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ANIMAL PATHOLOGY

Clinico-pathological and immunohistochemical findings in a case of bovine cutaneous angiomatosis in a Holstein heifer

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ABSTRACT: Bovine cutaneous angiomatosis (BCA) is a rare condition characterized by vasoproliferative skin lesions mainly affecting cattle in the northern hemisphere. An eight-month-old Holstein heifer showed two skin easy-bleeding nodules bulging from the epidermis in the forehead and close to the right prescapular region. Skin lesions were within the dermis and had an irregular surface and a soft heterogeneous reddish parenchyma composed of numerous variable-sized arterioles, veins, and capillaries surrounded by abundant collagenous fibrous tissue. Immunohistochemical assays evidenced endothelial cells lining proliferative vascular structure immunolabeled for CD31 and Von Willebrand factor, and vascular smooth muscle cells immunostained for smooth muscle actin. All clinical, pathological, and immunohistochemical features observed in the Holstein heifer were hallmarks of BCA. Considering its potential for hereditary spread, BCA must be included in the differential diagnosis of easily bleeding skin nodules that do not respond to routine topical wound treatments to prevent its spread in Brazilian herds.
Key words: angiomatosis, skin, blood vessels, cattle, herds.

Achados clínico-patológicos e imuno-histoquímicos em um caso de angiomatose cutânea bovina em uma novilha Holandesa

RESUMO: A angiomatose cutânea bovina (ACB) é uma condição rara caracterizada por lesões vasoproliferativas na pele que afetam principalmente bovinos do hemisfério norte. Uma novilha Holandesa de oito meses de idade apresentou dois nódulos cutâneos protuberantes na epiderme, que apresentavam fácil sangramento localizados na fronte e próximo à região pré-escapular direita. As lesões cutâneas dérmicas tinham uma superfície irregular e um parênquima avermelhado heterogêneo macio composto por numerosas arteríolas, veias e capilares de tamanho variável circundadas por tecido fibroso colagenoso abundante. O exame imuno-histoquímico evidenciou células endoteliais revestindo a estrutura vascular proliferativa imunomarcadas para CD31 e fator de Von Willebrand, e células vasculares de músculo liso imunomarcadas para actina de músculo liso. Todas as características clínicas, patológicas e imuno-histoquímicas observadas na novilha Holandesa foram características da ACB. Considerando seu potencial de disseminação hereditária, a ACB deve ser incluída no diagnóstico diferencial dos nódulos cutâneos de fácil sangramento que não respondem aos tratamentos tópicos de rotina para evitar sua disseminação nos rebanhos brasileiros.
Palavras-chave: angiomatose, pele, vasos sanguíneos, bovino, rebanhos.

INTRODUCTION

Angiomatosis is a complex of proliferative vascular lesions with several clinical and pathological manifestations, presenting with single or multifocal distribution and affecting the skin and other various organ systems (RICHARD et al., 1995; DIAZ-DELGADO et al., 2012; LUDWIG et al., 2015; HENDRICK, 2017; BARON et al., 2020; JACINTO et al., 2021). Although rare, cutaneous angiomatosis has already been reported in humans, domestic and wild animals (LUPPI et al., 2010; DIAZ-DELGADO et al., 2012; LUDWIG et al., 2015; HENDRICK, 2017; BARON, et al., 2020; JACINTO et al., 2021). Among these vascular lesions in domestic animals, bovine cutaneous angiomatosis (BCA), progressive angiomatosis in dogs and cats, and scrotal vascular hamartoma in dogs have been mainly reported (HENDRICK, 2017).

BCA shares significant histological similarities with the human pyogenic granuloma (HENDRICK, 2017) and has occurred in adult animals in Great Britain, France, and the United States, but it also has been recognized as a juvenile

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form likely affecting cattle under one year old (WATSON & THOMPSON, 1990; JACINTO et al., 2021). The etiology of cutaneous angiomatosis is not entirely determined in veterinary medicine, but chromosomal mutations (HENDRICK, 2017; JACINTO et al., 2021), infections, and inflammation have been related in the pathogenesis (YAGER et al., 2010). These vascular malformations may occur as a result of failure in angiogenesis during early embryonic development (BORST et al., 2020) or in the repair of traumatic injuries in the integumentary blood vessels (WATSON & THOMPSON, 1990). Furthermore, these vascular proliferations likely arise from the overgrowth of angioblastic cells over the proliferation of fibroblasts in exuberant granulation tissues (LUPPI et al., 2010).

Brazil has one of the most important commercial cattle herds worldwide, significantly contributing to the country's economy and international trade. Considering bovine cutaneous vasoproliferative conditions have never been reported in Brazil, the first identification of an unprecedented disease in Brazilian cattle herds may be of great relevance for the knowledge of cattle diseases, and considering its pathogenesis is likely related to abnormal chromosomal changes (JACINTO et al., 2021). Therefore, this report characterized the clinical and pathological aspects of BCA in a Holstein heifer.

Case description

An eight-month-old Holstein heifer from a dairy herd in Planaltina, Federal District, Brazil, was evaluated due to the development of two dermal easy-bleeding nodules. According to the handler, the nodules increased in size during the 40-days evolution. Clinical evaluation was unremarkable, and the only alterations were restricted to the skin. An irregularly-shaped dermal nodule was bulging from the epidermis in the forehead and medial to the frontal bone, measuring 2.1 x 1.6 x 1.6 cm; the second skin nodule of 2.0 x 3.0 x 2.2 cm was close to the right prescapular region (Figure 1). The gross aspect of cutaneous lesions consisted of nodules within the dermis with an irregular surface and a soft heterogeneous reddish parenchyma interspersed by discrete white trabecular fibrous tissues. Both nodules were surgically removed, fixed in a 10% buffered formalin solution (pH 7.0), routinely embedded in paraffin, and histological sections were stained with hematoxylin and eosin (H&E), and Masson's trichrome. The heifer was followed up for six months, and there was no recurrence of the skin lesions. Actually, three years later, based on the farm's animal records, the new veterinarian in charge stated that the animal had developed two pregnancies and lactation with no recurrences, and no other cattle had developed similar skin lesions in the herd.

Histologically, both vascular nodules were non-encapsulated and composed of numerous variable-sized arterioles, veins, and capillaries irregularly distributed within the superficial to deep dermis (Figure 2A), surrounded by abundant collagenous fibrous tissue (Figure 2B). Some vessels were tortuous and lined by flat or plump endothelial cells, and lumina was occasionally indistinct. Other vessels showed a thick wall with multiple layers of smooth-muscle cells or a single or two smooth-muscle cell layers. Mild to moderate lymphoplasmacytic inflammatory infiltrate was scattered surrounding some arterioles and capillaries. The epidermis was mostly intact and moderately hyperplastic, with some areas of erosion. Adnexal glands and cutaneous appendages were lacking in the affected superficial dermis and were unchanged in areas where vasoproliferative lesions arose only in the medium to deep dermis.

Immunohistochemical (IHC) assays were performed on the skin samples using the biotin–peroxidase–streptavidin method (ImmunoDetector DAB, HRP, BioSB Inc., Santa Barbara, CA, USA), and primary antibodies directed against smooth muscle actin (SMA, 1A4–Dako Corp., dilution1:200), endothelial cells, CD31 (JC70A–Dako Corp., dilution1:200) and Von Willebrand factor (VWF, F8/86–Dako Corp. dilution1:200) incubated overnight. Antigen retrieval was conducted in a pressure cooker for 3 min in citrate pH 6.0 at 125 °C. As negative controls, normal bovine skin histological sections were incubated with nonimmune rabbit serum (1:200), and primary antibodies were omitted. Layers of vascular smooth muscle cells in the proliferating vessels showed intense immunolabeling for SMA (Figure 3A). Marked immunostaining for VWF (Figure 3B) and CD31 (Figure 3C) was observed in the endothelial cells lining proliferative vascular structures.

Skin vascular proliferations and tumors may have distinct and complex morphological features, and their classification is challenging in humans and animals. Cutaneous angiomatosis in cattle displays at least two distinct forms: juvenile bovine angiomatosis affecting calves under one year old; and BCA, mainly reported in adult cattle but can also be observed in young animals (WATSON & THOMPSON, 1990; RUETTEN et al., 2014; HENDRICK, 2017; JACINTO et al., 2021). For the first time in Brazil, this report showed a case of BCA affecting an eight-month-old Holstein heifer.

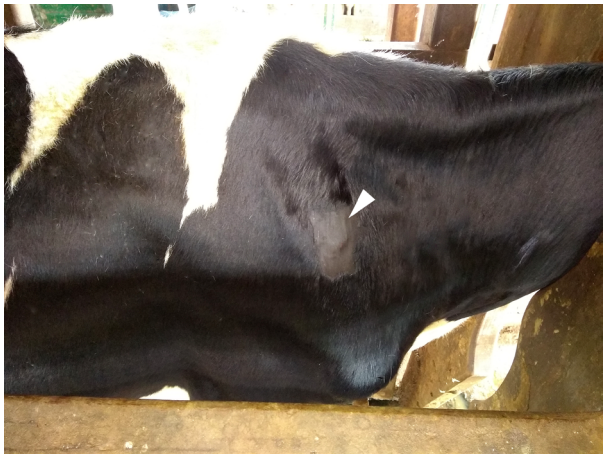

Figure 1 - Eight-month-old Holstein heifer. A dermal nodule was bulging from the epidermis close to the right prescapular region (white arrowhead).

Sporadic bleeding at both lesions was the only clinically significant change observed in the heifer, in addition to both lesions slightly bulging from the skin. Abundant bleeding in the skin lesions following spontaneous vascular rupture was observed in a Simental calf with generalized bovine juvenile angiomatosis (JACINTO et al., 2021). Lethargy, difficulty in locomotion, and local edema were reported in a cat with cutaneous angiomatosis (BARON et al., 2020). Exophthalmia, lagophthalmos, and elevated nictitating membrane have been observed in a horse with ocular angiomatosis (LUDWIG et al., 2015). As evidenced above, clinical signs in affected animals may be diverse and depend on the location of vascular proliferations.

Dermal soft vascular lesions with an irregular surface and heterogeneous red and white parenchyma detected in the heifer were the main gross aspect commonly reported in cases of BCA presenting

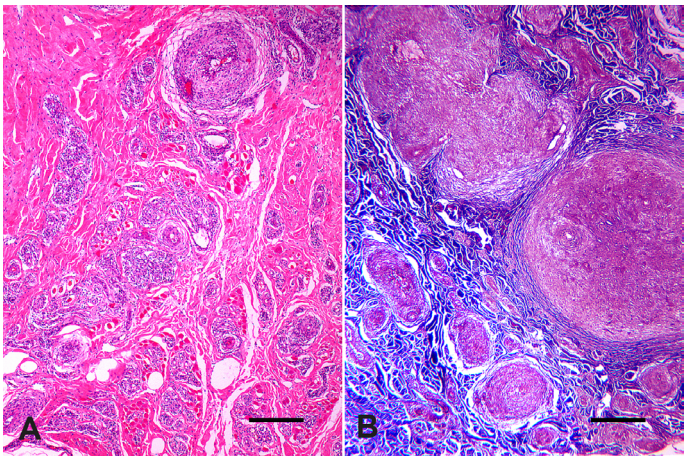

Figure 2 - Eight-month-old Holstein heifer. BCA, dermal nodule, histological findings. (A) A number of variable-sized arterioles, veins, and capillaries within the dermis (H&E, bar = 250µm). (B) Abundant collagenous fibrous tissue surrounding proliferative vessels (H&E, bar = 250µm).

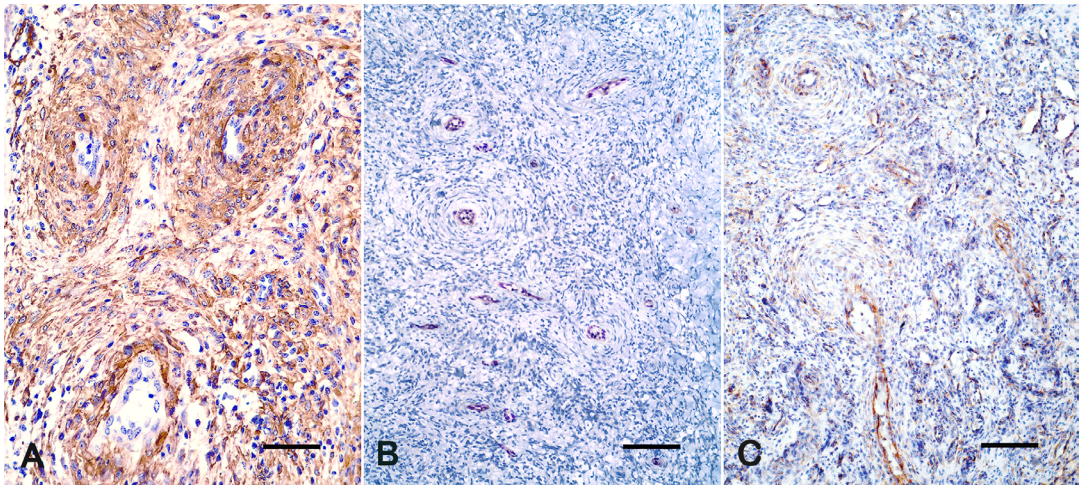

Figure 3 - Eight-month-old Holstein heifer. Bovine cutaneous angiomatosis (BCA), dermal nodule, immunohistochemical findings. (A) Vascular smooth muscle cells evidencing intense immunostaining for smooth muscle actin (SMA) (Immunoperoxidase, bar = 25µm). (B) Endothelial cells lining proliferative vascular structures show immunolabeling for Von Willebrand factor (VWF) (Immunoperoxidase, bar = 100µm). (C) Vasoproliferative lesion showing endothelial cells immunolabeled for CD 31 (Immunoperoxidase, bar = 100µm).

single or multiple lesions (WATSON & THOMPSON, 1990; RUETTEN et al., 2014; HENDRICK, 2017; JACINTO et al., 2021). Soft, pink, or reddish masses are usually located on the dorsum over the withers, back, and loin. They may be single or multiple and are always fragile (HENDRICK, 2017; JACINTO et al., 2021). Calves with juvenile bovine angiomatosis have shown a systemic form characterized by multiple vascular nodules affecting organs of the abdominal and thoracic cavities, in addition to cutaneous vasoproliferative lesions (WATSON & THOMPSON, 1990; JACINTO et al., 2021).

Microscopically, cutaneous proliferative masses composed of numerous arterioles, veins, and capillaries of varying sizes in the dermis surrounded by fibrous tissue and a mild to moderate lymphoplasmacytic inflammatory infiltrate evidenced in the Holstein heifer had similar histopathological features observed in cattle with cutaneous angiomatosis (WATSON & THOMPSON, 1990; RICHARD et al., 1995; RÖSTI et al., 2013; RUETTEN et al., 2014; HENDRICK, 2017; JACINTO et al., 2021). Epithelial and follicular hyperplasia (LUPPI et al., 2010) and a fibromyxomatous or myxoid stroma have also been reported in animals with angiomatosis in the skin (RICHARD et al., 1995; RUETTEN et al., 2014).

As observed in the present case, endothelial cells in the vascular lesions immunolabeled for CD31 and VWF in a calf with juvenile bovine angiomatosis (JACINTO et al., 2021) and in the cutaneous angiomatosis of a llama (LUPPI et al., 2010). Similarly observed in the heifer, a bull with cutaneous angiomatosis showed vascular proliferations immunostained for VWF and vascular walls immunolabeled SMA (RUETTEN et al., 2014).

Cutaneous angiomatosis has no well-defined pathogenesis, but some cases are related to inflammatory reactions, bacterial infections (YAGER et al., 2010), or congenital chromosomal abnormalities (HENDRICK, 2017; JACINTO et al., 2021). These vascular malformations may occur as a result of failure in angiogenesis during early embryonic development (BORST et al., 2020) or in the repair of traumatic injuries in the integumentary blood vessels (WATSON & THOMPSON, 1990). Furthermore, these vascular proliferations likely arise from the overgrowth of angioblastic cells over the proliferation of fibroblasts in exuberant granulation tissues (LUPPI et al., 2010).

Cutaneous angiomatosis must be differentiated from other vascular proliferations, such as vascular hamartoma, hemangioma, and hemangiosarcoma. Vascular hamartomas are congenital malformations with different histological features, unlike vascular lesions that arise months after the Holstein heifer's birth (LUDWIG et al., 2015). In contrast with the BCA observed in the heifer, hemangiomas and hemangiosarcomas are composed of endothelial

cells in different degrees of differentiation, and they are arranged in vascular spaces of varying sizes, clefts, and with or no solid areas (GROSS, 2005; STOCK et al., 2011; DIAZ-DELGADO et al., 2012; HENDRICK, 2017). In addition, angiomatosis can be differentiated from vascular neoplasms given the fact it is formed by mature vessels composed of different cell types, while in vascular neoplasms there is an uncontrolled proliferation of a single cell type (AFFOLTER et al., 2004; HENDRICK, 2017).

All clinical, pathological, and immunohistochemical features observed in the Holstein heifer were hallmarks of BCA. Considering that there are no consistent criteria for classifying these vascular proliferations in the literature, and the heifer did not present signs of visceral involvement (HENDRICK, 2017; JACINTO et al., 2021; RICHARD et al., 1995), the diagnosis of BCA was stated instead of the juvenile form. Furthermore, BCA has been reported in animals under one year old (HENDRICK, 2017). The owner was advised to avoid the use of this heifer in future breeding programs since BCA could be inherited as a genetic condition (JACINTO et al., 2021). BCA must be included in the differential diagnosis of easily bleeding skin nodules that do not respond to routine topical wound treatments. Additionally, bovine practitioners and researchers in the genetic improvement of cattle must be alert to cases of BCA to prevent its spread in Brazilian herds.

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DECLARATION OF CONFLICT OF INTEREST

All the authors declare no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed to the design and writing, and critically reviewed and approved the final version of the manuscript.

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