TOXOPLASMOSIS: MORPHOLOGICAL EVALUATION OF SPINAL CORD NEUROGLIA FROM NONSYMPTOMATIC SEROPOSITIVE DOGS

(Toxoplasmose: avaliação morfológica da neuróglia da medula espinhal de cães soropositivos assintomáticos)

Alessandra Cristina Francischini de Carvalho¹, Maria Rita Pacheco¹, Silvana Martinez Baraldi-Artoni¹, Annita Morais Girardi²*

¹ Departamento de Morfologia e Fisiologia Animal, Faculdade de Ciências Agrárias e Veterinárias – UNESP Campus de Jaboticabal.
² Departamento de Clínica e Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias – UNESP Campus de Jaboticabal. Autor para correspondência.

ABSTRACT

This paper aims to analyze the morphology of the cells that compose the neuroglia of cervical, thoracic and lumbar spinal cord of non symptomatic dogs seropositive for Toxoplasma gondii. Twenty adult, mongrel dogs were used; ten healthy dogs, with negative serology for Toxoplasma, used as the control group (group 1) and ten non symptomatic dogs but seropositive for Toxoplasma gondii (group 2). After microtomy, the histological sections were stained with hematoxylin-eosin (HE), Masson's trichrome (MT), silver impregnation and by peroxidase-antiperoxidase (PAP) method. The slides were analyzed under optical microscopy to verify the morphology of these cells. The morphological characteristics between the two groups were similar and in accordance with literature. Thus, it was concluded that toxoplasmosis does not cause changes, visible by optical microscopy, in the neuroglia of the spinal cord of non symptomatic seropositive dogs.

Key-words: canine, central nervous system, histology, Toxoplasma gondii

RESUMO

Este trabalho objetivou analisar a morfologia das células que compõem a neuróglia das regiões cervical, torácica e lombar da medula espinhal de cães assintomáticos soropositivos para toxoplasmose. Utilizou-se 20 cães sem raça definida, adultos, dos quais dez apresentaram sorologia negativa para toxoplasmose, utilizados como controle (grupo 1) e dez foram assintomáticos, mas soropositivos para toxoplasmose (grupo 2). Após microtomia semi-seriada, os cortes histológicos foram corados pelas técnicas da hematoxilina-eosina (HE), do tricrômico de Masson (TM), da impregnação pela prata e pelo método imunoenzimático indireto da Peroxidase Antiperoxidase (PAP). As lâminas foram analisadas à luz damicroscopia óptica para verificar a morfologia destas células. As características morfológicas entre os dois grupos foram semelhantes e em conformidade com a literatura clássica. Assim, concluiu-se que a toxoplasmose não causa alteração morfológica, visível à microscopia óptica, na neuróglia da medula espinhal de cães assintomáticos soropositivos. Palavras-chave: canino, sistema nervoso central, histologia, Toxoplasma gondii

*Endereço para correspondência:
E-mail: annitamgirardi@gmail.com
INTRODUCTION

Neuroglia comprises astrocytes, oligodendrocytes, microglial and ependymal cells. Astrocytes have shape similar to a star, with two main morphologic types: protoplasmic, found in gray matter, with thicker, shorter and profusely branched extensions; and fibrous, in white matter, with long, thin and less branched extensions. Ependymal cells are cuboid or prismatic, present in natural cavities of CNS, have epithelial arrangement, microvilli usually with cilia in their luminal wall and one extension or basal process that penetrates the nervous tissue around cavities. Oligodendrocytes, found in white and gray matter, are smaller than astrocytes, have few short extensions, and produce the myelin sheath of axons. Microglial cells are small and elongated, having few short extensions that come out from their edges with dense, elongated and irregular nucleus, covered by thin spikes, found in white and gray matter and have phagocytic functions (Hirano, 1985; Chrisman, 1997; George & Castro, 1998; Machado, 2002; Ross & Pawlina, 2011; Junqueira & Carneiro, 2013).

Encephalitis in dogs are often caused by *Toxoplasma gondii* (*T. gondii*) and *Neospora caninum* (Paixão & Santos, 2004). Dogs are considered very receptive animals for toxoplasmosis, probably due to their carnivorous eating habit, what facilitates the ingestion of tissues contaminated by cysts and their contact with sporulated oocysts in contaminated soil (Germano, 1985). The infection has been noticed in cats and dogs in many countries.

Severe and fatal cases have been reported although clinical manifestations are uncommon due to the efficiency of *T. gondii* parasite (Hass et al., 1989; Lindsay, 1990; Guimarães et al., 1992; Paixão & Santos, 2004). Although many animals are serologically positive for toxoplasmosis, few develop clinical signs of disease (Lappin, 2004; Dubey & Lappin, 2012). It is presumed that disease manifestation occurs by local or systemic immune deficit of host organism, so that immunosuppressed patients may have primary or recurrent infection (Swinger et al., 2009). The most common histopathologic changes in CNS toxoplasmosis are non suppurative meningoencephalomyelitis with vasculitis, necrosis, malacia and gliosis with possible involvement of peripheral nerves (Dubey & Lappin, 2012; Giraldi et al., 2002).

Histopathological evaluation of brains and spinal cords of *T. gondii*-infected mice revealed comparable pathological processes, with high counts of infiltrated inflammatory cells, presence of cysts mostly in the grey matter, neuronal degeneration and hemorrhage. Inflammatory foci and *T. gondii* cysts were widely recognized in the brain and the spinal cord without any preference for a specific area. Despite the presence of recruited inflammatory cells and generalized activation of resident cells, no obvious changes in the myelin staining intensivity or axonal density could be observed in the spinal cords. Resident microglia and astrocytes displayed a strong activation in the grey and white matter in the spinal cord, with control animals showing only very small foci of astrocytes whereas in infected animals, the background is filled with processes of activated astrocytes (Möhle et al., 2014). Carvalho et al. (2015) reported similar morphological characteristics in spinal neurons of dogs negative for toxoplasmosis and seropositive non symptomatic dogs, but morphometric results showed changes in neurons size, structure and loss of star shape in seropositive animals.

Considering the histopathological changes observed in the CNS with clinical
toxoplasmosis, and the absence of researches in dogs infected but without clinical signs, this study aimed to analyze the occurrence of histological alterations in neuroglia structures of the spinal cord of non-symptomatic dogs with high serological reactivity to toxoplasmosis.

MATERIAL AND METHODS

For the proposed objectives were used twenty indefinite-breed adult dogs, weighing from 7 to 15 kg, from the Zoonosis Control Center of Araraquara city, state of São Paulo, Brazil. The serology for *T. gondii* was performed by Enzyme-Linked Immunosorbent Assay (ELISA), with technique described by Domingues et al. (1998), in Department of Veterinary Pathology of School of Agrarian and Veterinary Sciences, São Paulo State University, Jaboticabal, Brazil. The reactivity of sera was analyzed in terms of ELISA levels (from 0 to 9) and the animals used in this study were those that presented reactivity 8 or 9. Ten dogs, with negative serology for *T. gondii*, were used as control group (group 1) and ten non-symptomatic dogs, with levels of reactivity for *T. gondii* over 8, detected by Enzyme-Linked Immunosorbent Assay (ELISA), used as reagent group (group 2).

After the formation of groups 1 and 2, fragments of spinal cord corresponding to cervical, thoracic and lumbar spinal cord were collected at post-mortem, being removed from the medullary canal using a blunt-pointed forceps, and fixed in Bouin’s solution for 24 hours and processed routinely for paraffin embedding.

After microtomy at interval of 100µm, histological sections of 5µm thickness were stained with hematoxylin-eosin (HE) and Masson's trichrome (MT) techniques. Slices of 15µm thickness were stained by silver impregnation. All three techniques were performed according to Tolosa et al. (2003). Slices of 5µm thickness were stained by the immunohistochemistry PAP (peroxidase antiperoxidase) technique using the primary antibody anti-glial fibrillary acidic protein (anti-GFAP, according to Lemos & Alessi (1999), to identify astrocytes.

The glass slides were examined by optical microscopy to verify the morphology of structures and were photographed using a photo microscope model Olympus BX50 (Olympus America Inc., New York, USA).

RESULTS AND DISCUSSION

The morphology of astrocytes, revealed by enzyme immunoassay of PAP, was characteristic and easily viewed because of the brown color of their cytoplasm. The astrocytes located in the white matter, known as fibrous, had long and less branched extensions (Fig. 1), and the protoplasmic astrocytes, located in the gray matter, presented short and profuse extensions (Fig. 2). The observations on the astrocytes recall the reports of Hirano (1985), Machado (2002), Carvalho et al. (2005) and Junqueira & Carneiro (2013), when they recognize two major morphologic types of this cell: protoplasmic and fibrous. The distinction of two types of cells based on their location and number of cytoplasmatic extensions agrees with Machado (2002), Ross & Pawlina (2011), and Junqueira & Carneiro (2013). The GFAP, subunit of the intermediary filaments of the cellular cytoskeleton, exists in the cytoplasm of astrocytes, so immunohistochemistry using primary antibody anti-GFAP is generally chosen to identify astrocytes in the CNS (Lemos & Alessi, 1999; Ross & Pawlina, 2011).

The ependymal cells from studied regions of the spinal cord of dogs presented simple epithelial arrangement, with cylindrical cells with cilia, in both groups.
(Fig. 3), matching what was written by George & Castro (1998), Machado (2002) and Junqueira & Carneiro (2013).

The histological sections, impregnated with silver, presented the microglia with elongated cellular body, being invisible the nucleus and the cytoplasmic processes, characteristics observed in both groups (Fig. 4), what facilitated the differentiation of other cells of neuroglia, which had spherical nucleus. The oligodendroglia showed, by the method of silver impregnation, round cellular body (Fig. 4), but nucleus and cytoplasmatic extensions were invisible. These observations were similar in the mentioned segments of the spinal cord of dogs in both groups. The evidences on oligodendroglia and microglia resemble the illustrations on Di Fiore et al. (1982) and the reports of Carvalho et al. (2005) when they mention, using the silver impregnation method, that the cellular body of oligodendroglia is round, basic characteristic, to differentiate from microglia, whose cellular body is elongated and short.

The cells of neuroglia were present, their morphology remained unchanged and with similar aspect in both groups, in the three spinal cord segments. Thus, it can be considered that highly reactive animals to toxoplasmosis by ELISA test, but asymptomatic, showed no morphological differences in neuroglia by optical microscopy compared to nonreactive animals.

There is a need for differential diagnosis of toxoplasmosis in dogs with nervous symptoms in relation to other diseases that also affect this system (Moretti et al., 2002). Plugge et al. (2011), after a study in which 21.08% of dogs with nervous signs were seropositive for T. gondii by means of the indirect fluorescent antibody test (IFAT), recommended serological tests in diagnosing neurological diseases in dogs. However, this present study indicate that an animal positive to toxoplasmosis does not necessarily exhibit neurological signs due to nervous lesions by this disease.

The morphological characteristics described disagree with Möhle et al. (2014), which observed, by histopathological examination of different segments of spinal cord of T. gondii-infected mice, high counts of inflammatory cells, cysts mostly in the grey matter, neuronal degeneration, hemorrhage, resident microglia and astrocytes displaying strong activation in grey and white matter, while control animals showed very small foci of astrocytes. These authors suggest rather altered function of the spinal cord neurons and less or no structural alterations (Möhle et al., 2014), situation that cannot be excluded in relation to neuroglia, but can only be evaluated using other scientific methods.

CONCLUSIONS

It was concluded that toxoplasmosis does not cause visible changes by optical microscopy in the neuroglia of the spinal cord of non symptomatic seropositive dogs. This observation is important for differential diagnosis of neurological diseases in dogs.

REFERENCES


Figure 1. Photomicrographies of astrocytes from canine spinal cord white matter of control (A) and seropositive for toxoplasmosis group (B), showing fibrous astrocytes (arrow). Peroxidase-antiperoxidase.
Figure 2. Photomicroographies of astrocytes from canine spinal cord gray matter of control (A) and seropositive for toxoplasmosis group (B), showing protoplasmic astrocytes (arrow). Peroxidase-antiperoxidase.

Figure 3. Photomicroographies of ependymal cells (arrow) from canine spinal cord central canal of control (A) and toxoplasmosis seropositive group (B). Masson's trichrome.

Figure 4. Photomicroographies of neuroglia from canine spinal cord gray matter of control (A) and toxoplasmosis seropositive group (B), showing oligodentrocyte (arrow) and microglia (arrow head). Silver impregnation.