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Case report

A Hidden Threat: A Case Report on Pheochromocytoma in a Horse (*Equus ferus caballus*)

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Abstract: Pheochromocytomas are rare neuroendocrine tumors in horses, originating from chromaffin cells within the adrenal medulla. This case report describes a 15-year-old Friesian horse that presented with progressive ataxia, muscular weakness, and lateral recumbency, leading to euthanasia. During necropsy, a 2x3 cm dark-red mass with focal necrosis was diagnosed in the left adrenal gland, along with perirenal hematoma and hemoperitoneu. Histopathological analysis confirmed an adrenal pheochromocytoma, characterized by nests of polygonal to spindle-shaped cells with small amount of cytoplasm and low mitotic activity.. The clinical signs were likely due to catecholamine hypersecretion and the acute hemoperitoneum was caused by the tumor rupture and hemorrhage. Pheochromocytomas, though often diagnosed post-mortem, can cause life-threatening cardiovascular and systemic effects, underscoring the importance of histopathological evaluation and the need for improved antemortem diagnostic techniques in equine practice.

Keywords: Pheochromocytoma; Equine neuroendocrine tumor Adrenal gland neoplasm; Histopathology

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1. Introduction

Pheochromocytoma, a rare neoplasm in horses [1,2], arises from chromaffin [3] cells located within the adrenal medulla [4]. Histopathologically, these tumors are characterized by the proliferation of neuroendocrine cells, which are responsible for the production and secretion of catecholamines [3]. While pheochromocytomas are uncommon in equine species, their discovery typically occurs either incidentally during necropsy or upon investigation of unexplained clinical symptoms [4,5]. From a pathologic perspective, pheochromocytomas in horses often present as well-encapsulated, lobulated masses, ranging from a few to several centimeters in diameter [6]. These tumors are typically located within or adjacent to the adrenal gland [4]. Grossly, pheochromocytomas may appear firm and reddish-brown, with areas of hemorrhage or necrosis, reflecting their variable growth rates and vascularization [6]. Histologically, pheochromocytomas are composed of polygonal to spindle-shaped cells arranged in nests or cords. These cells exhibit a finely granular cytoplasm due to the presence of catecholamine-containing secretory vesicles [7]. The nuclei are generally round to oval, with prominent nucleoli, and mitotic figures are rare,

indicating the typically slow-growing nature of these tumors. However, more aggressive variants can demonstrate higher mitotic activity [1] and local invasion into surrounding tissues, including blood vessels. Hemorrhage, necrosis, and vascular thrombosis are commonly observed.

Special histochemical stains, such as chromogranin A, synaptophysin, and neuron-specific enolase, are useful in confirming the neuroendocrine origin of the tumor cells. Immunohistochemical analysis further aids in differentiating pheochromocytomas from other adrenal or metastatic neoplasms, with pheochromocytomas showing positive staining for chromaffin markers [8]. Additionally, electron microscopy may reveal dense core granules within the cytoplasm, corresponding to the secretory vesicles laden with catecholamines[7].

Given the neuroendocrine origin and variable behavior of pheochromocytomas, their histopathological features are critical for diagnosing and determining potential malignancy. Although these tumors are generally low aggressive in horses, malignant pheochromocytomas can invade adjacent tissues or metastasize [9].

This case report explore the pathological and histopathological findings of a pheochromocytoma in a horse, highlighting its cellular morphology and histological characteristics. While histopathological evaluation contributes to a greater understanding of tumor biology, this report also highlights its potential to guide future advancements in ante-mortem diagnosis, prognosis, and treatment approaches.

2. Case description

A fifteen-year-old intact male Friesian horse (*Equus ferus caballus*) was referred to the Equine Clinic at the Cluj-Napoca Faculty of Veterinary Medicine for evaluation due to an array of progressive clinical signs, including abnormal gait, ataxia, muscular weakness, and, eventually, lateral recumbency. The horse's condition deteriorated rapidly despite supportive care, culminating in a state of recumbency.

The initial clinical examination was conducted with the patient in right lateral recumbency. Vital parameters showed a heart rate of 96 beats per/minute, which was irregular, and a respiratory rate of 16 breaths per minute, with eupnea. a body temperature of 38.6°C, a dehydration score of 10%, icteric mucous membranes in the mouth, icteric and congested subconjunctival mucosa, nystagmus, and a body condition score of 2-3/5. Peristalsis in the left abdomen was present. The patient had not defecated or urinated during the clinical examination, so urinary catheterization was performed. The urine was brownish-red in color and low in quantity, approximately 3.5 L.

For a more complex neurological assessment, the patient was suspended in a sling and attempts were made to assist them in rising with the help of an electric elevator. Using the sling, the patient was able to maintain a quadrupedal position for approximately 12 hours, showing normal appetite for both food and water throughout this period. At the end of this time, the patient presented with muscle fasciculations and was left in recumbency to rest. Subsequently, the patient's condition deteriorated and he was no longer able to maintain a quadrupedal position regardless of the support provided. For the next three days, the patient was supported through parenteral therapy (fluid therapy, parenteral nutrition, analgesia, anti-inflammatory, gastric and hepatic protectants).

Clinical Pathology

Table 1. Clinical parameters assessments

Biochemical examination:	Glucose	176 mg/dL [71-141 mg/dL]),
	ASAT	892 U/L [100-525 U/L]
	ALP	361 U/L [10-335 U/L]
	Total bilirubin	4.69 mg/dL [0-2.40 mg/dL
Hematological examination:	Neutrophils	11.88 10^9/L [2.3-9.5]
	Lymphocytes	11.1 10^9/L [17-68]
	Eosinophils	0.3 10^9/L [1-8]
Cerebrospinal Fluid (CSF) examination:	Cytology	No significant findings
	Microbiological exames	negative
	Proteins	0.16 g/dL [<0.005 g/dL].
Peripheral blood	*Babesia spp.*(piroplasmosis)	PCR positive

Biochemical examination: high levels were found for glucose (176 mg/dL [71-141 mg/dL]), ASAT (892 U/L [100-525 U/L]), ALP (361 U/L [10-335 U/L]), total bilirubin (4.69 mg/dL [0-2.40 mg/dL]), CK (3530 U/L [21-400 U/L]), GLDH (90 U/L [0-15 U/L]). The renal profile was within normal limits.

Hematological examination: the results showed , neutrophilia, lymphopenia and eosinopenia. Red blood cell and platelet counts were within physiological limits.

Cerebrospinal Fluid (CSF) examination: a CSF sample was collected, which appeared normal with characteristic color and no changes in cytological, microbiological (negative), or hematological findings. However, the biochemical examination revealed high protein levels of 0.16 g/dL [<0.005 g/dL].

Peripheral blood sample: a blood sample was taken to diagnose a potential infestation with *Babesia spp*.(piroplasmosis). The blood smear confirmed a positive diagnosis due to the presence of intracellular parasitic forms and the diagnosis was further confirmed by a positive PCR result.

Despite the treatments administered over the three days of recumbency, the patient's condition deteriorated significantly, with a comatose neurological status, accentuated nystagmus, and continued high liver and muscle values.

Postmortem examination

The case was deemed suitable for euthanasia, and a full necropsy was subsequently performed to ascertain the underlying cause of the animal's clinical decline.

On gross examination, the body was markedly anemic, with retroperitoneal massive hemorrhage spreading from the left perirenal area, acute hemoperitoneum (measuring 2 liters), prominent hepatic steatosis and mild lung congestion. Within the left adrenal gland, a dense, dark-hemorrhagic, focally necrotic mass measuring approximately 2x3 cm was observed within the left adrenal gland. The mass expanded from the adrenal`s medulla, was moderately compressive on the surrounding adrenal cortex and was moderately elevating the capsule (Figure 1).

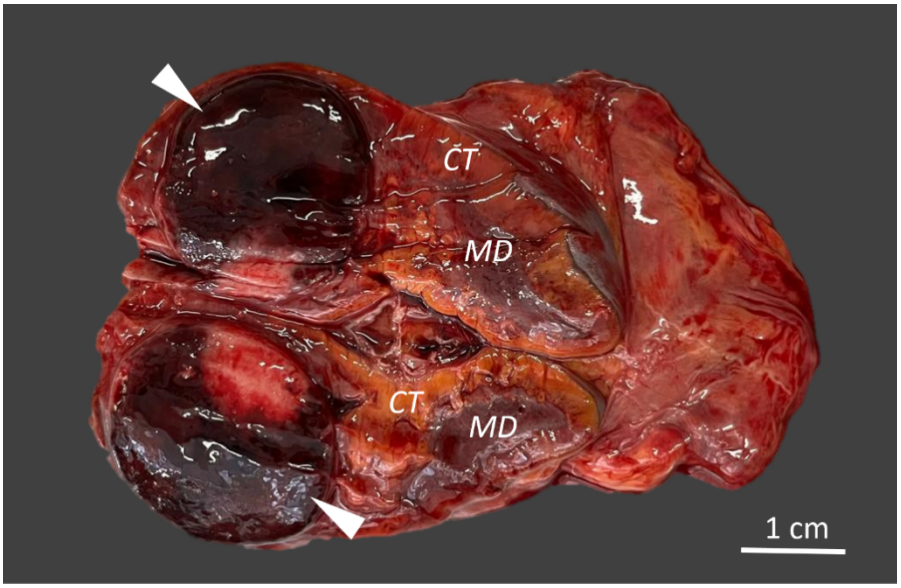

Figure 1. Left Adrenal Gland. Horse. An expansile mass with a focal area of necrosis compressinbg the surrounding adrenal tissue (arrow) is observed within the adrenal gland. CT-cortex, MD-medulla

Histological examination

The adrenal gland was fixed in 10% formalin for 48 hours, then dehydrated in ethanol, cleared in xylene, and infiltrated with paraffin at 58°C for 5 hours. Using a rotary microtome, 2 µm sections were cut, deparaffinized in xylene, and rehydrated through graded ethanol solutions. After rinsing in water, the sections were stained with hematoxylin for 5 minutes, rinsed, then stained with eosin for 2 minutes. The slides were dehydrated, cleared in xylene, mounted with Permount, coverslipped, and prepared for microscopic analysis.. Microscopic evaluation of the adrenal gland confirmed the presence of a neoplastic mass. The tumor was composed of nests and clusters of polygonal to spindle-shaped neoplastic cells. These cells were arranged in a well-defined trabecular pattern, supported by a fine fibrovascular stroma. The cytoplasm of the neoplastic cells was abundant and finely granular. Nuclei were round to oval, exhibiting a stippled chromatin pattern with occasional prominent nucleoli. The mitotic figures were rarely observed.. Additionally, the tumor displayed areas of extensive hemorrhage and necrosis, which correlated with the gross findings of hemorrhagic changes (Figure 2). The further histopathological analysis supported the diagnosis of pheochromocytoma, with the tumor arising from the chromaffin cells of the adrenal medulla. This histological

presentation, coupled with the observed hemorrhagic manifestations, shows that the pheochromocytoma rupture induced the acute hemoperitoneum and systemic symptoms observed before the animal's death.

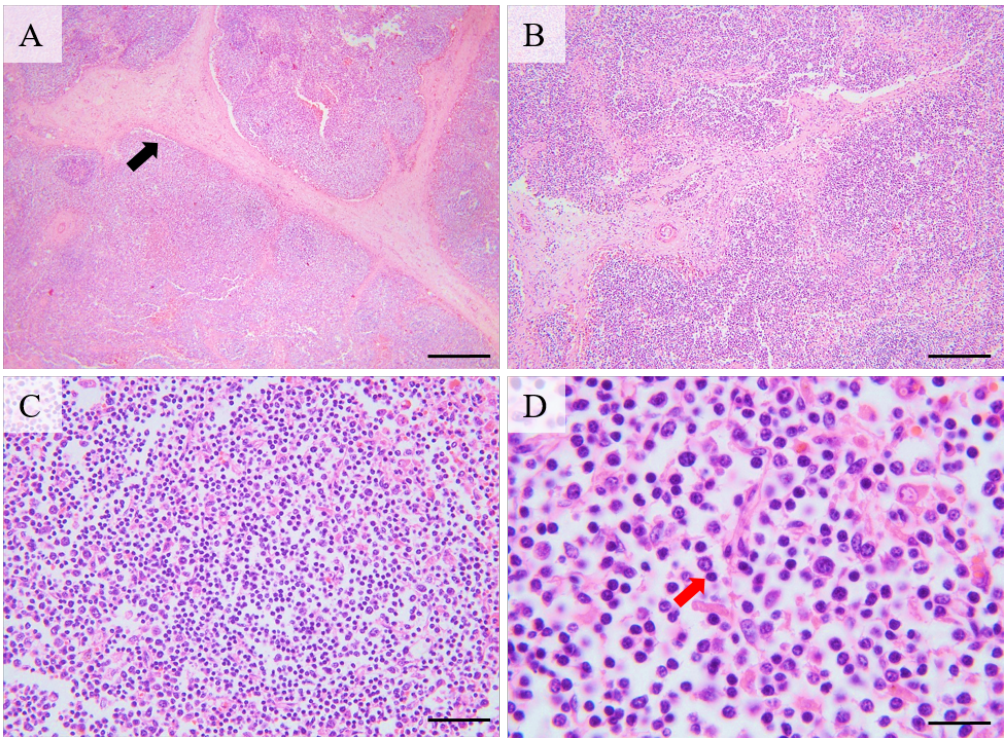

Figure 2. Histological features of a pheochromocytoma. Horse. The mass consists of polygonal to spindle-shaped cells arranged in a trabecular pattern, interspersed by a fine fibrovascular stroma (black arrow). The neoplastic cells display a small amount of eosinophilic cytoplasm (red arrow), while their nuclei range from round to oval, exhibiting a stippled chromatin pattern with occasional prominent nucleoli.. H&E stain. Ob. x4 (Image A), Ob. x10 (Image B), Ob. x20 (Image C), Ob. x100 (Image D). Barr 500 μm (Image A), 200 μm (Image B), 100 μm (Image C), 20 μm (Image D).

4. Discussion

Pheochromocytomas, though rare in equines, represent a critical pathology due to their potential to cause significant systemic effects, largely through catecholamine hypersecretion. This case highlights the importance of recognizing the neoplasm's pathologic and histopathologic presentations to guide accurate diagnosis and appropriate management. The presence of pheochromocytoma in this 15-year-old Friesian horse adds to the limited but growing body of literature documenting such tumors in horses, where they are often identified post-mortem or following a sudden onset of severe clinical signs [1,3]. The gross and histologic features observed in this case are consistent with previously reported descriptions of equine pheochromocytomas. The presence of a well-encapsulated, firm mass in the adrenal gland, with associated necrosis and hemorrhage, mirrors findings from earlier studies [6]. The dark-red color of the mass and its dense nature are suggestive of its high vascularization, which, in combination with areas of hemorrhage, reflects the potential for these tumors to cause acute, life-threatening hemorrhagic events, as seen in the severe hemoperitoneum and perirenal hematoma in this case. Histopathologically, the tumor displayed nests and trabecular arrangements of polygonal to spindle-shaped cells, with small amount of cytoplasm [7]. This cellular architecture is typical of pheochromocytomas, and the low mitotic index further supports its generally slow-growing nature [1]. However, the areas of necrosis and hemorrhage within the tumor may suggest a more aggressive variant, or the result of vascular compromise, which may be found in pheochromocytomas [9]. The clinical manifestations in this case, including progressive weakness, ataxia, and lateral recumbency, are not specific to pheochromocytoma but may reflect the cardiovascular and neuromuscular effects of excess catecholamine release. Elevated catecholamine levels can lead to neuromuscular dysfunction by increasing sympathetic stimulation, which can disrupt normal muscle tone and coordination. This may result in muscle weakness, impaired motor control, and uncoordinated movements, which could explain the observed symptoms of ataxia and recum-

bency in this case. However, these clinical signs were compounded by the presence of the acute hemoperitoneum, likely precipitated by hemorrhage from the adrenal mass. This scenario underscores the importance of considering pheochromocytoma in differential diagnoses when horses present with nonspecific but rapidly deteriorating clinical signs, especially in cases where significant cardiovascular disturbances are observed. From a diagnostic standpoint, pheochromocytomas in horses are often diagnosed incidentally, as was the case here, following euthanasia and necropsy. This delayed diagnosis highlights the challenges in identifying such tumors antemortem. Veterinary imaging, such as ultrasonography or advanced imaging techniques like CT and MRI, could offer valuable diagnostic insight when pheochromocytoma is suspected based on clinical or laboratory findings, such as unexplained hypertension [4]. Nonetheless, these diagnostic tools are rarely employed in equine practice due to financial and logistical constraints.

The histological findings in this case further confirm the neuroendocrine nature of the tumor, with the characteristic chromaffin cell morphology and staining properties. Special histochemical and immunohistochemical techniques, such as chromogranin A and synaptophysin staining, are valuable in distinguishing pheochromocytomas from other adrenal neoplasms or metastatic tumors [8].

This case report contributes to the limited pool of documented equine pheochromocytomas, emphasizing the necessity of a thorough histopathological evaluation to confirm the diagnosis. The tumor's potential to cause severe hemorrhage and the subsequent development of hemoperitoneum in this horse exemplify the life-threatening nature of pheochromocytomas when not promptly identified. Future studies should aim to improve the antemortem diagnostic techniques for pheochromocytoma in equines, which could enhance early detection and potentially improve clinical outcomes.

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