http://dx.doi.org/10.21708/avb.2023.17.4.11822

Metastatic epithelioid mesothelioma in a dog

Mesotelioma epitelioide metastático em um cão

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ABSTRACT: Mesothelioma is a neoplasm considered infrequent in canines. Its peritoneal location is not common in dogs when compared to the pleural origin. This study aims to report a case of metastatic trabecular epithelioid mesothelioma in a dog, diagnosed through anatomopathological and immunohistochemical examination. The patient was hospitalized and treated, but his clinical picture worsened, and euthanasia was chosen. At necropsy, the main findings were the abdomen and thorax with the presence of tumor nodules of soft consistency, and the cuts were pinkish-brown in color. Histologically, they were composed of malignant mesothelial cells, predominantly epithelioid cells arranged in cords. There was metastasis in the lungs, kidneys, and lymphatic vessels of the heart. Samples were submitted to immunohistochemical analysis and tumor cells showed cytoplasmic immunoreaction for anti-human mesothelial cell (HBME-1) and anti-cytokeratin (AE1/AE3), in addition to a moderate proliferative index evidenced by the reaction against the proliferation nuclear antigen cellular (PCNA). There was no staining for vimentin and anti-epithelial antigen (EMA). Thus, the anatomopathological and immunohistochemical analysis showed that it was a trabecular epithelioid mesothelioma with a moderate proliferative index and multiple metastatic sites.

KEYWORDS: Neoplasia; metastasis; histopathology; cytokeratin; HBME-1.

RESUMO: Mesotelioma é uma neoplasia considerada infrequente em caninos. A sua localização peritoneal não é comum em cães quando comparada à origem pleural. Este estudo objetiva relatar um caso de mesotelioma epitelioide trabecular metastático em um cão, diagnosticado através de exame anatomopatológico e imuno-histoquímico. O paciente foi hospitalizado e tratado, mas apresentou piora do quadro clínico, optando-se pela eutanásia. Na necropsia os principais achados foram abdômen e tórax com presença de nódulos tumorais de consistência macia e aos cortes eram de coloração pardo-rosada. Histologicamente eram constituídos de células mesoteliais malignas, predominando células epitelioides arranjadas em cordões. Havia metástase nos pulmões, rins e em vasos linfáticos do coração. Amostras foram submetidas à análise imuno-histoquímica e as células tumorais apresentaram imunorreação citoplasmática para anti-célula mesotelial humana (HBME-1) e anti-citoqueratina (AE1/AE3), além de moderado índice proliferativo evidenciado pela reação contra o antígeno nuclear de proliferação celular (PCNA). Não houve marcação para vimentina e anti-antígeno epitelióide (EMA). Assim, a análise anatomopatológica e imuno-histoquímica evidenciou tratar-se de mesotelioma epitelioide trabecular de moderado índice proliferativo com múltiplos sítios metastáticos.

PALAVRAS-CHAVE: Neoplasia; metástase; histopatologia; citoqueratina; HBME-1.

INTRODUCTION

Canine malignant mesothelioma originates from mesodermal cells that line the pleura, peritoneum, pericardial sac, testicular tunica vaginalis, and intrathoracic or intra-abdominal lymph nodes (Faraon *et al.*, 2010; Wilson, 2016; Zeira *et al.*, 2021). This neoplasm, considered rare in dogs, represents approximately 0.2% of all canine tumors (D'Angelo *et al.*, 2014). In this species, the neoplasm often originates in the pleural cavity, as in humans, and is rarely diagnosed in the peritoneal cavity (D'Angelo *et al.*, 2014; Frontario *et al.*, 2015). Clinical signs are nonspecific and are usually caused by the constant accumulation of intracavitary effusions leading to episodes of dyspnea, increased abdominal volume, and indisposition (Moberg *et al.*, 2022). Other clinical signs include weight loss, vomiting, and disseminated intravascular coagulation (Gumber *et al.*, 2011). The definitive diagnosis is

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Received: 05/06/2023. Accepted: 07/23/2023

based on the association of clinical signs, analysis of cavitary effusions, and through histopathological examination of the lesions or affected surfaces (Frontario *et al.*, 2015; Wilson, 2016). As most animals show nonspecific clinical signs, the treatment of mesothelioma often takes time to be instituted (Frontario *et al.*, 2015). This study reports a case of metastatic trabecular epithelioid mesothelioma in a dog, characterizing its anatomopathological and immunohistochemical aspects.

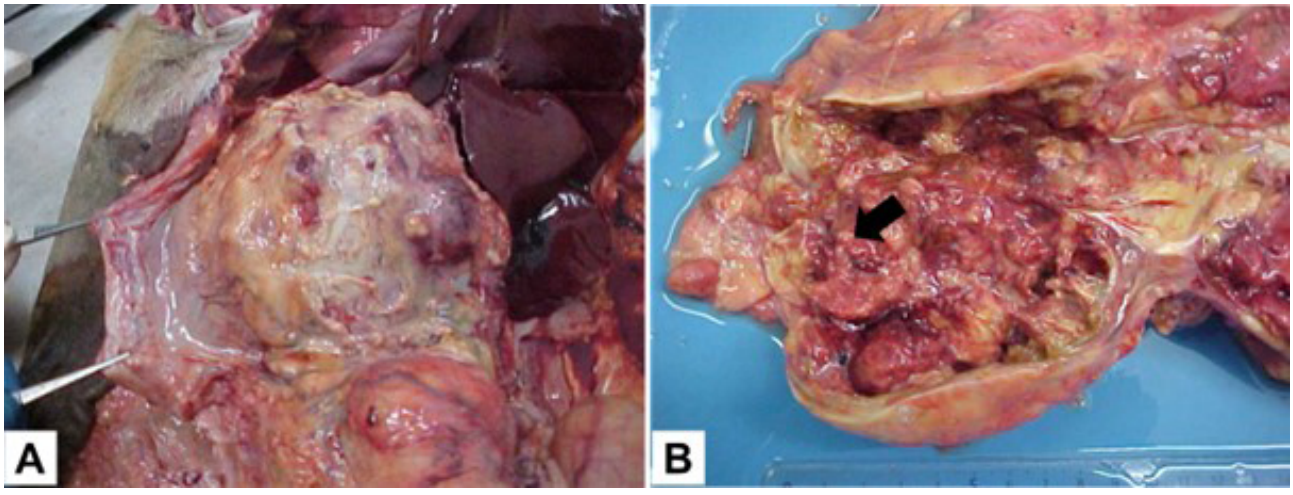
CASE REPORT

The case occurred in a male, six-year-old Boxer dog, who had suspected heart disease and a history of treatment with anti-inflammatory drugs and diuretics. The clinical examination revealed an increase in abdominal volume and pulmonary auscultation with muffled heart sounds. The radiographic examination showed pleural effusion and generalized abdominal radiopacity. The evolution of the clinical picture lasted two weeks. Supportive treatment was performed, but the patient did not improve, and euthanasia was authorized.

The cadaver was sent for a necroscopic examination. In the subcutaneous tissue, accentuated edema was observed in the pelvic limbs. Upon opening the thoracic cavity, a mild hydrothorax was observed; presence of multiple tumor-like nodules in the right parietal pleura, intercostal muscles, ribs, and cranial sternal lymph nodes. The lungs had a blackish wine color, which suggests congestion, in addition to edema. There was also serohemorrhagic fluid in the pericardial sac and right heart dilation. Upon opening the abdominal cavity, there was abundant serohemorrhagic fluid adhered to the right parietal peritoneum, an encapsulated tumor nodule, with a smooth surface, of soft consistency, which invaded the underlying muscles and measured 28x18x7 cm (Figure 1A). When cut, it was friable, pinkish-brown, or reddish in color, and with marked vascularization (Figure 1B). In the omentum, a tumoral nodule with an appearance similar to that observed in the peritoneum, measuring 10x8x5 cm, was observed. Tumor nodules were also found in the renal and inguinal lymph nodes. The liver was congested and with a marked accentuation of the lobular pattern. There were erosions and ulcerations in the gastric mucosa.

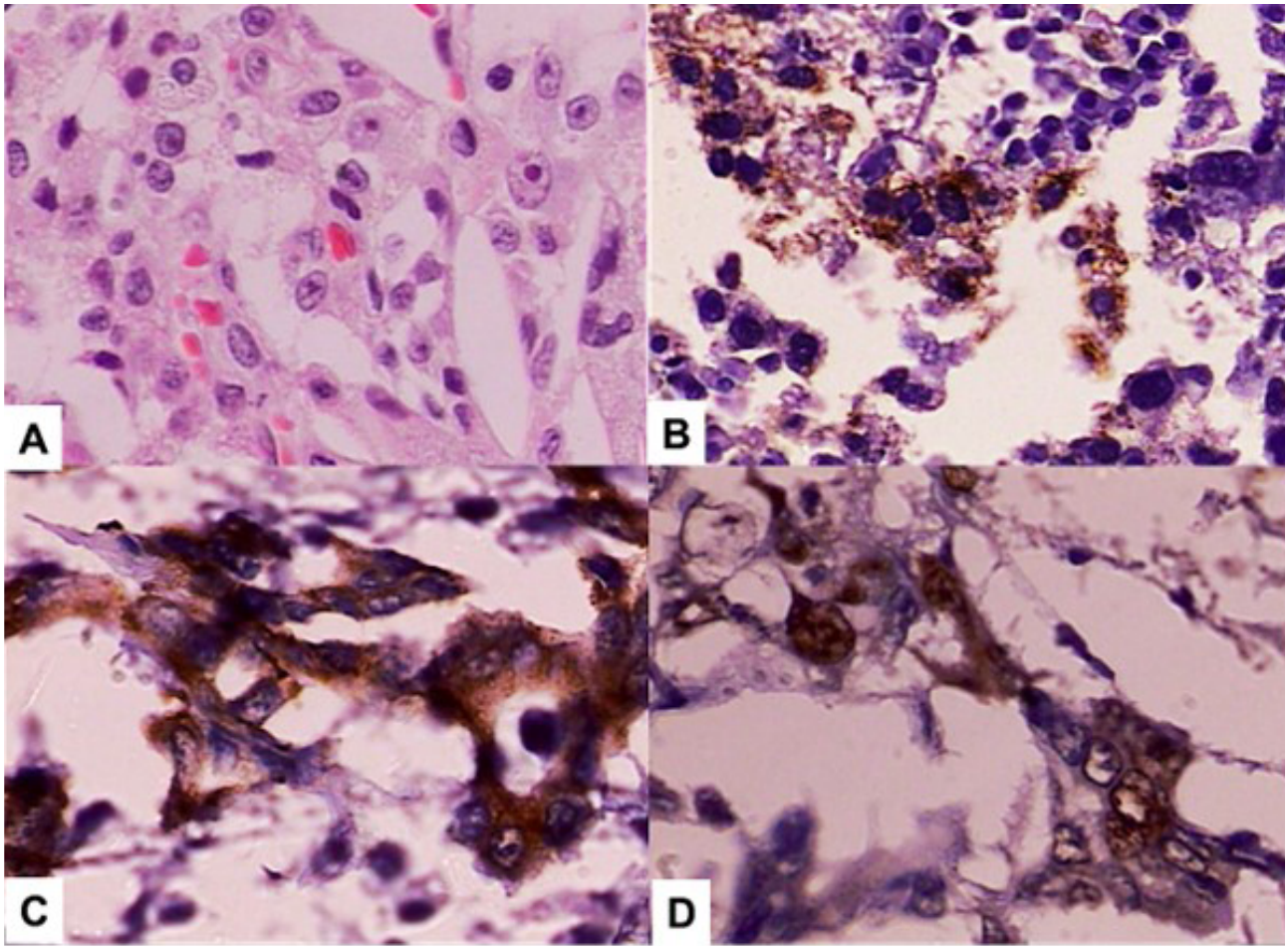
Samples from all organs were collected and fixed in 10% formalin and processed by conventional histochemical methods. Histologically, the aforementioned tumor nodules were made up of atypical mesothelial cells, predominantly epithelioid cells, sometimes with vacuolated cytoplasm (Figure 2A), organized in chordal arrangements, eventually forming irregular stratifications on a poorly organized connective tissue, sometimes outlining tubules, characterizing trabecular epithelioid mesothelioma. The mitotic index was 10 mitoses in a total microscopic area of 2.37mm2 (accrued in 400x fields). Tumor giant cells were also observed, in addition to foci of tumor necrosis, suppurative inflammation, and hemorrhage. There were also multifocal metastases without obstruction of vessels and areas of infarction in the lungs and kidneys, in addition to the presence of tumor cells in the lymphatic vessels of the heart.

Tumor samples were submitted to immunohistochemical analysis (IHC) using the streptavidin-biotin-peroxidase technique. Monoclonal antibodies were used: anti-human mesothelial cell (HBME-1), anti-cytokeratin (AE1/AE3), anti-epithelial antigen (EMA), vimentin (V9) and against proliferating cell nuclear antigen (PCNA). The cytoplasm of the tumor cells was positive for HBME-1 (Figure 2B) and AE1/AE3 (Figure 2C). There was no labeling for EMA and vimentin. The PCNA showed moderate to strong nuclear positivity and reached an average of 30% labeling index (Figure 2D). The PCNA response was estimated by calculating the negative and positive cells observed in a total microscopic area



Autores, 2023.

Figure 1. Metastatic trabecular epithelioid mesothelioma in a canine. **A)** Abdominal cavity, right parietal peritoneum. Encapsulated tumor nodule, with a smooth surface, of soft consistency, which invaded the underlying musculature. **B)** Cut the surface of encapsulated tumor nodule with friable, pinkish-brown, or reddish areas and marked vascularization.



Autores, 2023.

Figure 2. Metastatic trabecular epithelioid mesothelioma in a canine. **A)** Peritoneal nodule made up of atypical mesothelial cells, predominantly epithelioid cells, sometimes with vacuolated cytoplasm, organized in a chordal arrangement and supported by poorly organized connective tissue (HE, 400X). **B)** Malignant mesothelial cells with cytoplasmic immunostaining for HBME-1 (IHC, 400X). **C)** Malignant mesothelial cells with cytoplasmic immunostaining for AE1/AE3 (IHC, 400X). **D)** Malignant mesothelial cells with nuclear immunostaining for PCNA (IHC, 400X).

of 2.37mm2 (measured in 400x fields). Thus, the anatomopathological analysis and IHC showed that it was a trabecular epithelioid mesothelioma with moderate proliferation and multiple sites of metastasis.

DISCUSSION

Dogs with peritoneal mesothelioma have an average age of 10 years at the time of diagnosis, similar to what was observed in the present case (Munday *et al.*, 2016). There is no sex predilection for the development of mesotheliomas (Zeira *et al.*, 2021). Clinical signs are commonly related to the development of recurrent intracavitary effusions due to the expressive cellular exfoliation of the neoplasm (Valenciano; Rizzi, 2020; Moberg *et al.*, 2022).

Macroscopically, the mesothelioma in the present case consisted of masses and invasive tumor nodules with a primary site of probable origin in the peritoneum, considering the magnitude of the lesions in the abdominal cavity. As for the distribution, peritoneal mesotheliomas can present as a single focal mass, multifocal or diffuse thickening, and with variable macroscopic appearance (D'Angelo *et al.*, 2014; Munday *et al.*, 2016). The presence of metastases in organs such as the lung, kidney, and lymphatic vessels of the heart occurs due to the ability of mesothelioma to metastasize via the lymphatic route to these sites, although this condition is rarely reported (Head *et al.*, 2002). In addition, peritoneal mesotheliomas can penetrate through the diaphragm to the lungs (Frontario *et al.*, 2015).

Histologically, three main types of mesothelioma can be observed, the epithelioid being considered the most common in both animals and humans, followed by the fibrous/sarcomatoid and biphasic/mixed type (Husain *et al.*, 2009; Merlo; Rosciani, 2012; Gopal *et al.*, 2022). In the present case, the mesothelioma was classified as epithelioid by IHC confirmation with positivity for AE1/AE3 (Gopal *et al.*, 2022), of the trabecular subtype, because cells arranged in cords predominate in the histopathological analysis, similar to adenocarcinomas (Merlo; Rosciani, 2012).

Due to its similarity with other epithelial neoplasms, mesothelioma represents a diagnostic challenge for pathologists. In this context, performing the IHC is a fundamental diagnostic tool (Merlo; Rosciani, 2012; Frontario *et al.*, 2015). In this case, positive IHC staining for human mesothelial cells (HBME-1) and cytokeratins (AE1/AE3) enabled the definitive diagnosis of epithelioid mesothelioma. The evaluation of cytokeratin expression was carried out to distinguish epithelioid from fibrous mesothelioma since only epithelial cells are positive for this marker, and this marking is not expected in fibrous mesotheliomas, which are positive for vimentin (Sato *et al.*, 2005; Munday *et al.*, 2016). In the present case, the use of the HBME-1 marker was extremely important for the definitive diagnosis of mesothelioma, since it reacts specifically in mesothelial cells (Robinson *et al.*, 2005; Shih *et al.*, 2013).

In the present case, PCNA showed moderate to strong nuclear positivity and reached an average of 30% labeling index. As for the proliferative index markers, either PCNA or Ki-67, it is important to emphasize that reactive mesothelial cells can have a high mitotic index (Ramael *et al.*, 1994; Kumaki *et al.*, 2002; Zhang *et al.*, 2005; Taheri *et al.*, 2008; Toledo *et al.*, 2018). By way of comparison with carcinomas, for mesotheliomas, further studies are still needed to establish the prognosis concerning companion animals.

CONCLUSION

In addition to the anatomopathological and immunohistochemical findings that allowed the diagnosis of metastatic trabecular epithelioid mesothelioma, we believe that it brings concise findings that open doors for future studies.

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