



## Case Report

# Spinal cord meningioma in a Boxer breed dog

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Submitted November, 16<sup>th</sup> 2019, Accepted December, 10<sup>th</sup> 2019

## Abstract

Meningiomas are one of the most common primary tumors of the canine central nervous system. They usually grow within the cranial cavity but occasionally they are found in the spinal cord. A case of a Boxer breed with a spinal cord transitional meningioma is reported. Two years history presenting progressive neurological signs is described. The MRI examination revealed a focal, intradural extramedullary lesion within the right side of the spinal canal, compromising the C1 and C2 vertebral segments. Cytoreductive surgery with a dorsolateral laminectomy approach was performed to decompress the spinal cord and to obtain a definitive diagnosis. Histopathological studies diagnosed a grade I transitional meningioma. Immunohistochemistry and immunofluorescence analysis detected positive cells to vimentin (VIM), pan-cytokeratin (pCk), neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP) and doublecortin (DXC). Although apparently benign, positivity to pCk and DXC suggest a possible transition into a malignant tumor. Although variations in the behavior, histology and the immunoreactive profile of these tumors are reported, the latter constitutes a good indicator for the diagnosis of the patient.

**Key words:** transitional meningioma, canine, MRI, immunohistochemistry, immunofluorescence.

## Introduction

Meningiomas belong to the group of most common primary tumors of the canine central nervous system (7, 10, 15). They usually grow within the cranial cavity (82%) but occasionally they are found in the spinal cord (15%) or the retrobulbar space (3%) (12). In 95% of the cases, its incidence has been reported in 7 or more years old dogs affecting mainly large breeds, such as Boxer and Golden Retriever. So far, sex predilection has not been demonstrated (3, 12, 25).

Spinal cord meningiomas are solitary, well defined, thin, gray to pink neoplasms that originate in the meninges. They can develop in the dura mater, the pia mater and, quite frequently, in the hair cells of the arachnoid (28). They grow slowly, extending through the subarachnoid space. The clinical signs are those that denote the compression of the spinal cord. Very rarely,

they show metastasis and invasion of the nervous tissue. Neurological signs are related to the anatomical location of these tumors because, when compressing nervous tissue, they cause cell destruction, generate edema and, in many cases, induce the appearance of hemorrhages (2). Histologically, meningiomas are classified as meningotheelial, fibroblastic, transitional, psammomatous, angioblastic, papillary, granular, myxoid, and anaplastic (7, 21).

The aim of the present work was to describe a clinical case in which the patient was reported to veterinary clinic due to pain and neurological signs of compression in the cranial cervical region of the spinal cord. Semiological maneuvers, complementary studies for imaging diagnosis, surgical intervention, histopathological diagnosis, immunohistochemical and immunofluorescent studies to characterize the sample are described.

## Case report

In 2016, a 9-years-old, 23 kg weight male canine Boxer was examined due to a pain in the pelvic limbs and difficulty to get up. X-rays analysis of the hip and lumbar spine revealed a spondyloarthritis of the region. A chondroprotector was then prescribed (Artrin, Laboratório Brouwer, Argentina), observing a desirable evolution. Two years later, weakness of the pelvic members was observed. At clinical exam, the animal moved supporting itself with the dorsal portion of the right thoracic limb and with signs of pain. Injectable glucocorticoids (triamcinolone acetone 0,6 g - Holliday Scott, Argentina) were prescribed and the patient was referred for consultation with a neurologist.

### *Neurological examination*

The dog showed a proprioceptive delay of the right thoracic limb, cervical ventro-flexion, with the scapulae displaced laterally and the pelvic extremities open. Likewise, amble marching was detected, where the center of gravity moved laterally with oscillations (wriggling). At that time, a presumptive diagnosis of disc extrusion/protrusion or neoplasia was established. As a palliative treatment, 3 mg/kg tramadol (John Martin, Argentina) every 8h as a central action analgesic, 20 mg/day prednisolone (Holliday Scott, Argentina), as an anti-inflammatory, and ranitidine (John Martin, Argentina) as a gastric protector was indicated. Also, a cervical radiograph was requested. One week after the onset of treatment the clinical status of the patient substantially improved.

### *Radiographic study*

No apparent osteoarticular alterations were observed (Fig. 1A and B); therefore, a magnetic resonance study was indicated.

### *Magnetic resonance (MRI) study*

The MRI examination revealed a focal, intradural extramedullary lesion within the spinal canal on the right side, compromising the C1 and C2 vertebral segments (Fig. 1C-F). The lesion was hypointense on T2-weighted images and hyperintense on T1-weighted images, as compared with the gray matter of the spinal cord. The lesion's width and length were 12 and 21 mm, respectively, and it occupied > 50% of the spinal canal at C1. A thinning of the tumor was detected extending caudally from the mass. Taking into consideration the dog's signalment, the progression of clinical signs and

MRI findings, a neoplastic process, such as spinal meningioma or other mesenchymal or round cell tumor, was considered most likely. Given the compressive characteristics of the mass a reductive surgery was suggested.

### *Surgery*

Cytoreductive surgery with a dorsolateral laminectomy approach was performed to decompress the spinal cord and to obtain a definitive diagnosis. An extramedullary, intradural, nodular and yellowish lesion was identified. The tumor surface was sectioned, and a biopsy sample was obtained by aspiration. At that time, it was observed that the roots of the spinal C1 and C2 nerves were immersed in the tumor mass; therefore, they were meticulously preserved by blunt dissection. Complete resection of the tumor mass was then performed, showing little adherence to the spinal cord, from which it could be easily detached by dissection.

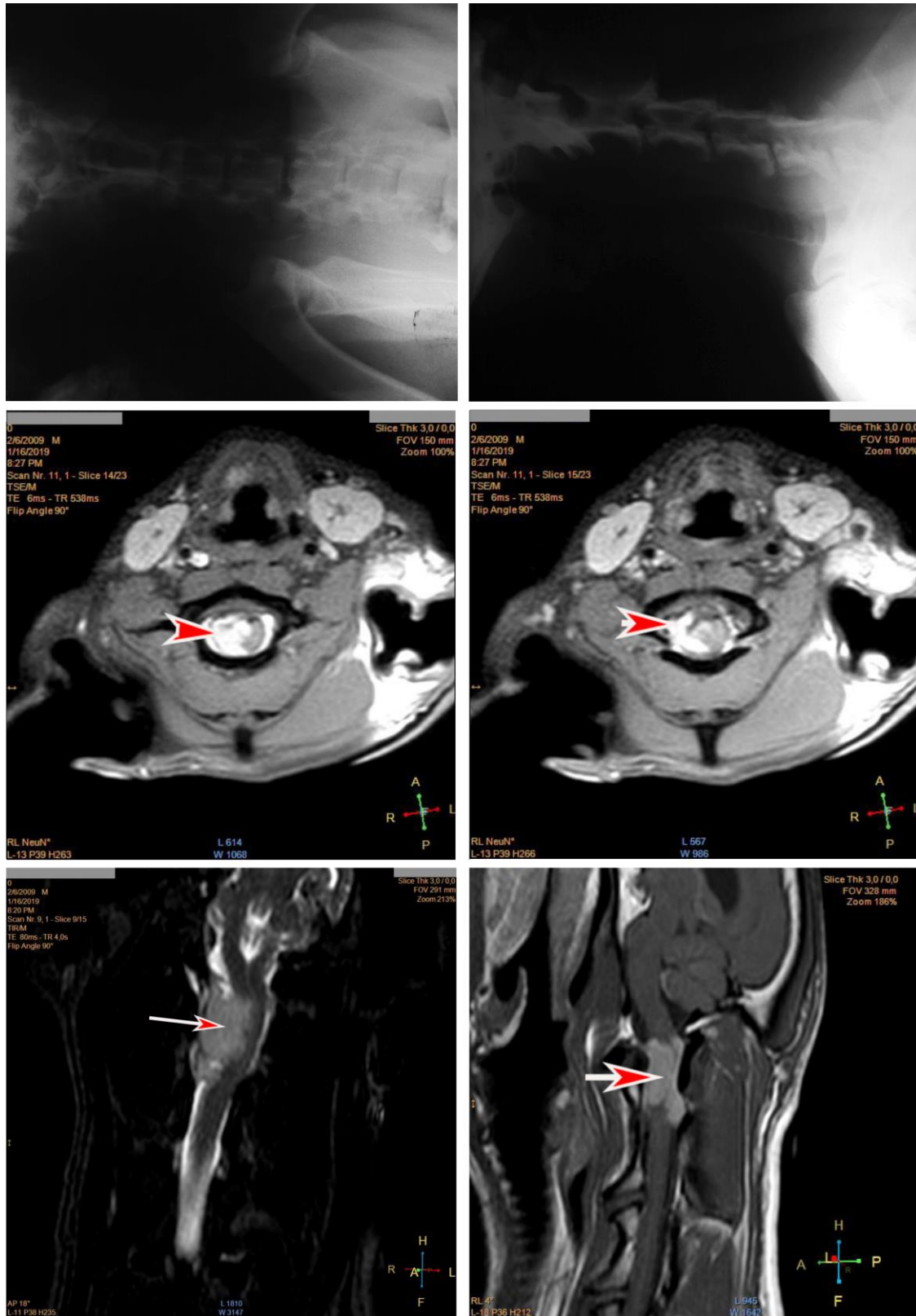
### *Histopathological study*

Three samples (1 x 0.3 x 0.3 cm each) of the excised mass were fixed in formalin for 24 h and then processed for their inclusion in paraffin. The material had an irregular surface and was solid. Histologically, a population of irregularly fusiform cells with diffuse limits was observed. In the most uniform areas, these cells were organized in sheets, while in other areas they surrounded small blood vessels, acquiring a syncytial and spiral arrangement (Fig. 2A and B). These cellular organizations were observed partially divided into lobes by connective tissue trabeculae.

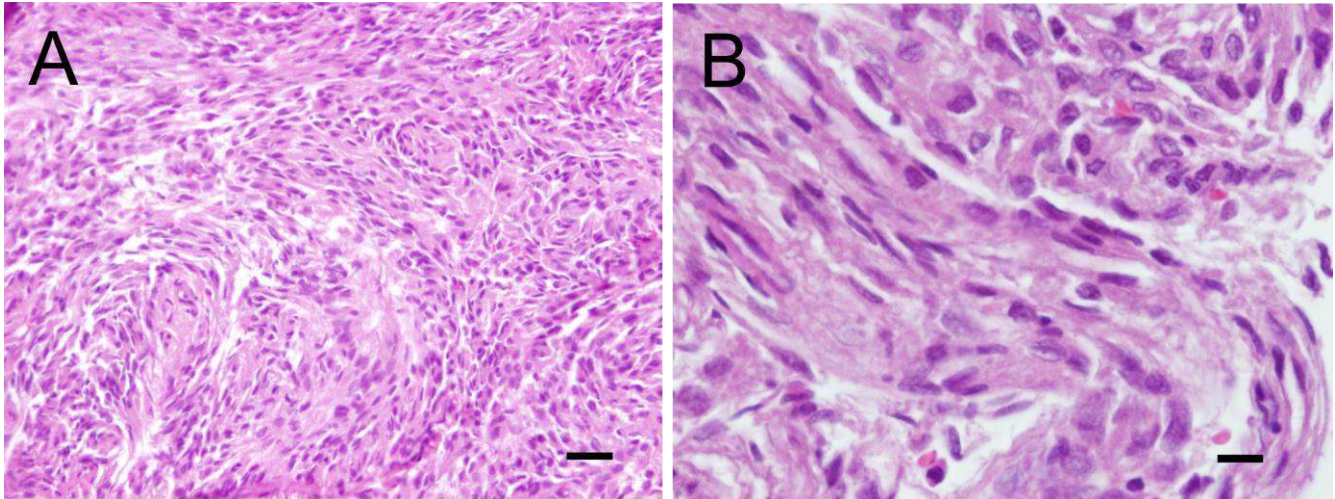
The cells had abundant cytoplasm and contained an ovoid or elongated nucleus with a delicate heterochromatic pattern. Many of these cells had evident nucleoli. Some cells were identified with a longitudinal intranuclear basophilic band through the folded nucleus and other occasional cells showed intranuclear cytoplasmic invagination, giving the image of empty inclusions. A very low mitotic index was observed. According to these characteristics, grade I meningioma, transitional (mixed) subtype was diagnosed.

### *Immunohistochemical and immunofluorescent studies*

To further characterize the tumor immunohistochemical (IHC) and immunofluorescent (IF) studies were performed following standardized protocols (23). Sections of the same material used for histopathological diagnosis were used.



**Figure 1.** Diagnostic imaging. **A.** Rx (dorsal view) of the cervical region of the patient. **B.** Rx (latero-lateral view) of the same patient. **C and D.** MRI of the cervical region (T1 sequence) showing the location of a hypotensive mass of approximately 12x21 mm, covering the spinal cord. **E.** MRI of the cervical region (T2 sequence) showing the location of a mass that covered the spinal cord and the vessels that irrigate it. **F.** MRI of the cervical region (T2 sequence) showing the location of a mass that covered the spinal cord between C1 and C2 segments.



**Figure 2.** Histological appearance of grade I meningioma, transitional (mixed) subtype. Hematoxylin and Eosin staining. Bar = 30  $\mu$ m (A), 10  $\mu$ m (B).

For the characterization of the cells and tissues components of the tumor the following primary antibodies were used: anti-vimentin (VIM, monoclonal, DakoCytomation, Carpinteria, CA, USA), anti-pancytokeratin (pCk, rabbit polyclonal, Dako, Carpinteria, USA), anti-neuron-specific enolase (NSE, rabbit polyclonal, Sigma-Aldrich, St Louis, MO, USA), anti-gial fibrillary acidic protein (GFAP, rabbit polyclonal, DAKO, Denmark) and anti-doublecortin (DXC, c-18, polyclonal goat, Santa Cruz Biotech, Dallas, TX, USA).

For detecting antibodies in the IHC samples the EnVision™ detection system + HRP system labelled anti-mouse or anti-rabbit polymer (DakoCytomation) and liquid 3,3-diaminobenzidine tetrahydrochloride (Vector Laboratories Inc., CA, USA) were used.

For the identification of antibodies in the IF samples, anti-mouse or anti-rabbit antibodies were used, as appropriate, labeled with Alexa 488 or Alexa 555 (Invitrogen, Thermo Fisher Scientific Inc.). All these samples were finally incubated with DAPI for the fluorescent identification of nuclei.

The IHC samples were observed in a research microscope (Olympus BX53). Images were captured using a digital video camera (Olympus DP-73) and processed by a digital image analyzer (cellSens v1.7, Olympus). Images of the fluorescent samples were digitalized using a confocal microscope (Olympus FV1000).

In all cases, the immunostaining of the tumor cells was cytoplasmic with a variable intensity, from weak to intense using different antibodies or even in some cells within the same sample. They also had a variable distribution in the tumor samples. Anti-pCk staining was distributed throughout the entire sample, although it was expressed in a few cells (Fig. 3A-C). Its intensity was moderate. Vimentin was identified in virtually all tumor cells and its intensity was also moderate (Fig. 3D-F). Doublecortin was expressed in isolation in some cellular

concentrations surrounding a blood vessel. Its appearance was observed in only one of the three samples submitted. Its labeling was the most intense of all the selected antibodies and its pattern of intracellular distribution alternated between homogeneous or in particulate accumulations (Fig. 3G and H). The anti-NSE antibody identified the cytoplasm of virtually all tumor cells, in a homogeneous or particulate pattern. In some cells the staining was more intense than in others (Fig. 3I-K). Finally, the anti-GFAP antibodies were located in some spiral accumulations of tumor cells and its intensity of staining was the weakest of all the analyzed antibodies.

In all the cases in which the IF labeling was performed, delimitation of the markers was more precise than with the conventional IHC.

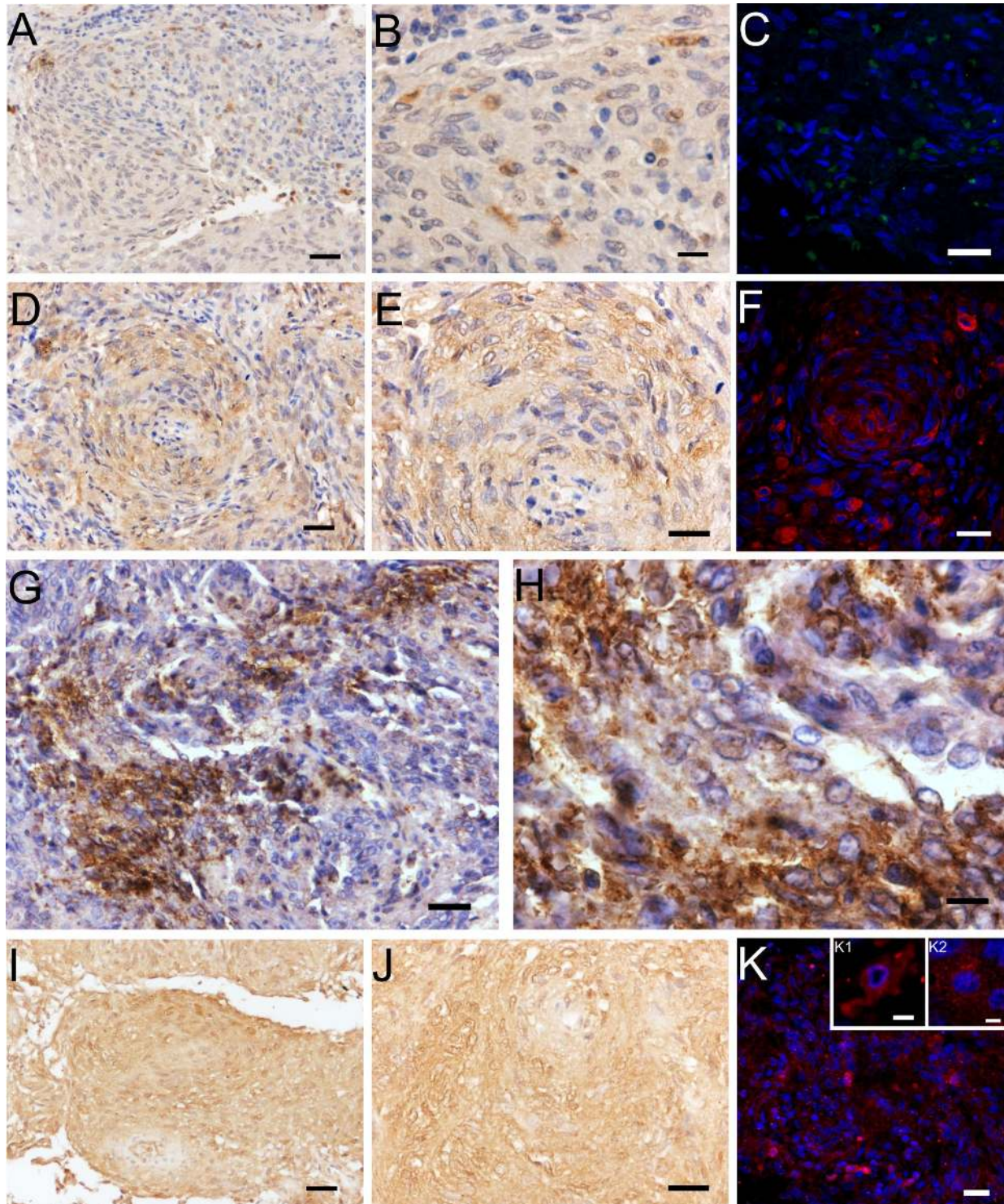
## Discussion

Extramedullary spinal cord neoplasms account for 85% of all spinal cord neoplasms while intramedullary spinal cord neoplasms comprise the remaining 15% (17, 24). Extramedullary spinal cord tumoral lesions may have a primary or secondary origin. The most common primary tumors are nerve sheath tumors and meningiomas (3, 25). Both are slow-growing, locally invasive, causing a progressive and compressive myelopathy, and have a low rate of metastasis.

Meningiomas are tumors arising from cells of the arachnoid membrane and pia mater of the nervous system (16). They are one of the most common primary neoplasms of the central nervous system in dogs and cats. In dogs, meningiomas are typically solitary, whereas in cats, meningiomas can be single or multiple (27). Although the thoracic spine is the most common location for spinal meningiomas in humans (80%), its location in canines is more frequent in the cervical spinal cord (68%) (5, 14). As described by Lacassagne et al. (17), most meningiomas are

located in the first three cervical segments, although some tumors were reported at the thoracic spine level and at even at the lumbar region. Interestingly, it is described that

grade I meningiomas show a strong predilection for the cervical spinal cord, as in our case, while grade II tumors are more frequent in the thoracolumbar region (25).



**Figure 3.** Immunohistochemical and immunofluorescent reaction of meningioma. **A-C.** anti-pCk; bar: 30  $\mu$ m (A), 10  $\mu$ m (B), 20  $\mu$ m (C). **D-F.** anti-VIM; bar: 30  $\mu$ m (D), 20  $\mu$ m (E), 20  $\mu$ m (F). **G-H.** anti-DXC; bar: 30  $\mu$ m (G), 10  $\mu$ m (H). **I-K.** anti-NSE; bar: 30  $\mu$ m (I), 20  $\mu$ m (J), 20  $\mu$ m (K). Insets show the NSE pattern distribution in tumoral cells. In K1 a homogeneous pattern is observed (bar = 5  $\mu$ m) while in K2 the pattern is particulate (bar = 5  $\mu$ m). The detail of the delicate heterochromatic pattern inside the nucleus is observed.

Predisposing factors for the appearance of meningiomas such as age and breed have been reported. Most canine tumors increase constant and regularly their incidence as the animal ages (9, 19). The Boxer breed is considered as a high-risk breed of several types of tumors (6, 8, 9). Moreover, according to Petersen et al. (25) Boxer breeds presents the greatest predisposition to intraspinal meningiomas.

The larger diameter of the spinal canal in the cervical region could also have a role in the lateralization of clinical signs given that more severe compression is needed to affect both sides of the spinal cord. Inflammatory or infectious processes (*e.g.*, meningomyelitis or discospondylitis causing vertebral instability or intervertebral disk protrusion) and some vertebral anomalies leading to degenerative changes of the articular facets or intervertebral disk (*e.g.*, cervical spondylomyelopathy -Wobbler syndrome) may also cause lateralization signs but develop with low frequency in geriatric dogs (1).

As mentioned by McEntee & Dewey (20), radiographs of the skull typically do not provide useful clinical information in cases of brain tumors. In our case was only useful to dismiss osteoarticular injuries that could justify the clinical signs. MRI images of meningiomas usually reveal an intradural/extramedullary mass with a broad-based dural fixation and a variable signal intensity in pre-contrast images weighted in T1 (T1W) and in T2 (T2W) (25), as was observed in our case. Meningiomas tend to displace, rather than invade the parenchymal tissue, however, many canine meningiomas do display some degree of invasiveness (20). Nevertheless, in our case no signs of invasiveness were observed.

MRI images could be used to differentiate meningiomas from other tumors. Thus, meningiomas have an extra-axial attachment, meaning that they arise from the periphery of the organ and move inward or axially. In contrast, gliomas, which in the Boxer breed represent 50% of reported cases (12), tend to arise from an intra-axial location (intra-organ growing moving outward), often lack distinctive tumor margins because they tend to infiltrate rather than displace normal tissue, and usually their contrast enhance is poor and non-uniform. Ependymomas tend to be intraventricular in location and often uniformly contrast enhance (20). However, those descriptions are guidelines only since different types of meningiomas could behave differently according to their histological characteristics.

According to Lacassagne et al. (17), the most effective treatment for most malignant tumors and meningiomas is a wide surgical excision. What has not been achieved so far is to improve the survival time after tumor resection, which does not exceed one year (10). In our case, survival after surgery was of only one day. Moreover, the general clinical condition was complicated by the presence of postoperative edema which spread to

the medulla, causing brainstem signs such as alterations in respiratory rate, heart rate and blood pressure.

The histological grades of meningiomas vary from I to IV, with grade I having low proliferative potential. Diagnosis of these tumors depends on many factors such as age, breed, and neuroanatomical location, together with neuroimaging findings. Nevertheless, despite the availability of algorithms that guide towards establishing a provisional diagnosis based on these various criteria, an accurate diagnosis currently depends on histopathological evaluation by experienced pathologists and immunochemistry for confirmation and differential diagnosis, often complemented with ultrastructural and molecular genetic studies (18, 27).

The most common meningioma grade I subtype in dogs (56%) are transitional, meningothelial, microcystic, and psammomatous, in descending order. Irrespective of their histologic classification, meningiomas are uniformly and robustly immunoreactive for vimentin expression (21, 22), as was observed in all tumoral cells of our case. As reported by Motta et al (22), some tumors have a variable, focal expression of low and/or high molecular weight cytokeratins. In our case, we found isolated pan-cytokeratin stained tumoral cells widespread all over the analyzed samples. Although carcinomas show high affinity for cytokeratins and lack that affinity for vimentin, the presence of the former in a meningioma would not indicate a tendency to malignancy (21, 22), while the presence of the latter will not ensure benignity (4). According to the studies of Ramos Vara et al. (27), the expression of neuron-specific enolase (NSE) is observed in 87% of cases, while GFAP is only expressed in 16% of canine meningiomas. In our case, NSE bound to the cytoplasm of almost all tumoral cells whereas GFAP expression was located in some spiral accumulations of tumor cells, and its intensity of staining was weak. According to the data provided by these authors, none of the 13 cases of transitional meningiomas were positive for GFAP. However, it should be mentioned that none of these tumors had a location in the spinal cord. However, the expression of GFAP was detected in a meningioma associated with the spinal cord, but in this case, it was anaplastic (4). In any case, according to our studies carried out in experimental animals, markers of the normal nervous system can change their affinity with aging and even in relation to other organs of the same system (11, 26). It can be assumed, then, that different subtypes of tumors, present in different organs of the nervous system, can behave differently in particular cases. Doublecortin (DCX) is highly expressed in invasive brain tumors, including malignant meningiomas; therefore, it was suggested as a good marker for invasion (13). Nevertheless, these authors also describe it in a few cases of grade I meningiomas. Although its possible presence in these types of tumors was not discussed, it led us to speculate with the possible transition of the tumor of our case to a more aggressive

stage, especially considering the presence of cytokeratin in some cells.

The results of this study suggest that it is necessary to use different laboratory techniques to arrive to a more conclusive diagnosis. A good histopathological analysis combined with a battery of immunohistochemical and immunofluorescent studies would allow a more appropriate way to diagnose this type of tumors. Although variations in behavior, histology and immunoreactive profile of meningiomas are reported, the latter is a good indicator for differential diagnosis. In addition, the use of immunofluorescence techniques is advisable since it allows a more precise delimitation of the markers. So far, clinical, imaging and surgical procedures are still indicated to deal with these types of patients.

### Acknowledgements

This work was supported by the National University of La Plata (grant V270-2019 to ELP). The collaboration of Natalia Scelsio (INIBIOLP-CONICET) for the performance of some histochemical stains is appreciated. The collaboration of Dr. Quiroga and Dr. Massone, from the Pathology service of the School of Veterinary Sciences, National University of La Plata, for the histopathological diagnosis of the tumor is gratefully acknowledged.

### Disclosure

Authors declare no Conflict of Interest for this article.

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