Slov Vet Res
DOI 10.26873/SVR-1670-2024
UDC 591.8:616-006.6:616-091.8:636.7
Pages: 61–7

Case Report

Undifferentiated Embryonal Rhabdomyosarcoma in a German Shepherd Dog: Macroscopic, Histopathologic and Immunohistochemical Features

Key words

embryonal rhabdomyosarcoma;
dog;
histopathology;
immunohistochemistry

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Abstract: This case report describes an 8-year-old female German Shepherd dog with undifferentiated embryonal rhabdomyosarcoma. The mass measured 13 x 12 x 9 cm, weighed 900 grams, and had an elastic consistency. Histopathologic examination revealed a large necrosis area in the center of the mass. We determined cells with spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged atypia, and mitosis around the necrosis. We noted long cells with nuclei arranged in a row and wreath-like multinucleated giant cells among these cells. In the immunohistochemical examination, neoplastic cells were stained with vimentin, desmin, skeletal muscle myosin, sarcomeric actin, and SMA positively, while Iba1, HLA-DR, pancytokeratin, S100B, SOX10, and GFAP were stained negatively. Myogenin was intranuclearly positive in approximately half of the cells. The case was diagnosed as RMS and was classified as undifferanted varyant of embryonal type on the basis of histopathologic and immunohistochemical findings. The original morphological and immunophenotyping structure of the tumor, along with the intriguing structure of the giant cells, led us to believe that sharing the case would be beneficial. This case will contribute to the pathomorphological knowledge of canine striated muscle tumors for studies in the field of veterinary oncology.

Received: 26 December 2022
Accepted: 17 October 2024

Introduction

Rhabdomyosarcoma (RMS) is arised in skeletal muscle, where they could be derived from the resting myoblasts, or satellite cells, they can arise in any part of the body, including sites that normally lack skeletal muscle. The cell of origin of RMS is still controversial and may differ for different subtypes. It has been suggested that in some cases they arise from primitive mesenchymal cells capable of differentiation into skeletal muscle cells (1). RMS in humans and dogs can resemble undifferentiated myoblasts or early embryonic myotubes. The term embryonal is a descriptive term for neoplasms exhibiting a range of cellular morphologies that resemble various developmental intermediates and often have a myxomatous stroma as seen in developing muscle (1-3). According to the international

classification system, human RMS is subdivided into embryonal, botryoid, alveolar, and pleomorphic (anaplastic) subcategories (1, 2). Classification of canine RMS closely parallels classification schemes in human medicine (1). The accurate classification of these tumors in humans is prognostically important, with the best outcomes associated with botryoid rhabdomyosarcoma and the worst with alveolar rhabdomyosarcoma. Embryonal forms have an intermediate prognosis. There is presently insufficient information on clinical outcome to make such prognostic predictions in animals, but development of such information will depend on accurate and consistent classification (2). RMSs in domestic animals are classified based on histopathological findings as embryonal, botryoid, alveolar and pleomorphic RMS (1, 2). Embryonal RMS includes three variants: myotubular, rhabdomyoblastic and spindyloid. Histology of myotubular variant consists of presence of characteristic multinucleated "strap cells", which form myutubes. The rhabdomyoblastic variant consist on histology of frequent round to polygonal cells with abundant eosinophilic cytoplasm. The spindle-cell variant of RMS is rare and a relatively new category. Histological aspect consists of thin spindyloid myoblast cells, usually with formation of bundles and myxoid stroma (1, 3). Botryoid RMS is considered a variant of embryonal RMS in both human and veterinary medicine. Macroscopically, it appears as a polypoid, grape-like mass and is encountered most commonly in the urinary bladder, where it can be seen protruding from the mucosa. Histological examination reveals many undifferentiated rhabdomyoblasts and/or strap cells suspended in a myxoid matrix, these being characteristic (1-3). Alveolar RMS is histologically subdivided in classic and solid variants. The classic variant is characterized by aggregates of small, poorly differentiated round cells. The solid variant in dogs consists of sheets of small round neoplastic cells divided by thin fibrous septa. This pattern is not always present, making the histologic architecture similar to rhabdomyoblastic embryonal RMS, thus the diagnosis is very difficult. Molecular genetic analysis has been proven efficient in this matter (1, 3). Pleomorphic RMS marks the least common variant in human medicine. In dogs, like in humans, is diagnosed typically in adults and is extremely rare in young patients. The tumour rises almost exclusively within skeletal muscle of the limbs. Typically, this variant occurs in large muscles of the limbs and histologically contains a very pleomorphic cell population (1-3).

RMS are relatively rare domestic animal neoplasms with a variety of gross morphologies, histologic variations, and cellular phenotypic variations. In veterinary medicine, the frequency of nonlaryngeal or noncardiac canine RMS is low, with 65 total case reports published (2). RMS cases have been most commonly reported at dogs (2-9), less so in other species; cows (10), cats (11), horses (12) and sheep (13). In dogs, the most common sites involved the skeletal muscle, tongue, larynx, lip, myocardium, urinary bladder and ovarium (2-4, 7, 8, 14). Cooper and Valentine (2) reported in a 20-year retrospective registry study at Cornell University that 58 of the approximately 83,000-neoplasia cases were diagnosed with rhabdomyoma or RMS. They noted that only 16 of the 58 cases could be confirmed by contemporary methods.

In this case report, it was aimed at defining undifferentiated embryonal RMS, which was diagnosed in an 8-year-old female German Shepherd dog by macroscopic, histopathological, and immunohistochemical methods.

Case Presentation

The animal owner provided informed consent. An 8-year-old female German Shepherd dog, with the complaint of the formation of a round mass, approximately 12 cm in diameter, within the borders of the ventro-caudal region of the scapula, sternum, and neck and closer to the left side, was admitted to the clinics of the Faculty of Veterinary Medicine, Kyrgyz-Turkish Manas University, Bishkek, Kyrgyzstan. The owner stated that the mass reached this size for approximately 6 months and did not cause any functional disorders in the animal. The dog was in good general condition, and the appetite was unchanged. Radiographs were taken, and blood analyses were performed. The radiographs revealed that the mass had no connection to deeper tissue. Thoracic radiographs did not show any metastatic lesions. The results of the blood analysis were within normal limits (Table 1).

As a result of the evaluations, it was decided to remove the mass by surgery. For the surgery, the area was shaved and disinfected (Figure 1A). Anesthesia was administered with

Table 1: The dog's blood test data

Parameters	Value	Units
WBC	9.1	x 10^9/ L
Lymph	1.7	x 10^9/ L
Gran	6.5	x 10^9/ L
HGB	195	g/L
RBC	7.51	x 10^{12}/ L
HCT	49.0	%
MCV	65.3	fL
MCH	25.9	Pg
MCHC	35,7	g/dL
PLT	376	x 10^9/ L
MPV	9.1	fL

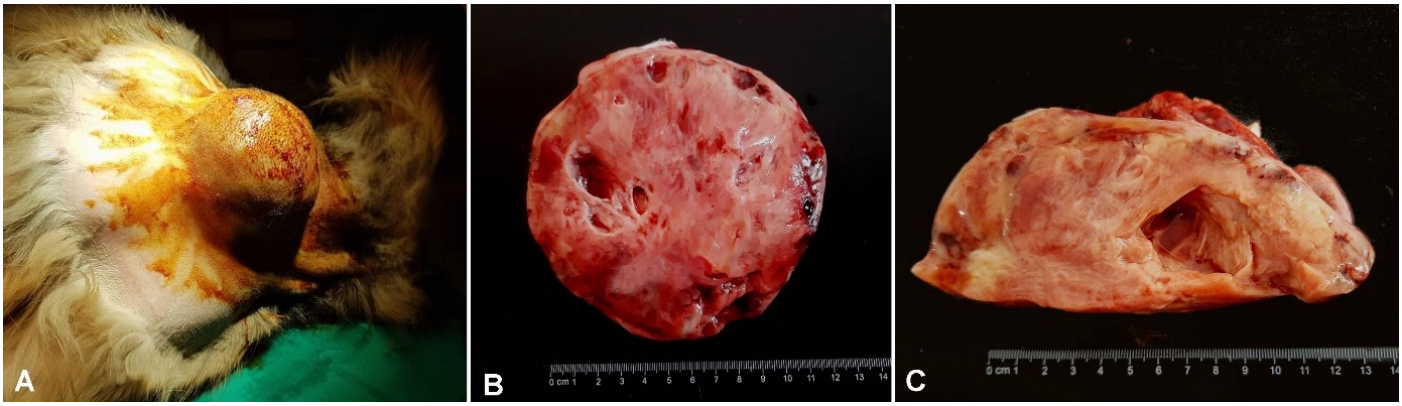

Figure 1: A. Preoperatif appearence of round mass, B- On cut-surface, in the middle of the mass, yellowish-gray colored, occasionally hard areas and slightly red colored structures in gelatinous-pelmetic structure. Melting areas of different sizes and irregular cavernous structures, C- The areas close to the wall of the mass harder consistency and dark red nodular structures with a diameter of 1-2 cm in these areas. A grayish-white thin fibrous capsule outside the mass

xylazine hydrochloride (Vetaxyl Vetaş, Istanbul, Turkey) (1 ml/10 kg) and ketamine hydrochloride (Ketamidor Vetaş, Istanbul, Turkey) (10 mg/kg). An oval incision was made into the skin. The mass was resected with at least 3 cm lateral margins and completely removed. Later, the muscles were closed with continuous sutures and the skin with simple separate sutures. The area was put into protective dressing. Postoperatively, ceftriaxone sodium (Novosef 1 g, Sanofi Istanbul, Turkey) was used for 7 days against secondary infections.

The surgically removed mass was sent to the pathology laboratory for diagnostic examinations. The mass was 13x12x9 cm in size, weighed 900 grams and had an elastic consistency. When the mass was sectioned, a yellowish coloured, viscous serous fluid was observed. Yellowish-gray coloured hard areas and red coloured jelly-like structures were seen in the middle of the mass. Melting areas of different sizes (5-40 mm) and irregular cavernous structures were noted in these areas (Figure 1B). It was noted that the areas close to the wall of the mass had a harder consistency and dark red nodular structures with a diameter of 1-2 cm in these areas. A grayish-white thin fibrous capsule was observed outside the mass (Figure 1C). According to the information obtained from the owner of the patient after the operation, it was learned that no new tumour

Table 2: For IHC staining primary antibodies

Primary Antibody	Company, product code	Dilution, incubation time /temperature
Vimentin	Abcam, ab28028	1/200, 2 hours/room temperature
Iba1	Wako, 019-19741	1/500, 18 hours/+4 °C
Desmin	DAKO, M0760	1/20, 2 hours/room temperature
SMA	DAKO, M085	1/100, 2 hours/room temperature
Anti-pan-Cytokeratin	Santa Cruz, SC-58830	1/50, 2 hours/37 °C
SOX-10	Santa Cruz, SC-365692	1/50, 2 hours/room temperature
S100B	DAKO, Z0311	1/500, 2 hours/room temperature
GFAP	Thermo Scientific, RB-087	1/50, 2 hours/room temperature
Sarcomeric actin	DAKO, M-0874	1/200, 2 hours/room temperature
Myogenin	Santa Cruz, SC-12732	1/50, 2 hours/room temperature
Skeletal Muscle Myosin	Santa Cruz, SC-32732	1/50, 2 hours/room temperature
HLA-DR	Santa Cruz, SC-53319	1/50, 2 hours/room temperature

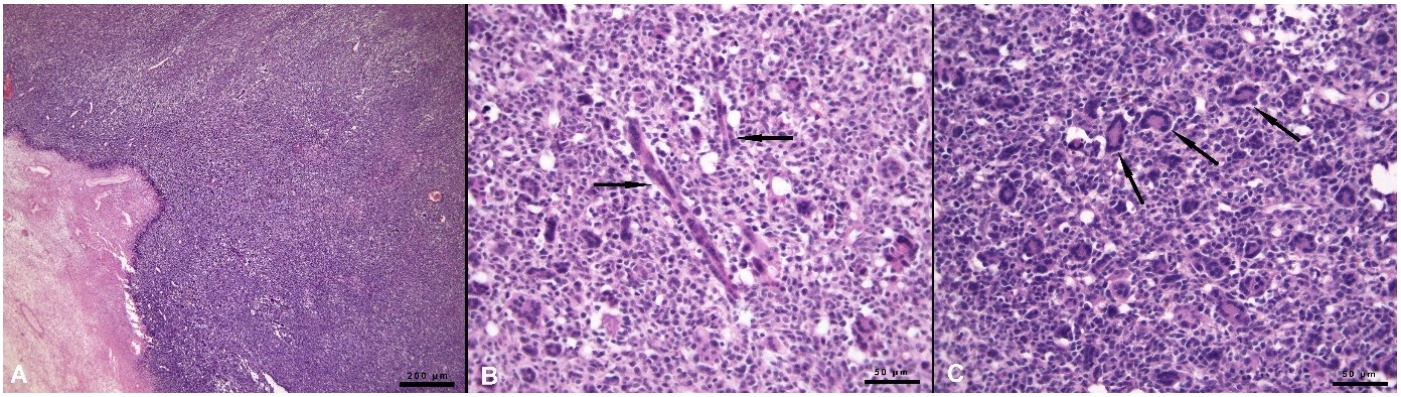

Figure 2: Microscopic view of neoplastic mass at low-power magnification; cell-rich neoplastic area around the necrotic center, H&E. Bar, 200 µm (A). The view of these neoplasic cells at high-power magnification: areas of spindle-oval-shaped cells with hyperchromatic nuclei and giant cells with nuclei lined up in a row (arrows) H&E. Bar, 50 µm (B), wreath-like multinucleated giant cells (arrows) at high-power magnification H&E. Bar, 200 µm (C)

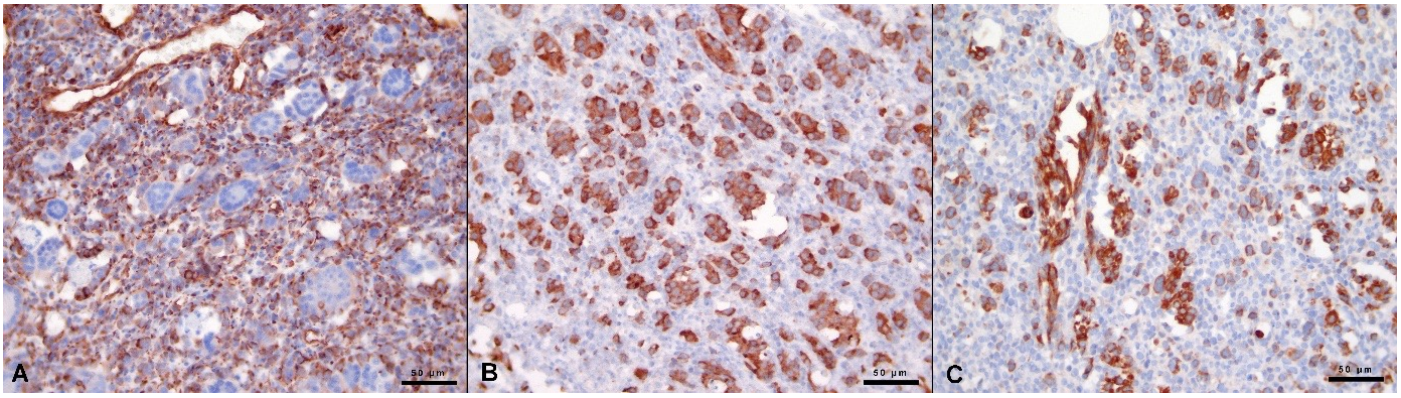

Figure 3: In immunohistochemical staining: vimentin (A), desmin (B) and skeletal muscle specific myosin (C) are positively stained. In wreath-like multinucleated giant cells vimentin is negative while desmin (A) and skeletal muscle specific myosin are positive (C). AEC chromogen, Gill's hematoxylin. Bars, 50 µm

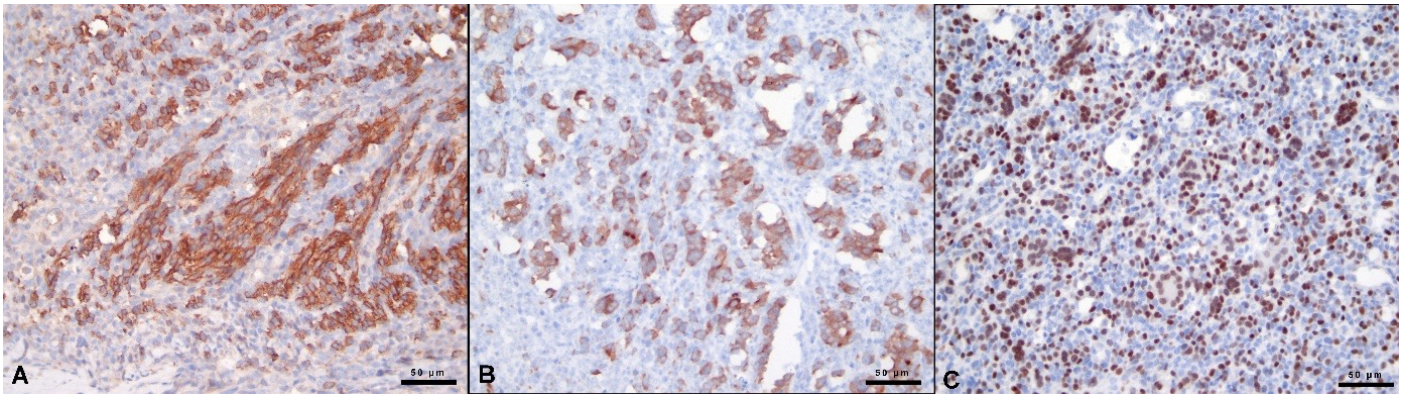

Figure 4: Sarcomeric actin (A), SMA (B) and myogenin (C) positivity in immunohistochemical staining. AEC chromogen, Gill's hematoxylin, Bars, 50 µm

or infection occurred in the area where the mass was removed. Likewise, it was confirmed that there was no mass formation in any other part of the body.

Tissue samples taken from different areas of the mass (close to the capsule, middle area, around the melting areas, hard and nodular structures) taken by the operation were fixed in 10% neutral buffered formalin solution. Fixed tissues were processed routinely and blocked in paraffin. Then, 4-5 micron thick sections were taken from the samples in paraffin blocks with a microtome to normal and silane-coated slides. Sections were stained with hematoxylin-eosin (H&E) and immunohistochemically. All stained sections were examined under a light microscope (Olympus BX 51, Japan).

Primary antibodies given in the Table 2 were used for IHC staining. For IHC staining method, Avidin-Biotin-Peroxidase Complex (ABC) method was used (15). ABC KIT (VECTASTAIN® Elite® ABC-HRP Kit, Peroxidase, PK-6100) were applied according to the user guide of the ABC-KIT. Anti-rabbit/mouse biotinized antibodies (1/100 dilution,

Boster bio- BA1007, BA1003) were dripped onto control sections instead of primary and secondary antibodies. 3-amino-9-ethylcarbazole (AEC) chromogen (TA-060-HA, AEC Substrate System, LabVision/ThermoScientific) which is a substrate of horse radish peroxidase enzyme, was used for 30 min. After that, non-alcoholic, 20% Gill's (III) hematoxylin was used for the background staining for 60 sec. The slides were covered with a coverslip by aqueous adhesive.

On histopathologic examination, there was a large necrosis area in the center of the mass. Around the necrosis, cells with spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged, atypia and mitosis were determined (Figure 2A). Among these cells, long cells with nuclei lined up in a row (Figure 2B) and wreath-like multinucleated giant cells (Figure 2C) were noted.

In the immunohistochemical examination, neoplastic cells were stained with vimentin (Figure 3A), desmin (Figure 3B), skeletal muscle myosin (Figure 3C), sarcomeric actin (Figure 4A), and SMA (Figure 4B) positively while Iba1, HLA-DR, Pancytokeratin, S100B, SOX10 and GFAP were stained negatively. Myogenin (Figure 4C) was intranuclear positive in approximately half of the cells. On the other hand, while the giant cells were negative with vimentin (Figure 3A), they were strongly stained cytoplasmically with desmin (Figure 3B), skeletal muscle myosin (Figure 3C), sarcomeric actin (Figure 4A), and SMA (Figure 4B).

The case was diagnosed as RMS, with wreath-like multinucleated giant cells, long cells with nuclei lined up in a row in the histopathology and immunohistochemistry findings.

Discussion

In the presented case, the tumor mass diagnosed was located in the neck region, which was reported the common localizations of the RMSs. The regions where RMS is most frequently observed in dogs have been reported as follows; skeletal muscle, tongue, larynx, lip, myocardium, urinary bladder and ovary (2-5, 7, 8, 14). In the study in which RMSs were evaluated (1), information about the localization of the tumor was stated as follows; the urogenital tract was the most common location (n=32/65; 49%), the head, neck, and face were common locations (n=24/65; 37%), less common locations included the limbs (n=5/65; 8%), in the hip and spine (n= /65; 3%), in the skin and mammary glands (n=2/65; 3%).

In the differential diagnosis, RMS, histiocytic sarcoma, and undifferentiated pleomorphic sarcoma are tumors compatible with morphology. Malignant melanoma, malignant peripheral nerve sheath tumor, and perivascular wall tumor should also be considered in the differential diagnosis, although they are less likely (16). Because SOX-10 and S100B were negative, we eliminated amelanotic malignant melanoma and malignant peripheral nerve sheath tumors, and Iba1 and HLA-DR negativity eliminated histiocytic sarcoma despite the presence of giant cells (16, 17). Desmin and, more specifically, sarcomeric actin, myogenin, and skeletal muscle myosin positivity distinguish this tumor from perivascular wall tumors (PWTs) (16, 18). Desmin and SMA can be positive in myopericytomas, which is one of the PWTs. But myosin is negative. Myosin (skeletal muscle-specific myosin) positivity in the case, as well as sarcomeric actin and myogenin positivity, distinguish it from this tumor (16). At the same time, Tuohy et al. (6) reported that myogenin positivity strengthens the diagnosis of RMS.

The histopathology and immunohistochemistry findings identified the present case as RMS, characterized by wreath-like multinucleated giant cells and long cells with nuclei lined up in a row. The wreath-like multinucleated giant cells, which are conspicuous in histopathology and positive with antibodies staining striated muscle, consisting of nuclei circumscribed like a wreath under the cytoplasmic membrane, were found interesting. These wreath-like multinucleated giant cells are similar to giant cells previously identified in a heifer (10) and some human rhabdomyosarcomas (19, 20). However, the available sources do not contain any reports of these giant cells in canine RMSs.

The myotubular variant of embryonal RMS is dominated by multinucleated "strap" cells forming myotubes, whereas the rhabdomyoblastic variant is dominated by round to polygonal cells with abundant eosinophilic cytoplasm. The spindyloid embryonal RMS, as the name implies, is composed of thin spindyloid myoblast cells forming bundles within a myxoid stroma (1). The mass's center displayed a large necrosis area. Around the necrosis, we identified tumor cells with spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged atypia, and mitosis. Long cells with nuclei lined up in a row and wreath-like giant cells were also noted among these cells.

Conclusions

The presented case is original in terms of giant cells, morphological appearance and immunophenotyping. The rhabdomyosarcoma in the present case was classified as undifferanted varyant of embryonal type on the basis of histopathologic and immunohistochemical findings. Important histopathological findings for this classification can be as follows; wreath-like multinucleated giant cells, spindle-oval and rounded morphology, hyperchromatic nuclei, unclear cytoplasmic borders, tightly arranged, atypia and mitosis, long cells with nuclei lined up in a row. In addition, crucial immunohistochemical findings for this classification listed as follows; neoplastic cells were stained with vimentin, desmin, skeletal muscle myosin, sarcomeric actin, and SMA positively while Iba1, HLA-DR, Pancytokeratin, S100B, SOX10 and GFAP were stained negatively. While the

giant cells were negative with vimentin, they were strongly stained cytoplasmically with desmin, skeletal muscle myosin, sarcomeric actin, and SMA. In veterinary pathology, striated muscle tissue tumors are not frequently encountered. According to the available literature, the morphology and even immunophenotyping of the tumor is not fully established. Examining the aforementioned cases and retroscopic studies reveals that the majority of the described striated muscle tumors exhibit distinct characteristics. The reviewed literature does not contain any similar cases exhibiting these findings. This case will contribute to the pathomorphological knowledge of canine striated muscle tumors for studies in the field of veterinary oncology.

Acknowledgements

Author contributions. All authors contributed to the study conception and design. FH: Conceptualization, Methodology, Validation, Writing- Original draft preparation, Writing- Reviewing and Editing. AT: Resources, Writing-Original draft preparation. MFB: Methodology, Visualization, Writing- Original draft preparation, Validation. AAK: Resources, Visualization. NK: Methodology, Visualization.

Ethical approval. Informed consent was obtained from the animal owner.

Funding. This research did not receive any specific grant from funding agencies in the public,commercial, or not-for-profit sectors

Conflict of Interest Statement. The authors declared that there is no conflict of interest.

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Nediferenciran embrionalni rabdomiosarkom pri nemškem ovčarju: makroskopske, histopatološke in imunohistokemijske značilnosti

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Izvleček: V tem poročilu primera je opisan primer osemletne samice nemškega ovčarja z nediferenciranim embrionalnim rabdomiosarkomom. Tumor je meril 13 x 12 x 9 cm, tehtal 900 gramov in imel elastično konsistenco. Histopatološki pregled je pokazal veliko območje nekroze v središču mase. Okoli nekroze smo določili celice z vretenasto ovalno in okroglo morfologijo, hiperkromatičnimi jedri, nejasnimi citoplazemskimi mejami, tesno razporejenimi atipijami in mitozo. Med temi celicami smo opazili dolge celice z jedri, razporejenimi v vrsto, in vencu podobne večjedrne orjaške celice. Pri imunohistokemijskem pregledu so se neoplastične celice pozitivno obarvale z vimentinom, dezminom, miozinom skeletnih mišic, sarkomernim aktinom in SMA, negativno pa z Iba1, HLA-DR, pancitokeratinom, S100B, SOX10 in GFAP. Miogenin je bil intranuklearno pozitiven v približno polovici celic. Primer je bil diagnosticiran kot RMS in na podlagi histopatoloških in imunohistokemijskih izvidov razvrščen kot nediferenciran varietetni embrionalni tip tumorja. Izvirna morfološka in imunofenotipska struktura tumorja, skupaj z zanimivo strukturo orjaških celic, nas je napeljala na misel, da bi bila objava tega primera koristna, saj bi lahko prispevala k patomorfološkemu znanju o tumorjih progastih mišic pri psih za študije na področju veterinarske onkologije.

Ključne besede: embrionalni rabdomiosarkom; pes; histopatologija; imunohistokemija