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Histopathological features of the brain extracellular matrix from dogs with canine distemper

[*Caracterização histopatológica da matriz extracelular do sistema nervoso central de cães com cinomose canina*]

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ABSTRACT

Canine distemper causes demyelinating leucoencephalitis, like human multiple sclerosis. The encephalic microenvironment, including the extracellular matrix, is an important key factor of this lesion, already described in multiple sclerosis but not proved in canine distemper. Thereby, the aim of this work is to characterize the extracellular matrix in the encephalon of dogs with canine distemper. Samples of cortex and cerebellum of 14 naturally infected dogs with canine distemper virus were collected after being sent for necropsy in the Animal Pathology Laboratory of the Veterinary Hospital of Uberlândia Federal University. The samples were processed as routine, stained with Hematoxylin and Eosin (H.E), Masson Trichrome (MT), Periodic Acid-Schiff (PAS) and Reticulin, and then described. Areas of demyelination and necrosis were quantified in percentage of stain. The TM samples showed blue stain around vessels and meninge, which indicates a higher deposition of collagen in lesioned areas. At necrotic areas, reticulin stain pointed to a disorganization in the vascular wall and PAS-stained pink granules in macrophages. We conclude that the extracellular matrix seems to participate in the pathogeny of canine distemper. More research should be done to better detail the involvement of these molecules in the course of this disease.

Keywords: neuropathology, histochemical, demyelination, canine Morbillivirus

RESUMO

A cinomose canina, assim como a esclerose múltipla, provoca leucoencefalite desmeilinizante. O microambiente encefálico, incluindo a matriz extracelular, atua como fator adjuvante na esclerose múltipla, porém não há comprovação do mesmo na cinomose canina. Portanto o objetivo neste trabalho é caracterizar a matriz extracelular do encéfalo de cães com cinomose canina. Foram coletados fragmentos de córtex frontal e cerebelo de 14 cães naturalmente infectados com cinomose canina encaminhados para necropsia no Laboratório de Patologia Animal do Hospital Veterinário da Universidade Federal de Uberlândia. Os fragmentos foram processados rotineiramente, corados com Hematoxilina e Eosina (H.E.), Tricrômico de Masson (TM), Ácido-Periódico de Schiff (PAS) e Reticulina e então foram descritas. As áreas com desmielinização e/ou necrose também foram quantificadas. A coloração de TM evidenciou marcação azulada em vasos e meninge, indicando maior deposição de fibras colágenas nas lesões. Nas áreas de necrose notou-se parede vascular desorganizada, a partir da reticulina, além de pigmentos róseos em macrófagos corados com PAS. Conclui-se que a matriz extracelular aparenta participar na patogenia da cinomose. Outros estudos são necessários para maiores detalhes da ação dessas moléculas na patogenia da cinomose canina.

Palavras-chave: neuropatologia, histoquímica, desmielinização, Morbillivirus canino

INTRODUCTION

Canine distemper virus (CDV) is a *Morbilivirus* from the *Paramyxoviridae* family that causes disease in canines but also reported in wild animals, including non-human primates (Loots *et al*., 2017). In the central nervous system (CNS) it causes mostly demyelination, sometimes associated with leukoencephalitis with intense lymphoplasmacytic inflammation. This lesion is similar to the described in measles and especially in human multiple scleroses. Thereby, the zoonotic potential of this disease is an increasing concern (Lempp *et al*., 2014).

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Demyelination in canine distemper (CD) has multiple causes, like injury in oligodendrocytes and astrocytes, and disturbs in blood and cerebrospinal fluids (Pan *et al*., 2013). Furthermore, Sheehusen *et al*. (2016) show an association of the lesion with extracellular matrix (ECM) degradation of the CNS caused by imbalance of metalloproteinases and their inhibitor.

The ECM from CNS is composed mainly of the basal membrane from the blood-brain barrier (Novak and Kaye, 2000). Therefore, the impairment of the matrix results in higher permeability of the barrier that favors the virus entry in the brain. (Lempp *et al*., 2014; Novak and Kaye, 2000). In addition to that, fibronectin aggregates and metalloproteinases imbalance can cause demyelination and prevent remyelination, proved in multiple sclerosis and post-infectious encephalitis caused by measles virus (Lempp *et al*., 2014; Seehusen *et al*., 2016; You and Gupta, 2018).

The ECM influence in demyelination and remyelination in canine distemper has few evidence, even though this disease shows similar lesions to multiple sclerosis. Therefore, the aim of this paper is to characterize the brain extracellular matrix in lesioned areas in dogs naturally infected with canine distemper in order to contribute to the understanding of the pathogeny of this lesion as well as the failure in remyelination in this disease.

MATERIAL AND METHODS

The sample collection followed the recommendation of the Ethics Committee on the Use of Animals (CEUA) from the Uberlândia Federal University (UFU) under the protocol number A005/02.

Fourteen dogs assisted in the Veterinary Hospital of UFU were referred to the Animal Pathology Laboratory of UFU. The animals were sent to necropsy, and they all had Canine Distemper as clinical suspicion, some also had positive serological test for the disease.

Samples of frontal lobe and cerebellum of all animals were collected, totalizing 28 fragments. All samples were submitted to immunohistochemical and reverse transcription-polymerase chain reaction (RT-PCR) tests to confirm the canine distemper diagnosis (Furtado *et al*., 2017).

To histopathology all samples were fixed in 10% buffered formaldehyde, processed in alcohol and xylol, embedded in paraffin and trimmed at 4μm. The stains used were Hematoxylin and Eosin (H.E.), Masson Trichrome (MT), Periodic-acid Schiff (PAS) and Reticulin. All staining methods followed the manufacturer recommendation.

After stained, all slides were scanned using Aperio Scanscope AT - Leica®, available at the Multiuser Laboratory of UFU (RELAM-UFU). Slide reading was made using the software ImageScope. Histopathological features were described individually to emphasize the main characteristics of each stain method. The data of the descriptive analysis was grouped to compare similar characteristics of the different stain methods. We used areas of the same slides that showed no lesions as our control group to compare the accuracy of the stains.

After the descriptive analysis the slides were classified according to the presence of demyelination and necrosis. For demyelination it was selected areas from cortex and cerebellum that showed the lesion in all staining methods, including H.E. In these samples and in all samples that showed necrotic areas, pictures in TIFF resolution were made for posterior analysis using the ImageJ® software. We also selected areas with no lesion of the same animal, as a control to comparison.

At ImageJ® software analysis, the first step was scale setting according to the picture snapped in ImageScope. After setting scale we removed the scale label. Then, we changed the picture to RGB stack to improve the grey scale. After that, three windows were disposed, each one showing one of the primary colors named as follow: Blue, Green and Red. Green window was selected as the standard color because it showed the highest contrast. The threshold was manually regulated according to the wanted stained areas, compared to the original slide in reason to diminish staining artifacts. To finish, the option “measure” was selected, and it indicated the percentage of marking for each stain in the analyzed area (Fig. 1 and 2). All percentage data was tabled to comparison and is available on Table 1 and 2.

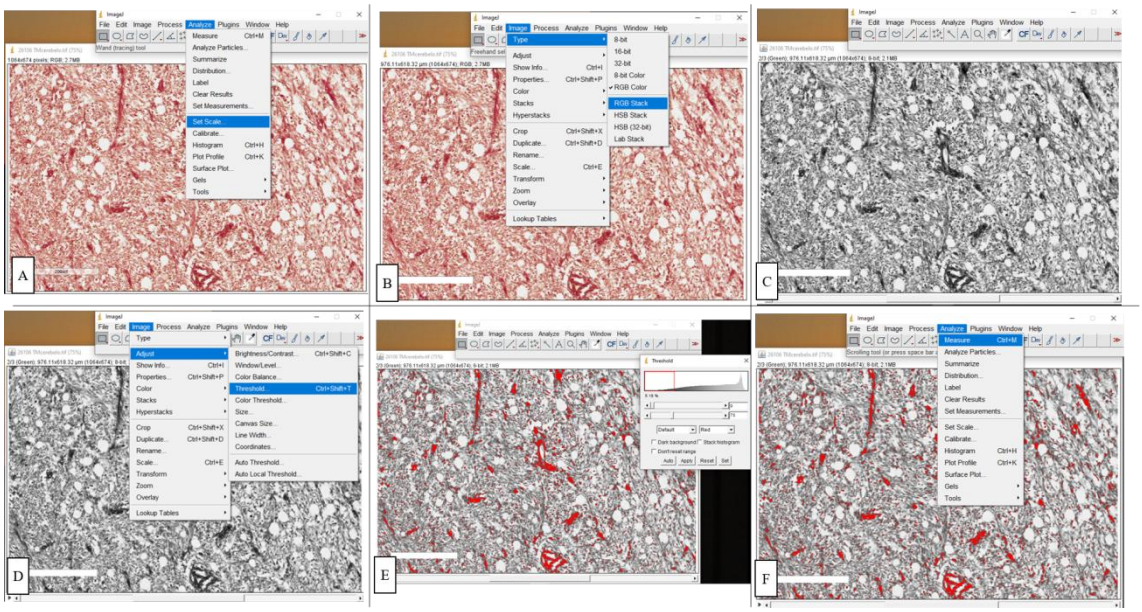

Figure 1. TM. Followed protocol to measure the percentage of marked area in the staining methods. A. The option "Analyze" was selected in the ImageJ software, followed by "Set Scale". The scale was modified to µm according to the scale bar from the ImageScope snapshot. After that the scale bar was removed. B. The option "Image" was selected, then "Type". The image was modified to RGB stack that opened three image channels (Red, Green, and Blue) C. Channel "Green" was elected for all images because it showed the highest contrast of marked areas D. The button "Image" was selected again and then "Adjust" and "Threshold". E. The threshold was manually selected, matching the measured area with the marking visualized in the slide. F. To finish, "Analyze" and "Measure" were selected, which resulted in a group of measurements that indicated the percentage of stained area.

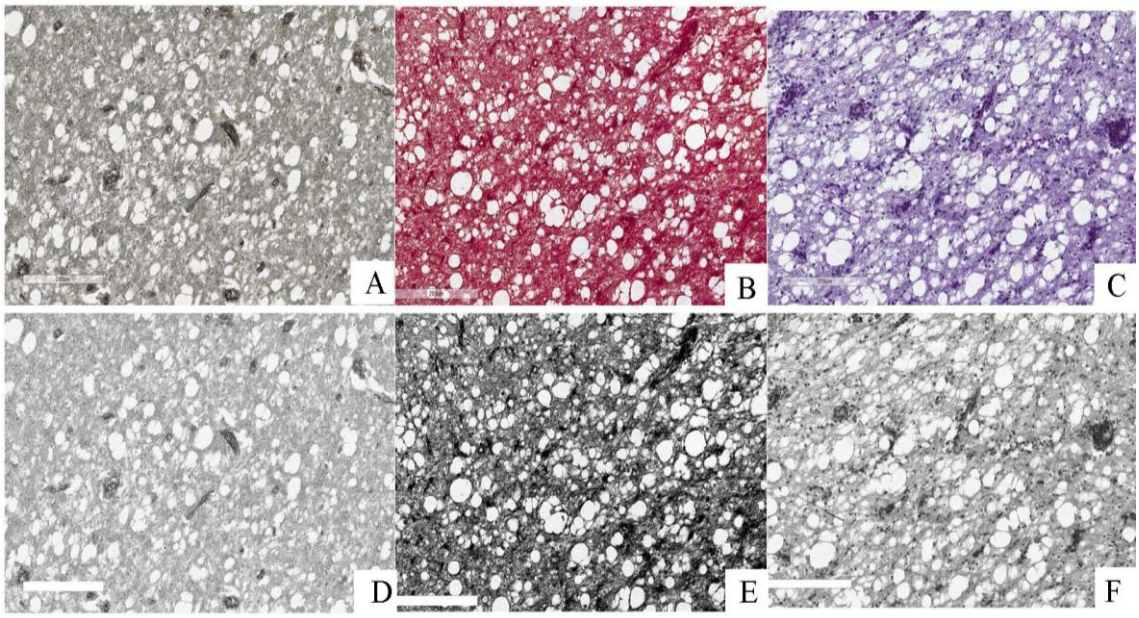

Figure 2. Reticulin (A and D), TM (B and E) and PAS (C and F). The lines below indicate the stains after the RGB stack selection in the software ImageJ. 100x.

Table 1. Percentage of marked area according to Reticulin, PAS and TM stain in areas of demyelination in analysis by the ImageJ software

	Reticulin	PAS	TM
25923 Cortex	0.397	7.772	15.584
25923 Cerebellum	3.470	3.594	28.077
25936 Cerebellum	2.620	13.297	3.023
25959 Cerebellum	4.024	14.462	5.177
26034 Cortex	13.146	35.169	11.379
26034 Cerebellum	47.842	27.342	13.965
26106 Cerebellum	1.928	12.022	8.832
26297 Cerebellum	3.342	7.164	10.403
26298 Cortex	8.544	4.816	5.237
26298 Cerebellum	2.991	10.660	2.638
26455 Cerebellum	0.722	8.377	5.086
Average	8.863	13.690	9.382
Standard deviation	13.689	9.715	7.417

Table 2. Percentage of marked area according to Reticulin, PAS and TM stain in necrotic areas in analysis by the ImageJ software

	Reticulin	PAS	TM
25821 Cortex	-	-	13.315
25821 Cerebellum	-	-	14.313
25923 Cerebellum	-	4.398	7.994
25936 Cerebellum	-	2.766	11.692
25959 Cortex	0.488	-	-
25959 Cerebellum	3.735	9.964	11.793
26106 Cerebellum	2.964	8.435	4.301
26188 Cerebellum	-	-	-
26267 Cerebellum	-	10.7	30.333
26297 Cerebellum	-	-	8.780
26298 Cerebellum	-	7.774	
Average	2.396	7.340	12.815
Standard deviation	1.696	3.135	7.780

RESULTS

The samples of cortex and cerebellum of all animals were analyzed after staining in Hematoxylin and Eosin (H.E.). The main lesions seen in this stain was demyelination (11/14 – 79%), followed by cortical laminar necrosis (9/14 – 64%) and perivascular cuffing (8/14 – 57%), indicating a frequent mononuclear inflammatory infiltrate (Fig. 3A-C), especially in the cortex.

Malacia was also another important lesion observed, and it was characterized by the presence of gitter cells and rarefaction of the cerebral parenchyma. This lesion was present in eight animals mostly in the cerebellum (Fig. 3D).

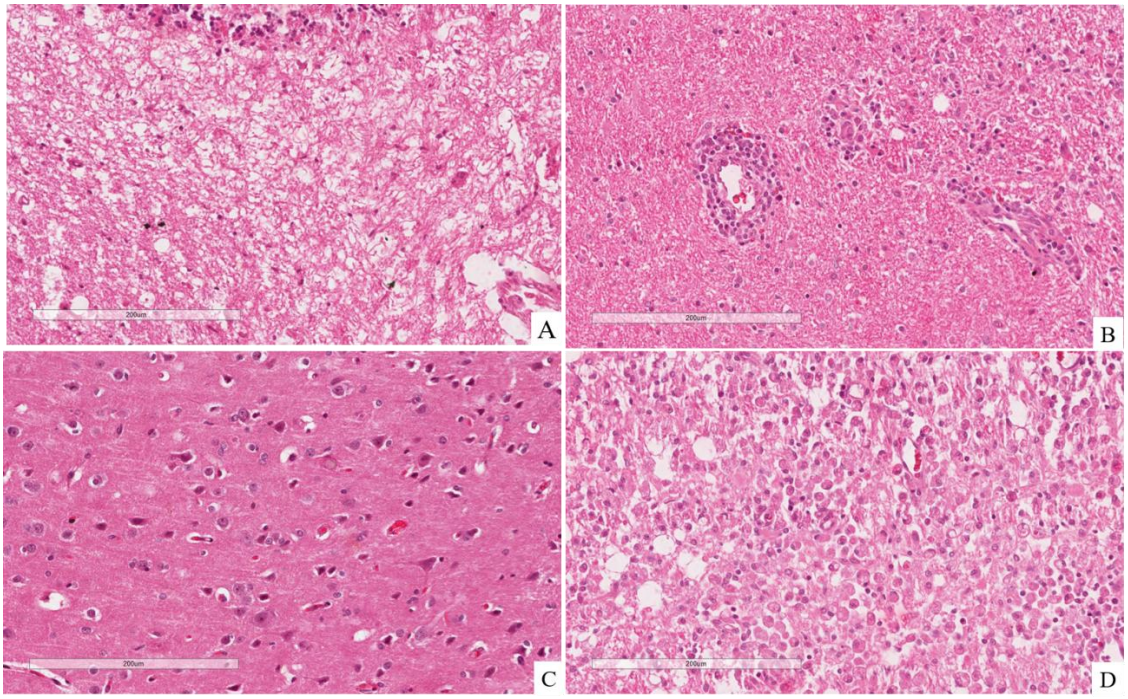

Figure 3. Samples of cortex and cerebellum in H.E. showing demyelination (A), perivascular cuffing (B), cortical laminar necrosis (C) and gitter cells in malacia (D) – 100x.

Finally, the H.E. stain also showed intranuclear inclusion bodies in astrocytes and neurons. They were in the cerebellum of eight animals, and in six of them the inclusions bodies were close to necrotic areas.

Trichrome Masson highlighted a red stain in the brain parenchyma. However, the blue stain was also present in some fragments, which indicated the presence of collagen fibers, located mostly in the meninges and around blood vessels of the parenchyma and meninges. The intensity of the red stain varied, showing some samples with lighter and some samples with darker red (Fig. 4D). Some slides also showed the brain parenchyma colored violet to blue (Fig. 4B).

Twenty-one samples showed the blue stain around blood vessels and meninges, and this was absent only in seven cortex samples (Fig. 4C). The blood vessels were the main location of the stain (17/21 – 81%), mostly the ones from the meninges, that counted 13 out of the 17 samples, and all located in the cerebellum. The staining was mainly discrete. The meninges also showed a blue stain in seven animals, and, in five of these, the blood vessels of the area were also marked (Fig. 4D).

TM was the stain that most identified necrotic areas (9/28 – 32%), when compared to H.E. The distribution of the lesion was also similar to the H.E. samples, where the cerebellum was the most affected area (8/9- 89%). After analyzing the staining intensity at the necrotic areas using the software ImageJ®, the TM stain showed a higher intensity of coloration than PAS and Reticulin, with an average of 12.13% of marked area. However, in the demyelinated areas, the intensity of stain was like Reticulin, and both were lower than PAS.

The areas without demyelination showed a higher average when compared to the demyelination areas in TM. In this staining method, the areas with no lesion had an average of 21.58% (SD±18,163) of marked area against 9.38% (DP±7,512) in the lesioned areas. In general, Reticulin and TM presented a higher intensity of coloration in the non-lesioned areas than in the lesioned areas. The opposite happened with PAS, where the lesioned areas had higher percentages of marking than the non-lesioned areas.

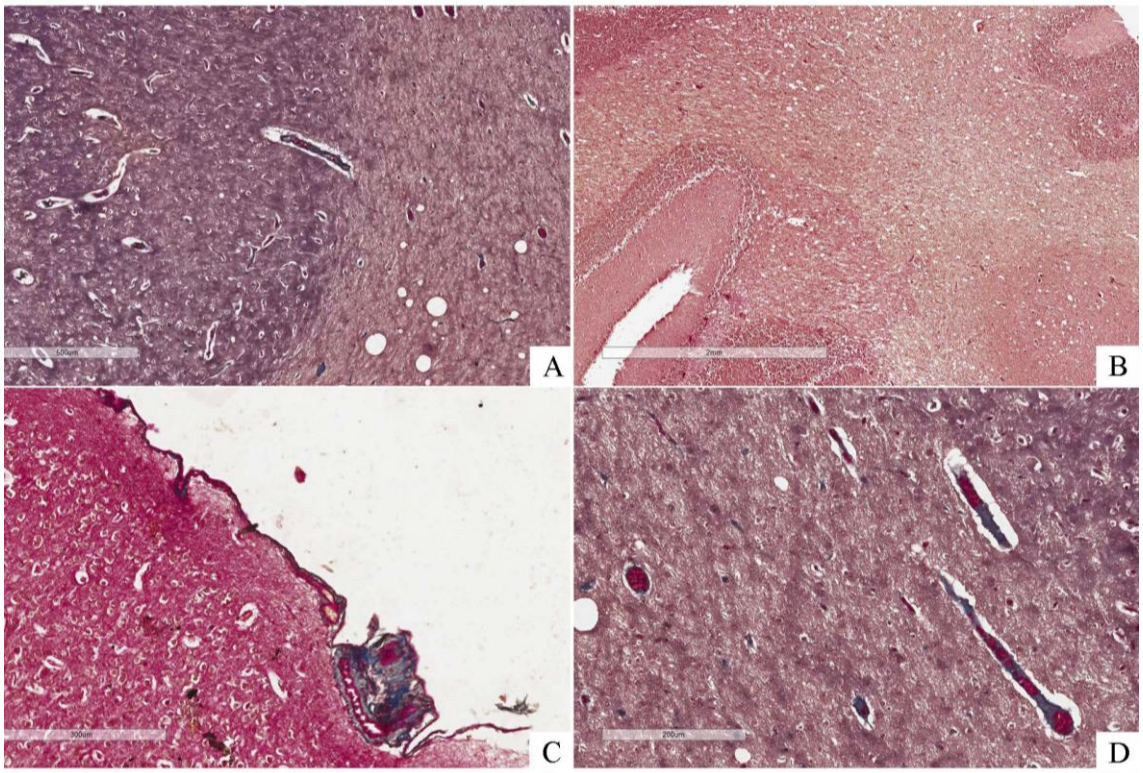

Figure 4. Samples of cortex and cerebellum in TM showing purple stain in the parenchyma (A), sometimes brighter (B). Meninges and blood vessels of the meninges (C) or parenchyma (D) showed a blue color indicating collagen fiber deposition. A and B- 50x. C and D –100x

Still about TM, in the necrotic area this stain showed an average of 12.81% (SD±7.78) of marked areas, against 21.23% (SD±16,574) in the normal areas. However, not all samples followed this decrease, and some presented higher percentage of marking in the necrotic areas. It is noteworthy to mention that the samples of PAS matched the increase and decrease of marking in the comparison of non-lesioned and demyelinated areas of the TM stain.

In all staining methods, the cortex showed a higher average of marked areas than the cerebellum.

The PAS stain highlighted pink granules in glial cells and macrophages. The necrotic areas were visible in six samples of cerebellum and were characterized by the presence of gitter cells. This stain identified only half of the necrotic areas showed with the H.E. stain. However, both stains indicated the presence of macrophages almost in all the same samples, except for one that was not visible in the PAS stain.

The marked structures in the cytoplasm of macrophages stained with PAS were round, sometimes punctual. In these areas the average percentage of marked area was 7.34% (SD±3.13), lower than TM and higher than Reticulin. Even though the macrophages showed this distinguish marking, the non-lesioned areas showed a higher percentage of staining, equal to 10.13% (SD±6.76).

In the demyelinated areas, PAS stain showed a higher average of marked area than the other two stains of 13.69% (SD±14.70). In this lesion there was a higher difference in PAS stain against Reticulin and TM, since innumerous areas that showed high marking in PAS had low percentages of staining area in the other stains. Only one sample varied, showing a higher percentage of marked area in Reticulin, of 47.84%, against 27.34% and 13.96% in PAS and TM, respectively.

The areas without demyelination were less stained (average of 10.9%, SD±9.92) than the

demyelinated regions in PAS. This was the only stain that showed this feature, and it was mostly present in cortex samples.

PAS was the stain that allowed more identification of demyelinated areas from all staining methods, including the routine H.E. From the 28 samples analyzed, 23 were positive for this lesion and most of them were seem in cerebellum (13/23 – 56.5%). They were classified mostly as moderate demyelination (11/23 – 47.8%) followed by discrete demyelination (10/23 – 43.5%). This was similar from the other staining methods that showed a prevalence of discrete demyelination.

Other specific marking of PAS in the encephalic parenchyma includes a pink staining in the blood vessels from 25 of the 28 samples. The presence of the stain varied from the blood vessel wall and the intravascular region (Fig. 5A), sometimes shown in both areas. The inflammatory cells in the perivascular cuffing were also marked. In this division the blood vessel walls were the most marked area, in 18 of the 25 samples. In five of these samples, the vascular wall was stained together with the intravascular area, in one sample the vascular wall and the perivascular cuffing were marked, in five fragments only the intravascular region was highlighted, and to finish, only two samples showed marking exclusively in the perivascular cuffing. These last two mentioned samples were also characterized with intense perivascular cuffing in H.E. stain. Marking in the perivascular cuffing was not seen concomitant with intravascular staining. Both cortex and cerebellum showed a similar vascular staining, and the graduation was majority discrete, followed by moderate and intense.

Other markings that stood out in PAS staining were in neurons, glial cells and macrophages (Fig. 5B-D). As said previously the PAS stain was highlighted in the interior of macrophages in all six necrotic areas. This marking was exclusive of this staining method, so both TM and Reticulin did not show a similar pattern of stain. However, the marking in neurons and glial cells were also present in the Reticulin stain. The TM stain showed pigmentation in neurons only in two samples of cortex. Still, PAS was the stain that showed the most intense staining in these cells, present in nine samples for neurons and 16 samples for glial cells. In both cells, the marking was present in the cytoplasm in round and dot shapes, in variable sizes but always occupying less than 50% of the cytoplasm. The neurons were marked equally in cerebellum and cortex, but the glial cells marking were more seen in cerebellum, in 10 samples, against six fragments of cortex. Some glial cells were located around demyelinated areas or in areas of astrocytosis, especially in cortex.

Besides that, the encephalic parenchyma showed 17 samples with pink dotted marking in the PAS staining, usually associated with areas of demyelination. Among these, 11 were from cerebellar region, whereas 6 were visualized in the cortex region. Only three samples marked exclusively the white matter, and the other 14 samples showed the marking in the grey and white matters. Two samples also showed the marking in the middle of the cells from the granular layer from the cerebellum, close to the demyelinated areas. The marking was mostly discrete (10/17 – 58.82%) even though it was frequent.

Finally, PAS was the staining that showed the less marking in the meninges, present only in eight of the 28 analyzed samples. From these, six were marking only the blood vessels of the meninges whereas two, in cerebellum, showed the marking in both meninges and its blood vessels.

In Reticulin the main feature and exclusive aspect of this stain was the disorganization of the vascular wall, located mostly in the demyelinated and necrotic areas. The most important marking in this staining was of the blood vessel wall, present in 100% of the samples. The samples varied in intensity of marking and vascular wall organization.

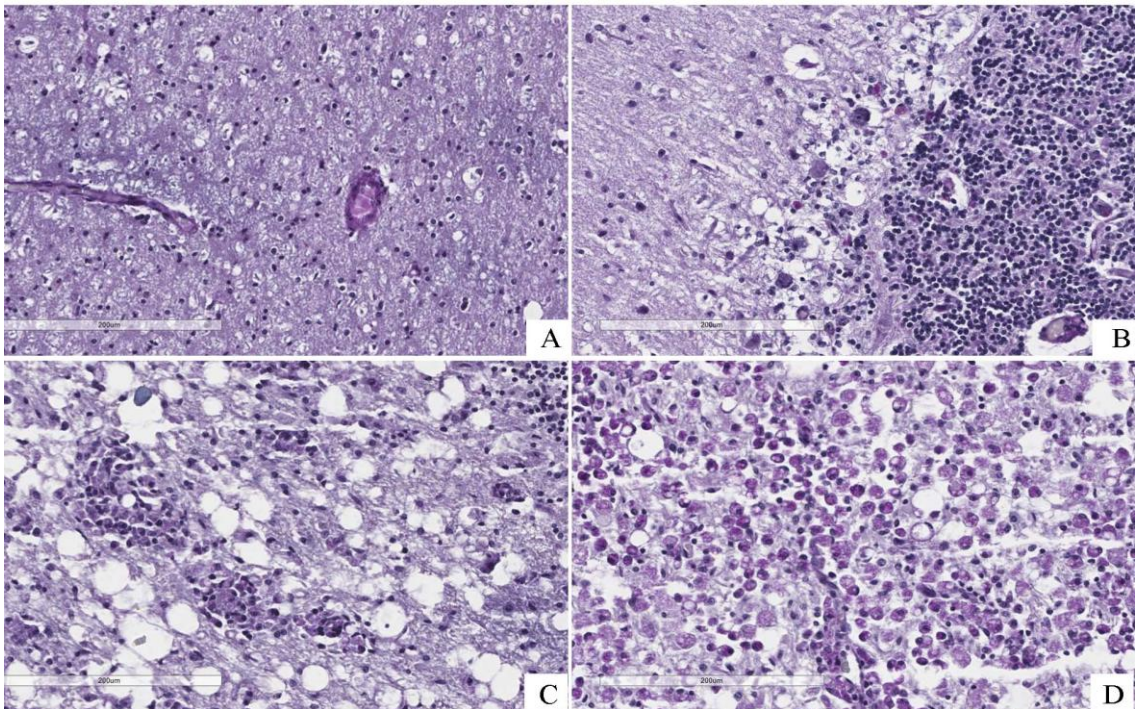

Figure 5. Samples of cortex and cerebellum showing changes identified with PAS stain. There was pink color in the blood vessels of the parenchyma, in the vascular wall and intravascular (A) Neurons and glial cells were also stained with pink in the cytoplasm (B). In necrotic areas there was pink stain in the glial cells and perivascular cuffing (C) as well as in macrophages (D). A- 100x. B, C and D –400x.

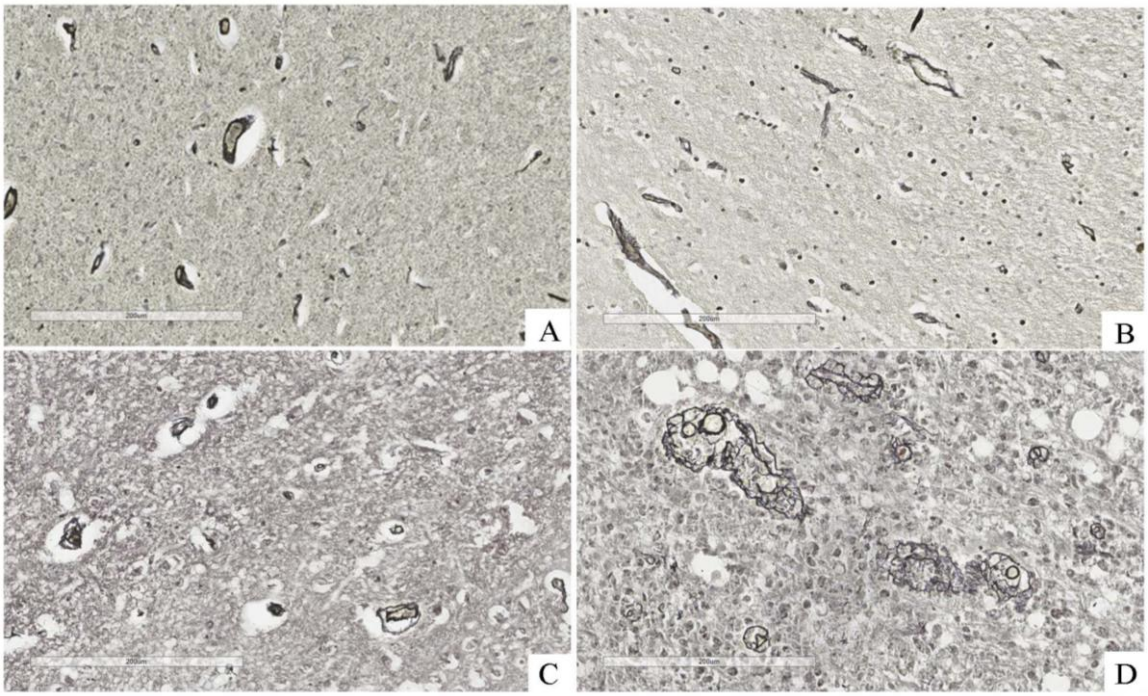

Figure 6. Samples of cortex and cerebellum showing reticulin stain in vascular walls. In this stain we noticed a disorganization of the vascular wall reticular fibers classified as: very organized (A), organized (B), disorganized (C) and very disorganized (D). 100x.

Besides the vascular walls, the meninges was also another marked area in the Reticulin stain, present in 25 of the 28 fragments. Only one sample of cerebellum and two cortexes did not show this marking. The intensity of the marking was similar, where eight were discrete, eight were moderate and nine were intense. The organization of the meninges was also pointed and 10 samples showed the meninges disorganized, whereas the very disorganized, organized and very organized were present in five samples each. The very disorganized meninges did not show an intense marking. This intensity of marking was mostly present in the disorganized classification. The cerebellum was the most affected region with disorganized meninges (7/10 – 70%). Now the cortex showed mostly an organized meninges (4/12 – 33.33%), but it was similar to the disorganized (3/12 – 25%) and very disorganized (3/12 – 25%).

Reticulin marked neurons and glial cells in five and 11 samples respectively. The neurons showed a brown to black color, mostly in the cytoplasm, but also present in the cellular membrane in one sample. Two marked samples were from cerebellum and three came from cortex. Only one sample showed neuronal marking in both PAS and Reticulin concomitant with cortical laminar necrosis in H.E.

In the glial cells the black color was distinguished in the nucleus with multifocal distribution. Only three samples with this staining in the cells did not show the same marking in PAS. This marking was mainly discrete in the cerebellum region, and discrete to moderate in cortex. Finally, only four of these 11 samples showed astrocytosis in the H.E stain.

The encephalic parenchyma was another place highlighted by the Reticulin stain. The marking presented different shapes, from punctual to bundles, distributed in the white and grey matters. Only one sample of cortex did not show any marking in these regions. This marking was classified in discrete, moderate, and intense, and moderate and intense were the most present, in 12 and 10 of the 27 samples, respectively. Still, the Reticulin stain showed the lower average of area marked, in all demyelinated, necrotic, and non-lesioned areas than TM and PAS. The samples with higher percentage of marked areas in Reticulin were associated with the higher marking in the parenchyma, sometimes showed as bundles following the format of the extracellular matrix.

To finish, Reticulin was the stain with less percentage of the marked area in the necrotic lesions, with an average of 2.40% (SD±1.69), but it was not different from the non-lesioned areas in the same samples (average of 2.14%±1.59). In demyelination, the Reticulin stain showed an average of 9.77% (SD±14.72), and it was lower than the non-lesioned areas (average of 11.40%±18.66). This increase in the percentage of marking in the non-lesioned areas followed the increase in the TM stain and it was predominant in the cortex. However, the PAS stain showed the opposite behavior, decreasing in the non-lesioned areas when compared to the demyelinated areas.

DISCUSSION

The main lesion of the encephalic samples in the studied canines was demyelination. The causes of this lesion are still debatable, even though it is the most described in this viral disease (Ecco *et al*., 2016). It seems that in the beginning of the acute infection the viral agent is the responsible for the lesion because of the lack of inflammatory cells in the region. Right after that, there is an increase of these cells that may contribute to the permanence of the lesion since the viral charge decreases in the cerebral tissue (Lempp *et al.,* 2014; Vandevelde and Zurbriggen, 2005). In this paper the demyelinated lesion exceeded the presence of perivascular cuffing, which indicates the acute phase in some animals.

The involvement of blood vessels was clear in all samples pointed by all staining methods, thus demonstrating the importance of the hematoencephalic barrier in the viral proliferation. This data is supported by other authors that indicate that in the beginning stage of the infection, sometimes before the demyelination, CDV is present in the encephalon, usually around blood vessels or in the cells of the choroid plexus (Pratakpiriya *et al*., 2017).

In TM some animals showed a higher marking of collagen fibers characterized by the blue color around blood vessels of the parenchyma and

meninges, indicating an involvement of these vessels in the pathogeny of the disease. An increase of marking of the collagen fibers in the basement membrane of blood vessels in the cerebral parenchyma is reported in cerebral injuries and occasionally in malformations in these vessels (Chen *et al*., 2020). According to Piera-Velazquez and Jimenez (2019), the endothelial cells may suffer conformational changes in some situations involving vascular, degenerative, inflammatory, and fibrotic diseases. That way, these cells lose some important features, like cellular junctions and polarity, mainly in the hematoenchephalic barrier, and they start showing mesenchymal features, like production of collagen fibers and even phenotypical changes presenting looser and with elongated shapes (Piera-Velazquez and Jimenez, 2019).

Krajewska *et al.* (2009) and Niesman *et al.* (2014) demonstrate a correlation between cerebral lesion, including neuronal necrosis and the TM stain using semiautomatic algorithms. In the lesioned areas and with a higher neuronal necrosis there is a decrease in the red stain and an increase in the shadow/penumbras around the lesion. This decrease in the coloration was also demonstrated in our paper, where necrotic areas had a lower average of marking area than the non-lesioned areas.

However, compared to the other stains TM showed the higher average of marking either in the lesioned or the non-lesioned areas. In this paper we used the color divided in the three primary color channels (RGB) and the color that showed the highest contrast was the green channel that was used in all staining samples. By choosing the green channel we excluded the red and blue colors, that way these colors showed less luminescence and higher contrast, which was measured by the ImageJ® software. Therefore, a higher area of marking indicates a higher presence of red or blue colors that are easily recognized in the TM stain.

Concomitantly with the higher marking in the TM stain we observed a lower average of the marked area in the PAS stain, also in the necrotic areas. The PAS stain showed two different marking patterns, where the cellular nucleus and cytoplasm stained in purple and the extracellular matrix stained in pink, varying in the intensity. However, in the necrotic areas the marking of the extracellular matrix was lower, which explains the decrease in the percentage of marking in these cases. This finding indicates that in necrosis the macrophages/gitter cells act in the phagocytosis of the extracellular matrix area, as also showed by Sobel and Ahmed (2001).

Other hypothesis, proved in lesions of multiple sclerosis is that the macrophages can act in the production of some proteoglycans of the extracellular matrix (Lau *et al*., 2012). Anyway, it is important to highlight the participation of the macrophages in the degeneration/regeneration of the extracellular matrix, an important factor for the success of the temptations of remyelination in the cerebral parenchyma (You and Gupta, 2018).

The PAS stain is responsible for the pink or magenta marking of carbohydrates or macromolecules rich in carbohydrates, especially represented by glycogen (Yamabayashi, 1987). That way the pink color in this stain can be explained by the fact that the extracellular matrix is made mainly by glycosaminoglycans, which are long chains of carbohydrates called polysaccharides (Novak and Kaye, 2000).

This stain was more accentuated in some animals, especially in the demyelinated areas in granular to round shapes. Some papers show an association among this marking with the presence of polyglucosan bodies that are structures formed by the accumulation of polysaccharides that were not degraded or were produced excessively. The presence of these bodies was already associated with innumerous cerebral lesions and degenerative diseases as Alzheimer in humans (Duran and Guinovart, 2015) as well as in elderly dogs, especially more than ten years old (Nešić *et al*., 2021). Astrocytes are cells that apparently have an important role in the degradation and storage of polysaccharides. The participation of the neurons has been investigated and it was already shown the accumulation of these substances in the cytoplasm of these cells, also seen in our experiment (Duran and Guinovart, 2015). Beside these two cells we observed the accumulation of these substances in the perivascular cuffing cells, endothelial cells or among these, indicating a possible role of these cells in the synthesis or

degradation of these carbohydrate rich substances.

The PAS stain was the one that marked more in the demyelinated areas than in the non-lesioned areas, probably characterizing the accumulation of polysaccharides in demyelination. Besides that, there was a higher intensity of stain in the cellular nucleus, mainly in the glial cells, and in some samples, there was an increase in the quantity of these cells.

Now, talking about Reticulin, this stain showed a strong marking in the vessels, which were expected since this stain highlights reticular fibers, present mainly in the basement membrane of the vessels (Novak and Kaye, 2000). However, we noticed that especially in the lesioned areas the same fibers lost their original conformation and organization around the blood vessels. Furthermore, these areas showed an increase in the marking of the reticular fibers loose in the parenchyma, not involved with the blood vessels. A similar finding was described by Seehusen *et al*. (2016) that indicated reticular fibers using Gomori stain. Other important finding of our paper is the intense reticulin marking in the meninges, which was not pointed by Seehusen *et al*. (2016).

That way we can indicate a relation between the disorganization of the reticular fibers either in the meninges or in the vessels, especially around the lesioned areas, with the canine distemper virus. Watanabe and Kakizaki (2017) identified a relation between the entry of neurotropic viral agents in the CNS with the inflammatory response of this organ. The authors indicate that in some systems the immune response activated by the virus facilitate the entrance in the CNS, and among these systems there was a higher production and conformational change of the reticular fibers, similar to the fibers found in lymph nodes that participate in the presentation of the agent to the inflammatory cells. In the CNS these cells present changes mainly in the basal membrane of blood vessels and in the meninges and this similarity indicate a participation of the reticular fibers in the entry of the agent in the CNS.

CONCLUSION

All stains used in this paper showed variable changes in the samples of central nervous system form dogs naturally infected with canine distemper. The main alterations were in the vascular wall, sometimes in the meninges, which showed a higher deposition of collagen fibers in TM and a higher disorganization of reticular fibers in Reticulin, especially in necrotic areas. Besides that, the PAS stain highlighted a possible storage of glycogen in the glial cells, sometimes in neuron bodies. Also, in areas of necrosis the cytoplasm of macrophages stained in pink. The features indicate a participation of the extracellular matrix in the pathogeny of Canine Morbilivirus in the central nervous system. However, more studies are needed to better detail this participation.

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