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Case Report

Primary and metastatic alveolar rhabdomyosarcoma: clinical case reports in canines from the Peruvian Amazon

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Abstract
This report presents four cases of alveolar rhabdomyosarcoma in canines, affecting the striated muscular tissues of the rostral skin, mandible, uterus, and multiple metastases. These cases were treated at veterinary clinics across the San Martín region (provinces of San Martín, Moyobamba, and Tocache), Peru, from a total of 118 neoplastic cases diagnosed histopathologically at the Animal Histopathology Laboratory of the Professional School of Veterinary Medicine, National University of San Martín, between 2020 and 2024. Diagnosing alveolar rhabdomyosarcoma requires a combination of histopathological analysis, immunohistochemical markers, and molecular techniques to distinguish it from other neoplastic variants. These cases highlight the importance of histopathological analysis, appropriate selection of chemotherapy or surgical resection, owner compliance, and the limitations of empirical therapies which, in the absence of a diagnostic foundation, often lead to recurrence and treatment failure in tumor management. In recent years, cytological and histopathological diagnosis has become a key resource in the San Martín region, contributing significantly to therapeutic decision-making and the prognostic evaluation of various types of canine neoplasms.

Keywords: muscle tissue, dogs, neoplasm, oncology

Introduction

Rhabdomyosarcomas (RMS) are malignant neoplasms originating from skeletal muscle precursor cells, subclassified into four distinct variants: alveolar, botryoid, embryonal, and pleomorphic (11, 26). Alveolar rhabdomyosarcoma (RMS) is histologically classified into two subtypes: the classic and solid variants, botryoid RMS is considered a subtype of embryonal RMS in both human and veterinary pathology; embryonal RMS includes three recognized histologic variants: myotubular, rhabdomyoblastic, and spindle cell types; pleomorphic RMS, although the least common subtype in human pathology, has also been reported in veterinary species. In dogs, as in humans, pleomorphic RMS is typically diagnosed in adult animals and is exceedingly rare in juveniles (7, 29).

The pleomorphic variant is the rarest form observed in domestic animals and can be particularly challenging to differentiate from the embryonal subtype (8). This neoplasm is characterized by its aggressive clinical behavior, a propensity for local recurrence following surgical excision, and a significant potential for metastasis (8).

Respect to the molecular pathogenesis of rhabdomyosarcoma (RMS), it has been proposed that the disease may originate from a developmental arrest in undifferentiated myoblastic cells (34). This phenomenon is associated with the PAX-FKHR gene fusion, which promotes uncontrolled cellular proliferation, aberrant cell cycle progression, inhibition of tumor suppressor genes such as *RB* and *p53*, and disruption of normal myogenic differentiation pathways (12). This gene translocation leads to the overexpression of *MyoD1*,

which enhances cellular proliferation, along with increased levels of *myogenin*, which promotes differentiation toward muscle lineages (33). Additionally, constitutive activation of *H-RAS* provides persistent mitogenic signals that further drive continuous cell cycle progression, ultimately resulting in the neoplastic growth characteristic of alveolar rhabdomyosarcoma (30).

Rhabdomyosarcoma is considered a rare neoplasm in veterinary medicine (18). When it does occur, it is most commonly observed in animals aged 2 to 3 years (8). The low incidence of this tumor may be attributed to its clinical, phenotypic, and morphological heterogeneity (7). A retrospective study conducted at the Laboratory of Histology, Embryology, and Veterinary Pathology of the Universidad Nacional Mayor de San Marcos reported a prevalence of 40.40 ± 1.3% (19/47) for alveolar rhabdomyosarcoma across various anatomical sites, based on a total of 1,125 neoplastic cases in canines over a nine-year period (20).

Rhabdomyosarcoma can arise in various anatomical locations, affecting tissues that may or may not contain striated muscle. It has been observed in regions such as the face (maxillofacial area), neck, and tongue (8), as well as the esophagus and larynx (4). The neoplasm has also been documented in internal organs including the heart (15), liver, urinary tract, uterus, and cervix (6). Furthermore, rhabdomyosarcoma has been reported in areas like the parmeningeal space (19) and perianal region (31). This locally invasive neoplasm has a marked potential for metastasis, with secondary spread observed in lymph nodes, spleen, lungs (9), and kidneys. Macroscopically, rhabdomyosarcomas typically present as large, pinkish, and highly vascular masses (32), exhibiting invasive growth patterns (9). The clinical signs associated with this neoplasm vary depending on its anatomical location and the extent of tissue involvement (25). For instance, large tumor masses in the legs may cause mobility issues (2); lesions in the oral cavity can lead to difficulty in food prehension and dysphagia (28); tumors in the maxillary region may result in edema and molar displacement (13); and nasal involvement may present as nasal discharge (17), among others.

The diagnosis of rhabdomyosarcoma primarily relies on histopathological examination. However, for a definitive diagnosis, supplementary techniques such as immunohistochemistry are essential (21). Immunohistochemistry plays a critical role in identifying the specific cellular origin of rhabdomyosarcoma. Markers such as myoglobin are particularly valuable, as they demonstrate increased sensitivity in detecting well-differentiated rhabdomyosarcomas, though they may be negative in poorly differentiated cases (22).

Markers such as myogenin are commonly used to identify rhabdomyosarcomas in humans, as they are associated with a family of transcription factors and gene products that play a critical role during the early phases of muscle cell differentiation and biogenesis (1, 5, 24). As a result, myogenin is widely regarded as a specific marker for rhabdomyosarcoma in human medicine (22). Although molecular techniques have been less extensively explored in canine cases, genetic mutation analysis employed in human medicine may provide a valuable standard for distinguishing between rhabdomyosarcoma subtypes, such as alveolar and embryonal (23). Unfortunately, the prognosis for this neoplasm is often poor due to its high metastatic potential and aggressive biological behavior (7).

Cases description

Between 2019 and 2024, a total of 118 neoplastic cases were submitted for histopathological diagnosis to the Animal Histopathology Laboratory at the Professional School of Veterinary Medicine, National University of San Martín, in San Martín, Peru. Of these, four cases of alveolar rhabdomyosarcoma were identified, originating from various anatomical sites: rostral cutaneous, mandibular, uterine, and cases with multiple metastases. These cases were diagnosed in canines treated at veterinary clinics in the San Martín region, including the provinces of San Martín, Moyobamba, and Tocache. Clinical evaluations, histopathological analyses, and immunohistochemical follow-ups were conducted for each case during the study period.

Tissue samples were collected from various anatomical locations, depending on their site of origin, either during surgical procedures or necropsy, as appropriate. The samples were fixed in 10% buffered formalin solution, routinely processed, and embedded in paraffin. Sections 4–5 µm thick were cut from the paraffin blocks using a microtome and mounted on standard glass slides. The sections were routinely stained with hematoxylin and eosin (H&E) and, in cases of rostral cutaneous alveolar rhabdomyosarcoma, additionally subjected to immunohistochemical staining for myogenin and myosin. All stained sections were examined under a light microscope (Nikon Eclipse Ei, Japan) equipped with a Tuopcam XCAM4K8MPA camera (Sony sensor, China) at the Animal Histopathology Laboratory, Faculty of Veterinary Medicine, National University of San Martín, Tarapoto, Peru.

Additionally, the cases were further reviewed at the Laboratory of Histology, Embryology, and Veterinary Pathology, Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos. Informed consent was obtained from the animal owners, who also approved the publication of the case reports presented in this study.

Alveolar rhabdomyosarcoma at the mandibular region

A 12-year-old female Rottweiler crossbreed presented with a mass in the jaw, initially diagnosed as a transmissible venereal tumor based on clinical examination. The patient underwent chemotherapy with vincristine (0.5 and 0.65 mg/m²), but despite an initial response, the tumor recurred. Surgical resection was performed, but the tumor reappeared, and

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the animal was treated again with chemotherapy. Unfortunately, this intervention failed to produce a significant therapeutic response. The entire treatment process spanned six months, after which the owners sought a second opinion at another veterinary clinic.

Upon reassessment, a mass measuring approximately 10 cm x 8 cm x 7 cm (length × width × height) was identified. Macroscopic examination revealed that the tumor involved the gingiva, resulting in tooth loss and showing evidence of bone involvement with a characteristic irregular surface and incrustation (Fig. 1). Clinical laboratory results showed severe leukocytosis, suggestive of an underlying infectious process, although other blood parameters were within normal limits. Given the aggressive nature of the lesion, Lateral mandibulectomy was the selected surgical technique.

Histopathological analysis revealed the presence of atypical, round to pleomorphic mesenchymal cells with faintly basophilic cytoplasm. The nuclei were round to irregular in shape, with several binucleated and trinucleated cells, large basophilic nucleoli (1–3 per cell), irregular nuclear membranes, and dense chromatin. The mitotic activity was high, with more than three mitotic figures per field at 40x magnification. These neoplastic cells were arranged in clusters or cords, embedded within a vascular connective tissue stroma, forming alveolar-like structures that extended into the deep dermis (Fig. 2A,B). Follow-up was not possible, as the animal died the day following the surgical procedure. A post-mortem examination was declined by the owners.

Alveolar rhabdomyosarcoma with multiple metastases

A 10-year-old female mixed-breed dog weighing approximately 12 kg was euthanized following a month-long clinical history of abdominal swelling, acute pain, anorexia, and lethargy. External examination revealed pallor of the oral mucous membranes, signs of periorbital dehydration, abdominal distension with acute pain, and marked cachexia. No laboratory or imaging diagnostics were conducted, as the owners declined further investigations before proceeding with euthanasia.

Figure 1. Alveolar rhabdomyosarcoma. Exuberant mass originating from the mandibular region, exhibiting invasion of the gingival mucosa and underlying bone, with concomitant loss of dental structures.

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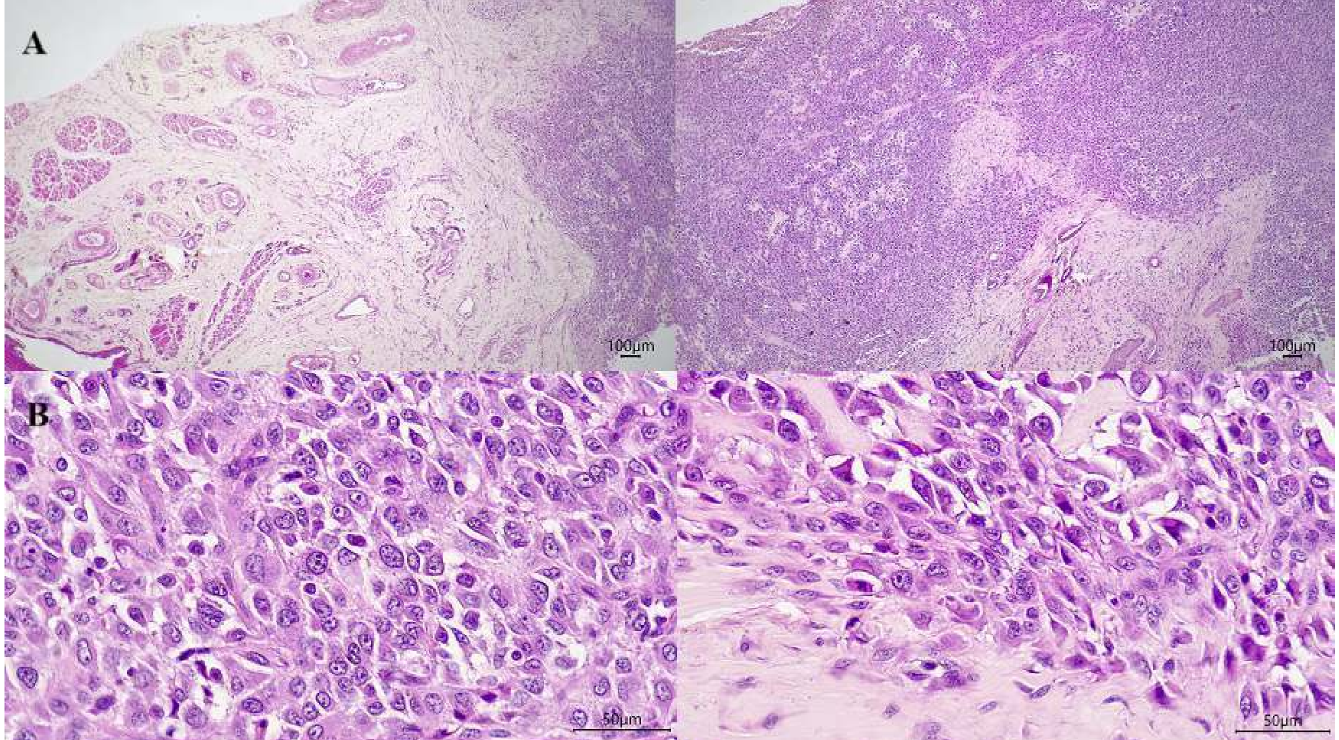

Figure 2. Alveolar rhabdomyosarcoma in the mandible. A. Atypical mesenchymal cells infiltrating the stromal tissue. HE. Scale bar =100 µm). B. Neoplastic cells displaying characteristic morphological features, including irregular nuclear membranes and prominent nucleoli HE. Scale bar =50 µm).

Necropsy findings included icteric subcutaneous tissue, lymphadenomegaly, and hemorrhagic foci, including purpura and ecchymosis in the lungs. The spleen showed tumor clusters ranging from 0.5 to 2 cm in diameter, with a dark reddish color, localized in the cranial portion of the organ (Fig. 3A). The liver exhibited postmortem biliary imbibition lesions on the diaphragmatic surface of the right lobe, alongside diffuse tumor masses of similar size (0.5–2 cm) and dark red coloration (Fig. 3B). Additionally, small reddish masses (~0.3 cm) were found on the abdominal surface of the diaphragm (Fig. 3C).

Histopathological examination revealed significant findings. In the spleen, the white pulp showed marked depletion of lymphoid follicles and proliferation of numerous atypical mesenchymal cells, which were round to pleomorphic in shape. These cells exhibited faintly basophilic cytoplasm, and their nuclei were circular to pleomorphic, with varying sizes and prominent nucleoli and many of them were binucleated, and they are along with a moderate number of multinucleated megakaryocytes (exceeding 12 µm in diameter) were observed. The red pulp displayed extensive hemorrhage, fibrin deposits, necrosis, and focal neutrophilic infiltration, some of which showed varying degrees of degeneration. A large hemorrhagic area interspersed with fibrin strands and numerous degenerate neutrophils was evident, with brown pigment suggesting hemosiderin. These findings were consistent with metastasis of alveolar rhabdomyosarcoma, accompanied by extramedullary hematopoiesis, hemorrhage, and necrosis (Fig. 4A).

In the diaphragmatic skeletal striated muscle, there was a proliferation of atypical mesenchymal cells, similar to those observed in the spleen, interspersed with severe hemorrhage and deposits of brown pigment indicative of hemosiderin. Alveolar-type rhabdomyosarcoma with necrosis and hemorrhage was identified (Fig. 4B). In the liver, areas of disrupted trabecular architecture were noted, along with individual hepatocytes and focal infiltration by atypical mesenchymal cells resembling those found in the diaphragm (Fig. 4C).

Rostral cutaneous alveolar rhabdomyosarcoma

A 5-year-old male Labrador retriever was presented to a veterinary clinic in the Tarapoto district with a nodular lesion resembling a wart on the right lower periorbital area, approximately 3 cm in diameter. Based on its morphological features, the veterinarian opted for excision of the lesion without prior histopathological evaluation. However, approximately three months post-surgery, the mass recurred in the same location, and an additional mass of similar characteristics appeared in the right nasal region, measuring approximately 2.5 cm in diameter (Fig. 5A).

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Figure 3. Tumor nodules identified in the spleen (A), liver (B), and diaphragmatic muscle (C), showing a diffuse distribution of neoplastic masses.

An excisional biopsy was performed for histopathological diagnosis, during which two neoplastic tissue samples were collected. Macroscopically, the lesions exhibited a dark reddish coloration, a smooth surface, and a solid, invasive consistency affecting the skin. Histopathological findings revealed no significant alterations in the epidermis, superficial dermis, and adnexa. However, the middle dermis was heavily infiltrated by atypical mesenchymal cells, ranging from round to pleomorphic in shape. These cells exhibited faintly basophilic cytoplasm and nuclei of varying sizes, many of which were prominent, with diameters exceeding 12 µm. Several cells were binucleated or trinucleated, with irregular nuclear membranes, dense chromatin, and 1 to 3 large basophilic nucleoli. Mitotic activity was marked, with more than three mitotic figures observed per field at 40x magnification. These neoplastic cells were arranged in clusters or cords, surrounded by a discrete vascular connective tissue stroma, forming alveolar-like structures. The mass infiltrated into the deep dermis, extending to the underlying striated skeletal muscle fibers, resulting in necrosis at the ventrolateral surgical margin. These histological features were consistent with a diagnosis of alveolar rhabdomyosarcoma with cutaneous necrosis (Fig. 5B).

To confirm the neoplasm's mesenchymal origin, immunohistochemical analysis was performed using specific markers for muscle cell identification. The anaplastic cells exhibited positive staining for myosin (Fig. 5C) and myogenin (Fig. 5D) in varying degrees, supporting the diagnosis of

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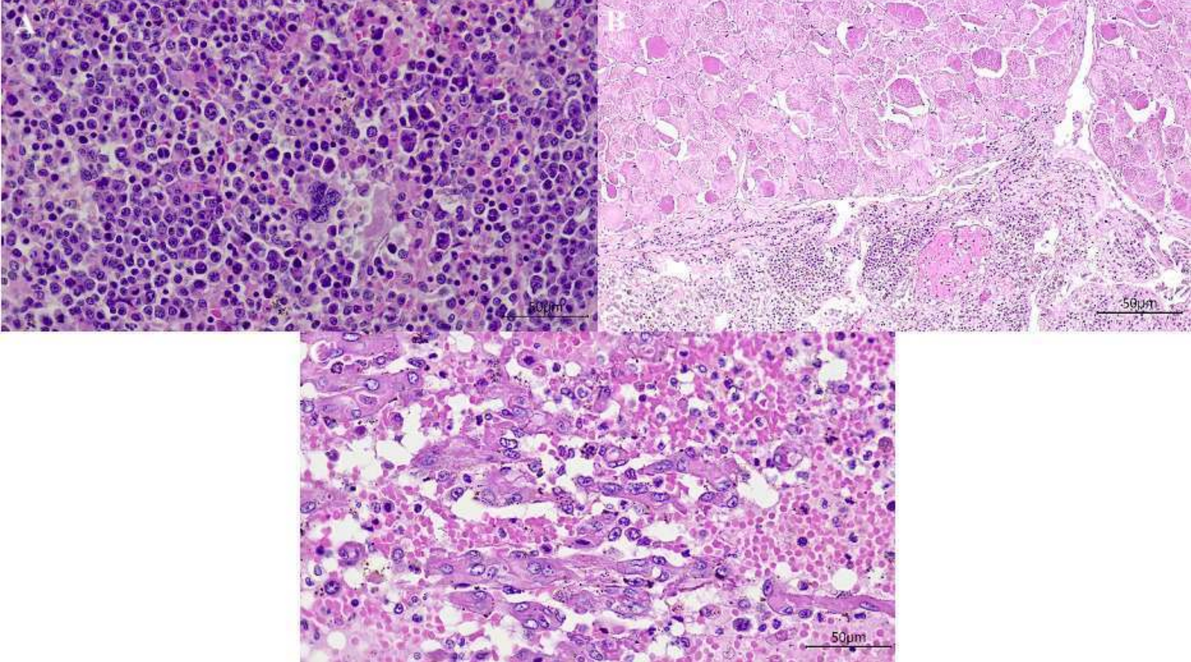

Figure 4. A. Spleen. Histopathological examination revealing infiltration by atypical mesenchymal cells with pleomorphic nuclei and dense chromatin, indicative of a neoplastic process. HE. Scale bar =50 µm. Liver. B. Disruption of the hepatic parenchyma with infiltration of atypical mesenchymal cells, leading to architectural distortion. HE. Scale bar =50 µm. C. Diaphragmatic muscle. Infiltration of atypical mesenchymal cells into the connective tissue surrounding skeletal muscle bundles, with evidence of necrosis in affected regions. HE. Scale bar =50 µm.

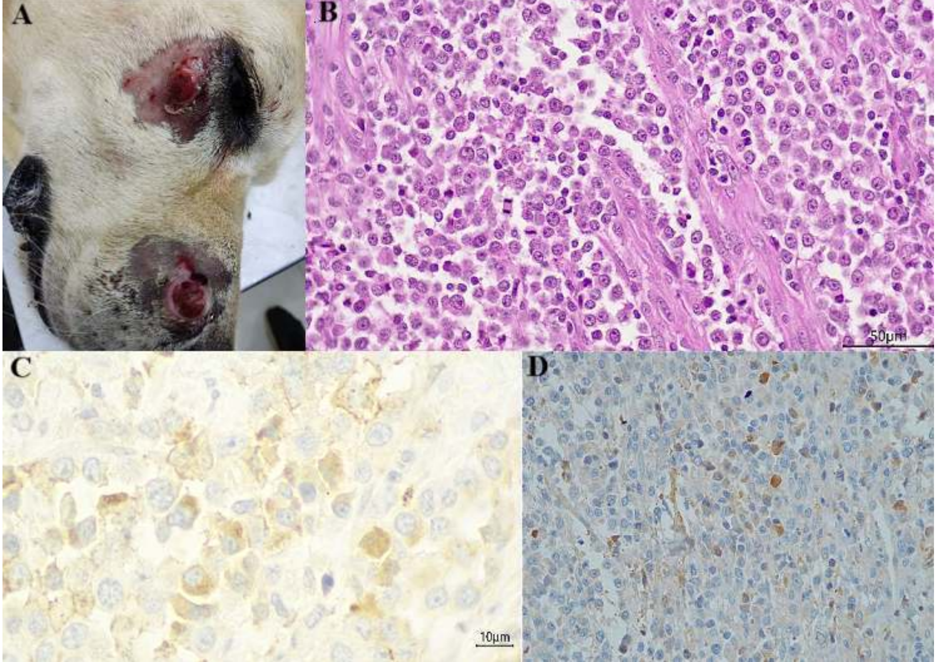

Figure 5. A. Neoplastic masses located in the right lower periorbital and nasal regions, both of which exhibit marked vascularization and invasive growth patterns. B. Alveolar rhabdomyosarcoma. Neoplastic cells with binucleation or trinucleation, irregular nuclear membranes, dense chromatin, and 1–3 large basophilic nucleoli. The stroma is finely structured and supports the neoplastic cell proliferation. HE. Scale bar =50 µm. C. Positive immunohistochemical staining for myosin in the cytoplasm of neoplastic cells, further supporting the diagnosis of rhabdomyosarcoma. Immunohistochemistry (IHC). Scale bar =10 µm. D. Positive immunohistochemical staining for myogenin in the nuclei of neoplastic cells, confirming their myogenic origin. Immunohistochemistry (IHC). Scale bar =10 µm.

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rhabdomyosarcoma. Despite the diagnosis, the owners chose not to pursue chemotherapy or any other form of treatment.

Follow-up was unfortunately not feasible, as the owners did not return to the veterinary facility after the diagnosis. Consequently, the clinical progression of the neoplasm could not be assessed.

Uterine alveolar rhabdomyosarcoma

This case presents a 3-year-old female Argentinian Dogo with a history of transmissible venereal tumor who underwent chemotherapy. After six months, the pet returned with copious vulvar discharge. Clinical examination revealed polycythemia (HGB: 21.9 g/dL, HTC: 51.1%) and leukocytosis (LYM: 19.7 x 10^9/L, MID: 9.3 x 10^9/L, NEUT: 24.6 x 10^9/L). The owners declined imaging studies prior to surgery due to economic limitations. The dog was then subjected to surgery due to suspicion of pyometra; however, a mass measuring 7 cm x 10 cm was found at the distal end of the left uterine horn (Fig. 6A).

In the histopathological diagnosis, the uterine mucosa appeared thin, with low cuboidal epithelium and sparse, dilated tubular glands lined by cuboidal epithelium containing acidophilic proteinaceous secretion. Additionally, there were obliterated glands surrounded by multifocal lymphocytic exudate, while the myometrium displayed normal-appearing smooth muscle. In four sections, a tissue composed of abundant atypical mesenchymal cells was identified; these cells were round to pleomorphic, with faintly basophilic cytoplasm and circular to pleomorphic nuclei of varying sizes, many prominent, exceeding 12 μm in diameter. Several cells were bi- or trinucleated, with irregular nuclear membranes, dense chromatin, one to three large basophilic nucleoli, and more than three mitotic figures per field at 40X magnification. These cells were organized in bundles or lines adhering to a discrete vascular connective stroma surrounding them in alveolar-like structures, extending to the ventrolateral surgical margin. Histopathological diagnosis indicated a mild non-suppurative or chronic mononuclear endometritis with alveolar-type rhabdomyosarcoma (Fig. 6B-D). At the six-month postoperative follow-up, diagnostic ultrasonography revealed no signs consistent with local tumor recurrence.

Discussion

Rhabdomyosarcoma is a malignant neoplasm originating from striated muscle, classified based on its histological characteristics into four subtypes: embryonal, botriod, alveolar, and pleomorphic. It is relatively infrequent in canines

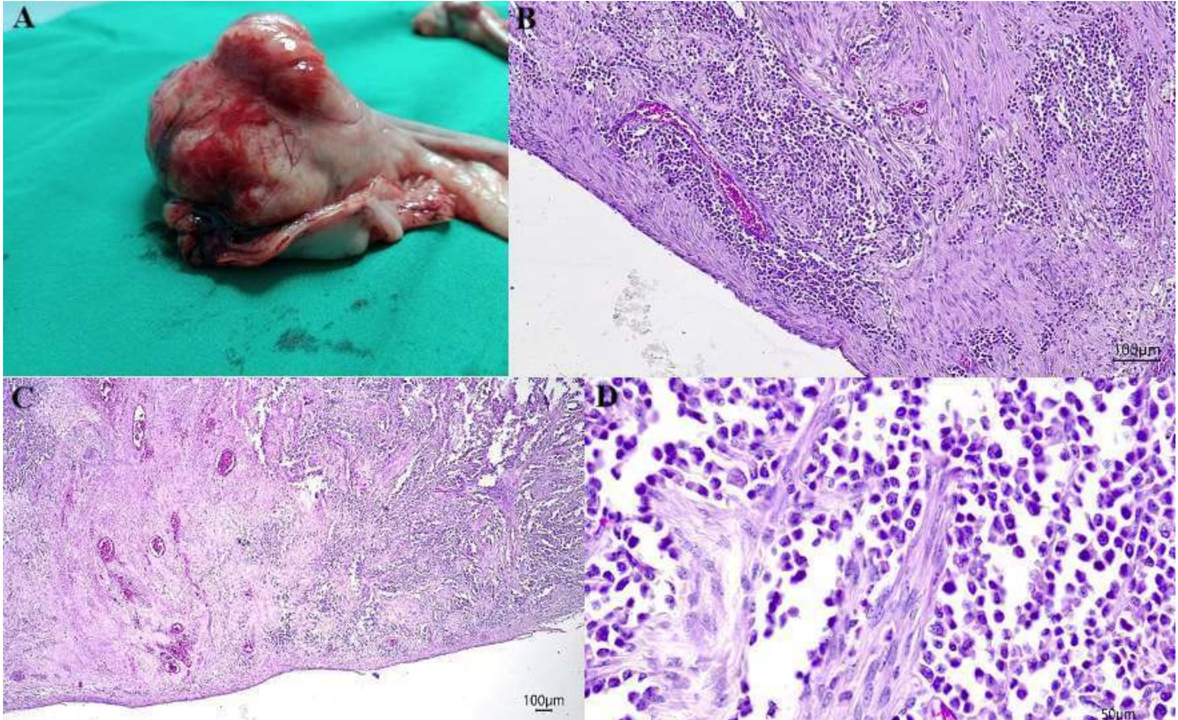

Figure 6. Alveolar rhabdomyosarcoma in the uterus. A. Macroscopic appearance of an exuberant tumor mass located at the distal end of the left uterine horn, exhibiting invasive growth characteristics. B. Tumor cell infiltration into the uterine tissue, replacing normal architecture. B. Complete absence of uterine glands and mucosal lining, consistent with neoplastic transformation. HE. Scale bar =100 μm. C. Distribution of tumor cells in dense clusters. HE. Scale bar =100 μm. D. Detailed view of the alveolar-like pattern of the neoplastic cells, characteristic of rhabdomyosarcoma. HE. Scale bar =50 μm.

(10, 11). Alveolar rhabdomyosarcoma is distinguished by the presence of atypical, round cells arranged in an alveolar pattern, with a supporting fibrous stroma (8). In Peru, a study conducted at a veterinary histopathological reference laboratory in Lima found that rhabdomyosarcoma accounted for 5.6 ± 1.3% of all diagnosed neoplasms in canines, based on a total of 1125 cases. Of these, 19 (40.4%) were identified as rhabdomyosarcoma over an eight-year retrospective evaluation period (21). Other reports have documented alveolar rhabdomyosarcoma in various anatomical locations, including the right forelimb, which is described as a firm, immovable mass (27). Additionally, the condition has been previously observed in the maxillofacial region of two juvenile canines, both of which succumbed to the disease approximately two months following surgical intervention (14).

In remote Amazonian regions, distant from the capital, it has been observed that veterinarians do not consistently request pathological examination for definitive diagnosis of neoplastic masses, opting instead for empirical treatment strategies such as surgical excision or chemotherapy. In the cases described, primary tumors located in the mandible, ovaries, and facial region were either empirically treated or surgically resected without prior histopathological assessment. As a result, subsequent recurrence with metastasis and more aggressive tumor behavior were observed, as exemplified by the case of multi-organ metastasis. This issue is widespread, as clinicians often delay or forgo diagnostic evaluations; when such evaluations are requested, they are frequently not performed due to issues related to owner consent or financial constraints.

Histopathological examination revealed the malignant behavior of rhabdomyosarcoma, characterized by the disruption of muscle fibers and loss of their structural integrity. Cellular aggregates exhibited an alveolar pattern, with muscle fibers losing their characteristic striations and transitioning into round cells displaying features of malignancy, including anisocytosis and anisokaryosis, which predominated in most of the microscopic fields. Avallone (3) emphasizes the critical role of specific marker-based techniques in accurately diagnosing these neoplasms. In the context of immunohistochemistry, Kobayashi et al. (21) demonstrated that the expression of markers such as myogenin and MyoD strongly correlates with the histopathological characteristics of myogenic neoplastic cells. However, Andrew (1) cautions that these markers may also be expressed in atrophied muscle fibers or muscle cells infiltrating other sarcomas, necessitating careful diagnostic evaluation. For the confirmation of histologically anaplastic cases, immunohistochemical techniques such as myogenin and myosin are employed (27), with the latter showing 95% sensitivity in human rhabdomyosarcoma cases (16).

In the case of cutaneous rhabdomyosarcoma, the immunohistochemical evaluation was positive for both markers, with varying intensity of reactivity. This variation can be explained by the fact that these neoplasms may lose receptor expression during their muscular transformation. However, the results confirmed that the sample was positive for a mesenchymal neoplasm of muscular origin, as demonstrated by this technique in conjunction with histopathology. Avallone (3) highlights the importance of using markers for accurately determining the appropriate surgical and chemotherapy protocols. Therefore, the evaluation of rhabdomyosarcoma must be approached from both clinical and laboratory perspectives, underscoring the need for greater awareness among clinical veterinarians regarding diagnosing and managing sarcomas in domestic dogs.

In conclusion, these case reports highlight the diagnostic and therapeutic challenges associated with alveolar rhabdomyosarcoma in canines from the Peruvian Amazon. The findings demonstrate the diverse regional and systemic presentations and dissemination patterns of this neoplasm, as well as the failure of empirical therapies lacking diagnostic support. These observations underscore the critical need for routine implementation of cytological or histopathological diagnostics in clinical practice, along with the application of immunohistochemical techniques using specific markers to enable accurate early diagnosis and, consequently, more effective clinical management. Furthermore, additional research is warranted to develop and strengthen investigative lines in histopathological, immunohistochemical diagnosis, and oncologic therapeutic approaches that can enhance understanding and treatment of this neoplastic disease in remote Amazonian regions of the country.

Conflict of Interest

The authors declare no competing interests.

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