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OCORRÊNCIA DE ANTICORPOS ANTI-*Toxoplasma gondii* E *Neospora caninum* EM CÃES COM E SEM SINAIS NEUROLÓGICOS EM CAMPO GRANDE, MATO GROSSO DO SUL

BETS-SABA NAATE NAUMANN CERQUEIRA LEITE

Campo Grande – MS
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OCCURRENCE OF ANTI-TOXOPLASMA GONDII AND NEOSPORA CANINUM ANTIBODIES IN DOGS WITH AND WITHOUT NEUROLOGICAL SIGNS IN CAMPO GRANDE, MATO GROSSO DO SUL

Orientadora: Profª Drª Mariana Isa Poci Palumbo

Dissertação apresentada ao programa de Pós-Graduação em Ciências Veterinárias da Universidade Federal de Mato Grosso do Sul, como requisito parcial para obtenção do título de Mestre em Ciências Veterinárias.

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“A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê.” (Arthur Schopenhauer)

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RESUMO

Neospora caninum e *Toxoplasma gondii* são protozoários intracelulares, com distribuição mundial e causam distúrbios neuromusculares em diversos animais, inclusive em cães. Essa espécie é definida como hospedeiro definitivo de *N. caninum,* e apesar de não serem hospedeiros definitivos de *T. gondii*, os cães são considerados portadores, sentinelas e possíveis veiculadores mecânicos deste agente. Existem poucos estudos a respeito da prevalência sorológica de *N. caninum* e *T. gondii* na população canina em Campo Grande, MS, bem como estudos referentes a prevalência destes protozoários em animais com e sem sinais neurológicos. Devido à importância da infecção por esses protozoários, este estudo teve como objetivo investigar a ocorrência de anticorpos contra *T. gondii* e *N. caninum* em cães com e sem sinais neurológicos, em Campo Grande, Mato Grosso do Sul. Foram coletadas 58 amostras de cães atendidos na rotina do hospital veterinário da Universidade Federal do Mato Grosso do Sul (HV-UFMS), provenientes de Campo Grande – MS (sendo 28 com e 30 sem sinais neurológicos). O diagnóstico sorológico para a verificação da presença de anticorpos para ambos protozoários no soro cães foi realizado através da Imunofluorescência Indireta (IFI). Títulos ≥ 1:25 e ≥ 1:16 foram considerados positivos para *N. caninum* e *T. gondii,* respectivamente. A associação da sorologia com as variáveis foi realizada através do cálculo de *odds ratio* (OR) com intervalo de confiança de 95%. Das 58 amostras de soro obtidas, 12 (20,69%) foram positivas para *T. gondii*, 13 (22,41%) positivas para *N. caninum* e três (5,17%) para ambos. Os títulos para *T. gondii* variaram de 1:16 a 1:4096 e para *N. caninum* de 1:25 a 1:100. Dos 28 (48,28%) animais com sinais neurológicos, sete (25%) eram soropositivos para *T. gondii*, nove (32,14%) sororreagentes para *N. caninum* e três (10,71%) soropositivos para ambos os agentes. As principais alterações neurológicas

observadas foram convulsões, andar compulsivo, déficit de nervos cranianos e inclinação da cabeça. Dos 30 animais sem sinais neurológicos, cinco (16,67%) foram positivos para *T. gondii* e quatro (13,33%) para *N. caninum*. Não houve associação e diferença estatística significativa entre as variáveis observadas e a soropositividade para *T. gondii* e *N. caninum.* Os resultados mostraram a presença de *N. caninum* e *T. gondii* na região de Campo Grande no Mato Grosso do Sul. Além disso, cães com e sem sinais neurológicos foram soropositivos para os dois agentes, ou seja, a toxoplasmose e neosporose devem ser inseridas no diagnóstico diferencial de cães com sintomatologia neurológica nesta região.

Palavras-chave: Campo Grande, imunofluorescência indireta, prevalência, neuropatias, sorologia.

ABSTRACT

Neospora caninum and *Toxoplasma gondii* are intracellular protozoa, with worldwide distribution and cause neuromuscular disorders in several animals, including dogs. This species is defined as the definitive host for *N. caninum* and although they are not definitive hosts for *T. gondii*, dogs are considered carriers, sentinels and possible mechanical carriers of this agent. There are few studies regarding the serological prevalence of *N. caninum* and *T. gondii* in the canine population in Campo Grande, MS, as well as studies regarding the prevalence of these protozoa in animals with and without neurological signs. Due to the importance of infection by these protozoa, this study aimed to investigate the occurrence of antibodies against *T. gondii* and *N. caninum* in dogs with and without neurological signs in Campo Grande, Mato Grosso do Sul. 58 samples of dogs seen in the routine were collected from the veterinary hospital of the Federal University of Mato Grosso do Sul (HV-UFMS), from Campo Grande – MS (28 with and 30 without neurological signs). The serological diagnosis to check for the presence of antibodies to both protozoa in the serum of dogs was carried out through Indirect Immunofluorescence (IFAT). Titers ≥1: 25 and ≥ 1:16 were considered positive for *N. caninum* and *T. gondii* respectively. The association of serology with variables was performed by calculating odds ratios (OR) with a 95% confidence interval. Of the 58 serum

samples obtained, 12 (20.69%) were positive for *T. gondii*, 13 (22.41%) positive for *N. caninum* and three (5.17%) for both. Titles for *T. gondii* ranged from 1:16 to 1:4,096 and for *N. caninum* from 1:25 to 1: 100. Of the 28 (48.28%) animals with neurological signs, seven (25%) were seropositive for *T. gondii*, nine (32.14%) seropositive for *N. caninum* and three (10.71%) seropositive for both the agents. The main neurological changes observed were seizures, compulsive walking, cranial nerve deficit and head tilt. Of the 30 animals without neurological signs, five (16.67%) were positive for *T. gondii* and four (13.33%) for *N. caninum*. There was no association and significant difference between the variables observed and seropositivity for *T. gondii* and *N. caninum.* In addition, dogs with and without neurological signs were seropositive for both agents, that is, toxoplasmosis and neosporosis should be included in the differential diagnosis of dogs with neurological signs in this region.

Keywords: Campo Grande, indirect immunofluorescence, prevalence, neuropathies, serology.

CAPÍTULO 1

1. INTRODUÇÃO GERAL

Nas últimas décadas tem aumentado a inter-relação entre animais, seres humanos e o meio ambiente, o que tem ocasionado desequilíbrios ecológicos, fato esse de grande relevância do ponto de vista da saúde pública, uma vez que aumenta a possibilidade da transmissão de zoonoses (ZINSSTAG *et al*., 2011). Desta forma, os cães, cada vez mais inseridos como membros da família a grupos familiares, podem desempenhar importante papel na manutenção e transmissão de agentes infecciosos, e atuarem como sentinelas de suas respectivas doenças (ULLMANN *et al*., 2008; BRASIL *et al.*, 2018).

A toxoplasmose e a neosporose são doenças com amplas distribuições geográficas, clinicamente semelhantes, causadas pelos protozoários *Toxoplasma gondii e Neospora caninum*, respectivamente, que são coccídeos intracelulares do filo Apicomplexa (DUBEY *et al*., 1988A). Apesar de serem bastante semelhantes geneticamente, estruturalmente e imunologicamente esses coccídeos possuem particularidades individuais, além de serem doenças biologicamente diferentes. Cães e gatos são os hospedeiros definitivos de *T. gondii e N. caninum*, respectivamente, portanto eliminam oocistos em suas fezes. Embora os felinos terem fundamental importância no ciclo biológico do *T. gondii*, por serem hospedeiros definitivos, os cães também podem mecanicamente transmitir oocistos ao homem, sendo assim, são considerados um risco potencial para a transmissão do agente (DUBEY *et al*., 2007). Além disso, a toxoplasmose é uma zoonose e uma enfermidade de grande relevância em felinos, ovinos e humanos, enquanto a neosporose afeta principalmente bovinos e cães (DUBEY, 2003). Ainda é incerto que *N. caninum* seja um protozoário zoonótico, apesar de alguns estudos demonstrarem que os seres humanos podem apresentar anticorpos contra esse agente (OSHIRO *et al*., 2015). Além disso, já foi detectada a presença deste coccídio em amostras de sangue de cordão umbilical através da reação em cadeia da polimerase (PCR) (DUARTE *et al*., 2020).

Assim como nos animais, nos seres humanos, a infecção por *T. gondii* ocorre principalmente pela ingestão de carne mal cozida ou crua contendo

cistos teciduais, pelo consumo de água ou alimentos contaminados com oocistos, assim como pela via transplacentária. Na maioria das vezes, essas infecções são assintomáticas ou autolimitantes, sendo os casos mais graves em imunossuprimidos. A primo-infecção durante a gestação pode causar sérios problemas de saúde ao feto, como nascimento prematuro, afecções neurológicas e oculares ou até mesmo aborto (JONES *et al.*, 2001). Da mesma forma, a toxoplasmose canina é considerada uma doença oportunista, os casos mais graves afetam principalmente animais com o sistema imune comprometido, caracterizada por alterações neuromuscular, respiratória e gastrointestinal, inclusive infecção generalizada (DUBEY e BEATIE, 1988; DA SILVA *et al.*, 2005).

Já foi relatada a infecção natural de cães com *N. caninum* no município de Campo Grande – MS (DE OLIVEIRA *et al*., 2004; ANDREOTTI *et al*., 2006). Além do mais, um estudo avaliou a soroprevalência de *T. gondii*, *Leishmania infantum* e *N. caninum* em Campo Grande – MS em gatos, por se tratar de área endêmica para Leishmaniose (SOUSA *et al*., 2014). Porém, são escassos os dados a respeito da prevalência sorológica de *T. gondii* na população canina nesta região, bem como estudos referentes à sua prevalência em animais com e sem sinais neurológicos. Alguns autores avaliaram a prevalência destas infecções em cães na região de Curitiba e observaram que 30,7% foram soropositivos para *T. gondii* e 11,5% para *N. caninum* e 7,7% para ambas (CONSTANTINO *et al*., 2016). Maior prevalência foi observada em outro estudo para as duas infecções, que utilizou animais oriundos de propriedades rurais limítrofes a uma reserva biológica no Espírito Santo (ACOSTA *et al*., 2016).

É relevante investigar a presença de anticorpos anti-*N. caninum* e anti-*T. gondii* em soros de cães naturalmente infectados. A carência de informações sobre as infecções por *Toxoplasma* e *Neospora* em cães com e sem sinais neurológicos reforça a necessidade de pesquisas a fim se conhecer a epidemiologia e fatores de risco para essas co-infecções na região e assim estabelecer possíveis estratégias de prevenção e saúde pública.

2. OBJETIVO

2.1 Objetivo geral

Avaliar a prevalência de anticorpos anti- *Toxoplasma gondii* e anti-*Neospora caninum* em uma amostra de uma população de cães com e sem sinais neurológicos em Campo Grande, Mato Grosso do Sul.

2.2 Objetivos específicos

- Identificar cães reagentes para *Toxoplasma gondii* e *Neospora caninum* e comparar a prevalência entre os grupos de animais com e sem sinais neurológicos.

- Avaliar associações de risco entre as variáveis estudadas e positividade para *T. gondii e N. caninum em cães.*

3. REVISÃO DE LITERATURA

Neospora caninum e *T. gondii* são parasitas comuns no território brasileiro. Estudos recentes demonstraram que em cães a soroprevalência de *T. gondii* varia, de acordo com a região, de 2,6% a 90% (DUBEY *et al*., 2012; DANTAS *et al.*, 2014; LANGONI *et al*., 2013; LOPES *et al*., 2015; RAIMUNDO *et al*., 2015). Animais errantes e que vivem em regiões periurbanas ou rurais, geralmente apresentam maior soroprevalência de *T. gondii* (RAIMUNDO *et al*., 2015), assim como animais idosos e habituados a comer carne crua ou mal cozida (BRITO *et al*., 2002).

A prevalência de anticorpos para *N. caninum* no Brasil varia de 1,98% a 67,6% (COIRO *et al.*, 2011; DUBEY, 2013). Estudos mostram que o livre acesso às ruas e açudes, viver em ambiente rural e idade avançada são os principais fatores de risco em cães (AZEVEDO *et al.,* 2005; BENETTI *et al*., 2008; PARADIES *et al.*, 2007; DANTAS *et al*., 2014). Além disso, Wouda *et al*. (1999) observaram grande correlação entre cães de fazendas soropositivos para *N. caninum* e a soropositividade em bovinos.

Toxoplasmose

A toxoplasmose é uma das zoonoses parasitárias mais comuns em humanos e animais de sangue quente. Em torno de um terço da população mundial foi já foi exposta a este parasita (HILL e DUBEY, 2002). Baseado em diferentes estudos epidemiológicos, considerando as altas prevalências em cães e gatos, a infecção por *T. gondii* é comum, entretanto a doença é incomum (DIAS e FREIRE, 2005). Tanto em humanos como em animais

domésticos, na maioria dos casos a infecção é assintomática, no entanto, na sua forma congênita ou em indivíduos imunocomprometidos é capaz de causar doença severa, como é o caso da infecção em animais jovens, idosos ou com doenças concomitantes (VIDOTTO, 1992; DIAS e FREIRE, 2005; DUBEY *et al*., 2012), onde a infecção pode ocasionar várias alterações neurológicas, oculares ou condições clínicas mais graves. Em animais de criação, a infecção leva ao aborto e à mortalidade de recém-nascidos (RAGOZO *et al.*, 2010).

O *T. gondii* é um parasita intracelular obrigatório e possui três estágios infecciosos, esporozoítos (presentes nos oocistos que são eliminados nas fezes de felídeos infectados), taquizoítos (forma de replicação rápida) e bradizoítos (forma de replicação lenta) em cistos teciduais. O ciclo de vida deste protozoário é do tipo heteróxeno facultativo, alterna entre os estágios sexual e assexual, que ocorre no hospedeiro definitivo e intermediário, respectivamente (RAGOZO *et al*., 2010). Os felídeos são hospedeiros definitivos e principalmente os gatos desempenham um papel importante na epidemiologia de *T. gondii*, por liberarem oocistos resistentes no meio ambiente. O ser humano e os outros animais são considerados hospedeiros intermediários ou paratênicos (VIDOTTO, 1992).

As principais vias de infecção são transplacentária, ingestão de carne crua ou mal cozida infectada com cistos teciduais proveniente de animais com infecção crônica e ingestão de alimentos e água contaminados com oocistos esporulados (DE MOURA *et al.*, 2006; RAGOZO *et al.,* 2010; DUBEY *et al.*, 2012). Pelo hábito alimentar carnívoro dos felídeos, o principal meio de infecção desses animais é pela ingestão de cistos teciduais, assim os bradizoítos penetram nas células epiteliais do intestino delgado, sofrem uma série de ciclos de reprodução assexuada (merogonia) para então passar pelo ciclo sexuado (gametogonia) de reprodução e posteriormente a liberação de oocistos nas fezes. Os gatos eliminam oocistos durante um curto período de tempo, de três a sete dias após ingestão dos cistos teciduais, entretanto pode ocorrer por mais de 20 dias, liberando até 10 milhões de oocistos nas fezes no pico de eliminação. No meio ambiente, estes oocistos tornam-se infectantes após o período de um a cinco dias e quando esporulados são bastante resistentes à maioria dos desinfetantes, podendo permanecer no meio

ambiente por meses a anos dependendo da umidade e temperatura (DUBEY e BEATIE, 1988; DUBEY *et al.*, 2012). O oocisto esporulado é uma das formas infectantes mais importantes da *T. gondii*, pela facilidade de contaminação de fezes no solo e alta resistência ambiental, assim tem importante capacidade de infectar herbívoros, onívoros, roedores, carnívoros e até o homem (VIDOTTO, 1992).

O diagnóstico definitivo de toxoplasmose *ante-mortem* é incomum, porque raramente o *T.gondii* é encontrado em amostras de tecidos, líquidos de lavagem broncoalveolares, humor aquoso e líquido cefalorraquidiano (LCR). Além disso, a detecção de occistos nas fezes de gatos com diarreia apenas sugere a toxoplasmose, uma vez que a infecção por *Besnoitia* e *Hammondia* produz oocistos com a morfologia semelhante (LAPPIN, 2017). Em cães e gatos, a detecção do DNA através da PCR e anticorpos específicos para *T. gondii* em teste sorológicos ocorre tanto em animais clinicamente doentes e saudáveis, portanto não devem ser realizados isoladamente. Dos testes séricos, a detecção do IgM é o que melhor tem correlação com a toxoplasmose clínica, pois não é comum ser detectado no soro de animais saudáveis (LAPPIN, 1999; POWELL *et al*., 2010). Desse modo, o diagnóstico *ante-mortem* é realizado com base na combinação dos sinais clínicos, detecção de anticorpos séricos, resposta positiva ao tratamento e exclusão de possíveis doenças semelhantes. A identificação de anticorpos específicos para *T. gondii* associada à detecção do DNA através da PCR é também considerada precisa para diagnosticar toxoplasmose ocular ou do sistema nervoso central (SNC) em gatos (LAPPIN, 2017).

Neosporose

O *N. caninum* teve seu reconhecimento pela primeira vez em cães na Noruega (BJERKÅS *et al*.,1984). Devido à semelhança morfológica e sintomática, este protozoário era diagnosticado como *T. gondii* até 1988, quando ocorreu a descrição de um novo gênero e espécie *N. caninum* (DUBEY *et al*., 1988A). Desde então, a neosporose emergiu como uma doença importante de cães e gados em todo o mundo e é considerada como uma das principais causas de aborto em bovinos (DUBEY, 2003; GOODSWEN *et al*., 2013).

No ciclo de vida deste coccídeo há três estágios infecciosos: os taquizoítos (localizados dentro de um vacúolo parasitóforo no citoplasma da célula hospedeira), os bradizoítos (contidos no interior dos cistos teciduais) e os esporozoítos (no interior dos oocistos). Os dois primeiros são encontrados nos hospedeiros intermediários e ocorrem intracelularmente (DUBEY *et al.,* 2002*)*. Além de serem hospedeiros intermediários, o cão doméstico (*Canis lupus familiaris*) (MCALLISTER *et al*., 1998), o coiote (*Canis latrans)* (GONDIM *et al*., 2004), o dingo (*Canis lupus dingo*) (KING *et al*., 2010) e o lobo cinzento (*Canis lupus*) (DUBEY *et al.*, 2011) são reconhecidos como hospedeiros definitivos de *N. caninum.* Neles ocorre a fase sexuada e eliminação de oocistos nas fezes, estágio morfologicamente semelhante aos oocistos de *T. gondii* e *Hammondia heydorni*, também bastante resistente às condições ambientais (MCALLISTER *et al*., 1998).

Neospora caninum pode ser transmitido vertical e horizontalmente. Na transmissão horizontal, carnívoros podem ser infectados através da ingestão de tecidos contendo cistos ou taquizoítos. Já em herbívoros e outros animais, a infecção geralmente ocorre através da ingestão de alimentos e água contaminada pelos oocistos esporulados. A transmissão vertical é comum em bovinos e a infecção transplacentária pode ocorrer quando a mãe é infectada durante a gestação, descrito como infecção transplacentária exógena. Além disso, fêmeas infectadas podem transmitir verticalmente em sucessivas gestações, devido à reativação da infecção durante a gestação, classificada como infecção transplacentária endógena (DUBEY *et al*., 2017, ANVARI *et al*., 2020).

Em cães, apesar da replicação do coccídeo ocorrer em diversos tecidos, a doença clínica reflete principalmente a infecção neuromuscular, com sinais como paralisia, ataxia entre outros sinais neurológicos de lesões multifocais do sistema nervoso central, onde o cerebelo, córtex e tronco cerebral são comumente afetados. Em alguns animais a doença é subclínica, entretanto, filhotes infectados congenitamente desenvolvem paralisia ascendente com hiperextensão dos membros posteriores, associado a atrofia muscular e tendem a apresentar quadros mais graves, assim como animais idosos e imunossuprimidos. Distúrbios pulmonares, miocárdicos, dermatológicos,

hepáticos e reprodutivos também podem ocorrer (ORDEIX *et al*., 2002; HOON-HANKS *et al*., 2013; ANVARI *et al*., 2020). Embora a encefalomielite e a miosite se desenvolvam em gatos infectados experimentalmente, quando são naturalmente expostos são soropositivos para *N. caninum*, porém não manifestam a doença clínica (DUBEY *et al.*, 1990; BRESCIANI *et al*., 2007).

O diagnóstico *ante-mortem* da neosporose é difícil e a associação da história clínica, idade e exames sorológicos são bastante pertinentes. Atualmente, há diversos métodos de diagnóstico, imunológicos e moleculares para detecção de *N. caninum* (DUBEY *et al*., 2017). A demonstração do parasito no líquido cefalorraquidiano (LCR) ou em outros tecidos fornece o diagnóstico definitivo, porém são raramente encontrados na citologia do LCR, impressões de lesões de pele e lavados broncoalveolar (GALGUT *et al.*, 2010). Os taquizoítos e/ou cistos do *N. caninum* podem ser identificados através de exames histológicos e de imuno-histoquímica, além de que a coloração imuno-histoquímica e a PCR podem ajudar a distinguir de outros parasitas relacionados, através de amostras de tecidos e fluidos corpóreos de animais e fetos infectados (DUBEY *et al*., 1988B). O parasito também pode ser isolado a partir de tecidos de animais infectados e inoculados em cultivo celular ou em animais de laboratório (LINDSAY e DUBEY, 2000).

A combinação dos sinais clínicos relacionados à neosporose com a sorologia positiva, excluindo outras causas que promovem síndromes clínicas semelhantes, fornece o diagnóstico presuntivo. Vários testes sorológicos podem ser usados para detectar anticorpos anti-*N. caninum*, incluindo reação de imunofluorescência indireta (RIFI), ensaio imunoenzimático (ELISA) e o teste modificado de aglutinação (N-MAT). A RIFI é considerada uma técnica bastante específica, com pouca reação cruzada com outros parasitos e por isso vem sendo considerada a prova padrão para o sorodiagnóstico de *N. caninum*. (YAMANE *et al*., 1993; BJÖRKMAN e UGGLA, 1999). Título maior ou igual a 1:25 aponta apenas a exposição do hospedeiro ao parasito, não necessariamente a doença (LANGONI *et al*., 2012) Estudos demonstraram que praticamente todos os casos confirmados de neosporose demonstraram títulos maiores ou iguais a 1:800, o que torna uma boa evidência quando um cão apresentar este título associado aos sinais clínicos esteja com neosporose

(BARBER e TREES, 1996). Todavia, alguns cães podem excretar oocistos e apresentar lesões compatíveis com neosporose, porém sem soroconversão, de modo a apresentar sorologia negativa, assim não significa que cão esteja livre da infecção por *N. caninum* (LINDSAY *et al.,1*999).

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CAPÍTULO 2

Artigo formatado conforme normas para publicação na revista Veterinary Parasitology

525 **Occurrence of anti-*Toxoplasma gondii* and anti-*Neospora caninum* antibodies in**
526 **dogs with and without neurological signs in Mato Grosso do Sul state, Brazil**

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546

547 **Abstract** This study aimed to investigate the occurrence of antibodies against
548 *Toxoplasma gondii* and *Neospora caninum* in dogs with and without neurological signs
549 in Campo Grande, Mato Grosso do Sul. Serum samples from 58 dogs were used for this

study. Serological diagnosis was performed using the indirect fluorescent antibody test (IFAT), and titers ≥ 1:25 and ≥ 1:16 were considered positive for *N. caninum* and *T. gondii*, respectively. Of the 58 serum samples, 12 (20.69%) were showed anti-*T. gondii*, 13 (22.41%) anti-*N. caninum* antibodies, and three (5.17%) for both antibodies. Anti-*T. gondii* antibody titers ranged from 16 to 4,096 while those of anti-*N. caninum* antibody ranged from 25 to 100. Of the 28 (48.28%) dogs with neurological signs, seven (25%) were seropositive for *T. gondii*, nine (32.14%) for *N. caninium* and three (10.71%) for both evaluated agents All seropositive dogs for both agents showed neurological changes. Of the 30 (51.72%) dogs without neurological signs, five (16.67%) were positive for *T. gondii* and four (13.33%) for *N. caninum*. There was no association between the following variables: neurological signs, vaccination, feeding, contact with carcasses, age, diseases, environment, breed, sex, and contactantes in the same environment, with seropositivity for *T. gondii* and *N. caninum*. The results showed the presence of *N. caninum* and *T. gondii* in Campo Grande, Mato Grosso do Sul. Both dogs with and without nervous symptoms were seroreactive, thus highlighting the important role of dogs in the epidemiology of these protozoa.

Keywords: indirect fluorescent antibody test, toxoplasmosis, neosporosis, neuropathies, serology.

1. Introduction

Neospora caninum and *Toxoplasma gondii* are intracellular protozoa, with wide geographic distributions, belonging to the phylum Apicomplexa that cause neuromuscular, gastrointestinal, respiratory, and reproductive disorders in several animals, including dogs (Dubey et al., 1988; Mineo et al., 2001). They are morphologically similar, being differentiated by ultrastructural and immunological

574 particularities (Dubey & Beattie, 1988; Lindsay & Dubey, 1989) and molecular (Sager
575 et al., 2006).

576 Dogs are increasingly adopted into families; therefore, they can contribute to
577 maintaining and transmitting infectious agents (Brasil et al., 2018). They are the
578 definitive and important hosts of *N. caninum* (McAllister et al., 1998; Dubey et al.,
579 2007). Dogs have also been described as a sentinel and possible risk factor for *T. gondii*
580 infection in humans due to their role in the mechanical transmission of oocysts (Schares
581 et al., 2005; Lopes et al., 2014).

582 Toxoplasmosis is a serious public health problem, with greater importance in
583 women who get infected during pregnancy and immunocompromised people, such as
584 HIV patients, who usually have neurological changes (Dubey et al., 2012). Children of
585 women who were first infected during pregnancy may have serious health
586 complications, such as neurological and ocular disorders, and even fetal death and
587 abortion (Dubey et al., 2009; Torgerson & Mastroiacovo, 2013).

588 The pathogenicity of *N. caninum* and the severity of the infection vary according
589 to the host species; is mainly a clinical disease of cattle and dogs (Dubey et al., 2017).
590 Neosporosis is one of the main causes of abortion in cattle, with estimated economic
591 loss in world livestock ranging from 2 to 5% per year in most farms, and reaching 20%
592 in some (Goodswen et al., 2013). Wouda et al. (1999) observed a strong correlation
593 between farms dogs seropositive for *N. caninum* and seropositivity in cattle from the
594 same farms. In addition, Lobato et al. (2006) reported the presence of *N. caninum*
595 antibodies in humans, especially in HIV-infected patients and patients with neurological
596 disorders, moreover was significantly associated with seropositivity for *T. gondii*.

However, the zoonotic potential of *N. caninum* has not yet been fully established (Bresciani et al., 2007; Duarte et al., 2020).

Previous studies have revealed the occurrence of *N. caninum* in dogs in the municipality of Campo Grande - MS (Oliveira et al., 2004; Andreotti et al., 2004; 2006). However, there is little data on the serological prevalence of *T. gondii* in the canine population in this region as well as studies on the prevalence of these protozoa in dogs with and without neurological signs.

Considering the limited regional data in the city of Campo Grande and the importance of *T. gondii and N. caninum* in dogs and other species, the aim of this study was to evaluate the association of *T. gondii* and *N. caninum* infection in dogs with and without neurological signs in Campo Grande, Mato Grosso do Sul.

2. Material and Methods

2.1 Ethics Committee

Dog owners authorized the use of their dogs by signing the Free Consent Form. This study was approved by the Ethics and Use of Animals Committee (CEUA) of the Federal University of Mato Grosso do Sul (UFMS) (protocol 511 1.034/2019).

2.2 Study area and *samples*

The present study was conducted in Campo Grande (20°26'16″ "S and 54°32'16" ″W), in the state of Mato Grosso do Sul, Brazil. According to the Köppen-Geiger climatic classification, the climate of this area is predominantly tropical, with dry-winter characteristics, subtype AW. A well-defined rainy season occurs during the summer months (November–March), while the dry period occurs in the other months of the year. It is characterized by high temperatures, from 18 - 28 °C, with thermal variation from 5 - 7 °C.

Fifty-eight blood samples were collected from dogs from various regions of the city of Campo Grande - MS treated during routine visits at the Federal University of Mato Grosso do Sul (UFMS) veterinary hospital from April 2019 to December 2019. The dogs were divided into two groups: control (without neurological signs) (WNN, n = 30) and case (dogs with neurological signs) (NS, n = 28). Animals with seizures, behavioral changes, cranial nerve deficits, paralysis or limb paraplegia were considered "cases". The owners responded to a questionnaire regarding their dogs with the objective of identifying possible factors associated with the occurrence of toxoplasmosis and neosporosis among the dogs in the study region, including sex, age, breed, place of habitation, presence of contactants, contact with wild or wandering animals, access to the streets, food, ingestion of raw meat, contact with carcass of other animals, morbid antecedents, and vaccination. The signs presented by dogs with neurological alterations were recorded as well as the lesion site, identified through neurological examination. Other diseases that promote neurological changes have not been excluded.

2.3 Serological exams

Serological diagnosis to verify the presence of anti-*N. caninum* and anti-*T. gondii* antibodies in the serum of dogs were performed using IFAT technique, according to the methods by Conrad et al. (1993) and Camargo (1974). Tachyzoites of the Nc-1 strain of *N. caninum* previously cultivated in VERO cells and tachyzoites of the RH strain of *T. gondii* previously inoculated in mice were used as the antigen and canine total anti-IgG conjugate (Sigma, USA) as the secondary antibody. As a control, sera from dogs known to be positive and negative were included in all the slides used. The samples for *N. caninum* and *T. gondii* were initially diluted at 1:25 and 1:16,

respectively, and then incubated in a humid chamber at 37 °C for 30 min. They were then washed three times in PBS for 5 min for *N. caninum* and 10 min for *T. gondii*. Sequentially, diluted conjugate was added to PBS and the incubation and washing process performed again. After drying the slides, they were mounted with glycerin (pH 9.0) and a cover slip and examined under an epifluorescence microscope (Nikon, Japan). Only samples that showed fluorescence of the entire surface of the tachyzoites were considered positive. Samples ≥ 1:25 and ≥1:16 were considered positive for *N. caninum* and *T. gondii*, respectively.

2.4 Statistical analysis

Association of serological test results with the variables used in the epidemiological questionnaire was performed by calculating the odds ratio (OR) with a 95% confidence interval, p-value <0.05 were considered significant. All analyses were performed using the Bioestat 5.0 program.

3. Results

Of the 58 canine serum samples analyzed using IFAT, 12 (20.69%) showed anti-*T. gondii* antibodies, 13 (22.41%) anti-*N. caninum antibodies*, and three (5.17%) showed both antibodies. Anti-*T. gondii* antibody titers for ranged from 1:16 to 1:4,096, while those of anti-*N. caninum* antibodies ranged from 1:25 to 1:100, as shown in Table 1. Of the 3 dogs positive for both protozoa, one dog (33.33%) had anti-*T. gondii* and anti-*N. caninum* antibody titers of 1:16 and 1:50, respectively, while the other two (66.67%) both had 1:64 and 1:100, respectively.

Of the 28 (48.28%) dogs with neurological signs, seven (25%) were seropositive for *T. gondii*, with antibody titers ranging from 1:16 to 1:4,096, while nine (32.14%) had antibody titers for anti-*N. caninum* which ranged from 1:25 to 1:100. Three

(10,71%) dogs were seropositive for both evaluated agents, with antibody titers ranging from 1:16–1:64 and 1:50–1:100 for anti-*T. gondii* and anti-*N. caninum*, respectively. The main neurological alterations observed were seizures, compulsive walking, cranial nerve deficits and head tilt (Table 2). Of the 30 (51.72%) dogs without neurological signs, five (16.67%) were positive for *T. gondii* and four (13.33%) for *N. caninium*, with antibody titers ranging from 1:16-1:4,096 and 1:25-1:50, respectively.

There was no statistically significant association or difference ($p > 0.05$) between the possible risk factors for toxoplasmosis (Table 3) and neosporosis (Table 4) analyzed in this study.

4. Discussion

In this study, the seroprevalence of anti-*T.gondii* antibodies in dogs was 20.69%, similar to that observed by De Souza et al. (2003), Coiro et al. (2011), and Langoni et al. (2013), in which 19.7%, 26.9%, and 20.8% of dogs in the state of São Paulo, respectively, were seropositive for *T. gondii*, . In Brazil, previous studies have demonstrated a wide variation in the frequency of seropositive dogs in other regions, such as 12.7% in the municipality of Natal, Rio Grande do Norte (Lopes et al., 2015), 15.6% in Patos, Paraíba (Dantas et al., 2014), 57.4% in Araguaína, Tocantins (Raimundo et al., 2015), and 88.5% in Jauru, Mato Grosso (Santos et al., 2009).

Presence of antibodies against *N. caninum* was observed in 22.41% of the canine blood samples evaluated, similar to those reported by de Souza et al. (2002), Fernandes et al. (2004), Oliveira et al. (2004), Andreotti et al. (2006), and Raimundo et al. (2015). Oliveira et al. (2004) and Andreotti et al. (2006) noted in the same region, values close to those of the present study, was verified seropositivity in 26.53% and 27.2% of dogs.

693 The seroprevalence obtained in the present study for *N. caninum* is in
694 accordance with that observed in the worldwide canine population (17.14%). There is
695 great variation in the occurrence of antibodies against *N. caninum* in dogs in Brazil and
696 worldwide, with a higher prevalence of *N. caninum* in dogs in Belgium (41.36%) and
697 Grenada (1.62%) (Anvari et al., 2020). In Brazil, there was also a wide variation in the
698 seroprevalence of *N. caninum*, with values from 2.6% in Bahia (Sicupira et al., 2012) up
699 to 67.6% in Mato Grosso (Benetti et al., 2009).
700 Only 5.17% of the evaluated dogs were seropositive for both protozoa; these
701 values were close to those found by Azevedo et al. (2005) in Campina Grande, Paraíba
702 (4.9%) and by Varandas et al. (2001) in the Northeast of the State of São Paulo (5.76%).
703 Variations in the prevalence of antibodies against *N. caninum* and *T. gondii* may
704 be due to several factors, such as sample size, characteristics of the population studied,
705 regional differences, study seasonality, as well as different serological assays, and cut-
706 off titers (Azevedo et al., 2005; Anvari et al., 2020).
707 Of the dogs with neurological signs, 25% were positive for *T. gondii*, 32.14% for
708 *N. caninum*, and 10.71% for both protozoa. There are only a few studies on the
709 seroprevalence of neosporosis and toxoplasmosis in dogs with neurological signs, since
710 most Brazilian studies as well as those in other countries have been conducted with
711 asymptomatic dogs. In this study, there was a higher occurrence of neurologic signs in
712 dogs positive for *N. caninum*, which differs from previous studies that demonstrated
713 that most dogs with neurological clinical signs were seropositive for *T. gondii* (Mineo et
714 al., 2001; Giraldi et al., 2002, and Plugge et al., 2011).
715 Toxoplasmosis, neosporosis and distemper promote similar clinical signs,
716 increasing the importance of the differential diagnosis of these diseases, in addition,
717 Brito et al. (2002) concluded that a toxoplasmosis has been associated with changes

718 combined with distemper, since this virus presents an important immunosuppressive
719 action. The dogs with clinical *N. caninum* infection usually have titers higher than 1:800
720 (Barber & Trees, 1996). However, lower titers were observed in dogs with neurological
721 signs, as observed by Plugge et al. (2011), which could be correlated with other diseases
722 that lead to neurological signs, such as leishmaniasis and ehrlichiosis, considering that
723 in the present study, these were not excluded or possibly because the dogs did not
724 undergo seroconvert.

725 Although carnivorism is the main form of infection by *T. gondii* in animals and
726 humans (Dubey & Beattie, 1988), this study did not find an association between the
727 type of feeding and seropositivity for *T. gondii*, corroborating observations in other
728 studies (Bresciani et al., 2007; Constantino et al., 2016; Rodrigues et al., 2016).
729 However, Moura et al. (2009) reported a higher occurrence toxoplasmosis in dogs
730 receiving a homemade diet. Carlos et al. (2010) also observed that there was no
731 statistical significance in relation to the consumption of raw meat; however, the
732 consumption of homemade food and meat were considered risk factors for *T. gondii*
733 infection.

734 Although all dogs had owners, the majority (60.34%) had free access to streets.
735 This variable had no statistically significant association with seropositivity for *T. gondii*,
736 which was also observed by Bresciani et al. (2007). However, Ferreira et al. (2016)
737 observed high seroprevalence in domiciled dogs which did not have free access to
738 streets. These findings demonstrate the environment shared between them can offer
739 conditions for *T. gondii* infection, reinforcing the important role of the dog as a sentinel
740 of toxoplasmosis.

741 There was no association regarding age and the presence of antibodies against *T.*
742 *gondii*, which is in accordance with previous studies (Varandas et al., 2001; Romanelli

et al., 2007; Langoni et al., 2013). However, Guimarães et al. (2009) and Dantas et al. (2014) observed that the probability of infection increases with age, possibly due to greater exposure to the agent, which was not noticed in the present study.

In this study, no correlation was observed between seropositivity for *T. gondii* and the racial pattern of the dogs, however Carlos et al. (2010) and Dantas et al. (2014) noted that mixed breed dogs are more likely to be seropositive for *T. gondii* than breed dogs. In addition, they suggested that these results are probably related to the management conditions in which these animals are submitted, as well as the characteristics of the owner, since in many times, the mixed animals belong to families with few economic resources, so they end up not having access to quality food and water, in addition they have free access to the street.

We did not observe association between sex and the environment with the presence of anti-*T. gondii* antibodies. Similar results were observed in previous studies (Varandas et al., 2001; Langoni et al., 2013; Acosta et al., 2016; Ferreira et al., 2016), contradicting those observed by Coiro et al. (2011), who found that female dogs had a 1.6 times higher chance of *T. gondii* infection than male dogs. Regarding the variable environment, Raimundo et al. (2015) verified a higher occurrence of seropositivity in dogs from rural areas than that in dogs from urban areas, even without statistical association, a fact possibly explained by the greater exposure of dogs from rural area is the contact with intermediated host.

The main route of *N. caninum* infection in dogs is by ingestion of food contaminated with tissue cysts. However, in the present study, both raw meat consumption and homemade feeding were not significantly associated with the presence of antibodies against *N. caninum*, similar to studies by Benetti et al. (2008) and Cañón-Franco et al. (2003). Thus, the infection of the animals in this study may have occurred

768 through factors that were not included, such as ingestion of contaminated water or feces,
769 since it is common for dogs to have the habit of coprophagia. Future studies should be
770 carried out in search of these factors, as a possible risk of infection.

771 Although in this study there was no association between street access and living
772 in a rural environment with seropositivity for *N. caninum,* other authors have reported a
773 strong association between the occurrence of anti-*N.caninum* antibodies and street
774 access (Gennari et al., 2002; Azevedo et al., 2005; Benetti et al., 2008).

775 There was no significant difference in *N. caninum* infection in terms of age,
776 breed, and sex, corroborating previous studies (Gennari et al., 2002; Romanelli et al.,
777 2007; Sousa et al., 2012, and Langoni et al., 2013). However, a study by Capelli et al.
778 (2004), in Italy, detected that older dogs had a higher risk of *N. caninum* infection, and
779 that purebred dogs had higher seropositivity (13.6%) than those without a defined breed
780 (7.1%). Oliveira et al. (2004) also observed an association between the presence of
781 antibodies against *N. caninum* with age, as did Raimundo et al. (2015); however, they
782 found a higher prevalence in dogs without a defined breed. Moreover, according to
783 Acosta et al. (2016), there was no significant difference between male and female dogs
784 from the rural areas of Espírito Santo. Although Oliveira et al. (2004) observed that
785 male dogs had higher seroprevalence (30.71%) than that of female dogs (20.95%), there
786 was no statistically significant difference, suggesting that male and female dogs are
787 exposed to the same risks.

788 **4. Conclusion**

789 The results showed the presence of *N. caninum* and *T. gondii* in the Campo
790 Grande region of Mato Grosso do Sul. Dogs with and without nervous symptoms were
791 seropositive, thus revealing their important role in the epidemiology of these coccids.

However, further studies with a larger sample are needed in order to understand the epidemiology and risk factors for these co-infections in the region.

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Table 1. Distribution of antibody titers for *Toxoplasma gondii* and *Neospora caninium* through IFAT in dogs treated at the veterinary hospital of Federal University of Mato Grosso do Sul, in the city of Campo Grande, MS, Brazil.

	Toxoplasma gondii				***Neospora caninum***		
Titers	**Number of positive dogs**	**% of total**	**% of positives**	**Titers**	**Number of positive dogs**	**% of total**	**% of positives**
16	3	5,17%	25,00%	25	7	12,07%	53,85%
64	5	8,62%	41,67%	50	3	5,17%	23,08%
4,096	4	6,90%	33,33%	100	3	5,17%	23,08%
Total	**12**	**20,69%**	**100,00%**	**Total**	**13**	**22,41%**	**100,00%**

Table 2. Frequency of neurological signs presented by dogs with neurological symptoms according to the results of serology for *Neospora caninum and Toxoplasma gondii.*

Neurological signs	Seronegative	Seropositive for *N. caninum*	Seropositive for *T. gondii*	Seropositive for both	Total
Seizure	7 (25%)	5 (17.86%)	1 (3.57%)	1 (3.57%)	14 (50%)
Compulsive walking	2 (7.14%)	1 (3.57%)	-	-	3 (10.71%)
Deficits in cranial nerves	2 (7.14%)	1 (3.57%)	-	-	3 (10.71%)
Head tilt	2 (7.14%)	-	-	1 (3.57%)	3 (10.71%)
Flaccid quadriplegia	1 (3.57%)	-	1 (3.57%)	-	2 (7.14%)
Flaccid tetraparesis	-	-	1 (3.57%)	1 (3.57%)	2 (7.14%)
Head pressing	2 (7.14%)	-	-	-	2 (7.14%)
Walk in circles	2 (7.14%)	-	-	-	2 (7.14%)
Spastic quadriplegia	-	-	1 (3.57%)	-	1 (3.57%)
Spastic paraplegia	-	1 (3.57%)	-	-	1 (3.57%)
Spastic tetraparesis	1 (3.57%)	-	-	-	1 (3.57%)
Proprioceptive deficits	-	-	1 (3.57%)	-	1 (3.57%)
Involuntary spasms	-	1 (3.57%)	-	-	1 (3.57%)
Loss of balance	-	-	-	1 (3.57%)	1 (3.57%)
Amaurosis	-	1 (3.57%)	-	-	1 (3.57%)
Atrophy of the temporal muscles	-	1 (3.57%)	-	-	1 (3.57%)
Pleurototonus	1 (3.57%)	-	-	-	1 (3.57%)
Opisthotonus	1 (3.57%)	-	-	-	1 (3.57%)
Tremor of intention	1 (3.57%)	-	-	-	1 (3.57%)
Positional strabismus	1 (3.57%)	-	-	-	1 (3.57%)
Nystagmus	-	-	-	1 (3.57%)	1 (3.57%)
Trismus	1 (3.57%)	-	-	-	1 (3.57%)
Vocalization	1 (3.57%)	-	-	-	1 (3.57%)
Compulsive tail chasing	1 (3.57%)	-	-	-	1 (3.57%)
Hypermetry	1 (3.57%)	-	-	-	1 (3.57%)

1011 **Table 3.** Distribution of different variables with serology for *Toxoplasma gondii* in dogs
1012 treated at the veterinary hospital of Federal University of Mato Grosso do Sul in Campo
1013 Grande, MS, Brazil.

Variable	Results IFAT (IgG)		Total	OR	Interval	p
	Positive	Negative				
Neurological signs						
Yes	7 (12,07%)	21 (36,21%)	28 (48,28%)	1.6667	0.4606 - 6.0303	0.6466
No	5 (8,62%)	25 (43,10%)	30 (51,72%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			
Vaccination						
Yes	10 (17,54%)	39 (68,42%)	49 (85,96%)	0.7692	0.1344 - 4.4029	0.8632
No	2 (3,51%)	6 (10,53%)	8 (14,04%)			
TOTAL	12 (21,05%)	45 (78,95%)	57 (100%)			
Raw meat intake						
Yes	3 (5,36%)	11 (19,64%)	14 (25,00%)	1	0.2290 - 4.3672	1
No	9 (16,07%)	33 (58,93%)	42 (75,00%)			
TOTAL	12 (21,43%)	44 (78,57%)	56 (100%)			
Contact carcass of other animals						
Yes	2 (3,45%)	9 (15,52%)	11 (18,97%)	0.8222	0.1526 - 4.4291	0.8530
No	10 (17,24%)	37 (63,79%)	47 (81,03%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			
Age						
Up to 5 years	5 (8,62%)	22 (37,93%)	27 (46,55%)	0.7792	0.2155 - 2.8173	0.9553
Older than 5 anos	7 (12,07%)	24 (41,38%)	31 (53,45%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			
Disease History						
Yes	5 (8,93%)	13 (23,21%)	18 (32,14%)	1.7033	0.456 - 6.3620	0.6539
No	7 (12,50%)	31 (55,36%)	38 (67,86%)			
TOTAL	12 (21,43%)	44 (78,57%)	56 (100%)			
Rural environment						
Yes	2 (3,45%)	2 (3,45%)	4 (6,90%)	4.4000	0.5515 - 35.1069	0.3897
No	10 (17,24%)	44 (75,86%)	54 (93,10%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			
Breed						
Mixed	3 (5,26%)	22 (38,60%)	25 (43,86%)	0.3485	0.0833 - 1.4583	0.2483
Defined	9 (15,79%)	23 (40,35%)	32 (56,14%)			
TOTAL	12 (21,05%)	45 (78,95%)	57 (100%)			
Sex						
Male	6 (10,53%)	12 (21,05%)	18 (31,58%)	2.75	0.7417 - 10.1958	0.2319
Female	6 (10,53%)	33 (57,89%)	39 (68,42%)			
TOTAL	12 (21,05%)	45 (78,95%)	57 (100%)			
Contactants						
Yes	9 (15,52%)	29 (50,00%)	38 (65,52%)	1.7586	0.4178 - 7.4026	0.6635
No	3 (5,17%)	17 (29,31%)	20 (34,48%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			
Contact wild / wandering animals						
Yes	2 (3,45%)	13 (22,41%)	15 (25,86%)	0.5077	0.0977 - 2.6390	0.6551
No	10 (17,24%)	33 (56,90%)	43 (74,14%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			
Homemade food						
Yes	7 (12,07%)	28 (48,28%)	35 (60,34%)	0.9000	0.2474 - 3.2741	0.8639
No	5 (8,62%)	18 (31,03%)	23 (39,66%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			
Street access						
Yes	7 (12,07%)	28 (48,28%)	35 (60,34%)	0.9	0.2474 - 3.2741	0.8639
No	5 (8,62%)	18 (31,03%)	23 (39,66%)			
TOTAL	12 (20,69%)	46 (79,31%)	58 (100%)			

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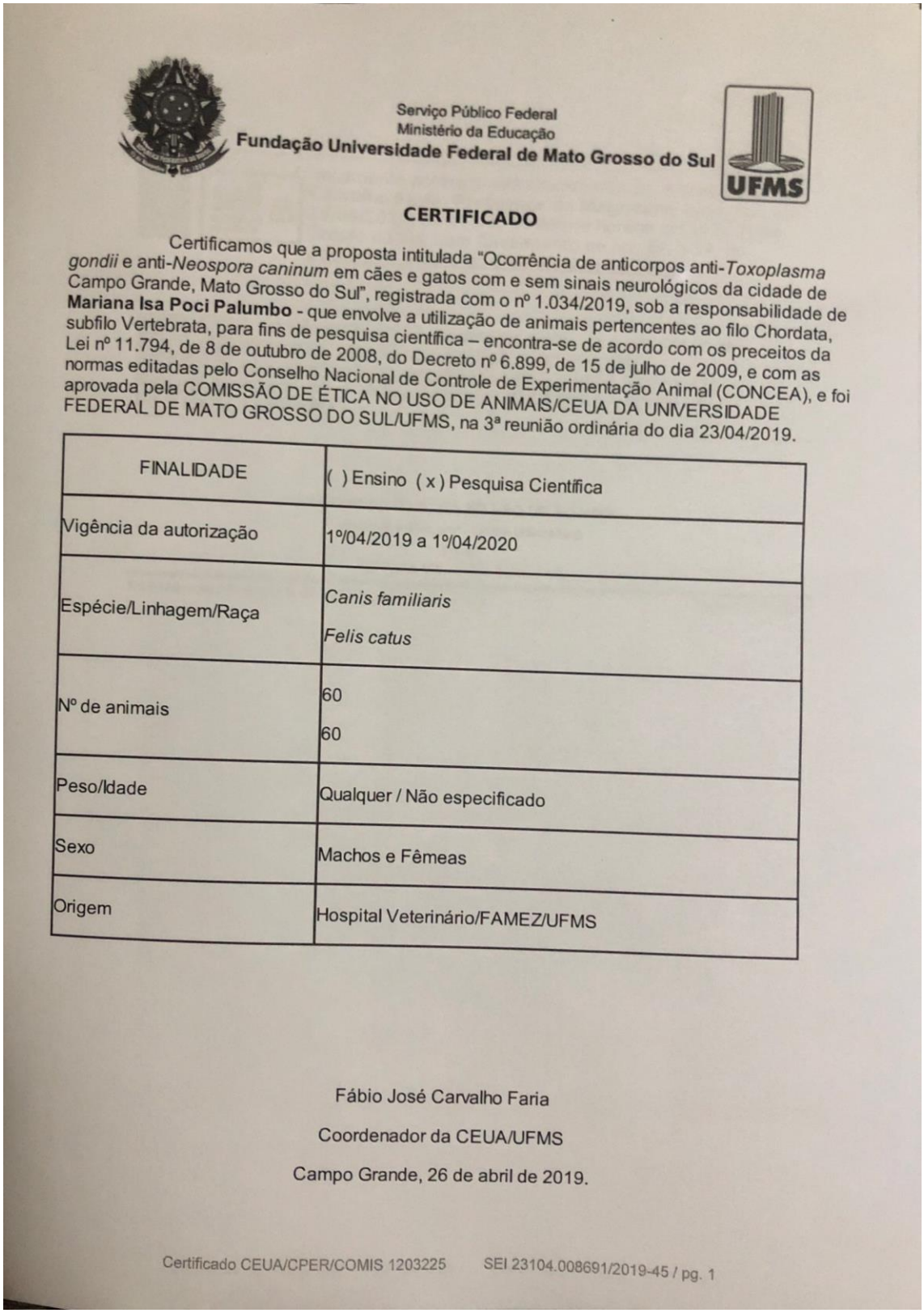
Table 4. Distribution of different variables with serology for *Neospora caninium* in dogs treated at the veterinary hospital of Federal University of Mato Grosso do Sul in Campo Grande, MS, Brazil.

Variable	Results IFAT (IgG)		Total	OR	Interval	p
	Positive	Negative				
Neurological signs						
Yes	9 (15,52%)	19 (32,76%)	28 (48,28%)	3.0789	0.8241 - 11.5030	0.161
No	4 (6,90%)	26 (44,83%)	30 (51,72%)			
TOTAL	13 (22,41)	45 (77,59%)	58 (100%)			
Vaccination						
Yes	12 (21,05%)	37 (64,92%)	49 (85,96%)	2.2703	0.2530 - 20.3700	0.768
No	1 (1,75%)	7 (12,28%)	8 (14,04%)			
TOTAL	13 (22,81%)	44 (77,19%)	57 (100%)			
Raw meat intake						
Yes	6 (10,71%)	8 (14,29%)	14 (25,00%)	3.75	0.9880 - 14.2329	0.1
No	7 (12,50%)	35 (62,50%)	42 (75,00%)			
TOTAL	13 (23,21%)	43 (76,79%)	56 (100%)			
Contact carcass of other animals						
Yes	2 (3,45%)	9 (15,52%)	11 (18,97%)	0.7273	0.1363 - 3.8804	0.9779
No	11 (18,97%)	36 (62,07%)	47 (81,03%)			
TOTAL	13 (22,41%)	45 (77,59%)	58 (100%)			
Age						
Up to 5 years	7 (12,07%)	20 (34,48%)	27 (46,55%)	1.4583	0.4225 - 5.0338	0.7772
Older than 5 anos	6 (10,34%)	25 (43,10%)	31 (53,45%)			
TOTAL	13 (22,41%)	45 (77,59%)	58 (100%)			
Disease History						
Yes	6 (10,71%)	12 (21,43%)	18 (32,14%)	3.3	0.8484 - 12.8363	0.1572
No	5 (8,93%)	33 (58,93%)	38 (67,86%)			
TOTAL	11 (19,64%)	45 (80,36%)	56 (100%)			
Rural environment						
Yes	1 (1,72%)	3 (5,17%)	4 (6,90%)	1.1667	0.1110 - 12.2624	0.6222
No	12 (20,69%)	42 (72,41%)	54 (93,10%)			
TOTAL	13 (22,41%)	45 (77,59%)	58 (100%)			
Breed						
Mixed	6 (10,53%)	19 (33,33%)	25 (43,86%)	1.1278	0.3254 - 3.9088	0.8979
Defined	7 (12,28%)	25 (43,86%)	32 (56,14%)			
TOTAL	13 (22,81%)	44 (77,19%)	57 (100%)			
Sex						
Male	5 (8,77%)	13 (22,81%)	18 (31,58%)	1.4904	0.4096 - 5.4223	0.7886
Female	8 (14,04%)	31 (54,39%)	39 (68,42%)			
TOTAL	13 (22,81%)	44 (77,19%)	57 (100%)			
Contactants						
Yes	7 (12,07%)	31 (53,45%)	38 (65,52%)	0.5269	0.1495 - 1.8573	0.5004
No	6 (10,34%)	14 (24,14%)	20 (34,48%)			
TOTAL	13 (22,41%)	45 (77,59%)	58 (100%)			
Contact wild / wandering animals						
Yes	2 (3,45%)	13 (22,41%)	15 (25,86%)	0.4476	0.0869 - 2.3044	0.5353
No	11 (18,97%)	32 (55,17%)	43 (74,14%)			
TOTAL	13 (22,41%)	45 (77,59%)	58 (100%)			
Homemade food						
Yes	8 (13,79%)	27 (46,55%)	35 (60,34%)	1.0667	0.3006 - 3.7853	0.8243
No	5 (8,62%)	18 (31,03%)	23 (39,66%)			
TOTAL	13 (22,41%)	45 (77,59%)	58 (100%)			
Street access						
Yes	9 (15,52%)	26 (44,83%)	35 (60,34%)	1.6442	0.4401 - 6.1429	0.6732
No	4 (6,90%)	19 (32,76%)	23 (39,66%)			
TOTAL	13 (22,41%)	45 (77,59%)	58 (100%)			

ANEXOS

Anexo A – Aprovação do comitê de ética de uso de animais



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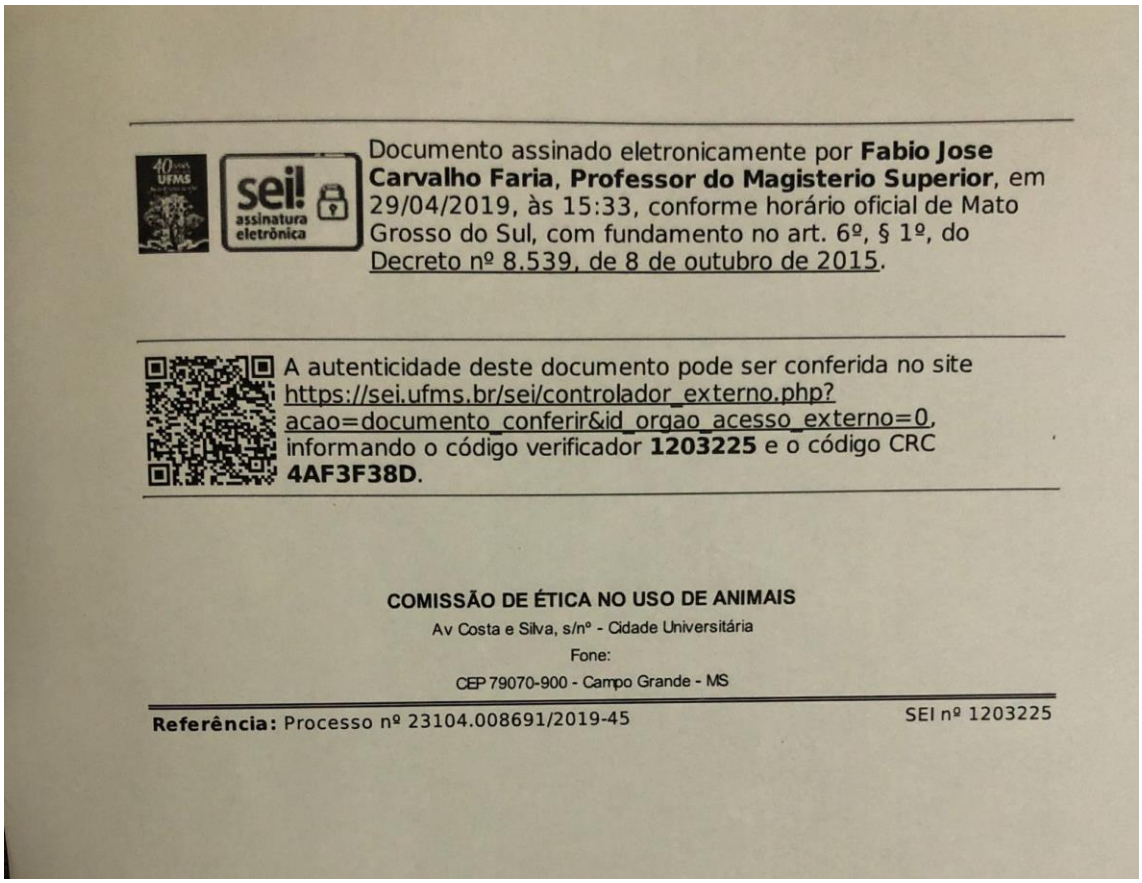
CERTIFICADO

Certificamos que a proposta intitulada "Ocorrência de anticorpos anti-*Toxoplasma gondii* e anti-*Neospora caninum* em cães e gatos com e sem sinais neurológicos da cidade de Campo Grande, Mato Grosso do Sul", registrada com o nº 1.034/2019, sob a responsabilidade de **Mariana Isa Poci Palumbo** - que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata, para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS/CEUA DA UNIVERSIDADE FEDERAL DE MATO GROSSO DO SUL/UFMS, na 3ª reunião ordinária do dia 23/04/2019.

FINALIDADE	( ) Ensino (x) Pesquisa Científica
Vigência da autorização	1º/04/2019 a 1º/04/2020
Espécie/Linhagem/Raça	*Canis familiaris* *Felis catus*
Nº de animais	60 60
Peso/Idade	Qualquer / Não especificado
Sexo	Machos e Fêmeas
Origem	Hospital Veterinário/FAMEZ/UFMS

Fábio José Carvalho Faria
Coordenador da CEUA/UFMS
Campo Grande, 26 de abril de 2019.

Certificado CEUA/CPER/COMIS 1203225 SEI 23104.008691/2019-45 / pg. 1



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COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Av Costa e Silva, s/nº - Cidade Universitária

Fone:

CEP 79070-900 - Campo Grande - MS

Referência: Processo nº 23104.008691/2019-45 SEI nº 1203225

Anexo B - Termo de declaração de livre consentimento

DECLARAÇÃO DE LIVRE CONSENTIMENTO

DADOS DO ANIMAL

Nome:_____ **Raça:**_____
Sexo:_____ **Prontuário:**_____

Espécie: _____**Idade:** _____ **Data:** _____**/**_____**/**_____

DADOS DO PROPRIETÁRIO

Nome:
_____

Rg: _____

Cpf:_____

Tel: _____

Declaro, para os devidos fins, que estou ciente e autorizo a coleta de sangue para realização de sorologia para toxoplasmose e neosporose de meu animal, bem como a divulgação dos resultados em trabalhos científicos.

Data:_____

Assinatura do proprietário:
_____

Anexo C – Questionário epidemiológico

QUESTIONÁRIO AO TUTOR

- Qual/ a cidade onde habita?

- O animal habita área urbana ou rural? Já visitou ou frequentou outras localidades? Quais?

-Ambiente onde vive (piso cimentado, gramado ou terra)

- O animal possui contactantes? Se sim, qual a espécie? Qual a condição clínica deles? O contactante tem acesso à rua?

- O animal é vacinado? A vacinação está atualizada? Quais vacinas?

- O animal possui acesso à rua? Sozinho ou com proprietário?

- Qual a alimentação do animal (ração comercial/caseira/mista)? Come carne crua?

-Contato com carcaça de outros animais?

- O proprietário já viu se o animal teve alguma vez contato com animais silvestres, errantes ou não?

- O animal possui antecedentes mórbidos? Qual?

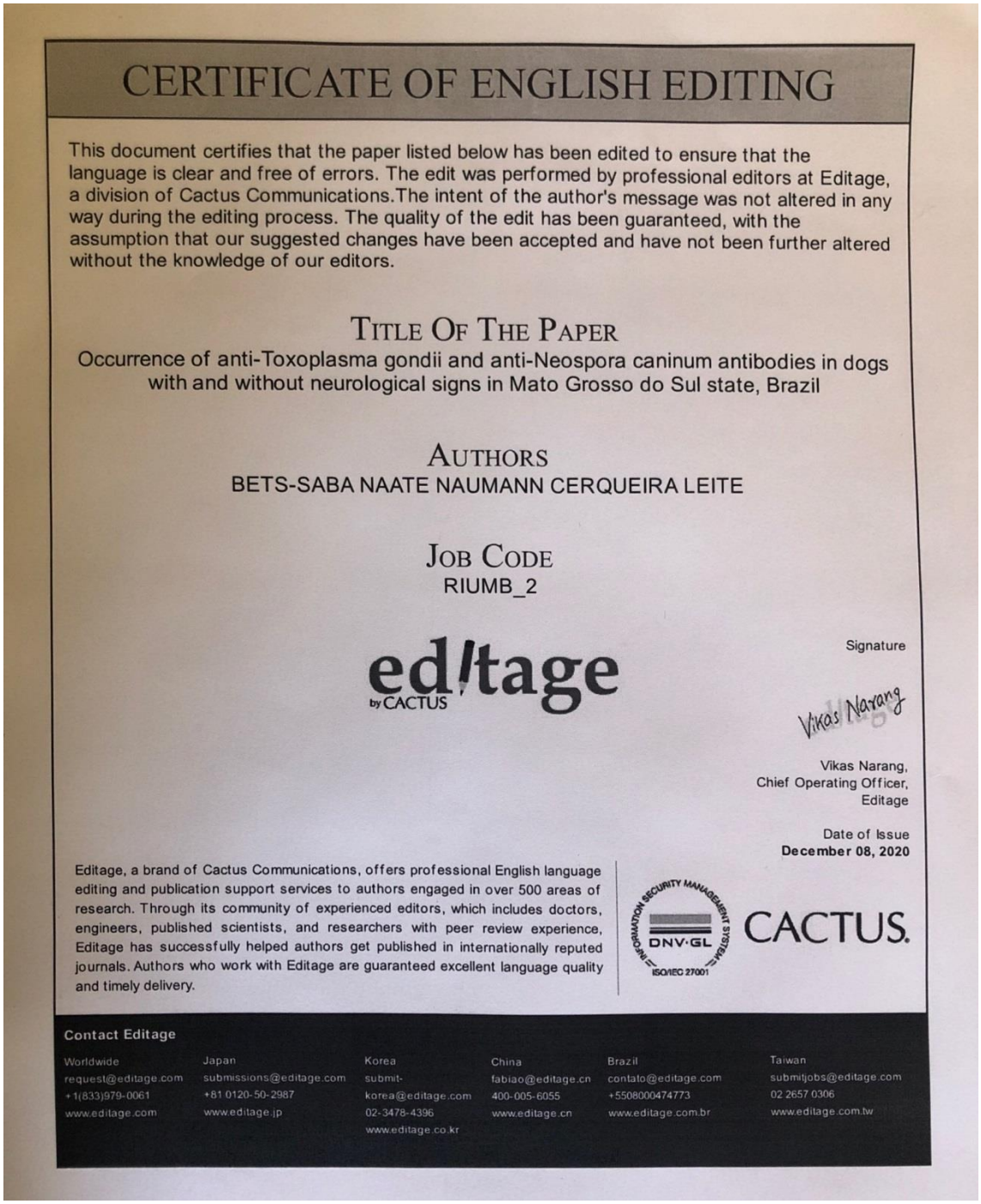
- O animal faz ou fez uso de medicamentos? Se sim, quais?

-Já realizou sorologia para leishmaniose? Faz prevenção da doença?Como?

Para médico veterinário:

Descrever os sinais neurológicos e localização da lesão:

Anexo D – Certificado de revisão em inglês



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Occurrence of anti-Toxoplasma gondii and anti-Neospora caninum antibodies in dogs with and without neurological signs in Mato Grosso do Sul state, Brazil

AUTHORS

BETS-SABA NAATE NAUMANN CERQUEIRA LEITE

JOB CODE

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Chief Operating Officer,
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Keywords (indexing terms), normally 3-6 items. Please refer to last index (Vol. 100/3-4).

Introduction

Material studied, area descriptions, methods, techniques

Results

Discussion

Conclusion

Acknowledgments and any additional information concerning research grants, etc.

References

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3. Equations should be numbered serially at the right-hand side in parentheses. In general only equations explicitly referred to in the text need be numbered.

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Indicate each footnote in a table with a superscript lowercase letter.

Artwork

Electronic artwork

General points

• Make sure you use uniform lettering and sizing of your original artwork.

• Embed the used fonts if the application provides that option.

• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.

• Number the illustrations according to their sequence in the text.

• Use a logical naming convention for your artwork files.

• Provide captions to illustrations separately.

• Size the illustrations close to the desired dimensions of the published version.

• Submit each illustration as a separate file.

• Ensure that color images are accessible to all, including those with impaired color vision.

A detailed guide on electronic artwork is available.

You are urged to visit this site; some excerpts from the detailed information are given here.

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Please do not:

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• Supply files that are too low in resolution;

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4. Each table should occupy a separate page of the manuscript. Tables should never be included in the text.

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Weatherley, A.J., Hong, C., Harris, T.J., Smith, D.G., Hammet, N.C., 1993. Persistent efficacy of doramectin against experimental nematode infections in calves. In: Vercruysse, J. (Ed.), Doramectin – a novel avermectin. Vet. Parasitol. 49, 45–50.

c. For books

Blaha, T. (Ed.), 1989. Applied Veterinary Epidemiology. Elsevier, Amsterdam, 344 pp.

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Wilson, M.B., Nakane, P.K., 1978. Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies. In: Knapp, W., Holubar, K., Wick, G. (Eds.), Immunofluorescence and Related Staining Techniques. North Holland, Amsterdam, pp. 215–224.

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