

## Extraskelatal Osteosarcoma in the Duodenum of a Dog

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### ABSTRACT

**Background:** Osteosarcoma is the most observed primary bone tumor in dogs, and may affect the appendicular and axial skeletons. In addition, it may be present in extraskelatal form, accounting for only 1% of cases. As shown by few reports in the literature, the involvement of the intestinal region by is rare. The objective of this study was to report the case of a 13-year-old Yorkshire dog, submitted to an exploratory laparotomy for suspected partial intestinal obstruction, diagnosed with extraskelatal osteosarcoma.

**Case:** A 13-year-old dog, Yorkshire Terrier, male, presented clinical signs of gastrointestinal abnormalities. An ultrasound examination was performed and was found a mass in small intestine region with wall and lumen invasion. Then, was realized exploratory laparotomy and detected intestinal obstruction due to a mass with approximately 5.0 x 6.0 x 4.4 cm localized in duodenum. Surgical removal was performed and the sample sent to the veterinary diagnostic laboratory for histopathological examination. The sample had an irregular surface and firm consistency. In addition, when cut, the mass enveloped the intestinal layers and sometimes obstructed the lumen. Then, the sample were processed routinely for histopathology. After that, in microscopy evaluation was detected cell proliferation, affecting all layers of intestine. In detail, cells were elongated with pleomorphism marked and atypical mitosis. In addition, there was production of cartilage and bone matrix. So, due the absence of others sites, the neoplasm was considered primary of intestine. After that, to evaluate the expression of KI-67 and COX-2 was performed, and the cell proliferation index was 54.0% and the COX-2 expression was moderate in less than 10% of neoplastic cells. After the surgery, the patient was hospitalized for a week and continue the treatment in home. Afterwards, the tutor received the diagnosis, but even though he was instructed on the severity of the case, he chose not to undergo chemotherapy. After three months, the patient presented abdominal fluid and nodules in your liver, suggesting metastasis, but without diagnosis confirmation. The patient died five months after the diagnosis of extraskelatal osteosarcoma. However, no necropsy was realized, impossibility the diagnosis confirm.

**Discussion:** The frequency of extraskelatal osteosarcoma in dogs remains unknown, with the mammary glands being the most affected site. In the present study, osteosarcoma affects the duodenal region and no reports of this neoplasm in the duodenum of dogs have been found in the literature. The clinical sign of dyschezia was important for the tutor to refer the animal to the veterinarian and perform the ultrasound in an attempt to elucidate the case, as the tumor mass is not always palpable. Histopathological examination and immunohistochemistry were necessary for the differential diagnosis and to establish the prognosis, although after the surgery the tutor chose not to perform chemotherapy. Extraskelatal osteosarcoma are usually highly metastatic, mainly affecting the lymph nodes and liver. In this case, the patient presented a liver nodule three months after the tumor removal surgery, but unfortunately, there was no diagnostic confirmation. Such neoplastic type is rarer and more aggressive than appendicular and axial osteosarcoma, with an average survival of 1 to 3 months. In this case, as a necropsy was not obtained, we cannot attribute the survival time to the disease. The survival rates of osteosarcomas in dogs are few months, but in the present case, although the patient died five months after surgery, the failure to perform a necropsy compromises the attribution of survival time to extraskelatal osteosarcoma.

**Keywords:** canine, neoplasm, bone tumor, histopathology, immunohistochemistry.

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## INTRODUCTION

Osteosarcoma (OS) is a mesenchymal malignant neoplasm, characterized by production of osteoid, rapid growth and invasive and destructive features. Such neoplasm comprises about 80% of the bone tumors affecting dogs [1]. The prevalence of OS is higher in large and giant breed dogs and the common age of the disease onset in canines is, on average, 7.5 to 9 years old [10]. Is a highly metastatic tumor, with the lungs and other bones being the main sites of metastasis. However, less commonly, regional lymph nodes and abdominal organs can be also affected [13]. OS diagnosis is based on anamnesis, physical examination, diagnostic imaging, such as radiography, and cytology. However, the confirmation is achieved through biopsy and histopathological examination [3,5].

OS predominantly manifests in the appendicular and axial skeleton, being the first one the most affected. In addition to affecting the axial and appendicular skeleton, OS may also affect extra-skeletal organs, which is the rarest form of the lesion, but the frequency in canine is not known [8]. In the extra-skeletal osteosarcoma (EOS) there is no primary periosteal or bone involvement and was reported in the spleen, liver, mammary gland, skin and subcutaneous, as well urinary and gastrointestinal tracts [4,6]. The prognosis of EOS is unfavorable [2]. The present study aimed to report a rare case of EOS found in the small intestine - duodenum - of a small male dog.

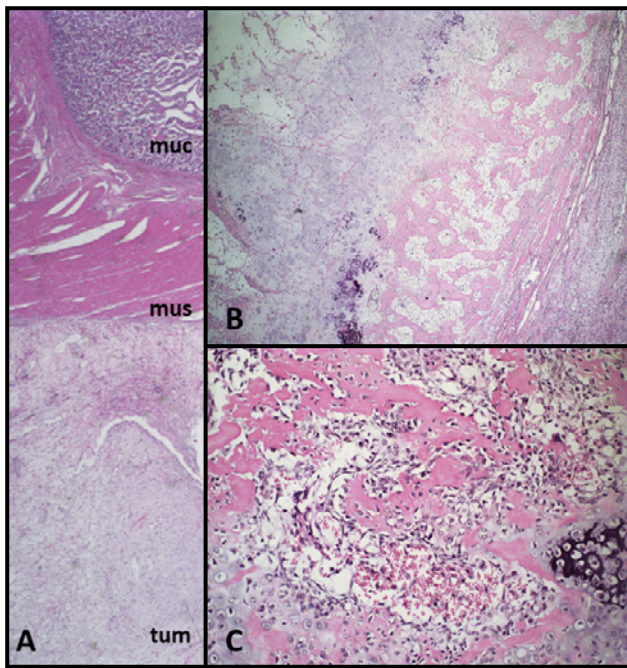
## CASE

A 13-year-old male Yorkshire Terrier dog, after presenting difficulty to evacuate, underwent an abdominal ultrasound examination (UE), in which abnormalities in the intestinal loops were observed. The organ had several segments with pleating of its walls and discrete amount of free fluid, which are indicative of inflammatory process. A mass with approximately 4.5 cm in diameter was observed in jejunum, invading from the wall to the intestinal lumen. The mass had an oval shape, heterogeneous echotexture parenchyma, irregular and poorly defined edges. After UE, 3 possible differential diagnoses were considered; neoplasia, granuloma or enteritis. The patient was referred for an exploratory laparotomy, due to the suspicion of partial intestinal obstruction, during which a mass measuring 5.0 x 6.0 x 4.4 cm was found in the duodenum and removed. Then, a terminal-terminal enteroanastomosis

was performed and the sample was sent to the Veterinary Diagnostic Center - CELULAVET, for histopathological examination.

In macroscopy, the intestinal sample measured 9.0 x 6.0 x 4.4 cm and contained a nodule of 5.0 x 6.0 x 4.4 cm with an irregular surface and firm consistency. When cut, the mass was shown to envelop the entire intestinal wall, sometimes obstructing the intestinal lumen. A solid surface was present, with creaking areas to the cut, which were light brown with foci of brownish color. The tissue fragments were routinely processed for histopathology and the histological sections of the tumor were stained with hematoxylin and eosin (HE)<sup>1</sup>. Histopathological examination revealed intestine fragment presenting intense cell proliferation, with high cellularity, affecting the submucosal, muscular and serous layers. Cells were arranged around atypical mineralized cartilaginous and bone matrix, as well as around areas with solid arrangement. In some areas, such cells exhibited elongated cytoplasm, rounded to oval hyperchromatic nuclei with large and evident nucleoli. Pleomorphism was pronounced, as were anisocariosis and anisocytosis, 13 mitoses were counted in 10 high power fields (400x), with the presence of atypical mitoses. Multifocal areas of necrosis and hemorrhage were also present (Figure 1). The final diagnosis was EOS based on the detection of osteoid matrix. Due to the absence of tumors in other places, the neoplasm was considered primary of the intestine.

Immunohistochemistry was performed to evaluate the expression of COX-2 and Ki-67 (Figure 2). Cuts of 3 µm were obtained from the paraffin blocks for the technique. Histological sections mounted on gelatinized slides were dewaxed in xylol and hydrated in decreasing alcohol graduations. Antigenic retrieval was induced by pressurized wet heat (125 DHLC) with antigen retrieval solution with pH 6.0 (Invitrogen - Thermo Fisher Scientific)<sup>2</sup>. Blocking of endogenous peroxidase was performed with hydrogen peroxide solution (H<sub>2</sub>O<sub>2</sub>) at 10% methyl alcohol. The slides were incubated at 4°C for 14 to 16 h with anti-Ki-67 mouse monoclonal antibody (Clone MIB-1, 1:50, DAKO)<sup>3</sup> and anti-COX-2 primary monoclonal antibody (Clone Sp21, 1:50, Invitrogen - ThermoFisher)<sup>2</sup>. Immunoreactivity was visualized by incubating the slides with diaminobenzidine chromogen (DAB Substrate System, DAKO) for 3 min and the counter staining performed with Mayer's hematoxylin. Antibodies were previously



**Figure 1.** Extraskelatal Osteosarcoma (EOS) in the duodenum of a dog. A- Intestine fragment showing neoplastic formation located below the muscle layer [HE; obj.20x]. B- Intestine fragment presenting loss of tissue architecture with replacement by cell proliferation, with high cellularity, affecting the submucosal, muscular and serous layer [HE; obj.4x]. C- Proliferation of neoplastic cells arranged around mineralized and atypical cartilaginous and bone matrix [HE; obj.20x]. Muc = Mucosal; Mus = muscle; Tum = tumor.

standardized for canine tissues, demonstrating specificity. The result of immunohistochemistry evaluation revealed moderate COX-2 expression in less than 10% of neoplastic cells [7] and cell proliferation index, assessed by Ki-67 immunopositivity, at 54.0% of those [9].

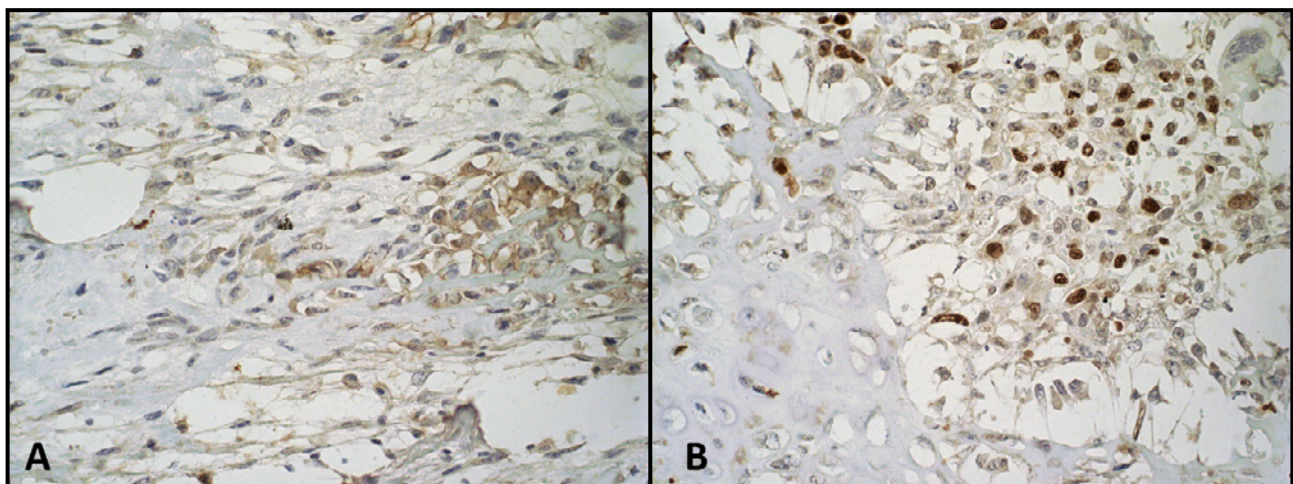
The patient was hospitalized for 1 week after surgery, remaining apathetic but responsive, and received forced feeding in the first days. Afterwards, it

progressed to voluntary feeding, with normal urination and pasty feces. The medical release was carried out at tutor's request with prescription for treatment at home with Trihydrate Amoxicilin + Potassium Clavulanate (Agemoxi CL<sup>®</sup>)<sup>4</sup> and Scopolamine Butylbromide.

Upon receiving the results of the histopathological examination with a diagnosis of EOS, the tutor was clarified and alerted about the severity of the condition and the need for follow-up with an oncologist. However, the tutor decided not to carry out any type of treatment due to the age and condition in which the patient was. After 3 months of surgery, the tutor reported a progressive worsening in the clinical condition of the animal, which was referred to a new ultrasound examination. The image exam evidenced presence of large amount of free fluid with high cellularity distributed inside the abdominal cavity, with increased echogenicity and amorphous and multinodular structures. The patient was also diagnosed with liver damage, which suggested possible metastasis. A small amount of abdominal fluid was collected, whose appearance was intensely bloody. After the examination, a computed tomography and a 2<sup>nd</sup> exploratory laparotomy were suggested to better define the patient's clinical condition. However, no complementary tests were performed for definitive diagnosis. After 5 months of the diagnosis of EOS, the dog died and no necropsy was performed.

## DISCUSSION

A retrospective study of 216 dogs diagnosed with OS revealed that 16.7% were diagnosed with EOS with most cases involving the mammary gland [4]. However, none of the cases involved the small intestine.



**Figure 2.** Extraskelatal Osteosarcoma (EOS) in the duodenum of a dog. A- Moderate cytoplasmic immunolabeling for COX-2 in neoplastic cells [Obj. 40x]. B- Nuclear Immunolabeling for Ki-67 in neoplastic cells [Obj. 40x].

In the literature, studies of EOS in the duodenal region of dogs were not found. To the authors' knowledge, in veterinary medicine, just a cat was diagnosed with EOS in duodenum [12]. The clinical signs of neoplastic lesions vary according to the site on which the neoplasm is installed. In the present case, there was dyschezia, due to partial intestinal obstruction caused by the tumor mass. In some cases, the tumor is palpable, but when it isn't, its presence is observed only after some imaging examination [11].

Histological findings confirmed the diagnosis of EOS, with the duodenum being the primary site of the lesion, due to the presence of osteoid matrix and absence of tumors in other sites. In addition, immunohistochemistry was performed to evaluate the expression of COX-2 and Ki-67. Such methods are of great importance for the final diagnosis of the neoplasm, since those help to define how to proceed with treatment and what the disease prognosis may be [15].

In general, OS presents great predisposition to metastasis, with the lungs being the main metastatic site of such tumor [1]. In contrast, EOS metastases are not frequent in the lungs, being more commonly found in lymph nodes and liver [14]. Therefore, patients are

instructed to continue following treatment and medical observation, even after surgical removal of the primary tumor. The guardian of the present case reported did not submit the animal to medical monitoring, so a possible metastasis in the animal's liver after removal of the primary tumor could not be confirmed. Such neoplastic type is rarer and more aggressive than appendicular and axial OS, with an average survival of 1 to 3 months [2]. In this case, as a necropsy was not obtained, we cannot attribute the survival time to the disease. After the result of the histopathological and immunohistochemistry performed in the Laboratory of Comparative Pathology (LPC), the final diagnosis of the patient was EOS, a rare neoplasm in animals.

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