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Case Report

Canine myxoid mesothelioma with clinical presentation of *Pseudomixoma peritonei*

Acácia Eduarda de Jesus Nascimento[1], Beatriz Ostertag[2], Nayara Toledo da Silva[1], Marina Possa dos Reys[3], Geovanni Dantas Cassali[3], Ayisa Rodrigues de Oliveira[1]*

[1]Setor de Patologia Animal, Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[2]Clínica Amigo de Patas, Belo Horizonte, MG, Brazil
[3]Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

*Corresponding author: ayisa.rodrigues@gmail.com
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Abstract
Myxoid mesothelioma is a rare variant of mesothelioma, characterized by a myxoid component, which refers to a gelatinous extracellular matrix containing mucin. This study describes a case of myxoid mesothelioma in a 13-year-old Yorkshire Terrier that presented with sudden abdominal distension, pain, and respiratory discomfort. Imaging exams revealed an extensive amount of amorphous, homogeneous, and viscous material in the abdominal cavity. Due to unsuccessful drainage attempts, exploratory laparoscopy was performed, uncovering large volumes of gelatinous material and areas of mesenteric thickness. Despite intensive care, the animal died post-surgery and was submitted for necropsy. Gross examination revealed diffuse gelatinous material coating the omentum and mesentery, along with firm micronodular areas. Microscopic analysis showed neoplastic sarcomatoid to round cells supported by delicate fibrovascular connective tissue with abundant myxoid stroma. Marked anisocytosis, anisokaryosis and cellular pleomorphism were observed; the myxoid nature of the stroma was confirmed by intense Alcian blue staining. Immunohistochemistry revealed positive vimentin and WT1 immunoreactivity, confirming the mesothelial origin of the tumor, while pancytokeratin, carcioembryonic antigen, high molecular weight cytoqueratin, and calretinin were negative. These findings support the diagnosis of myxoid-variant mesothelioma with clinical presentation of *Pseudomyxoma peritonei*.

Keywords: gelatinous extracellular matrix, mesenchymal neoplasms, mesothelial, myxoid.

Introduction

Myxoid mesothelioma is a rare variant of mesothelioma, a neoplasm originating from mesothelial cells lining the pleural, peritoneal, and pericardial cavities (4,12,13,21). This subtype is distinguished by the presence of a myxoid component, characterized grossly by a gelatinous extracellular matrix rich in mucin (20,29). In companion animals, mesotheliomas are typically reported in the pleural and peritoneal cavities, with no apparent breed, sex, or age predilection (18,12,21). Although mesotheliomas have been reported in various species, including canines (21), felines (4), birds (16) and bovines (11), the myxoid subtype represents a rare manifestation of this neoplasm in veterinary practice. The clinical presentation often involves nonspecific signs that varies according to the development site, such as abdominal distension, respiratory distress and apathy, making diagnosis challenging and reliant on complementary diagnostic tools, such as image exams and histopathology (13,15,30). In myxoid mesothelioma, the abundant mucin-rich stroma may obscure conventional histological features, being special staining and specific immunohistochemical markers essential

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for a definitive diagnosis (1,20,24). This comprehensive approach is critical for characterizing the tumor and guiding precise management strategies, given the variable biological behavior of these neoplasms (1,17).

Pseudomyxoma peritonei is a rare condition in both humans and animals, characterized by the progressive accumulation of mucinous material within the peritoneal cavity due to mucin-producing neoplasms (14,25,32). Although more documented in human medicine, veterinary reports remain scarce, with occasional cases described in dogs (3,19,22,32). The underlying neoplasms are typically of gastrointestinal or ovarian origin, although primary peritoneal tumors may also be involved (14). Due to its insidious progression, *P. peritonei* is often diagnosed at an advanced stage, complicating early detection and therapeutic intervention. In both human and veterinary medicine, *P. peritonei* is closely associated with mucinous tumors, which may exhibit myxoid characteristics due to the excessive production of extracellular mucin, creating a gelatinous and highly viscous environment within the peritoneal cavity (2,22).

To the best of the authors' knowledge, this is the first report of myxoid mesothelioma in dogs associated with clinical manifestation of *P. peritonei*. This report aims to describe the clinical, pathological, and immunohistochemical features of a rare case of myxoid mesothelioma in a Yorkshire Terrier, contributing to the broader knowledge of mesothelial tumors in veterinary medicine.

Case description

A 13-years-old female Yorkshire dog, weighing 1.78 kg and not spayed, was attended at a private veterinary clinic located in Belo Horizonte, Minas Gerais, Brazil, due to acute abdominal distension. During clinical examination, in addition to marked abdominal distension, were observed mild tachypnea (45 respiratory movements (RM)/minute [reference range: 20-30 RM/min (9)]), congested mucous membranes, and pain on abdominal palpation. Clinical signs of pain and respiratory discomfort enhanced when positioned in dorsal recumbency. Ultrasound exams revealed a free abdominal hyperechogenic and homogeneous liquid with moderate density, an increased echogenicity of the adjacent mesenteric tissue and difficulty in isolating the abdominal organs (Fig. 1A). Effort was made to drain the fluid, being collected only a small amount due to its high viscosity. Fluid analysis revealed a marked viscous material, making quantitative chemical evaluation impossible. Therefore, a semi-quantitative analysis was conducted. Nucleated cells were present, predominantly intact neutrophils and atypical lymphocytes, with proteins (100 mg/dL) and glucose (50 mg/dL).

A complete blood count (CBC) was conducted, showing normocytic normochromic anemia with reduced values of erythrocytes (4.29 x 10^6/µL [reference: 5.65 - 8.87 M/µL (27)]), hematocrit (28.7% [reference: 37.3 - 61.7% (27)]), and hemoglobin (10.7 g/dL [reference: 13.1 - 20.5 g/dL(27)]). Also, was observed marked leukocytosis (33.48 x10^3/µL [reference: 5.05 - 16.76 x10^3/µL (27)]) with neutrophilia (28.01 x10^3/µL [reference: 2.95 - 11.64 x10^3/µL (27)]) and monocytosis (4.95 x10^3/µL [reference: 0.16 - 1.12 x10^3/µL (27)]), associated with lymphopenia (0.49 x10^3/µL [reference: 1.05 - 5.10 x10^3/µL (27)]) and eosinopenia (0.02 x10^3/µL [reference: 0.06 - 1.23 x10^3/µL (27)]). Blood biochemical test was performed, which showed an elevated Blood Urea Nitrogen (BUN) value (BUN 37 mg/dL [reference: 7 - 27 mg/dL (27)]), with an elevated BUN/creatinine ratio (76 [reference: ≤ 23 (27)]). Antibiotics, anti-inflammatories and analgesics were recommended, however, the active drugs

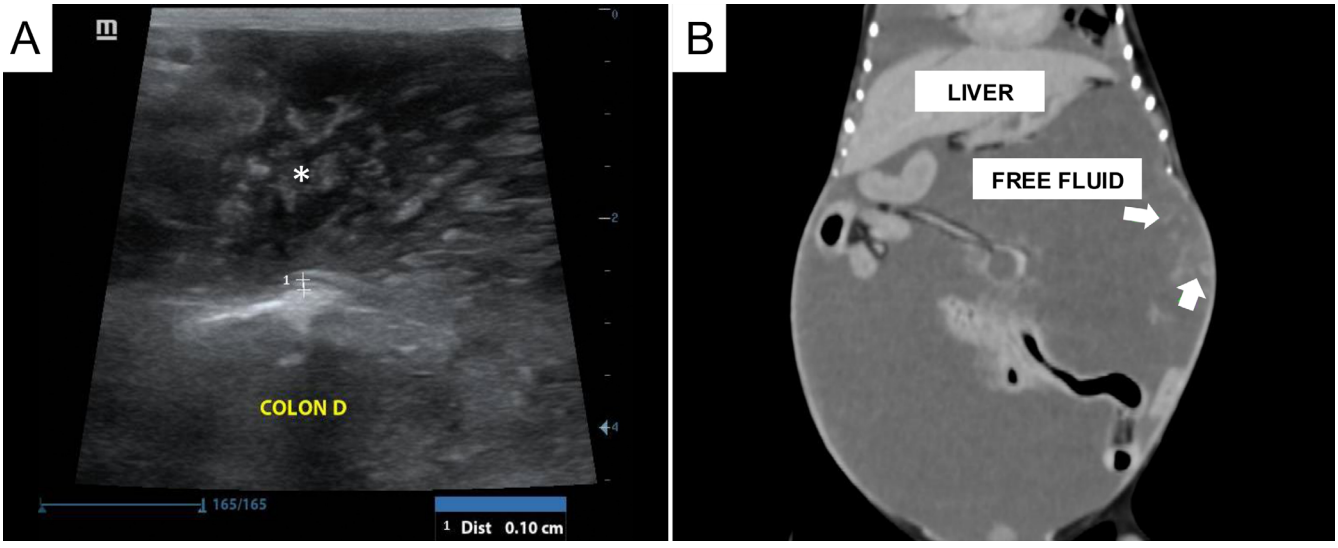

Figure 1. Canine myxoid mesothelioma with clinical presentation of *Pseudomyxoma peritonei.* A: Ultrasound exam. Free abdominal hyperechogenic and homogeneous liquid of moderate density, with increased echogenicity in mesenteric tissue (*). B: Computed tomography (CT). Large amount of free fluid in the peritoneal cavity and a focal area of hyper uptake (arrows).

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and dosages used were not reported. After two weeks, the animal returned to the clinic, with anorexia and worsening abdominal distension. New blood tests were conducted, with no significant changes.

Due to the inability to perform a thorough evaluation of the abdominal structures, a computed tomography (CT) scan was performed, revealing pleural effusion and pulmonary atelectasis, along with a large amount of free fluid in the peritoneal cavity, areas of hyper uptake in the mesentery and cholelithiasis (Fig. 1B). Due to the worsening clinical condition of the patient and the CT findings, an exploratory laparotomy was indicated. During the procedure, a large amount of gelatinous material was removed from the abdominal cavity and was observed a thickness on the intestine wall and mesentery. At the end of the surgery the animal died due to a cardiorespiratory arrest. The body was frozen and sent for necropsy one week later.

Grossly, about 15 mL of free, red-brown, marked viscous, and dense fluid was observed in the abdominal cavity (Fig. 2A). The omentum and mesentery were diffusely and intensely covered with gelatinous, amorphous, translucent, shiny, viscous, and slightly reddish material, with multifocal millimetric solid areas (Fig. 2B). This material covered the spleen, pancreas, greater curvature of the stomach, and part of the duodenum. In the thoracic cavity was observed about 80 mL of serous fluid compatible with transudate and interpreted as hydrothorax. The pulmonary lobes were diffusely and marked hypocrepitant and dark red (compressive atelectasis), with moderate amounts of white, frothy fluid flowing upon incision, extending to the bronchial bifurcation, compatible with edema. Additionally, there was intense pallor of the mucous membranes and viscera, as well as mild internal hydrocephalus.

Samples of omentum, mesentery, mesenteric lymph nodes, spleen, liver, intestine, pancreas, lung, kidneys, brain, skin, and heart were collected during necropsy, fixed in 10% buffered formalin, and processed using routine paraffin embedding techniques. Histological sections of 4 µm were stained with hematoxylin and eosin (HE) and Alcian blue for microscopic analysis. Microscopically, in the omentum and mesentery, as well as in the serosa of the spleen, liver, intestines, and pancreas, there were a loose proliferation of neoplastic cells, ranging from fusiform to round, organized into bundles, often individualized, supported by abundant finely fibrillar myxoid stroma marked blue at Alcian blue stain, compatible with mucin (Fig. 3A-D). This stroma was associated with a delicate fibrovascular connective tissue with intense neovascularization. The cells had mild vacuolated cytoplasm, elongated to round nuclei with coarse, hyperchromatic chromatin, and one or two prominent nucleoli. There were marked anisocytosis and anisokaryosis, high cellular and nuclear pleomorphism, frequent bi- or multinucleated cells, occasional karyomegaly, and slight nuclear molding. Twelve typical and atypical mitotic figures were identified in 2.37 mm².

For immunohistochemistry (IHC), sections of 4 µm of thickness were prepared of primary tumors and mounted on gelatin-coated slides. The antigen was immunodetected using the detection system anti-mouse/anti-rabbit (Novolink Polymer Detection System, Leica Biosystems, Newcastle Upon Tyne, Reino Unido) according to the manufacturer's instructions. Endogenous peroxidase activity was blocked with a 10% hydrogen peroxide solution diluted in methyl alcohol. Reagents were manually applied, and immunoreactivity was visualized by incubating the slides with the chromogen diaminobenzidine (DAB Substrate System, Dako, Carpinteria, CA, USA) for 3 minutes. The sections were counterstained with Harris hematoxylin. The negative control was obtained by replacing the primary antibody with phosphate-buffered saline (PBS). Details of the antibodies, dilutions, antigen retrieval procedures and incubation times are shown in Table 1.

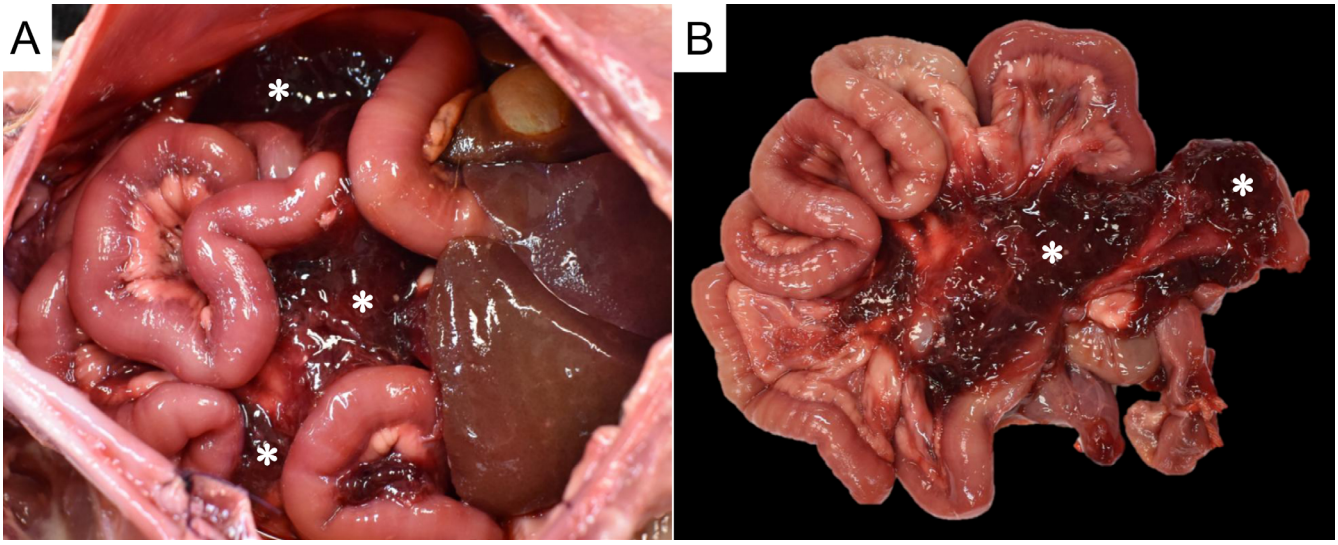

Figure 2. Canine myxoid mesothelioma with clinical presentation of *Pseudomyxoma peritonei*. Gross features. A: Abdominal cavity with gelatinous, red gelatinous, shiny, and translucent material (asterisks). B: The same material was also observed adhered to the intestines and mesentery (asterisks).

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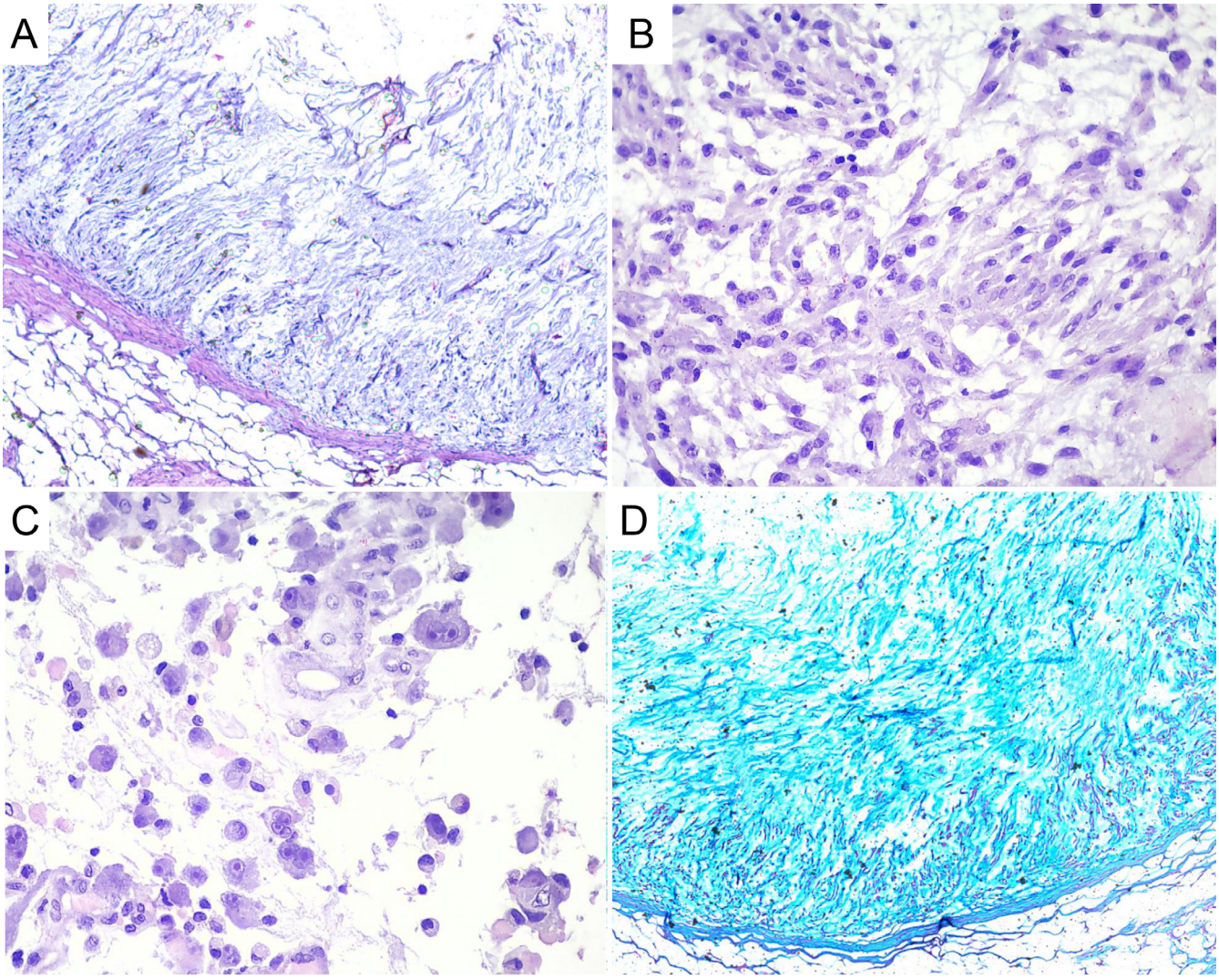

Figure 3. Histopathology of canine myxoid mesothelioma with clinical presentation of *Pseudomyxoma peritonei.* A: Abdominal proliferation of neoplastic cells supported by an abundant, finely fibrillar myxoid stroma (H&E, 100X). B-C: Neoplastic cells features vary from fusiform and stellate arranged in disorganized bundles (B) to round and individualized (C). Both features presented cells with big and central nuclei, one evident nucleolus and bi to multinucleations (H&E, 400X). D: Myxoid stroma associated with neoplastic cells, intensely blue-stained by Alcian Blue, indicating the presence of mucin (Alcian Blue, 100X).

Table 1. Primary antibody details of the immunohistochemistry panel used for the diagnoses of mesothelioma.

Target Antigen	Clone	Manufacturer	Dilution	Antigen Retrieval	Incubation Time (h)/ Temperature (° C)
Pancytoqueratin	AE1/AE3	Dako®	1:500	Pressurized Heat (125°C/20min) with citrate buffer pH 6.0	14-16h / 4° C
Vimentin	V9	Novocastra®	1:500	*Water-bath* Heating (95°C/20min) with citrate buffer pH 6.0	14-16h / 4° C
Cytoqueratin	34βE12	Bio SB®	1:40	*Water-bath* Heating (95°C/20min) with citrate buffer pH 6.0	14-16h / 4° C
Carcioembryonic Antigen (CEA)	II-7	Dako®	1:100	Pressurized Heat (125°C/20min) with citrate buffer pH 6.0	14-16h / 4° C
Calretinin	DAK-CALRET 1	Dako®	Ready to use	Pressurized Heat (95-99°C/20min, in Pt Link®) with EDTA pH 9.0	14-16h / 4° C
WT1	6F-H2	Invitrogen®	Ready to use	Pressurized Heat (95-99°C/20min, in Pt Link®) with EDTA pH 9.0	14-16h / 4º C

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Neoplastic cells had marked and diffuse positive cytoplasmatic immunostaining for vimentin (Fig. 4A) and nuclear immunostaining for WT1 (Fig. 4B). Immunostaining for pancytokeratin, carcioembryonic antigen (CEA), high molecular weight cytoqueratin and calretinin were negative. These findings support the mesothelial origin of the neoplastic cells, consistent with the diagnosis of mesothelioma with myxoid variation.

Discussion

Based on clinical, imaging, histopathological, and immunohistochemical findings, the diagnosis of myxoid mesothelioma was established. This is the first reported case of myxoid mesothelioma in veterinary medicine, specifically in a dog, with clinical manifestation of *P. peritonei*.

Mesothelioma is a mesenchymal neoplasm characterized by the malignant proliferation of mesothelial cells, a layer of mesenchymal-derived cells lining organs and body cavities (13,21,26,30). In general, based on their histological characteristics, mesotheliomas can be divided into three main subtypes: epithelioid, sarcomatoid, and biphasic, according to the morphological features of the tumor cells (1,4,11). The epithelioid subtype, characterized by uniform cuboidal cells, typically has a better prognosis, whereas the sarcomatoid subtype, composed of elongated fibroblast-like cells, is associated with a more aggressive clinical course. The biphasic subtype which combines elements of both presents an intermediate prognosis, depending on the proportion of epithelioid and sarcomatoid components, but is frequently reported as aggressive (1,5,6).

In the present case, the neoplastic cells predominantly exhibited sarcomatoid features. Furthermore, the tumor displayed a prominent myxoid stroma, marked stained by Alcian blue, indicative of mucin deposition, which justifies its classification as a myxoid mesothelioma. This rare histological variant is distinguished by an abundant extracellular matrix rich in mucin and myxomatous material, which contributes to its distinctive histological appearance. In veterinary medicine, reports on this variant remain scarce (8,20,23,31). Importantly, Alcian blue staining, which highlights mucin, was crucial to confirm the presence of this extracellular matrix component, as described in previous studies (20,24).

Clinical signs and laboratory findings of this condition are nonspecific, including abdominal distension, respiratory distress, general malaise, anemia, and neutrophilia, complicating an early diagnosis (4,7,10,21,28). Initial abdominal ultrasound revealed a homogeneous, viscous fluid with increased echogenicity in the mesentery. This finding, coupled with difficulty isolating other abdominal organs, suggested the presence of a significant effusion, likely due to an underlying neoplastic process. Similar findings have been reported in cases of abdominal effusion associated with mesothelial neoplasms, as seen in this case (20,28). The echogenicity of the fluid and the difficulty in isolating adjacent abdominal organs raised suspicion for an atypical peritoneal process, diverging from the typical characteristics of mesothelioma-related effusions (28,32). Unlike the more common presentation of mesotheliomas, which often involve serosanguineous or proteinaceous effusions, the abundant mucinous component observed in this case is more consistent with *P. peritonei*. The increased echogenicity of the fluid can be attributed to its high viscosity and mucin content rather than cellularity alone, distinguishing it from conventional neoplastic effusions (14,16,28). In this case, the dog presented with acute and severe abdominal distension, along with respiratory distress, with rapid clinical progression leading to death, corroborating the aggressive nature of this disease (4,28).

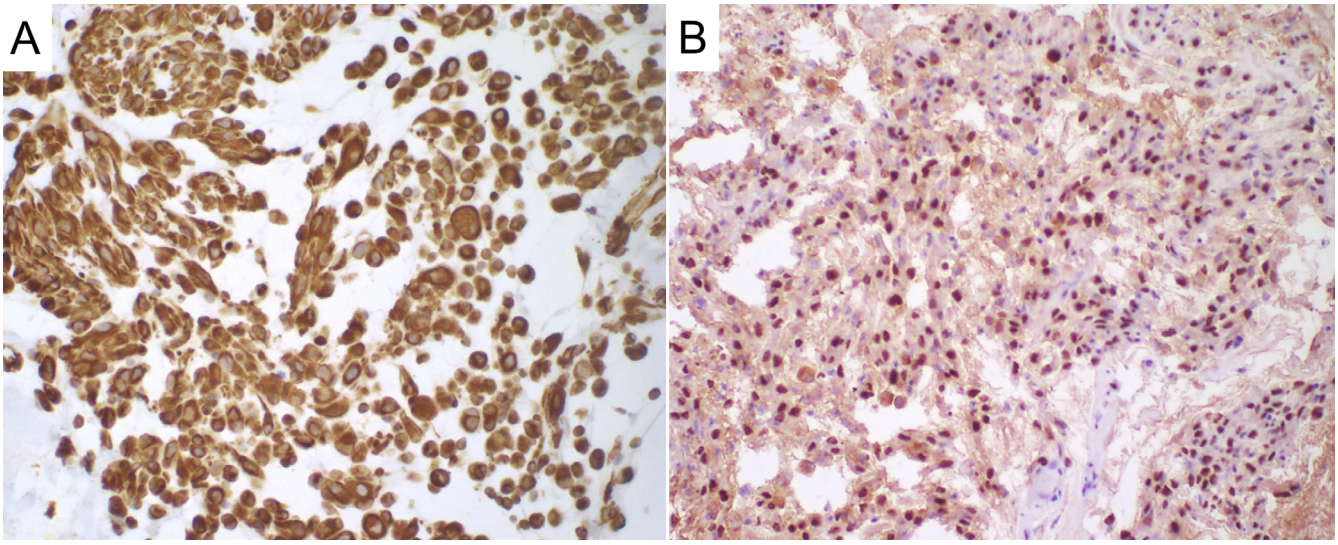

Figure 4. Immunohistochemistry of canine myxoid mesothelioma with clinical presentation of *Pseudomyxoma peritonei*. A: Neoplastic cells showing marked and diffuse positive cytoplasmic immunostaining for vimentin (DAB, 400X). B: Neoplastic cells with marked and diffuse positive nuclear immunostaining for WT1 (DAB, 400X).

The IHC findings in this study were consistent with a mesothelial origin of the neoplastic cells. The neoplastic cells exhibited strong immunoreactivity for vimentin and WT1, while pancytokeratin, CEA, cytoqueratin 34βE12, and calretinin were negative. These results align with previous studies that emphasize the utility of IHC panels in distinguishing mesothelial proliferations from other neoplastic or reactive processes (5,18). WT1, a transcription factor expressed in mesothelial cells, has been widely validated as a marker to differentiate mesothelial cells from carcinoma, showing consistent positivity in both reactive and neoplastic mesothelium (18). Similarly, vimentin, a mesenchymal marker, has shown reliability in confirming the spindle-cell morphology observed in certain mesotheliomas, including rare subtypes like the myxoid variant (5,18,28).

The negative immunostaining for pancytokeratin, CEA, and high molecular weight cytoqueratin suggests the absence of epithelial differentiation or metastatic carcinoma, aiding in narrowing the differential diagnosis (18). Calretinin, while often expressed in mesothelial proliferations, can exhibit variable sensitivity in certain mesothelioma subtypes, which might explain its lack of expression in this case (5,18). This variability underscores the importance of using a comprehensive IHC panel for accurate diagnosis.

Conflict of Interest

The authors declare no competing interests.

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