

Metastatic Vulvar Squamous Cell Carcinoma in a Mare

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ABSTRACT

Background: Squamous cell carcinoma (SCC) is a common cutaneous neoplasm in horses, which mainly affects the external genitalia, oral cavity, and periocular region. The development of SCC metastases is rare in these animals, and the most common occurrence is a marked local infiltration. Exposure to ultraviolet rays and skin depigmentation are the main etiological factors of SCC. Definitive diagnosis of the neoplasm is performed through histological examination of lesions. The present report describes the clinical, anatomopathological, and immunohistochemical (IHC) aspects of a case of metastatic SCC, with the vulva as the primary site in a mare.

Case: A 17-year-old mare, mixed breed, was referred to the Veterinary Hospital of the Universidade de Passo Fundo (UPF), with history of areas of depigmented skin (pinto coat), and clinical history of anorexia, frequent episodes of colic, and recumbency. During clinical evaluation, heart rate (68 bpm), rectal temperature (38.4°C), and respiratory rate (48 rpm) were elevated, and the oral mucosa was pale. The mare also had an ulcerated tumor mass involving the vulva, which extended to the inguinal region and involved the mammary gland. A cytological aspirate of the vulvar tumor was performed, in which no neoplastic cells were found. Next, a biopsy of 2 distinct areas of the vulva was performed. The material was sent for anatomopathological examination, which showed markedly pleomorphic malignant squamous cells, with individual keratinization and high mitotic index, organized in trabeculae with rare keratin pearl-like formations. The exam allowed the diagnosis of SCC Grade II. Due to the poor prognosis and high cost of treatment, the owner consented to euthanasia and necropsy examination. During necropsy, the vulvar tumor mass was grayish, firm to hard, infiltrative, and had friable areas. Tumor foci suggestive of metastasis were also observed in inguinal, mesenteric, mediastinal and renal lymph nodes, adrenal glands, lung, pericardium, medullary canal, intercostal muscles, right 15th rib, and tissue surrounding the azygos vein. Samples from all organs were collected for anatomopathological examination. Diagnosis of metastatic vulvar SCC was confirmed through histological and IHC studies, which evaluated the expression of cytokeratins (AE1/AE3), as well as the proliferative activity of neoplastic cells through the PCNA marker.

Discussion: The diagnosis of metastatic SCC was obtained through the observed clinical, necroscopic, histological, and IHC characteristics. This neoplasm usually appears in depigmented regions exposed to ultraviolet light, and older animals are more likely to be affected. The mare in the present case had areas of depigmented skin (pinto coat). The animal was kept in a paddock outdoor and exposed to constant solar radiation. Although rare in horses, manifestation of anorexia, progressive weight loss, and frequent colic episodes and recumbency may be closely related to the multiple sites of metastasis in the present case. The main histological findings of the neoplasm were the dense proliferation of malignant squamous cells with individual keratinization, arranged in a trabecular pattern and with rare formations of keratin pearls, in line with previous studies. During IHC evaluation, the neoplastic cells showed expression of cytokeratins (AE1/AE3), as well as high proliferative activity evidenced by the PCNA marker. Given this background, the present report describes the clinical, anatomopathological, and IHC aspects of a case of metastatic SCC with a primary site in the vulva of a mare.

Keywords: equine, genital neoplasm, tumor embolism, squamous cell carcinoma, metastasis, immunohistochemistry.

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INTRODUCTION

The squamous cell carcinoma (SCC) is the second most common tumor in horses and the most common neoplasm of their external genitalia [9,21]. Some predisposing factors associated with SCC development are exposure to high ultraviolet radiation levels, unpigmented areas of skin or mucous membranes, hairless regions, and infection with papillomavirus [6,9,17-19]. It usually appears in adult horses and can affect the oral cavity, including lips, hard palate, tongue, oral mucosa, and mandible. SCC can also invade the nasal cavity and paranasal sinuses and lead to the formation of regional lymph node metastases [6,13,17]. Ocular, penis/foreskin, vulva/perianal, and skin are the most common regions of occurrence in order of prevalence [21].

Neoplasms tend to remain localized. However, dissemination to other sites such as liver and lungs may occur, by tumor infiltration and subsequent hematogenous dissipation, followed by generalized metastasis to other tissues [10]. Abdominal metastasis can cause unspecific clinical signs, like anemia, anorexia, apathy, and mild and intermittent colic episodes, thus hindering diagnosis [15]. The definitive diagnosis of abdominal neoplasia is usually performed at necropsy. Due to the cost of some exams and poor prognosis of treatment options, most owners elect euthanasia as the best option in this scenario [20].

The present report aims to describe a rare case of metastatic vulvar SCC diagnosed through anatomopathological and immunohistochemical (IHC) examination.

CASE

A 17-year-old mare, mixed breed, was referred to the Veterinary Hospital of the Universidade de Passo Fundo (UPF), with history of areas of depigmented skin (pinto coat), anorexia, and progressive weight loss for more than 2 months, and a mass on the perineal region with fast growth during the last month. In the clinic consult, the owner complained that the animal started to present frequent episodes of colic and recumbence.

The mare had a normal body condition (score = 3/5) and mild dehydration, and it was depressed but responsive upon admission. The heart rate (68 beats per min), rectal temperature (38.4°C), and respiratory rate (48 breaths per min) were elevated, and the mucous membrane was pale. The blood exam revealed anemia with $5.55 \times 10^6/L$ of erythrocytes, 9.26 g/dL of hemoglobin, and a packed cell volume (PCV) of 29%.

A tumor mass with foci of ulceration was present surrounding the vulva and perineal region, extending to the mammary gland. This mass extended to the inguinal portion involving the enlarged mammary gland, with an irregular and deformed surface presenting ulceration and serohemorrhagic secretion.

A cytological aspirate of the vulva was performed but did not confirm any diagnosis. Next, a biopsy was taken from 2 areas of the vulva. The material was fixed in 10% formalin solution, processed by conventional histochemical methods, and stained with hematoxylin and eosin (HE)¹. The samples showed infiltrative proliferation of malignant squamous cells markedly pleomorphic and predominantly organized in multiple trabeculae surrounded by fibrovascular stroma, sometimes organized in solid nests. Numerous neoplastic cells were keratinized individually, and rare trabeculae exhibited keratin pearl formations/sketches. The cells had ample, fibrillar, and eosinophilic cytoplasm; large, hyperchromatic, rounded, or oval nuclei with loose and coarsely speckled chromatin and/or forming aggregates; evident, hyperchromatic, and multiple nucleoli in most cells. The mitotic index was 67 mitoses in 10 high-magnification fields. Moderate to severe tumor necrosis was observed, in addition to moderate to severe chronic suppurative inflammatory infiltrate, which was multifocally distributed through the tumor stroma and tissue parenchyma. There was also extensive epithelial ulceration associated with necrosis and accentuated diffuse suppurative inflammatory infiltrate, with the presence of basophilic structures suggestive of bacterial cocci. These findings confirmed the diagnosis of Grade II SCC. Due to poor prognosis, treatment cost, and animal condition, the owner consented to euthanasia.

At necropsy, a tumor mass with foci of ulceration was observed surrounding the vulva, perineal region, and extending to the mammary gland (Figure 1A). In the sections, the vulvar mass had a grayish color and firm consistency, with yellowish areas and abscess formation extending ventrally to the mammary gland. Other tumor masses, suggestive of metastases, were present in various organs such as inguinal, mesenteric, mediastinal and renal lymph nodes, adrenal glands, lung, pericardium, medullary canal, intercostal muscles, right 15th rib (Figure 1B), and surrounding the azygos vein. During necropsy, samples from the vulvar tumor mass and all organs were collected and processed as described above.

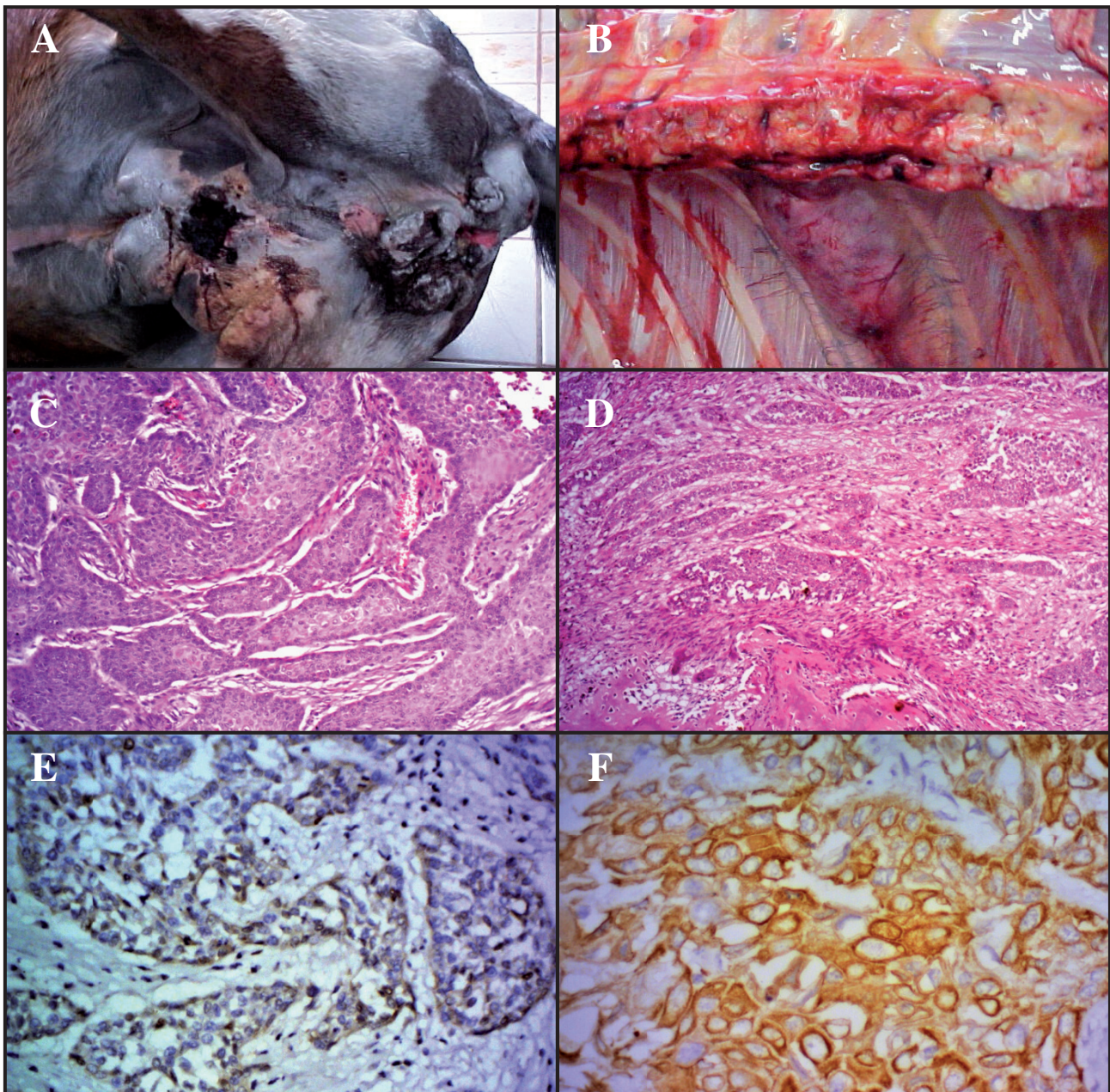


Figure 1. Metastatic vulvar squamous cell carcinoma in mare. A- Tumor mass with foci of ulceration involving the vulva, perineal region, and extending to the mammary gland. B- Intercostal muscles and right 15th rib with infiltrative and well-vascularized tumor mass, suggestive of SCC metastasis. C- Vulvar tissue exhibiting dense infiltrative proliferation of malignant squamous cells, organized into numerous trabeculae surrounded by fibrovascular stroma, and multiple neoplastic cells exhibiting individualized keratinization [HE; Obj.10x]. D- Intercostal muscles and right 15th rib showing loss of tissue architecture associated with numerous malignant squamous cells organized in trabeculae [HE; Obj.10x]. E- Vulvar tissue with malignant squamous cells displaying numerous nuclei immunostained with PCNA antibody [PCNA; Obj.20x]. F- Vulvar tissue with malignant squamous cells expressing intense cytoplasmic immunostaining for cytokeratins [AE1/AE3; Obj.40x].

All tissues with tumor masses were analyzed histologically and showed loss of tissue architecture associated with dense and infiltrative proliferation of malignant epithelial squamous cells that were markedly pleomorphic, organized in multiple trabeculae surrounded by fibrovascular stroma (Figure 1C-D). Numerous neoplastic cells showed individual keratinization, and rare trabeculae exhibited keratin pearl-like formations. The index averaged seven mitoses per high

power field. There was also moderate multifocal tumor necrosis, and moderate chronic suppurative inflammatory infiltrates distributed throughout the tumor stroma and tissue parenchyma. These findings confirmed the diagnosis of Grade II SCC and that the tumor masses were metastases.

For the IHC evaluation of the neoplasm, the proliferative cell nuclear antigen (PCNA) marker (Clone PC10, Zeta)² and the anti-cytokeratin marker

(clone AE1/AE3, Dako)³ were used through the streptavidin-biotin-peroxidase method, including positive controls. The vulvar mass (primary tumor site) showed neoplastic squamous cells with intense nuclear immunostaining by PCNA (Figure 1E), demonstrating a high proliferative index. Samples of vulva, rib, and lymph nodes with tumor masses were submitted to an anti-cytokeratin marker, and there was intense immunostaining of neoplastic cells (Figure 1F), confirming the epithelial origin of the neoplasm.

DISCUSSION

This study reports an unusual form of aggressive Grade II SCC with high proliferative and metastatic activity. In the present case, metastasis development was probably due to the long period between the beginning of the tumor and the diagnosis since SCC is a slow-growing carcinoma that usually presents a low metastatic potential [1]. According to the literature, moderately differentiated (Grade II) or undifferentiated (Grade III) SCC have a metastasis rate of up to 18.6% [8], in addition to a faster and more aggressive growth factor [1,3].

Clinical signs observed in this case confirm the challenge of diagnosing abdominal neoplasms since they usually present nonspecific clinical signs. In most cases, the owners choose euthanasia due to the cost of treatment and poor prognosis [4,16,20]. SCC prognosis is good in cases where extraction and adjunctive therapy are instituted early as these procedures help prevent recurrence and metastasis [7].

The mare had a pinto coat, advanced age, and its vulva was located in a non-pigmented region. These characteristics are predisposing factors associated with SCC development [6]. Exposure to ultraviolet radiation, hairless regions, and infections by papillomavirus can also contribute to the appearance of this type of tumor [9,18,19]. The evidence of an extensive tumor involving the vulva and spreading to the ventral portion of the body confirms the invasive characteristic of this type of neoplasm. However, this behavior is most commonly evidenced in poorly differentiated tumors (Grade III) [1,3,14].

Many authors describe metastasis from SCC involving regional lymph nodes [12-14], especially those surrounding SCC from external genitalia. Some reports show metastasis for other organs such as the lungs, liver, and peritoneum [5,11,12]. The normal route of metastasis is through lymph. In theory, when other organs are committed by metastasis, this might indicate the presence of tumor emboli [13]. To the author's knowledge, this is the first report of a case describing invasion and destruction of the rib bone and intercostal muscles, causing obstruction of the azygos vein and invading the medullary canal due to metastasis from a Grade II vulvar SCC.

The cytopathological analysis of the vulvar tumor mass (primary site of SCC) did not reveal that it was a neoplasm, which may have occurred due to failure in collection or secondary lesions, such as inflammation and necrosis. The tumor of the present study was classified as Grade II due to the prevalence of trabecular pattern, rare keratinization, high mitotic activity, and presence of nuclear pleomorphism, which is in line with previous studies [1,2]. However, it showed aggressive behavior as in Grade III SCC. IHC examination of the primary vulvar lesion and metastasis foci showed positive immunostaining for anti-cytokeratin (AE1/AE3) and PCNA antigens. This immunostaining confirmed the epithelial origin and the high proliferative activity of the neoplasm, respectively. According to the literature, neoplasms with a high proliferative index present an unfavorable prognosis and imminent risk of extensive local infiltration and development of metastases [1,8], aspects observed in the present case.

It is demonstrated the ability of the neoplasm to invade and destroy adjacent tissues, as well as to metastasize to different organs of the abdominal and thoracic cavities.

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