologic	50	7	14%	IFAT	1:25	*T. gondii*	Langoni et al. (2012)
São Paulo	Domiciled	342	17	4.9	IFAT	1:25	Age, sex, breed, *T. gondii*	Langoni et al. (2014)
São Paulo	Rural	93	6	6.5	IFAT	1:50	*Leishmania, T. gondii, Ehrlichia* spp., *Babesia canis*	Paulan et al., (2013)
São Paulo	Kennel	167	37	22.1	IFAT	1:25	*T. gondii, Leishmania*	Seabra et al. (2015)
	Clinic	133	36	27.0	IFAT	1:25	*T. gondii, Leishmania*	
Tocantins	Rural	99	43	43.4	ELISA	A,C	**Age, breed**, area, *T. gondii*	Raimundo et al. (2015)
			31	31.3	IFAT	1:25		
	Urban	105	45	42.9	ELISA	A,C		
			31	29.5	IFAT	1:25		

Bold=statistically significant risk factor, Area = urban, peri urban, rural; Habitat=stray, domiciled.

Table 4. Serologic studies of *N. caninum* antibodies in cattle from Brazil.

State	No. tested	Type	No. herds	No. positive	% positive	Test	Cut-off	Remarks[a]	Reference
Bahia	447	Dairy	14	63	14.0	IFAT	1:200	-	Gondim et al. (1999a)
Goiás	444	Dairy	11	135	30.4	IFAT	1:250	-	Melo et al. (2006)
Goiás	30	Mixed	1	13	43.3	IFAT	1:250	-	Melo et al. (2006)
Goiás	456	Beef	9	135	29.6	IFAT	1:250	-	Melo et al.(2006)
Maranhão	812	Dairy	27	412	50.7	IFAT	1:200	-	Teixeira et al. (2010)
Mato Grosso	932	Dairy	24 farms	499	53.5	IFAT	1:200	-	Benetti et al. (2009)
Mato Grosso do Sul	197	NS	6	66	33.5	ELISA	J	-	Chahan et al. (2003)
Mato Grosso do Sul	87	Beef	NS	26	29.9	IFAT	1:25	-	Ragozo et al. (2003)
Mato Grosso do Sul	23	Dairy	NS	5	21.7	IFAT	1:25	-	Ragozo et al. (2003)
Mato Grosso do Sul	90	Beef (history of abortions)	NS	38	43.0	ELISA	E	-	Andreotti et al.(2004)
Mato Grosso do Sul	60		Heifers	18	30.0	ELISA	E	-	Andreotti et al. (2004)
Mato Grosso do Sul	91		Healthy	7	7.7	ELISA	E	-	Andreotti et al. (2004)
Mato Grosso do Sul	2448	NS	205	449	14.9	IFAT	1:50	Production system (Dairy/beef)	Oshiro et al. (2007)
Mato Grosso do Sul	275	Beef	2 farms	81	29.5	cELISA	F	-	Paz et al. (2007)
Mato Grosso do Sul	392	Dairy/beef	4 farms	43	9.1	IFAT	1:50	-	Mello et al. (2008)
Mato Grosso do Sul	1098	Beef	1 farm	687	62.5	IFAT	1:50	Reproductive failure, 15% higher in seropositive cows.	Andreotti et al. (2010)
Minas Gerais	584	Dairy	18	109	18.7	ELISA	E	-	Melo et al. (2001)
Minas Gerais	126	Dairy	2	43	34.4	IFAT	1:25	-	Ragozo et al. (2003)
Minas Gerais	36	Beef	NS	4	11.1	IFAT	1:25	-	Ragozo et al. (2003)
Minas Gerais	576	Dairy	18	106	18.4	ELISA	E	-	Melo et al. (2004)
Minas Gerais	243	Dairy	2	41	16.8	ELISA	I	-	Mineo et al. (2006)
Minas Gerais	559	Dairy	18	510	91.2	IFAT	1:200	Farm size, number of cows lactating, milk production per day	Guedes et al. (2008)
Minas Gerais	575	Dairy	Abattoir	559	97.2	IFAT	1:200	Farm size, number of cows lactating, milk production per day	Guedes et al. (2008)
Minas Gerais	503	Dairy	Abattoir (fetuses)	64	12.7	IFAT	1:25	Farm size, number of cows lactating, milk production per day	Guedes et al. (2008)
Minas Gerais	1,204	Dairy	40 farms	260	21.6	IFAT	1:200	Reproductive failure	Bruhn et al. (2013)
Pará	500	beef	500	260	52	IFAT	1:128	Region, *T. gondii*	Silva et al. (2017)
Pará	40	Dairy	4 farms	7	17.5	IFAT	1:100	-	Minervino et al. (2008)
Pará	120	Beef	12	23	19.2	IFAT	1:100	-	Minervino et al. (2008)
Paraná	172	Dairy	1	60	34.8	ELISA	E	Abortion	Locatelli-Dittrich et al. (2001)
Paraná	15	Beef	NS	4	26.7	IFAT	1:25	-	Ragozo et al. (2003)
Paraná	75	Dairy	NS	16	21.3	IFAT	1:25	-	Ragozo et al. (2003)
Paraná	623	Dairy	23 farms	89	14.3	IFAT	1:25	Breed, presence of dogs, age, feed	Guimarães et al. (2004)
Paraná	385	Dairy	90 farms	45	12.0	IFAT	1:200	-	Ogawa et al. (2005)

[a]Statistically significant risk factors.

Table 4. Continued...

State	No. tested	Type	No. herds	No. positive	% positive	Test	Cut-off	Remarks[a]	Reference
Paraná	1263	NS	77 farms	423	33.0	ELISA	E	-	Locatelli-Dittrich et al. (2008)
Paraná	159	Beef	Abattoir	24	15.1	ELISA	E	-	Marques et al. (2011)
Paraná	309	Dairy	15 farms	63	20.4	IFAT	1:100	Feed, wild animal access, artificial insemination	Martins et al. (2012)
Paraná	76	Beef	4	23	30.3	ELISA	E	-	Nascimento et al.(2014)
Pernambuco	469	Dairy	20 farms	163	31.7	IFAT	1:200	Veterinary assistance, nutritional condition, presence of wetlands, manipulation of newborn calves, destination of cows that had aborted, abortion history, abortions period.	Silva et al. (2008)
Pernambuco	316	Dairy	25 municipalities	31	19.6	IFAT	1:200	Transplacental transmission	Ramos et al. (2016)
				57	36.0	ELISA	B, C		
Rio Grande do Sul	223 (abortion history)	Dairy	5	25	11.2	IFAT	1:200	Reproductive failure	Corbellini et al. (2002)
Rio de Janeiro	75	Dairy	NS	17	22.7	IFAT	1:25	-	Ragozo et al. (2003)
Rio de Janeiro	75	Beef	NS	5	6.7	IFAT	1:25	-	Ragozo et al. (2003)
Rio de Janeiro	563	Dairy	57 farms	131	23.2	ELISA	E	Breed	Munhoz et al. (2006, 2009)
Rio Grande do Sul	70	Dairy	NS	13	18.6	IFAT	1:25	-	Ragozo et al. (2003)
Rio Grande do Sul	70	Beef	NS	15	21.4	IFAT	1:25	-	Ragozo et al. (2003)
Rio Grande do Sul	1,549	Dairy	60 farms	276	17.8	IFAT	1:200	several	Corbellini et al. (2006)
Rio Grande do Sul	781	Dairy/beef	NS	89	11.4	ELISA	D	-	Vogel et al. (2006)
Rondônia	1011	Dairy	50	114	11.2	IFAT	1:25	Farm size, number of cows	Aguiar et al.(2006)
Rondônia	584	Beef	11 farms	56	9.5	IFAT	1:25	-	Aguiar et al. (2006)
Rondônia	514	Mixed	25 farms	50	9.7	IFAT	1:25	-	Aguiar et al. (2006)
Rondônia	621	Dairy	63 farms	66	10.6	IFAT	1:100	Abortion, birth of weak calves	Boas et al. (2015)
Santa Catarina	1518	Dairy	72 farms	466	30.6	IFAT	1:100	Presence of dogs	Fávero et al. (2017)
Santa Catarina	130	Dairy	29 farms	57	43.8	IFAT	1:200	Age, no. of pregnancies	Klauck et al. (2016)
São Paulo and Minas Gerais	600	NS	NS	101	16.8	IFAT	1:200	Area	Costa et al. (2001)
São Paulo	150	NS	NS	41	27.3	IFAT	1:25	-	Ragozo et al. (2003)
São Paulo	521	Dairy	NS	82	15.9	IFAT	1:200	-	Sartor et al. (2003)
São Paulo	521	Dairy	NS	159	30.5	ELISA	E	-	Sartor et al. (2003)
São Paulo	777	Beef	8 Farms	121	15.5	IFAT	1:200	-	Hasegawa et al. (2004)
São Paulo	505	Beef	11 Herds	101	20.0	ELISA	M	Production system	Sartor et al. (2005)
São Paulo	408	Dairy	6 herds	145	35.5	ELISA	M	*N. caninum* significatively higher in dairy cattle	Sartor et al. (2005)
São Paulo	1027	Dairy	3 farms	107	10.4	IFAT	1:100	High degree of association between *N. caninum* serological status of dams and daughter	Cardoso et al. (2012a)
Tocantins	192	Dairy	10 farms	48	25.0	IFAT	1:200		Martins et al. (2011)

[a]Statistically significant risk factors.

Table 5. Serological studies of *N. caninum* in buffaloes from Brazil.

State	No. of farms	No. tested	No. positive	% positive	Test	Cut-off	References
Bahia	4	117	42	35.9	IFAT	1:200	Gondim et al. (2007)
Pará	3	196	139	70.9	IFAT	1:25	Gennari et al. (2005)
Pará	26	500	195	39	IFAT	1:128	Silva et al. (2017)
Pará	4	212	187	88.2	ELISA	E	Viana et al. (2009)
Paraíba	14	136	26	19.1	IFAT	1:200	Brasil et al. (2015)
Rio Grande do Sul		164	24	14.6	ELISA	D	Vogel et al. (2006)
São Paulo	11	222	117 142	53.0 64.0	NAT IFAT	1:40 1:25	Fujii et al. (2001a, b)
São Paulo	12	411	230	56.0	IFAT	1:200	Souza et al. (2001)
São Paulo	5	192	169	88.0	IFAT	1:50	Chryssafidis et al. (2015)
Northern Brazil-13 provinces		4,796	2,665 2,345	55.5 48.8	ELISA IFAT	G, H 1:40	Silva et al. (2014)

Table 6. Serological studies of *N. caninum* in sheep from Brazil.

State	Type	No. tested	No. positive	% positive	Test	Cut-off	Remarks	Reference
Alagoas	26 farms	343	33	9.6	IFAT	1:50	**Small farms**, **water supply**	Faria et al. (2010)
Federal District	32 farms	1028	90	8.8	IFAT	1:50	Titers up to 51,200	Ueno et al. (2009)
Maranhão	5 farms	64	3	4.7	IFAT	1:25	**Food supplement**, **reproductive failure**	Moraes et al. (2011)
Mato Grosso do Sul	1 farm	441	136 141	30.8 32.0	IFAT ELISA	1:50 A, L	Comparison of techniques	Andreotti et al. (2009)
Minas Gerais	12 farms	334	27	8.1	IFAT	1:50	**Abortion history**	Salaberry et al. (2010)
Minas Gerais	2 farms	155	73 41	47.1 26.4	IFAT ELISA	1:50 A, C	Age	Rossi et al. (2011)
Minas Gerais	63 farms	488	64	13.1	IFAT	1:50	Age, **area**	Andrade et al. (2012)
Paraná	9 farms	305	29	9.5	IFAT	1:50	Sex, age, breed, reproductive fails, presence of dogs	Romanelli et al. (2007)
Pernambuco	23 farms	81	52	64.2	IFAT	1:50	**Age**	Tembue et al. (2011)
Pernambuco	Rural villages	179	39	21.8	IFAT	1:50	**Region, age,** sex, breed	Arraes-Santos et al. (2016)
Piauí	Rural villages	153	8	5.2	IFAT	1:50	**Region, age,** sex, breed	Arraes-Santos et al. (2016)
Rio Grande do Norte	35 farms	409	7	1.8	IFAT	1:50	Sex, presence of dogs, reproductive fails	Soares et al. (2009a)
Rio Grande do Sul	4 counties	62	2	3.2	ELISA	D		Vogel et al. (2006)
Rondônia	15 farms	141	41	29.0	IFAT	1:50	Titers up to 1:25,600; Reproductive problems, presence of dogs, source of water	Aguiar et al. (2004)
São Paulo	Meat breeds	597	55	9.2	IFAT	1:50	Age and presence of dogs	Figliuolo et al. (2004a)
São Paulo	16 farms	1497	120	8.0	IFAT	1:25	**Water supply, presence of dogs, reproductive problems**	Machado et al. (2011)
São Paulo	8 farms	382	49	12.8	IFAT	1:25	Tested for *T. gondii*	Langoni et al. (2011)
São Paulo/ Rio Grande do Sul	Abattoir	596	353	59.2	IFAT	1:25	**Sex**, **breeding system, breed, area,** age	Paiz et al. (2015)

Bold=statistically significant risk factors.

Table 7. Serological studies of antibodies to *N. caninum* in goats from Brazil.

State	Type	No. tested	No. positive	% positive	Test	Cut-off	Remarks	Reference
Bahia	9 herds	384	58	15.1	IFAT	1:100	**Breed**	Uzêda et al. (2007b)
Maranhão	5 farms	46	8	17.4	IFAT	1:25	Reproductive failure	Moraes et al. (2011)
Minas Gerais	90 herds	667	71	10.7	IFAT	1:50	Maximum titer 1:3,200	Andrade et al. (2013)
Paraíba	Abattoir	306	10	3.3	IFAT	1:50	Gender	Faria et al. (2007)
Pernambuco	23 farms	319	85	26.6	IFAT	1:50	**Age**	Tembue et al. (2011)
Pernambuco	Rural Villages	174	5	2.9	IFAT	1:50	Region, breed, age, sex	Arraes-Santos et al. (2016)
Piauí	Rural Villages	202	4	2.0	IFAT	1:50	Region, breed, age, sex	Arraes-Santos et al. (2016)
Rio Grande do Norte	14 farms	381	4	1.0	IFAT	1:50	Gender, reproductive fails, presence of dogs	Lima et al. (2008)
Santa Catarina	57 cities	654	30	4.6	IFAT	1:50	**Age, abortion, diet**	Topazio et al. (2014)
São Paulo	19 farms	394	25	6.4	IFAT	1:50	Maximum titer 1:12,800; Age, presence of dogs	Figliuolo et al. (2004b)
São Paulo	17 farms	923	161	17.7	NAT	1:25	**Presence of dogs**, Age, gender, reproductive problems	Modolo et al. (2008)

Bold=statistically significant risk factors.

Table 8. Serological studies of *N. caninum* antibodies in swine from Brazil.

State	Type	No. tested	No. positive	% positive	Test	Cut-off	Remarks	Reference
Paraíba	Abattoir	130	4	3.1	IFAT	1:50	Sex, Also tested for *T. gondii*	Azevedo et al. (2010)
Paraíba	Abattoir	190	6	3.2	IFAT	1:50	Also tested for *T. gondii*	Feitosa et al. (2014b)
Mato Grosso do Sul	Free living, wild	83	9	10.8	IFAT	1:50	**Sex**, age	Soares et al. (2016)

Bold=statistically significant risk factor.

Table 9. Serological studies of *N. caninum* in cats from Brazil.

State	Type	No. tested	No. positive	% positive	Test	Cut-off	Remarks	Reference
Bahia	Indoor, outdoor	272	8	2.9	IFAT	1:50	Also tested *Sarcocystis neurona*	Meneses et al. (2014)
Maranhão	Outdoor access	200	54	27.0	IFAT	1:25	Also tested *T. gondii*	Braga et al. (2012)
Mato Grosso do Sul	Free roaming and domiciled	151	10	6.6	IFAT	1:50	Also tested *T. gondii* and *L. infantum*	Sousa et al. (2014)
Paraíba	Free roaming and domiciled	201	0	0.0	IFAT	1:50	-	Feitosa et al. (2014a)
Pernambuco	Rural villages	32	2	6.2	IFAT	1:50	Region, breed, age, sex	Arraes-Santos et al. (2016)
Piauí	Rural villages	3	0	0.0	IFAT	1:50	Region, breed, age, sex	Arraes-Santos et al. (2016)
São Paulo	Free roaming and domiciled	502	60	11.9	NAT IB	1:40 B, C	NAT + (1+ at 1:800) 10 IB + Also tested *S. neurona*	Dubey et al. (2002)
São Paulo	Domiciled	400	98	24.5	IFAT	1:16	4 IFAT + 1:256	Bresciani et al. (2007a)
São Paulo	Domiciled	70	0	0.0	IFAT	1:16	-	Coelho et al. (2011)

Table 10. Serological studies of *N. caninum* in wild animals from Brazil.

Host species	Type	No. tested	No. positive	% positive	Test	Cut-off	Reference
Fam. Felidae							
Caracal (*Caracal caracal*)	Zoo	1	1	100.0	IFAT	1:25	André et al. (2010)
Fishing cat (*Prionailurus viverrinus*)	Zoo	1	1	100.0	IFAT	1:25	André et al. (2010)
Jaguar (*Panthera onca*)	Wild	11	7	63.6	IFAT	1:25	Onuma et al. (2014)
	Zoo	13	8	61.5	IFAT	1:25	André et al. (2010)
Jaguarundi (*Puma yagouaroundi* syn. *Herpailurus yagouaroundi*)	Zoo	25	5	20.0	IFAT	1:25	André et al. (2010)
Lion (*Panthera leo*)	Zoo	9	1	11.1	IFAT	1:25	André et al. (2010)
Little-spotted-cat (*Leopardus tigrinus*)	Zoo	35	11	31.4	IFAT	1:25	André et al. (2010)
Ocelot (*Leopardus pardalis*)	Zoo	42	30	71.4	IFAT	1:25	André et al. (2010)
Pampas cat (*Oncifelis colocolo*)	Zoo	3	3	100.0	IFAT	1:25	André et al. (2010)
Puma-cougar (*Puma concolor*)	Zoo	18	5	27.8	IFAT	1:25	André et al. (2010)
Serval (*Leptailurus serval*)	Zoo	1	1	100.0	IFAT	1:25	André et al. (2010)
Tiger (*Panthera tigris*)	Zoo	6	4	66.7	IFAT	1:25	André et al. (2010)
Fam. Canidae							
Crab-eating fox (*Cerdocyon thous*)	Wild	15	4	26.6	IFAT	1:50	Cañón -Franco et al. (2004)
			1	6.6	NAT	1:40	
	Captivity	2	0	0.0	IFAT	1:50	Melo et al. (2002)
Hoary fox*(Pseudalopex vetulus)*	Wild	30	0	0.0	IFAT	1:50	Cañón-Franco et al. (2004)
					NAT	1:40	
Maned wolf (*Chrysocyon brachyurus*)	Captivity and wild	48	0	0.0	IFAT	1:50	Melo et al. (2002)
	Zoo and preserves	59	5	8.5	IFAT	1:25	Vitaliano et al. (2004)
Pampas fox (*Lycalopex gymnocercus*)	Wild	12	5	41.6	IFAT	1:50	Cañón-Franco et al. (2004)
					NAT	1:40	
Fam. Didelphidae							
Opossum (*Didelphis marsupialis*)	Feral	396	84	21.2	IFAT	1:25	Yai et al. (2003)
Fam. Bovidae							
Barbary sheep (*Ammotragus lervia*)	Zoo	17	4	23.5	IFAT	1:50	Morikawa et al. (2014)
Fam. Cervidae							
Brazilian dwarf brocket (*Mazama nana*)	Captive and zoo	40	7	17.5	IFAT	1:50	Tiemann et al. (2005b)
	Captive	22	1	4.5	ELISA	E	Zimpel et al. (2015)
Brown brocket deer (*Mazama gouazoubira*)	Captive and zoo	66	29	43.9	IFAT	1:50	Tiemann et al. (2005b)
Marsh deer (*Blastoceros dichotomus*)	Captive	6	1	16.7	ELISA	E	Zimpel et al. (2015)
Pampas deer (*Ozotoceros bezoarticus*)	Wild National Park	23	3	13.0	IFAT	1:50	Tiemann et al. (2005a)
	Pantanal	16	12	75.0	IFAT	1:50	
Red brocket deer (*Mazama americana*)	Captive PR	4	0	0.0	ELISA	E	Zimpel et al. (2015)
	Captive and zoo	29	18	62.0	IFAT	1:50	Tiemann et al. (2005b)
Rodon (*Mazama rondoni*)	Captive and zoo	8	3	37.5	IFAT	1:50	Tiemann et al. (2005b)
Small red brocket (*Mazama bororo*)	Captive and zoo	3	2	66.6	IFAT	1:50	Tiemann et al. (2005b)
Fam. Caviidae							
Capybaras (*Hydrochaeris hydrochaeris*)	Wild	213	20	9.4	IFAT	1:25	Yai et al. (2008)
	Wild	63	1	1.5	IFAT	1:25	Valadas et al. (2010a)
	Wild	170	0	0.0	IFAT	1:50	Abreu et al. (2016)

Table 11. Serological studies of *N. caninum* antibodies in horses from Brazil.

State	No. tested	Type	No. positive	% positive	Assay	Cut-off titer or test	Remarks	Reference
Mato Grosso	200	Healthy	30	15.0	IFAT	1:50	Highest titer 1:400 in 1 horse.	Laskoski et al. (2015)
Pará	411	Healthy horses	28	6.8	IFAT	1:50	No risk factors detected.	Norlander (2014)
Paraná	72	Mares	28	38.8	IFAT	1:50	2 foals had pre-colostral antibodies	Locatelli-Dittrich et al. (2006)
Paraná	14	Pregnant mares	12	85.7	IFAT	1:50	Highest titer 1:400	Hoffmann Kormann et al. (2008)
Paraná	97	Healthy horses	14	14.4	IFAT	1:50	Highest titer 1:200 in 2 horses	Villalobos et al. (2012)
Paraná and Santa Catarina	112	Mares from 5 breeding farms	14	12.5	IFAT	1:50	25.7% (9/35) prevalence in mares with reproductive problem versus 6.4% (5/77) without problems. Highest titer only 1:50.	Abreu et al. (2014)
Rio Grande do Sul	241	Cart horses and Crioula breed	34	15.9	IFAT	1:50	-	Toscan et al. (2011)
Rio Grande do Sul	181	Pregnant mares	39	21.5	ELISA	A, C	9.3% of their paired foals had pre-colostral anti-*Neospora* antibodies.	Pivoto et al. (2014)
Rio Grande do Sul	197	Abattoir	77	39.1	IFAT	1:50	Tested for *Sarcocystis* spp. and *T. gondii*	Portella et al. (2017)
Rio Grande do Sul, São Paulo, Rio de Janeiro	101	Race horses	0	0.0	NAT	1:25	-	Dubey et al. (1999)
Santa Catarina	615	Healthy	25	4.1	IFAT	1:50	72 with history of neurological and reproductive problems.	Moura et al. (2013)
São Paulo	325 483	Healthy Diseased	19 73	5.8 15.1	IFAT	1:50	Highest titer 1:400	Villalobos et al. (2006)
São Paulo	26	History of ataxia.	15	57.6	IFAT	1:2	26 cerebrospinal fluids negative.	Stelmann et al. (2011)
South	203	Mares	129	63.3	IFAT	1:50	Of 129, 34.8% gave birth to seropositive foals.	Antonello et al. (2012)
10 states	961	Old horses from abattoirs	24	2.5	ELISA	C, K	-	Hoane et al. (2006)

Table 12. Detection of *N. caninum* antibodies in avian species from Brazil.

Host	Tested	%positive	DNA or Antibodies	Test	Cut-off / Method	Reference
Chicken (*Gallus gallus domesticus*)	200 (outdoor)	47 (23.5)	Antibodies	IFAT	1:50	Costa et al. (2008)
	200 (indoor)	3 (1.5)	Antibodies	IFAT	1:50	
	10 positive	6 (60.0)	DNA	PCR	3/4 of brain Direct PCR for Nc5	
	100 farm chickens	17 (17.0)	Antibodies	IFAT	1:50	Gonçalves et al. (2012)
		6 (6.0)	DNA	PCR	Np21/Np6	
Sparrow (*Passer domesticus*)	40	3 (7.5)	DNA	PCR	Heart and brain. Nested PCR of Nc5 and sequencing of ITS	Gondim et al. (2010)
Several species	294	0 (0.0)	Antibodies	IFAT	1:50	Mineo et al. (2011)

In one survey, 802 serum samples of female cattle from 55 dairy and beef farms from six Brazilian states (SP, RJ, MG, PR, RS, MS) were assayed by IFAT (cut-off 1:25) and 23.6% were seropositive; association between positivity to *N. caninum* and state of origin, age and production purpose was analyzed using uniform methodology (RAGOZO et al., 2003). Although seroprevalence was higher in animals older than 24 months, this difference was not statistically significant. Conclusion is not definitive because of the selection of low cut-off of 1:25. Among the six states, RJ had the lowest prevalence and MG the highest and dairy cattle had higher prevalence than beef cattle. In another large study from MS, *N. caninum* antibodies were found in cattle from 143 of 205 herds (IFAT 1:50); the cows were older than two years (OSHIRO et al., 2007). Overall seroprevalence was 14.9% (Table 4).

Even after using a higher cut-off titer (IFAT 1:200) than used most of the surveys listed in Table 4, high prevalence were recorded from MG, where *N. caninum* antibodies were detected in 23 of 24 herds with individual seroprevalence of 21.6% (BRUHN et al., 2013); and MT, where antibodies to *N. caninum* were found in 499 (53.5%) of 932 cattle samples, with at least one positive in each farm (BENETTI et al., 2009). In another report from MG, very high (91.2%; 510/559) prevalence of antibodies to *N. caninum* was found among 18 farms (GUEDES et al., 2008), revealing that *N. caninum* is well spread in the southern region of the state. They also recorded 97.2% (559/575) prevalence in cows from an abattoir.

There is an unconfirmed report that the quality of milk might be affected by neosporosis (MELO et al., 2001); an association was reported between the type of milk, classified as A, B and C according to its quality, produced in the farms and positivity to *N. caninum* antibodies, with higher occurrence in farms of MG that produce milk grade A/B than grade C; and the authors discussed about the production technology used indifferent farms, animals stress and commercialization. One possible cause is that the production of types A/B milk is higher and requests more from the animals, generating stress, which could be responsible for the higher prevalence.

An association between seroprevalence and age of cattle (older animals presenting higher prevalence) was detected, while feed produced on the farm was negatively associated with *N. caninum* infection in PR (GUIMARÃES et al., 2004). Also in PR, production of food in the farm, absence of artificial insemination and access of domestic and wild animals to feed facilities were associated with infection (MARTINS et al., 2012).

A strong association of *N. caninum* infection was found in farms of RS, which had presence of dogs close to the livestock and which also fed calves with colostrum pooled from several cows (CORBELLINI et al., 2006). In this study, the size of the farm was inversely associated with the presence of positive animals.

An association between beef herds and infection by *N. caninum* was reported in the Amazon region, RO, while comparing beef, dairy and mixed herds (AGUIAR et al., 2006). In that region, farms with more than 25 animals were also a risk factor. However, reproductive problems, contact with forest areas and presence of dogs were not associated with the coccidian infection.

Significant association related to animal management was found in PE (SILVA et al., 2008). Veterinary assistance, nutritional status, presence of wetlands, manipulation of the newborn calves (use of gloves when handling aborted fetuses), and destination of cows that aborted (higher in the ones that were treated with antibiotics than the ones that were discarded) were risk factors for the infection.

Pure bred Holstein cows had a higher exposure rate than mixed breeds (MUNHOZ et al., 2009) and an association between reproductive abnormalities (Table 4) (repeated estrus, repeated miscarriages and temporary anestrus) and seropositivity to *N. caninum* has been reported (BRUHN et al., 2013).

N. caninum is considered a primary pathogen and not influenced by concurrent BHV1, BVDV infections in dairy herds (MELO et al., 2004; MINEO et al., 2006).

Antibodies can fluctuate during pregnancy as reported, and increase in titer is not always associated with abortion (CARDOSO et al., 2009). A prospective longitudinal study was carried out in three farms in SP, during two consecutive years and the reproductive parameters were analyzed in those herds (CARDOSO et al., 2012a). In only one of the three herds the relative risk of abortion between *N. caninum* positive and negative cows was higher in the positive animals. No difference was observed regarding gestational age at abortion, repeated abortion, number of inseminations, and calving interval. A high association between *N. caninum* serological status of dams and daughters were observed in a longitudinal study carried out in three farms from SP, confirming the importance of vertical transmission, but there was no difference in the culling rate between positive and negative cows (CARDOSO et al., 2012b). A significant relationship between seropositivity of cattle and their offspring was also found in PE (RAMOS et al., 2016), which had a rate of transplacental transmission of 72.2% (13/18) for adults and 69.2% (9/13) for heifers by IFAT and 43.5% (17/39) for adults and 50.0% (9/18) for heifers by ELISA, concluding that vertical transmission is the major form of infection in this region.

Buffaloes: Studies with serum samples from Brazilian buffaloes (Table 5) showed occurrence of *N. caninum* antibodies varying from 14.6% to 88%. However, despite the high occurrence values, no reproductive disorders were reported in those groups (FUJII et al., 2001a, b; GENNARI et al., 2005; GONDIM et al., 2007)

Sheep: Seroprevalences ranged from 1.8% to 64.2% (Table 6). Some surveys stated as risk factors: abortion in the flock, presence of dogs, extensive husbandry systems and breed of the sheep. Great part of the studies also evaluated the presence of *T. gondii* infection, which usually showed a higher prevalence. In 2017, Filho et al. (2017) studied the vertical transmission rate of 50 naturally infected sheep, analysed by in house ELISA for six months. The initial prevalence of infection was 26.0% (13/50) and by the end of the study it had increased to 72% (36/50), being the vertical transmission rate 11%, which one sheep out of nine from a group gave birth to two infected ewes (IFAT 1:25).

Goats: In goats, serological surveys found rates between 1.0% (LIMA et al., 2008) to 26.6% (TEMBUE et al., 2011) of prevalence (Table 7). A study to evaluate infections by *T. gondii, N. caninum* and caprine arthritis-encephalitis virus (CAEV) was conducted, finding a prevalence of 37.81%, 23.62% and 17.23%, respectively (COSTA et al., 2012). The results indicate that CAEV does not predispose goats to infection by *T. gondii* or *N. caninum.* However, when CAEV/ *T. gondii* or CAEV/ *N. caninum* infection were detected, occurrence of reproductive failure was higher, maybe related to poor husbandry conditions. Differential diagnosis in cases of abortions in small ruminants is highly desirable.

Also, *N. caninum* DNA was found in brains of goats from BA. Silva et al. (2009) analyzed brains, hearts and tongues of 102 goats from slaughterhouses, and found a frequency of 1, 96% (2/102), using primers for ITS-1 region (JS4/CT2b; CT1/CT2).

Pigs: The prevalence of anti-*N. caninum* antibodies is low in pigs, and further studies are needed to evaluate the role of this species in the epidemiology of the parasite, including attempts to isolate viable parasites. Table 8 summarizes the studies with pigs from Brazil.

N. caninum antibodies were also found in feral pigs (*Sus scrofa*) in 10.8% (9/83) of samples from Pantanal, MS, with titers up to 1:800 by IFAT (SOARES et al., 2016).

Cats: In Brazil, studies determining the prevalence of antibodies in serum of cats (Table 9) found results that range from 0%, in cats from Andradina, SP, and Patos, PB (COELHO et al., 2011; FEITOSA et al., 2014a) to 27% in cats from MA (BRAGA et al., 2012). In animals from SP, NAT antibodies were found in 60 of 502 (12%) (DUBEY et al., 2002). The samples with titers greater than 1:80 were also examined by IB, as a confirmatory test, being ten of the 24 cats (41.6%) positive for both tests.

Wild animals: *N. caninum* antibodies were detected in sera of wild animals kept in captivity or trapped in the wild of the Families: Canidae, Felidae, Didelphidae, Bovidae, Caviidae and Cervidae (Table 10).

Among wild herbivores, *Neospora* has been better documented in cervids from Pantanal region (MS) (TIEMANN et al., 2005a). In that study, serum samples from 23 pampas deer (*Ozotoceros bezoarticus*) from the National Park of Emas, in GO, and 16 captured in bovine's farms, from Pantanal region, in MT, were tested for the presence of *N. caninum* antibodies. They found 13% and 75% positivity, respectively, for the deer that live inside the park and the ones from Pantanal, which is close to farms indicating that this proximity of wild and domestic animals could increase the occurrence of *N. caninum* infection among deer. From the Zoo of Curitiba, PR, 17 samples from Barbary sheep (*Ammotragus lervia*), from the Bovidae Family, were examined and four (23.5%) presented *N. caninum* antibodies (MORIKAWA et al., 2014).

Yai et al. (2003) tested 396 feral opossums (*Didelphis marsupialis*) samples from different regions of the city of São Paulo, and 21.2% (84/396) were positive.

Sera from 14 species of wild felids from zoos were tested for the presence of *N. caninum* (Table 10) antibodies and 12 species had at least one positive animal (ANDRÉ et al., 2010). In addition, 11 serum samples from free range jaguars (*Panthera onca*) from Pantanal were examined and seven (63.6%) were positive (ONUMA et al., 2014). In both studies, IFAT with a cut-off of 1:25 and anti-cat commercial conjugate was used for IgG antibody determination.

Despite the importance of wild canids in the epidemiology of *N. caninum*, few studies are available in Brazil. Crab-eating fox (*Cerdocyon thous*) is one of most common canids of South America, (Gennari, own observations) and four of these foxes were fed with masseter muscle and brain of two *N. caninum* seropositive bovines (IFAT >200). The foxes received the inoculum in two consecutive days. Two animals excreted *Hammondia heydorni* oocysts on eight and nine dpi but not *Neospora* oocysts (SOARES et al., 2009b). By means of molecular techniques, Nascimento et al. (2015) identified *N. caninum* in brain of six from 49 (12.2%) hoary foxes (*Pseudalopex vetulus* syn. *Lycalopex vetulus*) from PB. The molecular identification of the amplified products by sequencing reaction, using Nc-5 gene, presented 99% similarity with *N. caninum*.

Similarly, Muradian et al. (2012) tested wild urban rodents tissues (Family Muridae), but did not detect *N. caninum* DNA in four mice (*Mus musculus*), 20 brown rats (*Rattus novergicus*), and 193 black rats (*Rattus rattus*) from São Paulo city. Regarding capybaras, the first study done (YAI et al., 2008) tested animals from 11 counties in SP by IFAT (1:25) and found a prevalence of 9.4% (20/213), suggesting that they can serve as a source of *N. caninum* infection for wild canids. Also in SP, 63 capybaras were examined for *N. caninum* by IFAT (1:25) and other diseases and found two positive animals and one of them was positive for both *T. cruzi* and *N. caninum*, but no association was observed (VALADAS et al., 2010a). Recently, 170 samples of capybaras from SP were analyzed, but none were positive, although 17 (10%) were positive for *T. gondii* (ABREU et al., 2016). DNA of *N. caninum* was found in capybaras from PR (TRUPPEL et al., 2010), in 23% (6/26) of the studied animals. Parasite DNA, aiming the Nc5 gene was found in the liver and lymph nodes and ITS-1 was found in blood, liver, heart and lymph nodes.

Horses: At present, it is uncertain if horses are infected with both *N. caninum* and *N. hughesi* (DUBEY et al., 2017). Serosurveys on horses conducted in Brazil are summarized in Table 11. *N. caninum* antigen was used in all studies with exception of Hoane et al. (2006), that used *N. hughesi* SAG1 (NhSAG1) in an in-house ELISA.

A low rate of infection (0.4%, IFAT 1:100) was found among 500 donkeys (*Equus asinus*) sampled in BA; positive cases were confirmed by IB (GALVÃO et al., 2015). In a previous limited survey carried out in PA, no seropositive donkeys (n=6) or mules (n=9) were found (NORLANDER, 2014). Recently, a 2% seroprevalence was reported in donkeys (n=333) from five northeastern states (AL, PB, PE, PI and RN) using IFAT (1:50). In all these studies, *N. caninum* NC-1 strain was used as antigen source (GENNARI et al., 2016).

In PR, antibodies to *Neospora* were detected in two foals (LOCATELLI-DITTRICH et al., 2006), but the information is not definitive because of the low titer (1:50) detected. In addition, Antonello et al. (2012), by IFAT (1:50, *N. caninum* antigen), reported a high prevalence (63.3%) of *Neospora* antibodies in sera of 203 thoroughbred mares and their foals before suckling in two farms from Southern Brazil. A high percentage (34.8%) of seropositive mares gave birth to seropositive foals. Additionally, 8% of seronegative mares gave birth to seropositive foals. Mare sera were titrated further to 1:200 dilution and the seropositivity decreased to 33%, but foal sera were not titrated. In another study performed in RS, *Neospora* antibodies were found in 21.5% of 181 mares and in 9.3% of their foals in pre-suckling sera (PIVOTO et al., 2014); in this case, antibodies were assayed using an in-house indirect

ELISA, with NC-1 strain and soluble protein from tachyzoites maintained in CV-1 cells as antigen. Low levels of maternal IgG can cross the placenta in mares. Therefore, further studies are needed to confirm results of these investigations from Brazil (DUBEY et al., 2017).

Avian species: Table 12 summarizes the surveys of detection of *N. caninum* antibodies detection in avian species from Brazil. Seroprevalence was higher in free range chickens; 23.5% of 200 outdoors and 1.5% of 200 indoors chickens (*Gallus gallus*), in BA were seropositive (COSTA et al., 2008). In the same study, the authors found positive results for PCR from 6/10 (60%) seropositive chickens. 40 of 293 wild sparrows (*Passer domesticus*) from BA and PE were seropositive and *N. caninum* DNA by PCR was detected in three (7.5%) of brain and heart from 40 animals (GONDIM et al., 2010).

In a study by serological and histological methods none of the 294 wild and captive birds from nine avian orders had *N. caninum* antibodies. However in two psittacine birds, Apicomplexa-like tissue cysts were found and were immunostained positive with *N. caninum* antisera (MINEO et al., 2011).

Results of the experimental infection in birds from Brazil are summarized in Table 13. In the first Brazilian study (FURUTA et al., 2007) 50 chickens were inoculated with *N. caninum* tachyzoites, using different doses; chickens seroconverted but remained healthy. In 15 euthanized chickens, *N. caninum* tachyzoites were reported to be present in different tissues by IHC, at 15 dpi; however, no illustrations were provided. At the termination of the experiment (60 dpi), all chickens were seronegative by IFAT (<1:20) *N. caninum* was not found by IHC. In laying hens, no evidence of vertical transmission was found. However, 50% of embryonated chicken eggs inoculated with *N. caninum* died and chickens that hatched 21 dpi had neurological signs. Dogs fed chorioallantoid membranes and whole infected eggs seroconverted and excreted *N. caninum* oocysts in their feces, as confirmed by PCR (Nc5) (FURUTA et al., 2007).

In an unconfirmed report, four littermate two-month old dogs fed with chorioallantoid membranes previously infected *in ovo* with 10^6NC-1 strain tachyzoites were euthanized 3, 4, 5 and 6 dpi (MUNHOZ et al., 2013). The authors reported immunoreactivity to *N. caninum* in lesions in lungs, spleen, and small and large intestine but strangely *N. caninum* DNA was not detected in affected tissues by conventional PCR. The gross lesions depicted resemble bacterial septicemia and the results need confirmation. The dogs did not excrete oocysts. In a follow up study, the authors, infected 90-days old chickens and embryonated eggs with 10^6 *N. caninum* tachyzoites using NC-1 strain and fed three dogs with infected organs but the dog did not excrete oocysts (MUNHOZ et al., 2014). Although there is no confirmation by IHC and microscopy, *N. caninum* DNA was found in the spleen and pectoral muscles of one of the birds born from the inoculated eggs (MUNHOZ et al., 2014).

An attempt to infect quails (n=58) with doses of 3.5×10^6 and 5×10^6 of *N. caninum* tachyzoites (NC-Bahia) was largely unsuccessful (OLIVEIRA et al., 2013). Although there was evidence of transitory infection (seroconversion and finding of parasite DNA) at 14 dpi, the quails became seronegative at 30 dpi with no demonstrable parasite DNA or antigen in their tissues; two dogs fed quail tissues did not excrete oocysts.

Experimental infection was also conducted in pigeons (*Columba livia*) inoculated with the 10^7 NC-1 tachyzoites (MINEO et al., 2009). The pigeons developed transitory *N. caninum* antibodies starting at 5 dpi but at the end of the experimental period of

Table 13. Detection of *N. caninum* in avian from experimental studies in Brazil.

Host	No. animals	Dose	Tests	Results	Reference
Chickens					
	7-days old	10^3, 10^4, 10^5, 10^6	Killed 15,30,45,60 dpi	No mortality; Dpi 15:IFAT+ IHC+	Furuta et al. (2007)
	Laying eggs	10^8	Bioassay, PCR, Histopathology	Dpi 60: IFAT – IHC -	
(*Gallus gallus domesticus*)	Embrionated eggs	10^3, 10^4, 10^5, 10^6	Histopathology Dogs bioassay	Eggs- Mortality: 18-21 days incubation Dogs shed oocyst	
	90-days old	3×10^6	IFAT, IHC, Histopathology, Bioassay	IFAT+	Munhoz et al. (2014)
	Embrionated eggs	1×10^2	2 embryos died	PCR+ spleen, muscles (1bird)	
Pigeons					
(*Columbia livia*)	4	1×10^7 Blood samples each 5 days	IFAT	1:20; peak 10-20dpi	Mineo et al. (2009)
	4	control	IHC +	Lungs, heart, CNS	
Quails					
(*Coturnix coturnis japonica*)	40	3.5×10^6	Histopathology	+	Oliveira et al. (2013)
	8	5.0×10^6	IHC	+	
	10	control	PCR	+	

45 days, all birds were seronegative. One infected pigeon died at 25 dpi and *N. caninum* and lesions were found by IHC in lungs, heart, central nervous system, liver, spleen and kidney.

Rezende-Gondim et al. (2017) cultivated *N. caninum*, using a chicken cell line, and temperatures between 39 °C and 41.5 °C. Multiplication of *N. caninum* tachyzoites in vitro was inhibited at temperatures similar to those of chickens. The authors concluded that the avian body temperatures may be one of the reasons that isolation of the parasite is difficult in avian species.

In summary, avians are a poor host for *N. caninum*, based on failure to isolate viable parasite from naturally infected tissues and failure to induce chronic infection in experimentally infected birds.

Clinical infections

Dogs*:* There are two confirmed reports of clinical neosporosis in adult dogs. A seven-year-old male Collie from Salvador, BA developed incoordination and rear limb paralysis (GONDIM et al., 2001). The dog was found to have a *N. caninum* IFAT titer of 1:1600. In spite of medication with Clindamycin (22 mg/kg) for 14 days the dog died. A necropsy was performed. Histologically there was encephalitis associated with tachyzoites and tissue cysts and the diagnosis was confirmed by IHC examination. Live tissue cysts were found in squash preparations from the dog brain. Tissue cysts were found in the brains of gerbils inoculated with brain homogenate of the dog. Viable *N. caninum* was propagated in cell cultures seeded with infected gerbil brain.

The second case was from a 10 year old dog from RS (MANN et al., 2016). Persistent dermal lesions with multifocal ulcerative nodules on the neck and pelvic limbs were observed. The dog had an IFAT *N. caninum* antibody titer of 1:6400. Cytological examination of the exudates from nodules showed pyogranulomatous inflammation with tachyzoites and *N. caninum* was identified by PCR. The dog was medicated with Clindamycin (6 mg/kg) for 28 days with resolvement of lesions. However, the dermal lesions with identifiable tachyzoites reappeared 12 days after the cessation of treatment, perhaps due to a very low dose of Clindamycin used; the usual treatment is 20-25 mg/kg.

In addition to these confirmed reports, Langoni et al. (2012) isolated *N. caninum* by bioassay in gerbils inoculated with brains of two of seven dogs with neurological disorders. No other details were given about these dogs or the strain of *Neospora,* therefore, not included in Table 1.

Cattle*:* Reports of confirmed neosporosis abortion from six states are summarized in Table 14. Apparently, all of these cases were sporadic abortion. Corbellini et al. (2006) investigated 161 bovine abortions from 149 farms bovine abortions during 2001 and 2003 from RS. Causes of abortion were identified in 83 (51.5%) cases. *N. caninum* was the most important cause, and identified in 37 fetuses; in 34 fetuses, the diagnosis was confirmed by IHC examination. Overall, 37 of 161 (23%) fetuses were infected solely with *N. caninum*. In six cases, there was concurrent *Leptospira* sp. infection. Most aborted fetuses were 4.4 months gestational age. Cows aborting a *Neospora* infected fetuses were 2.4 times likely to have aborted previously.

In a follow up publication, more detailed investigation involved 258 aborted fetuses from RS and SC. Lesions indicative of neosporosis were found in 89 (34%) of these 258 submissions based on histopathology. The diagnosis was confirmed by IHC in 55 of these 89 cases (PESCADOR et al., 2007). A striking feature was the distribution of lesions; myositis in 92%, myocarditis in 76% and pneumonitis and encephalitis in 75%. Two of these fetuses had grossly visible pale white foci in lungs, indicative of necrosis. Overall, *N. caninum* was the predominant (21.3% of 258) cause of abortion in this investigation. A similar conclusion was reached by Cabral et al. (2009), who combined histopathology, IHC, and PCR to diagnose *N. caninum* in 24.8% of 105 fetal samples from the state of SP. They detected *N. caninum* DNA in the brains of 22% (16 of 72), placenta of 20.0% (4 of 20), heart and liver of 16.3% (8 of 49) and pool of kidney, lungs, and spleen of 10.9% (7 of 64).

Additional provisional evidence for neosporosis was based on higher seropositivity in aborting versus non-aborting cows on a given farm (Table 15). However, most of these reports were provisional and not a case controlled study. In PR, samples from a herd collected over a nine-yr follow-up period were analyzed for *Neospora* infections (LOCATELLI-DITTRICH et al., 2001). They found that the proportion of abortions was 20% and 8%, respectively for the seropositive and seronegative animals.

As mentioned in Table 1, Locatelli-Dittrich et al. (2003, 2004) isolated viable *N. caninum* from a seven-month gestational age fetus and a three-month old blind calf. However, the etiology was not confirmed because histological examination was not performed.

Goats*:* Fatal neosporosis has been reported from MG and RS (Table 16). Hydrocephalus was detected in a day old goat kid, which had a high titer of antibodies on IFAT (1:400) for

Table 14. Reports of *N. caninum*-associated abortion in cattle from Brazil.

State	No. aborted	No. positive (%)	Diagnosis			Reference
			Histo	IHC	PCR	
Bahia	1	1	Yes	Yes	ND	Gondim et al. (1999b)
Goiás	195 dead fetuses from abattoir	40 9(20.5)	No	No	Yes	Brom et al. (2014)
Paraná	34	8 (23.5)	Yes	Yes	ND	Santos et al. (2005)
Rio Grande do Sul	30	1 (3.3)	Yes	Yes	ND	Corbellini et al. (2000)
Rio Grande do Sul	46	22 (47.8)	Yes	Yes	ND	Corbellini et al.(2002)
Rio Grande do Sul	161	37 (17.4)	Yes	Yes	ND	Corbellini et al. (2006)
Rio Grande do Sul, Santa Catarina	258	55 (21.3)	Yes	Yes	ND	Pescador et al. (2007)
São Paulo	105	26 (24.7)	Yes	Yes	Yes	Cabral et al. (2009)

Table 15. Seropositivity as evidence of abortion in cattle from Brazil.

Total number of cattle/ farms examined	Aborting			Non aborting or control			Reference
	Test cut-off	No. tested	No. seropositive (%)	No. tested	No. seropositive (%)	Risk of abortion indicated by odd ratio (OR), significant association (SA), remarks	
223 dairy cows, 5 herds	IFAT, 1:200	NS	NS (23.3)	NS	NS (8.3)	OR 3.3	Corbellini et al. (2002)
2448 cattle from 205 herds, beef, dairy	IFAT, 1:50	-	55/68 herds (80.9)	-	84/134 herds (62.7)	OR 2.5	Oshiro et al. (2007)
1256, 41 aborted	IFAT, 1:200	41	24 (58.5)	1215	199 (16.4)	OR 7.2	Hein et al. (2012)
1204 dairy cows from 40 farms	IFAT, 1:200	NS	NS (31.1)	NS	NS (17.7)	OR 1.98	Bruhn et al. (2013)
621 cattle, 63 farms, 36 farms with abortion	IFAT, 1:100	-	26/36 farms (72.2)	-	12/27 farms (44.4)	SA	Boas et al. (2015)
3428 cattle from 174 herds	IFAT, 1:100	-	99/108 herds (91.7)	-	11/52 herds (21.1)	SA	Chiebao et al. (2015)
1273 cattle from 6 dairy herds	IFAT, 1:200	305	122 (40.0)	968	40 (4.1)	SA	Pessoa et al. (2016)

Table 16. Fatal neosporosis in goats from Brazil.

State	Case no.	History	Diagnosis				Reference
			Serology	Histology	Immuno	PCR	
Minas Gerais	1- day old	Born weak, unable to nurse	Doe, IFAT 1:400	White matter absent, mild necrosis, perivasculitis, only tissue cysts 9.8-20.5 µm in diameter.	Positive	Not done	Varaschin et al. (2012)
	Day of birth	New born kid, late term	IFAT doe 1:800, presuckling kid 1:400	No lesions in placenta	Negative	Positive in placenta.	
	Abortion	A chronically infected aborted 4 fetuses, 87 days after mating.	IFAT doe 1:6,400 at abortion day.	Positive in 1.	Brain positive in 1 fetus	PCR positive brain in the first and heart in the second fetus	Mesquita et al. (2013)
	2 stillborn	Stillborn on 148 days after mating of a chronically infected doe	IFAT doe 1:3,200 at parturition.	Positive in 2.	Positive in both.	Positive in CNS of both.	
Rio Grande do Sul	1- day old	Born weak, unable to nurse, ataxic, euthanized day 3	No data	Encephalitis, more severe in mid brain, many intact and degenerating tissue cysts of 12.4-32.2 µm in diameter.	Positive	Not done	Corbellini et al. (2001)

N. caninum and no antibodies for *T. gondii* (VARASCHIN et al., 2012). The cerebral hemispheres of the animal were asymmetrical the gyri were swollen, and ventricles were expanded. Histologically, there were only tissue cysts in the brain, no tachyzoites were observed and no lesions were observed in histological examination of placenta. Corbellini et al. (2001) described another case in southern Brazil, with a kid presenting neurological signs as ataxia and opistothonos, which got more severe when it was three days old, and was euthanized. Brain, heart, lungs and liver had microscopic lesions, but no grossly lesions were not observed.

Humans: Currently, there is no evidence of the zoonotic character of *N. caninum*, although antibodies in humans were found in immune compromised and normal patients in different parts of the world (DUBEY et al., 2017).

In Brazil, three studies were conducted. In MG, serum samples from HIV-infected patients (Group I), patients with neurological disorders (Group II), newborns (Group III) and a healthy control (Group IV) by IFAT (1:50) were assayed for *N. caninum* antibodies by indirect ELISA to detect immunoglobulin G (IgG), utilizing soluble antigen and IB as a confirmatory test, with lysed tachyzoites as antigen (LOBATO et al., 2006). They found IB positive patients in all groups, and HIV-infected patients and those with neurological disorders patients presented significantly higher prevalence. Combining all the tests, 37.7% (23/61) of group I, 18% (9/50) of group II, and 5% (5/91) from groups III had antibodies to *N. caninum.*

In the second study, antibodies to *N. caninum* (IFAT,1:50) were sought in Human Immunodeficiency Virus (HIV) patients; which 26.1% (81/310) patients from MS and 31.2% (10/31) patients from PR were seropositive and one patient had a titer of 1:400 (OSHIRO et al., 2015). In the third report, *N. caninum* antibodies (IFAT 1:100) were detected in 7 of 67 (10,5%) humans from 24 farms of MT (BENETTI et al., 2009).

Prospective and areas of future research

It is evident from this review that *N. caninum* infection is widely prevalent throughout Brazil. However, nothing is known of the prevalence of *N. caninum* oocysts in soil or in canine feces. It is also uncertain if there are additional definitive hosts, other than the domestic dog and some wild canids, as stated before. Overall, little is known of clinical neosporosis, particularly in cattle. The few reports pertain to sporadic cases of abortion with no information on epidemics or storms of abortion. There is need for a national survey in cattle using defined parameters. Future researches should focus on molecular characterization of *N. caninum* strains, possibility of vaccine production and relationship between wildlife and livestock epidemiology.

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