

Congenital Cutaneous Hemangioma in a Newborn Lamb

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ABSTRACT

Background: Hemangioma is a benign dermal or subcutaneous endothelial cell tumor composed of vascular spaces of varying sizes filled with erythrocytes and lined with a single layer of uniform endothelial cells. Although the pathogenesis is not well defined, these tumors are considered to result from an imbalance in angiogenesis, leading to uncontrolled proliferation of vascular elements. To the best of our knowledge, there are no reports of congenital cutaneous hemangioma in sheep. This report describes the clinical, laboratory, and pathological findings of a case of congenital hemangioma affecting a newborn lamb.

Case: A 5-day-old crossbred (Dorper x Santa Inês) lamb presenting with an ear nodule that expanded in the right ear was necropsied. An expansive subcutaneous nodule was observed macroscopically; it occupied approximately 90% of the right ear and had a crusty, irregular surface. The cut surface had multiple cavitations delimited by firm fibrous tissue and a light yellow-to-translucent content. Microscopically, it showed focally extensive subcutaneous neoplastic proliferation and moderate cellularity; it was formed of vascular beds of varied sizes and supported by moderate fibrocollagenous stroma. Suppurative inflammation was observed in the neoplastic vascular beds with large amounts of free basophilic coccoid bacteria inside macrophages. Immunohistochemistry analysis was performed to confirm the diagnosis. Strong cytoplasmic labeling was observed in neoplastic endothelial cells for CD31 and factor VIII. The Ki67 proliferation marker was positive in approximately 5% of neoplastic cells. The cells did not express smooth muscle actin (1A4) or pan-cytokeratin (AE1/AE3). Histological characteristics and immunohistochemistry findings were consistent with those of congenital cutaneous hemangioma, a rare neoplasm in sheep.

Discussion: The association of clinical, anatomopathological, and immunohistochemical data enabled the diagnosis of congenital cutaneous hemangioma in the 5-day-old lamb. Reports of vascular tumors in sheep are not frequent in literature and usually involve adult animals with no anatomical site predilection. In sheep, the occurrence of nasotracheal hemangioma in a 2-year-old ewe and gingival hemangioma in a 5-year-old sheep have already been described. A cutaneous extra-neural hemangioblastoma was diagnosed in the ear of a 1-month-old lamb. IHC was also used to confirm the diagnosis of hemangioblastoma. Macroscopically, hemangiomas can present as well-delimited and encapsulated masses that when cut, show a reticulated pattern similar to honeycombs that separate the blood-filled cavities. The present case showed a similar conformation but without enough erythrocytes to result in a bloody appearance. Tumor drainage and the predominance of blood serum in the content possibly made it macroscopically translucent. Microscopically, the hemangioma was classified as cavernous. This morphological variation forms large channels separated by fibrous connective tissue stroma, which may contain inflammatory cells. IHC confirmed the endothelial lining of the cystic cavities and was crucial in excluding differential diagnoses. Thus, factor VIII-related antigen was used as a marker for normal and neoplastic cells, as well as for tumoral and reactive neovascularization, in which neoplastic cells were immuno-expressed for CD31 and Factor VIII. In domestic animals, the association between CD31 and Factor VIII is considered more specific for vascular endothelial cells, differentiating them from cells of lymphatic origin. Congenital cutaneous hemangioma occurs in sheep, and its diagnosis and differentiation can be based on histopathology associated with conventional immunohistochemical panels for vascular neof ormation.

Keywords: sheep, mesenchymal neoplasm, vascular tumour, small ruminant, histopathology, immunohistochemistry.

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INTRODUCTION

Hemangioma is a benign dermal or subcutaneous endothelial cell tumor composed of vascular spaces of varying sizes filled with erythrocytes and lined with a single layer of uniform endothelial cells [1,6,9]. Hemangiomas are common in dogs with no breed predisposition [12]. In some cases, they can be induced by solar radiation and occur in the distal limbs of foals and the scrotum of pigs [6]. Additionally, congenital hemangiomas have been reported in cattle, horses, and pigs [6]. Although the pathogenesis is not well defined, these tumors are considered to result from an imbalance in angiogenesis, leading to uncontrolled proliferation of vascular elements [1,6,8]. Even in offspring, the pathogenesis of this tumor is still unclear. Some studies have reported its onset through the aberrant proliferation of a mutant progenitor endothelial cell, possibly of fetal origin, associated with environmental factors, and without evidence of hereditary factors [16]. To the best of our knowledge, there are no reports of congenital cutaneous hemangioma in sheep. Here, we report the clinical, laboratory, and pathological findings of the first case of congenital hemangioma affecting a newborn lamb.

CASE

According to the practitioner, the lamb was born unassisted, nursed correctly, and healthy. A small nodule on the right ear was the only alteration upon physical evaluation. The practitioner opted to drain the nodule, obtaining a moderate amount of translucent fluid. After this, the nodule enlarged, expanding the right ear, and its consistency altered from floating to firm. The following day, the lamb presented with apathy and reduced sucking reflex. Two days later, the lamb was found unresponsive on lateral recumbency. Therefore, the lamb was referred for hospital care.

Upon clinical evaluation, the 5-day-old cross-bred (Dorper x Santa Inês) lamb weighed 6.0 kg, and was unresponsive to external stimuli (comatose), tachycardic (160 beats per min), feverish (39.3°C), and dehydrated. The right ear was severely enlarged, presenting with a 10.0 × 8.0 × 5.0 cm firm mass with floating and ulcerated points that drained fetid and brownish content. The lamb died soon after the blood samples were collected. Hematology parameters presented left shift (1,022 [9%] band cells), toxic neutrophils, and hyperfibrinogenemia 1000 mg/dL (normal range: 240-315 mg/dL) [14]. Serum biochemistry profile showed hypoproteinemia 36

g/L (normal range: 58-63 g/L), hypoalbuminemia 11.6 g/L (normal range: 26-32 g/L), hypoglobulinemia 24.4 g/dL (normal range: 27-36 g/dL), increased urea 366 mg/dL (normal range: 36-62 mg/dL) and creatinine 5.2 mg/dL (normal range: 0.9-1.2 mg/dL) levels, but serum gamma-glutamyl transferase activity was within the reference range (76 UI/L; RI: 52-88 UI/L) [3,4].

During necropsy, an expansive and infiltrative subcutaneous nodule that occupied approximately 90% of the right ear, with a crusty and irregular surface, measuring 10.0 x 8.0 x 5.0 cm, was found. The cut surface contained multiple cavitations (honeycomb appearance) delimited by firm fibrous tissue and light yellow to translucent fluid (Figure 1A). The cranial lung lobes presented with multiple focally extensive dark red and hypocreptant areas and focal suffusion in the accessory lobe pleura. A slightly light brown mucous content was noted when cutting the trachea and main bronchi. Fragments of several organs and ear nodules were collected and fixed in a 10% buffered formalin solution, routinely processed for histopathology, cut into 5-micron thick sections, and stained with hematoxylin and eosin (H&E)¹.

Microscopically, the nodule consisted of a focally extensive and moderately cellular subcutaneous neoplastic proliferation formed by multifocal to coalescent vascular beds of varied sizes, lined by spindle cells, and supported by fibrocollagenous stroma walls. The spindle cells had thin eosinophilic cytoplasm and basophilic fusiform nuclei with indistinct and single nucleoli (Figure 1B). No mitotic figures were observed. Larger vessels, a deep dermis, and subcutaneous tissue were sometimes filled with large amounts of intact and degenerated neutrophils (dermatitis and abscessed vasculitis) and dense aggregates of free basophilic coccoid bacteria in the cytoplasm of macrophages, which were also present in the fibrous stroma.

Immunohistochemistry (IHC) was used to confirm the diagnosis. The sections were superimposed onto previously silanized slides. Table 1 shows the antibodies^{2,3} used with dilutions, their respective antigen retrievals, incubation, and positive controls. A dextran-peroxidase-HRP system (Dako En Vision™ FLEX+® kit)² was used for development, and the reaction was visualized using 3,3 diaminobenzidine². Harris hematoxylin¹ was used for counterstaining. Neoplastic endothelial cells presented strong cytoplasmic labeling for CD31 (Figure 1C) and Factor VIII (Figure 1D), and a positive Ki67 proliferation marker was present in approximately 5% of neoplastic cells. Cells did not express 1A4 or AE1/AE3.

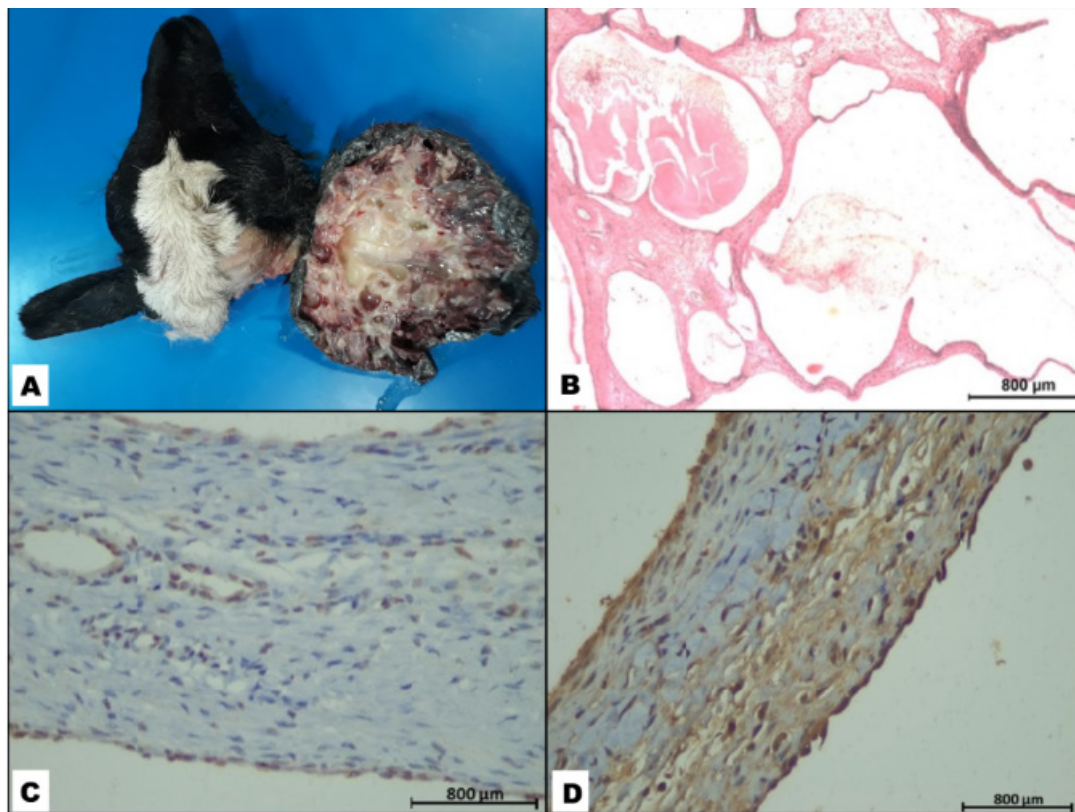


Figure 1. Congenital cutaneous hemangioma in a lamb. A- Cut surface of a right ear nodule with multiple cavitations (honeycomb appearance) delimited by fibrous tissue and a light yellow to translucent content. B- Proliferation of well-differentiated vessels lined with spindle cells with thin, sparse, eosinophilic cytoplasm and basophilic spindle-shaped nuclei with indistinct nucleoli [H&E, Obj.5x]. C- Lining cells express nuclear and cytoplasmic immunostaining for CD31 [Harris hematoxylin counterstaining. DAB.; Obj.5x]. D- Endothelial cells express Factor VIII-positive cytoplasmic immunostaining [Harris hematoxylin counterstaining. DAB; Obj.5x].

Table 1. Immunohistochemical panel for the characterisation of cutaneous haemangioma in a lamb.

Antibody	Antibody	Clone/Code	Concentration	Antigen retrieval	Incubation	Manufacturer	Positive control
Smooth muscle actin		1 to 4	1:200	Citrate (pH 5.6)	Overnight	Dako	Appendix
Factor VIII		F8/86	1:400	Citrate (pH 5.6)	Overnight	Dako	Placenta
Ki67		MIB-1	1:300	EDTA (pH 8,9)	Overnight	Dako	Tonsil
CD31		EP78	1:200	Citrate (pH 5.6)	Overnight	Cellmark	Tonsil
Pan-cytokeratin		Ae1/Ae3	1:300	Citrate (pH 5.6)	Overnight	Dako	Skin

DISCUSSION

The association of clinical, anatomopathological, and immunohistochemical data enabled the diagnosis of congenital cutaneous hemangioma in the 5-day-old lamb. Reports of vascular tumors in sheep are not frequent in literature and usually involve adult animals with no anatomical site predilection. In sheep, the occurrence of nasotracheal hemangioma in a 2-year-old ewe [15] and gingival hemangioma in a 5-year-old sheep [11] have already been described. A cutaneous extra-neural hemangioblastoma was diagnosed in the ear of a 1-month-old

lamb. IHC was also used to confirm the diagnosis of hemangioblastoma [17].

There was no predilection for sex or age among the goats with vasoproliferative lesions. Of the 4 animals diagnosed with hemangioma, a 4-year-old animal presented with an ear lesion, and only 1 animal, aged < 1 year, presented with a hemangioma on the skin of the thoracic region [2]. In the present report, dermatitis and abscessed vasculitis associated with bacteria triggered septic shock after ear drainage, as observed by the clinical (comatose status) and laboratory (left shift, toxic neutrophils, and acute renal failure) findings.

Macroscopically, hemangiomas can present as well-delimited and encapsulated masses that when cut, show a reticulated pattern similar to honeycombs that separate the blood-filled cavities [5,6]. The present case showed a similar conformation but without enough erythrocytes to result in a bloody appearance. Tumor drainage and the predominance of blood serum in the content possibly made it macroscopically translucent.

Microscopically, the hemangioma was classified as cavernous. This morphological variation forms large channels separated by fibrous connective tissue stroma, which may contain inflammatory cells. However, this classification has been mostly used in dog hemangiomas [6] and is considered inadequate for congenital vascular abnormalities in human medicine [13]. To the best of our knowledge, there is no consensus on the classification of congenital cutaneous lesions in farm animals; thus, the diagnosis of cavernous hemangioma prevailed [5]. In the present case, it was difficult to delimit lining endothelial cells with minimal mitotic index and atypia, differentiating them from hemangiosarcoma.

Hamartoma and chronic fibrosing inflammatory changes were included in the differential diagnosis. Hamartomas are lesions that result from an embryological development error, characterized by the abnormal arrangement of tissues belonging to an organ, often confused with malformations [13]. Hamartomas have different tissues in their composition that belong to that organ, and the present case showed a large number of vascular beds and absence of intratumoral dermal components, such as adnexal glands. A recent case of congenital cutaneous vascular hamartoma in a lamb presented with a lesion that could be differentiated from a hemangioma because it was already present at birth, being macroscopically well delimited, and having well-differentiated vascular structures [10]. The present case has similar characteristics but with greater tumor infiltration and mainly mitotic activity (5%) confirmed through Ki67 immunoexpression. This is a characteristic of benign tumors, unlike hamartomas, which present no mitotic activity [2,10]. In inflammatory conditions, blood vessel proliferation may be present in certain areas, resembling a hemangioma, but such changes contain abundant connective tissue and predominant inflammation [2]. Although abundant connective tissue was found, the exudate was located adjacent to the surgical drainage area, probably originating from secondary bacterial infection. Inflammatory changes tend to be less expansive and contain rudimentary and narrow vascular

beds due to angiogenesis [2]. On the other hand, chronic inflammation with these characteristics would have to originate in fetal life and thus, could have resulted in an abortion [7].

IHC confirmed the endothelial lining of the cystic cavities and was crucial in excluding differential diagnoses. Thus, factor VIII-related antigen was used as a marker for normal and neoplastic cells, as well as for tumoral and reactive neovascularization, in which neoplastic cells were immuno-expressed for CD31 and Factor VIII. In domestic animals, the association between CD31 and Factor VIII is considered more specific for vascular endothelial cells, differentiating them from cells of lymphatic origin [5]. Congenital apocrine cysts and hamartomas may contain epithelial components such as glands and mesenchymal components such as myoepithelial cells from the skin of origin [8]. These differential diagnoses were excluded by negative labeling for smooth muscle actin and pan-cytokeratin. Smooth muscle actin (1A4) identifies myoepithelial cells intermingled with epithelial cells in benign skin adnexal proliferative lesions, helping in the differentiation of malignant lesions and being used as a smooth muscle marker in spindle cells and pleomorphic tumors [8]. Smooth muscle actin (1A4) negativity also indicates the absence of a muscle layer that should be present in the wall of larger-caliber arteriovenous formations. Immunohistochemical analysis was also useful in determining the level of cell proliferation, growth, and malignant characteristics of the tumor.

Congenital cutaneous hemangioma occurs in sheep, and its diagnosis and differentiation can be based on histopathology associated with conventional immunohistochemical panels for vascular neof ormation.

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