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SHORT PAPER

# Cutaneous Angiomatosis in a Llama (*Lama glama*)

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## Summary

Cutaneous angiomatosis was diagnosed in an adult female llama (*Lama glama*). Lesions were raised or plaque-like, erythematous, firm to soft in consistency and were observed on the face and skin of the axillary, abdominal, perineal and inguinal regions. The lesions were not painful or pruritic. Microscopical examination revealed an irregular parakeratotic lamellar hyperkeratosis associated with diffuse proliferation of arterioles and venules in the superficial dermis. Immunohistochemical analysis determined that the cells forming these vessels and perivascular cells expressed factor VIII-related antigen, vascular endothelial growth factor (VEGF), CD31 and smooth-muscle alpha-actin. These studies confirmed the diagnosis of cutaneous angiomatosis.



*Keywords:* angiomatosis; immunohistochemistry; *Lama glama*; llama

Angiomatosis is a benign vasoproliferative change that may occur as a consequence of abnormal and disordered repair of tissue in response to primary injury. In man, such lesions may be congenital or may arise as a reactive, tumour-like vascular proliferation known as bacillary angiomatosis (Kumar *et al*., 2004). The pathogenesis of this disease is largely unknown. Bacillary angiomatosis has been reported in juvenile and young adult cattle (Cotchin and Swarbrick, 1963; Richard *et al*., 1995) as well as in dogs (Kim *et al*., 2005). Angiomatosis has also been diagnosed in various organ systems in cats and horses (Kipar *et al*., 2001; Lamm and Njaa, 2007). Microscopical lesions appear as areas of non-encapsulated vascular proliferation consisting of arteries, veins and capillaries. The lesions are sometimes associated with mononuclear inflammatory infiltration or fibroplasia with collagen deposition. The microscopical appearance is similar to that of granulation tissue, but without the classic perpendicular orientation of vessels to collagen. Treatment of these lesions usually consists of surgical removal or, in some cases, cauterization of the affected tissue (Cotchin and Swarbrick, 1963). Spontaneous regression does not occur. Cutaneous angiomatosis has not been documented previously in the llama.

An adult female llama (*Lama glama*) kept in captivity at the Fundação Zoo-Botânica de Belo Horizonte, Minas Gerais, Brazil was examined following the development of multiple skin lesions. These were ovoid or linear and ranged from 1–40 cm in diameter. Lesions affected the face and skin of the axillary, abdominal, perineal and inguinal regions. The lesions appeared as erythematous papules to plaques that were variably firm or soft in consistency. The lesions were not painful or pruritic. Biopsies of the lesions and normal skin were fixed in 10% neutral buffered formalin and processed routinely for embedding in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE). To further characterize the lesions, additional sections were stained with Masson's trichrome stain and subjected to immunohistochemistry (IHC) with primary antisera specific for the endothelial cell markers CD31 (rabbit polyclonal antibody, 1 in 20; Dako, Carpentaria, California), factor VIII-related antigen (murine polyclonal antibody, 1 in 400; Dako) and VEGF (rabbit polyclonal antibody, 1 in 200; Santa Cruz Biotechnology, Santa Cruz, California). An antiserum specific for smooth-

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muscle alpha-actin (murine monoclonal antibody clone 1A4, 1 in 200; Dako) was also employed. These reagents have been previously documented to show cross-reactivity with molecules from different animal species (Serakides *et al.*, 2000; Dickerson *et al.*, 2005).

Prior to incubation with primary reagents, sections were subjected to antigen retrieval by immersion in a commercially available solution of 0.05% proteinase K (Proteinase K-Fungal; Invitrogen, São Paulo, Brazil) for 30 min and incubation with $H_2O_2$ 3% in methanol to block endogenous peroxidase activity. A serum blocking solution (30 min) was included before incubation with primary antibodies in a humidified chamber overnight. The appropriate secondary antibodies (Dako) were biotinylated and used in conjunction with streptavidin–peroxidase (LSAB kit; Dako). The chromogen (3,3′-diaminobenzidine; Dako) was applied for 1 min and sections were finally counterstained with Mayer's haematoxylin. Normal vessels of the skin were used as an internal positive control. Negative control sections were incubated with phosphate buffered saline (pH 7.4, 0.01 M) in place of primary antibody. VEGF expression was compared between lesional and normal skin from the same body site.

Microscopical examination revealed intense and diffuse vascular proliferation in the superficial and mid dermis (Fig. 1A) with irregular parakeratotic lamellar hyperkeratosis; this morphology differed to that of the normal skin (Fig. 1B). The vascular proliferation consisted of numerous arterioles, venules and capillaries of variable diameter. Each of these was lined by a single layer of well-differentiated endothelium. Discrete haemorrhagic foci were present, but there was no significant inflammation. Some endothelial cells were supported by a single to double layer of smooth-muscle cells, which were immunohistochemically labelled for smooth-muscle actin expression (Fig. 2A). Trichrome staining revealed a collagenous matrix supporting and separating individual vessels (Fig. 2B). The cells lining the vascular channels expressed factor VIII-related antigen (Fig. 2C) consistent with an endothelial origin (Sadler, 1991). Perivascular cells expressed CD31 (Fig. 2D), a molecule that can be expressed in endothelial cells as well as by inflammatory cells and platelets (Stockinger *et al.*, 1990; Albelda *et al.*, 1991). There was intense and diffuse epidermal and dermal expression of VEGF (Fig. 2E), but in normal skin there was only focal epidermal VEGF expression (Fig. 2F). These

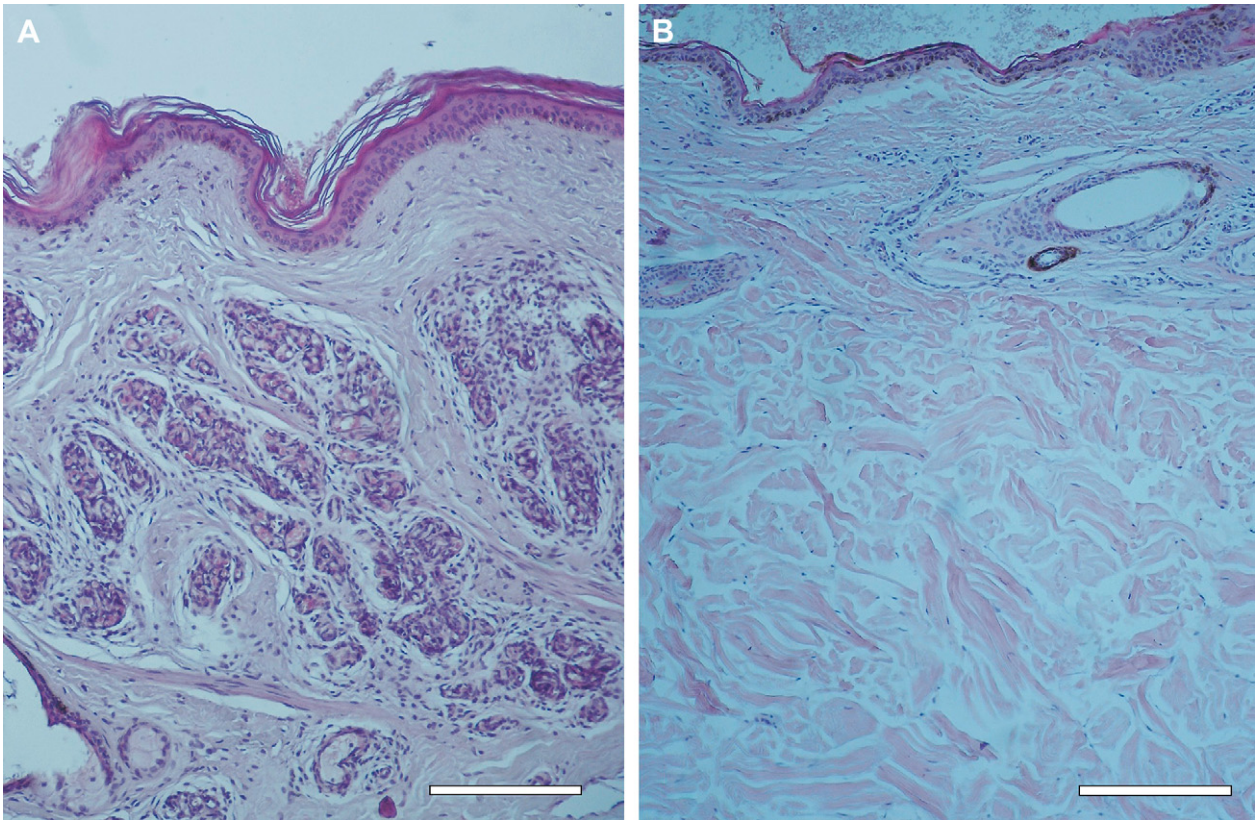


Fig. 1. (A) Diffuse vascular proliferation in the superficial and mid dermis of the inguinal skin. (B) Normal skin from the same region. HE. Bar, 107 μm.