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# Clinical, pathological, and immunohistochemistry characterization of toxoplasmosis in dogs with distemper in the semiarid region of Paraíba, Brazil

## Caracterização clínica, patológica e imuno-histoquímica da toxoplasmose em cães com cinomose no semiárido da Paraíba, Brasil

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### Abstract

The objective of this study was to describe the clinical, pathological, and immunohistochemistry characteristics of five cases of toxoplasmosis, an infection often associated with distemper in dogs. From January 2000 to December 2012, a retrospective study was performed analyzed dogs with distemper in the semiarid region of Paraíba. We evaluated this sample to focus on individuals who presented with concomitant structures in protozoa characteristics, and performed immunohistochemistry (IHC) tests using polyclonal anti-*Toxoplasma gondii* antibody. In all cases, the clinical signs were similar including digestive changes, as well as respiratory, neurological, and ocular lesions, suggesting an infection of canine distemper virus. The diagnosis of distemper was confirmed on histopathological analysis depending on the presence of intranuclear and intracytoplasmic eosinophilic inclusion bodies in different tissues. Histopathological examination also revealed the characteristic presence of parasitic cysts *T. gondii* in the brain in four cases, and in the lung in one case. The brain cysts were associated with multifocal areas of malacia and lung there was alveolar septa thickening due to infiltration of macrophages, lymphocytes, and plasma cells, with moderate proliferation of type II pneumocytes and coalescing multifocal areas of necrosis. These cysts are characterized by round and strongly basophilic structures, measuring approximately 5 to 70 µm, delimited by thin wall, stained by hematoxylin and eosin, and immunomarked as brown by immunohistochemistry (IHC) using the chromogen DAB. The diagnosis of toxoplasmosis associated with infection by canine distemper virus in the five case studied was based on microscopic findings and confirmed by immunohistochemistry. Toxoplasmosis should be included in the differential diagnosis of dogs with severe progressive systemic signs, especially when respiratory and neurological involvement is suspected.

**Key words:** Dog, distemper, toxoplasmosis, immunohistochemistry

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## Resumo

Objetivou-se com este trabalho descrever as características clínicas, patológicas e imuno-histoquímica de cinco casos de toxoplasmose associados à cinomose em cães no semiárido da Paraíba. Para isso, foi realizado um estudo retrospectivo durante o período de janeiro de 2000 a dezembro de 2012, sendo identificados e selecionados os casos de cães com cinomose. Destes, foram avaliados os que apresentavam concomitantemente estruturas características de protozoários e realizada a imuno-histoquímica (IHQ) com anticorpo policlonal anti-*Toxoplasma gondii*. Em todos os casos os sinais clínicos foram semelhantes e sugestivos de infecção pelo vírus da cinomose canina, variando de alterações digestivas, respiratórias, neurológicas e lesões oculares. O diagnóstico de cinomose foi confirmado na histopatologia através da presença de corpúsculos de inclusões virais eosinofílicos intranucleares e intracitoplasmáticos em diferentes tecidos. O exame histopatológico revelou ainda a presença dos cistos parasitários característicos de *T. gondii* no encéfalo em quatro casos e no pulmão em um caso. No encéfalo os cistos estavam associados a áreas multifocais de malacia e no pulmão havia espessamento dos septos alveolares por infiltrado de macrófagos, plasmócitos e linfócitos, com proliferação moderada de pneumócitos tipo II e áreas multifocais a coalescentes de necrose. Esses cistos caracterizaram-se por estruturas arredondadas fortemente basofílicas medindo aproximadamente de 5 a 70 µm, delimitadas por uma fina parede pela hematoxilina e eosina e na IHQ foram fortemente imunomarcados em marrom para *T. gondii*. O diagnóstico de toxoplasmose associado à infecção pelo vírus da cinomose nos cinco casos estudados foi baseado nos achados microscópicos e confirmado através da imuno-histoquímica. A toxoplasmose deve ser incluída no diagnóstico diferencial de cães com sinais sistêmicos progressivos severos, principalmente quando há envolvimento respiratório e neurológico.

**Palavras-chave:** Cão, cinomose, toxoplasmose, imuno-histoquímica

## Introduction

*Toxoplasma gondii* protozoan infections are widely prevalent in humans and animals. These infections affect several species of mammals and birds and are found on all continents (DUBEY, 2004; FIALHO et al., 2009). In Brazil, serological studies show a high prevalence of this disease in the canine populations in the states of Mato Grosso (SANTOS et al., 2009), Paraná (GIRALDI et al., 2002), Pará (VALADAS et al., 2010), Paraíba (AZEVEDO et al., 2005), and São Paulo (BRITO et al., 2002). Infection rates vary by state, measured at 88.5%, 82.5%, 69.9%, 45.1%, 32.5% of the populations, respectively.

The dog has epidemiological importance, since it acts as an infection sentinel, and can determine a risk factor for public health by the mechanical transmission of *T. gondii* for humans (LINDSAY et al., 1997; ULLMANN et al., 2008).

Immunity does not eliminate an infection established by *T. gondii*, and tissue cysts persist for several years after an acute infection. However,

in immunocompromised individuals, the infection can be reactivated by the dissemination conversion of tachyzoites and bradyzoites in tissue (DUBEY, 2004), generally associated with canine distemper virus (AGUIAR et al., 2012; MORETTI et al., 2002; SILVA et al., 2007).

This study describes five cases of toxoplasmosis associated with distemper in dogs in the semiarid region of Paraíba and their clinical, pathological, and immunohistochemical characteristics.

## Material and Methods

We reviewed the necropsy records of dogs diagnosed with canine distemper in the Animal Pathology Laboratory of the Federal University of Campina Grande, Campus of Patos, Paraíba, between January 2000 and December 2012.

The cases that had concurrently cystic structures showing intralésional characteristics of protozoa morphologically compatible with *T. gondii* were separated. These cases were assessed via the clinical

signs, as well as macroscopic and microscopic changes described in necropsy reports.

For confirmation, immunohistochemistry (IHC) was performed using the anti-*T. gondii* antibody with tissues embedded in paraffin blocks. The criterion for selection of the blocks was the presence of intralesional protozoal characteristic structures in tissue stained with hematoxylin and eosin.

We cut 3- $\mu$ m sections of the tissue in paraffin blocks and applied them to slides (ImmunoSlide-EasyPath). Then, the sections were de-paraffinized and rehydrated. The endogenous peroxidase was blocked by incubating the tissues twice for 10 minutes in a 3% hydrogen peroxide solution in distilled water and subsequently washing them in distilled water. Antigen retrieval was performed using citrate buffer (pH 6.0) in a microwave oven for 10 minutes at full power. Soon after, the slides were cooled for 10 minutes in a cold-water bath. To decrease non-specific binding (background), the sections were treated for 30 minutes with 5% powdered and skimmed milk (Molico) in distilled water. The sections were incubated in a humid chamber at 37° C for 60 minutes with the primary polyclonal antibody anti-*T. gondii* produced in rabbit diluted to 1:100 in antibody diluent (PBS - pH 7.4, EasyPath). The sections were then treated

with biotinylated secondary reagent followed by streptavidin-peroxidase (LSAB+System-HRP, Dako) and incubated at room temperature for 30 minutes in a humid chamber; they were then marked by adding the chromogen 3,3'-diaminobenzidine (DAB; Dako) for 2 minutes, followed by inactivation with water. The sections were washed and lightly counterstained with Harris hematoxylin, and then washed in running water for 10 minutes, dehydrated in alcohol graduations, clarified in xylol, and mounted with coverslips and Entellan. As a negative control, the same sections were used, replacing the primary antibody with a PBST.

## Results

From January 2000 to December 2012, 1022 dogs were necropsied, and 132 (12.9%) were diagnosed as cases of canine distemper. Of those, five cases of distemper were associated with characteristic parasitic cysts of *T. gondii* intralesional (3.7%), with four cases in the brain and one in the lung.

Clinical signs were similar in all cases and also suggestive of infection by canine distemper virus. These signs included digestive disorders, as well as respiratory, neurological, and ocular lesions (Table 1).

**Table 1.** Epidemiology and clinical of toxoplasmosis cases associated with distemper in dogs in the semiarid region of Paraíba during the period from January 2000 to December 2012.

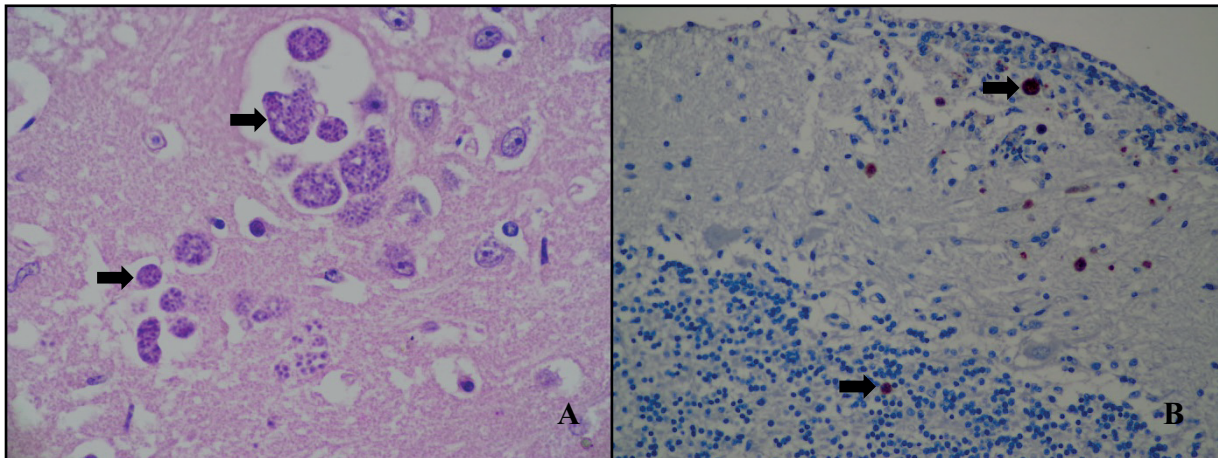
Case	Breed/Sex/Age	Clinical signs
1	Belgian shepherd, Female, 5 months	Eye discharge, salivation, diarrhea, myoclonus
2	Mixed breed, Male, 7 years	Anorexia, cough, unsteady walk, tenesmus, prostration, strabismus, myoclonus
3	Mixed breed, Female, 8 years	Eye discharge, diarrhea, myoclonus
4	Pinscher, Female, 3,7 years	Fever, eye and nasal discharge, diarrhea, pulmonary rales, circling
5	Mixed breed, Male, 6 months	Anorexia, eye and nasal discharge, salivation, vocalization, myoclonus

The five dogs had a clinical diagnosis of distemper, confirmed by presence of bodies of eosinophilic intranuclear and intracytoplasmic viral inclusion in different tissues on histopathological analysis, which also revealed the presence of characteristic parasitic cysts of *T. gondii* in the brain in cases 1, 2, 3 and 4, and only in the lung in case 5.

Microscopically in the brain, the lesions were characterized by multifocal areas of malacia associated with rounded, strongly basophilic structures, measuring approximately 5 to 70µm, and containing a number of basophilic structures

corresponding to bradyzoites delimited by a thin wall, all *T. gondii* characteristics (Figure 1A). Encircling these areas were mononuclear cell infiltrates composed predominantly of lymphocytes, plasma cells, macrophages and rare neutrophils. In two cases, the lesions were seen in the cortex and cerebellum (cases 1 and 2). In case 3, the lesions were restricted to the cerebellar peduncle. In case 4, the lesion was present in the frontal cortex, basal ganglia, thalamus, hippocampus, cerebellum and cerebellar peduncle, with loads of gitter cells and mononuclear leptomenigitis with infiltrate composed of macrophages, lymphocytes, and plasma cells.

**Figure 1.** Toxoplasmosis in dogs. A) Toxoplasmosis, cerebral cortex (gray matter). Observed are strongly basophilic round structures multiple delimited by a thin wall, cysts of *Toxoplasma gondii* features (arrows). HE. Obj. 40x. B) Toxoplasmosis, cerebellum. Positive immunomarking for *T. gondii*, the protozoan showing multiple exemplary in the granular and molecular layers (gray matter) (arrows). Immunohistochemistry, method of streptavidin-biotin-peroxidase, counter-stained with Harris hematoxylin. Obj. 20x.



In case 5, the parasitic cysts were observed only in the lung. The lesion was characterized by thickening of the alveolar septa by mononuclear inflammatory infiltrate composed of macrophages, lymphocytes and plasma cells, with moderate proliferation of type II pneumocytes. Some had multifocal areas of necrosis coalescing with macrophages associated with *T. gondii*.

The histological findings compatible with distemper were characterized mainly by non-suppurative encephalitis, demyelination and perivascular cuffing discrete, associated with intranuclear eosinophilic inclusion bodies in astrocytes, and intracytoplasmic and intranuclear in neurons and ependymal cells. Bodies of inclusion were also observed in extra-neural organs, such as



the main and parietal cells of the gastric mucosa, the urothelium of the bladder and renal pelvis, the bronchus and bronchiole epithelium, and the epithelium of pancreatic acinar ducts and mononuclear cells.

In the five cases described here, immunohistochemical analysis of tissue that had cystic structures of *Toxoplasma gondii* showed marking positive (Figure 1B). Tachyzoites of characteristic structures were also observed in the cytoplasm of macrophages and endothelial cells of vessels.

## Discussion

The diagnoses of toxoplasmosis associated with infection by canine distemper virus in the five case studies were based on microscopic findings and confirmed by immunohistochemistry.

Immunohistochemical analysis is important to differentiate *T. gondii* and *Neospora caninum*, since these protozoa are related and structurally similar. However, *T. gondii* is almost always associated with concomitant disease such as distemper in dogs, while the *N. caninum* appears to be a primary pathogen (MAXIE; YOUSSEF, 2007; LAPPIN, 2008; ZACHARY, 2013).

The clinical disease caused by *Toxoplasma gondii* is rare. Similarly, it is widely known that severe clinical manifestation of toxoplasmosis occurs in immunosuppressed dogs (MORETTI et al., 2002). Additionally, there are reported cases of dogs with neurological disorders and the presence of *T. gondii* cysts in which lesions consistent with distemper were absent (GIRALDI et al., 2002).

Of the five cases of *T. gondii* associated with infection by canine distemper virus, four had involvement of the brain. These two conditions can occur concomitantly, and the nervous signs observed are indistinguishable. There are also high mortality rates associated with the simultaneous

occurrence of these two agents in dogs (NELSON; COUTO, 1994; MORETTI et al., 2002).

Silva et al. (2007) demonstrated that toxoplasmosis was the most frequently observed co-infection in dogs with a neurological form of distemper, with a case of *T. gondii* in the brain, and four with visceral toxoplasmosis.

Information about contact frequencies with cats was not included in the original case history of the dogs. Despite the lack of data regarding the history of these animals, we suggest that the absence of immunoprophylaxis for distemper may have led to occurrence of the same, with consequent infection or reactivation of *T. gondii* being fatal. Important risk factors for the occurrence of toxoplasmosis in dogs are eating raw meat, free access to the street, food or water contaminated by feces, and/or cohabitation with cats (MORETTI et al., 2002; DUBEY, 2004; AZEVEDO et al., 2005).

## Conclusions

Toxoplasmosis should be included in the differential diagnosis of dogs with severe progressive systemic signs, especially with respiratory and neurological involvement, causing worsening of the clinical frame and disease progression to death.

The occurrence of fatal disease in immunosuppressed dogs in the home environment reinforces the importance for monitoring public health action to control this zoonosis.

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