DOI: 10.5433/1679-0359.2019v40n6Supl3p3781

Cytological, histopathological, histochemical, and immunohistochemical findings of multiple cutaneous nodules in a bovine

Achados citológicos, histopatológicos, histoquímicos e imuno-histoquímicos de múltiplas nodulações cutâneas em um bovino

Telma de Sousa Lima[1*]; Ruy Brayner de Oliveira Filho[2]; Mônica Shinneider de Sousa[3]; Nayadjala Távita Alves dos Santos[1]; Rubia Avlade Guedes Sampaio[1]; Ricardo Barbosa Lucena[4]

Abstract

This case study describes the cytological and histopathological findings of cutaneous masses in a bovine, including a peripheral nerve sheath tumor (PNST), vaccine-associated granulomatous inflammation, and eosinophilic inflammation due to parasitosis. A six-year-old undefined cow (SRD) presented with heterogeneous cutaneous lesions including multiple nodules in the left paralumbar fossa, bilaterally at the withers, and scattered along the dorsum, limbs and near the tail; some lesions were associated with ticks. Cytology of these nodules showed benign mesenchymal neoplasia (paralumbar fossa), granulomatous and pyogranulomatous inflammation (withers) and keratin (dorsum). Histopathology, in this order, confirmed PNST, post-vaccination granuloma, and eosinophilic dermatitis. A peripheral nerve sheath tumor was suspected based on the histological findings, showing a well-delineated proliferation of fusiform cells arranged in plexiform structures, which appeared red by Masson's Trichrome stain. The diagnosis was confirmed by immunohistochemistry (anti-S100 antibody). Vaccine reaction often occurs in cattle, and cytological examination is sufficient to determine the inflammatory process. Eosinophilic dermatitis is usually accompanied by perivascular inflammation and reflects the exfoliative process by the oral apparatus of the parasite.

Key words: Dermatopathies. Eosinophilic inflammation. Granulomatous inflammation. Peripheral nerve sheath tumor. Ruminant.

Resumo

Descrevem-se os achados citológicos e histopatológicos do tumor de bainha de nervo periférico (TBNP), da reação vacinal e da inflamação eosinofílica decorrente dapicada de carrapatoem um bovino. Uma vaca sem raça definida (SRD) de seis anos de idade foi apresentada com diferentes lesões cutâneasnodulares localizadas na fossa paralombar esquerda, bilateralmente na cernelha e dispersos no dorso, membros e próximo à cauda, por vezes associado a carrapatos. Realizou-se citologia e biópsia desses nódulos. Na citologia verificou-se neoplasia mesenquimal benigna (fossa paralombar), inflamação

[1] Discentes de Mestrado, Universidade Federal da Paraíba, UFPB, Areia, PB, Brasil. E-mail: telmasousava@hotmail.com; nayadjalat@gmail.com; rubia_avlade@yahoo.com.br
[2] Médico Veterinário, Hospital Veterinário, UFPB, Areia, PB, Brasil. E-mail: ruybrayner@gmaill.com
[3] Discente de Doutorado, Universidade Federal de Campina Grande, UFCG, Patos, PB, Brasil. E-mail: monica_shinneider@hotmail.com
[4] Prof., Pesquisador, UFPB, Areia, PB, Brasil. E-mail: lucena.rb@gmail.com
[*] Author for correspondence

Received: Oct. 16, 2018 - Approved: Sept. 25, 2019

granulomatosa e piogranulomatosa (cernelha) e ceratina (dorso). Na histopatologia, confirmou-se que esses nódulos correspondiam, nessa ordem, a tumor de bainha de nervo periférico, granuloma vacinal e dermatite eosinofílica. O diagnóstico do TBNP foi estabelecido com base nos achados histológicos, que caracterizaram-se por uma proliferação bem delimitada de células fusiformes arranjadas em estruturas plexiformes, corados em vermelho pelo Tricômico de Masson, e confirmado por imuno-histoquímica (anticorpo anti-S100). A reação vacinal ocorre frequentemente em bovinos e o exame citológico é suficiente para determinação do processo inflamatório. Dermatite eosinofílica em geral é acompanhada de inflamação perivascular e perianexal e reflete a ação esfoliativa do aparato bucal do parasita.
Palavras-chave: Dermatopatia. Inflamação eosinofílica. Inflamação granulomatosa. Ruminante. Tumor de bainha do nervo periférico.

Introduction

A variety of agents cause dermatopathies and subsequent economic losses in the bovine species, especially infectious, parasitic, photosensitizing, and neoplastic conditions as well as granulomatous and hypersensitive reactions (WHITE; EVANS, 2006). In general, these conditions are poorly reported in the literature, especially in Brazil where cutaneous nodular lesions in production animals are rarely investigated.

Peripheral nerve sheath tumors (PNST) are comprised of Schwann cell tumors, perineural fibroblasts, or both. They are described in a number of animal species (SCHÖNIGER; SUMMERS, 2009). There is some controversy regarding the nomenclature of these tumors, and distinction can be made using immunohistochemistry.

Post-vaccination granuloma refers to the nodular inflammatory response to vaccine adjuvant compounds in oily vaccines, such as aluminum hydroxide, saponins, or polysaccharide derivatives (SPICKLER, 2003). These processes can be diagnosed by cytology, a method that can define the morphological pattern of the lesion (WIEDMEYER et al., 2014) and stipulate the severity of the lesion. Unfortunately, this process is not commonly employed.

Tick infestation can cause nodular lesions of variable distribution in young and adult cattle. The exfoliative action of the parasite on bovine skin can lead to inflammation, hyperkeratosis, acanthosis, and collagen degeneration. This contributes to depreciation of the leather as it becomes hard, opaque, perforated, and rough (GASHAW; MERSHA, 2013).

The aim of this case study is to describe multiple cutaneous nodular lesions in a bovine, including PNST, eosinophilic dermatitis and vaccine-associated nodules, highlighting the cytological, histochemical and immunohistochemical findings.

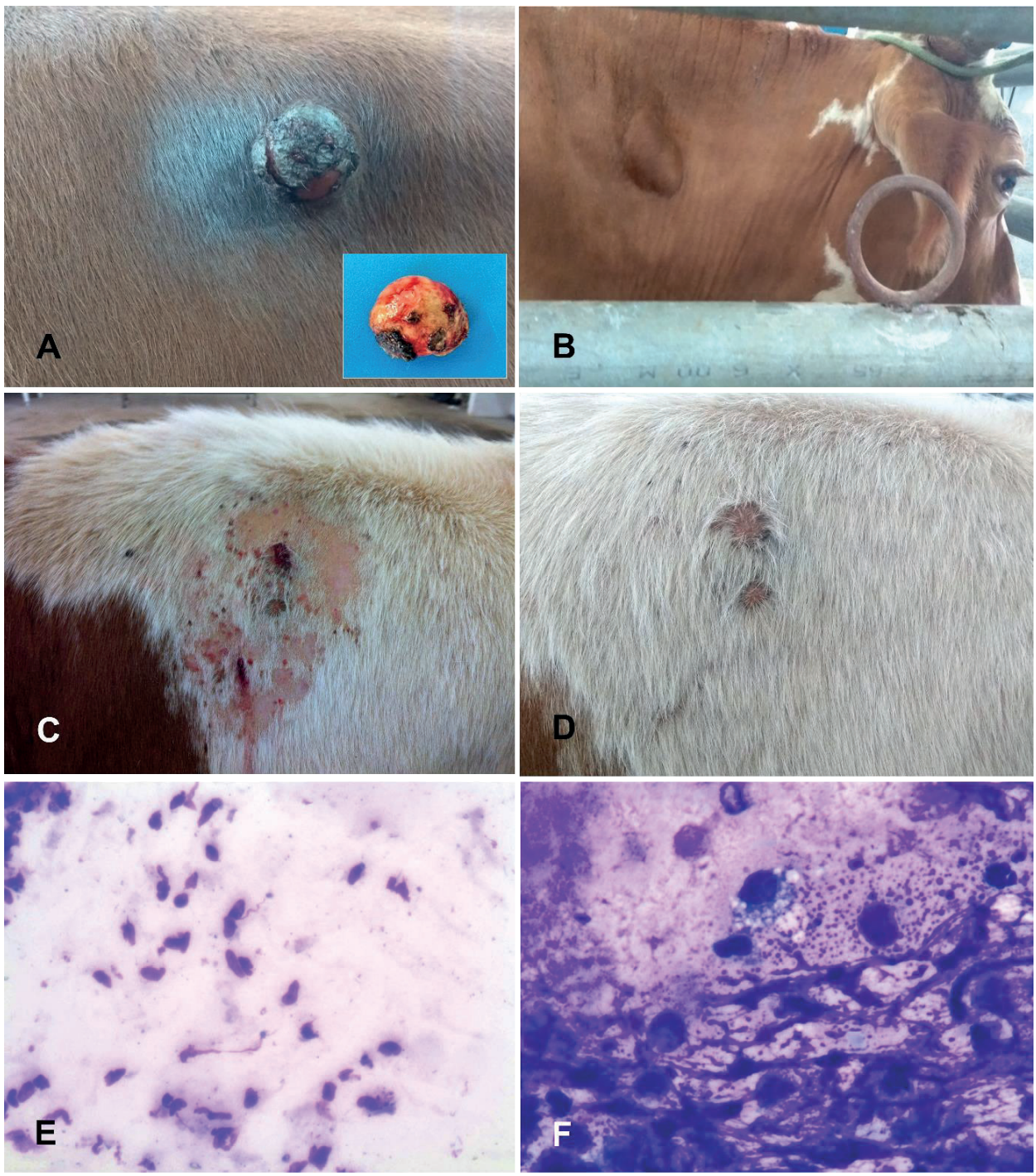
Case report

A six-year-old mixed-breed cow was admitted to the Veterinary Hospital of the Federal University of Paraíba (HV-UFPB) to confirm pregnancy. No significant changes were observed upon general clinical examination, but the dermatological examination showed multiple skin lesions throughout the body.

A firm, ulcerated exophytic nodule (nodule A) was present in the left paralumbar fossa, measuring 5.0×4.0×2.0 cm in size (Figure 1A). It exhibited a smooth, firm and yellowish cut surface. Bilaterally, at the withers (Figure 1B), there was an irregular, non-ulcerated, soft-coated skin elevation ranging from 3.0×2.0×1.0 cm in size (nodule B, left withers) to 16.0×11.0×4.0 cm in size (nodule C, right withers). Nodule B was soft and filled with a granular caseous material suggestive of an abscess, while nodule C was broad, soft, and contained serous, translucent, and slightly granular liquid. There were also irregular, slightly elevated, reddish, and crusty areas (nodule D) in the scapular region and extending to the back, ranging from 0.2

to 1.5 cm in length (Figure 1C and 1D). Multiple ticks were scattered on the skin, mainly along the back, limbs and near the tail; they were sometimes associated with these lesions.

Figure 1. Multiple cutaneous nodules in a cow. **A.** Cutaneous Neurofibroma. Note the firm exophytic nodule with marked ulceration in the paralumbar fossa. Inset: Nodule after complete surgical excision. **B.** Vaccine-associated granuloma at the withers. **C** and **D.** Tick bite reaction. Note multifocal areas of irregular, elevated, reddish and crusty skin along the back associated with parasites of the Ixodidae family. **E-F.** Cytologic findings of peripheral nerve sheath tumor **(E)** and vaccine-associated granuloma **(F)**. Rapid panoptic, 20x objective.



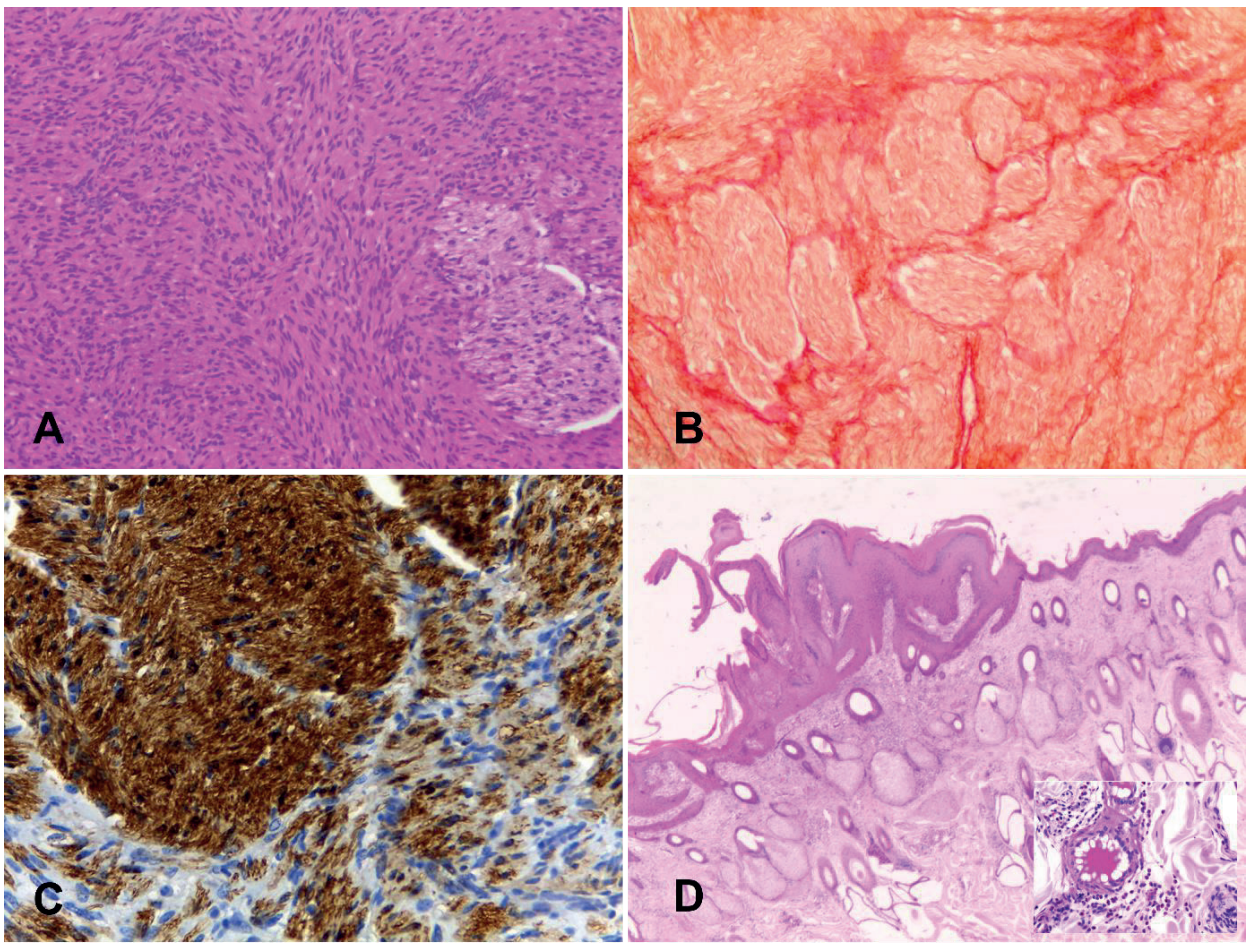
Cytological data were collected using fine needle aspiration (FNA) methods (samples A, B and C) and scarification (sample D). Excisional biopsy was performed on lesions A and D, and nodule A was subsequently submitted for Masson's Trichome staining. The sample was finally submitted for immunohistochemistry (IHC) by heat recovery in 10 mM citrate solution (pH 6.0) in a pressure pan (PascalR, Dako Santa Clara, California, United States of America). Subsequently, the slides were kept at room temperature for 20 minutes to cool and washed with deionized water. After antigen recovery, endogenous peroxidase blockage was achieved by immersing the slides in hydrogen peroxide (Peroxide Block, Cell Marque, 925B-09). After this procedure, the sections were washed in TRIS solution (pH 7.4) and non-specific sites were blocked with non-specific reaction blocking solution (proteinblockserum-free-DAKO®, Santa Clara, California, United States of America, ref. X0909). The slides were incubated with primary antibody (S-100, rabbit polyclonal, Dako®, Santa Clara, California, United States of America) for 18 hours at 4°C. As an amplification and detection system Envision Dual Link (DAKO®, Santa Clara, California, United States of America, K4065) and chromogen diaminobenzidine (DAKO®, Santa Clara, California, United States of America, K3468) were used. The slides were counterstained with Harris Hematoxylin.

The definition of the cytological method varied according to the morphology of the lesion and anatomical site. The slides were stained by Rapid panoptic (Interlab, Pinhais, Paraná, Brazil). The cytological examination was verified. Sample A was a hypocellular specimen, characterized by spindle cells whose cytoplasm was elongated and basophilic, with central to paracentral nucleus containing 1 to 3 evident nucleoli and discrete pleomorphism. This suggested a benign mesenchymal neoplasm (Figure 1E). Samples B and C were characterized by inflammatory infiltrates consisting of macrophages and occasional lymphocytes, neutrophils and fibrin. In both samples, there were globular and granular basophilic structures in the middle of the infiltrate and inside the cytoplasm of macrophages, suggestive of vaccine substance. The process was classified as marked focally extensive granulomatous inflammation (Figure 1F). Lesion D included only one hypocellular sample composed of red blood cells, neutrophils and occasional keratin plaques.

On the histopathological examination of nodule A, there was a well-delineated, non-encapsulated mass composed of a plexus of pleomorphic spindle cells and supported by delicate fibrovascular stroma. Cells ranged from a fusiform to globose shape with scarce and slightly eosinophilic cytoplasm with elongate to oval nuclei with loosely dotted, normochromatic chromatin, with 1 to 2 evident nucleoli, moderate anisocytosis and anisokaryosis, and rare mitotic figures per high magnification field (Figure 2A). In TM, the mass was diffusely colored red (Figure 2B), proving no intra- or peritumoral connective tissue was present. Immunoblotting was positive for S-100 antibody, evidencing strong labelling of neoplastic cells (Figure 2C). These findings were consistent with the diagnosis of peripheral nerve sheath tumor.

Lesion D was comprised of multifocal areas with focally extensive inflammatory infiltrate consisting predominantly of eosinophils that permeated the collagen fibers and involved both the attachments and the blood vessels. In addition, a focally extensive area of acanthosis was observed, forming papillary projections associated with moderate hyperkeratosis. These characteristics suggested a moderate multifocal eosinophilic dermatitis associated with acanthosis, hyperkeratosis, and folliculitis (Figure 2D).

Figure 2. Histopathology of multiple cutaneous nodules from a cow. **A-C.** Cutaneous Neurofibroma. Note the mesenchymal cell proliferation forming plexiform beams. Hematoxylin-Eosin, objective 20X. **A.** Evidence of nerve plexuses, stained intensely red in Masson's trichrome, obj. 20X **B.** Positive immunoblotting for S-100, counterstained with Harris Hematoxylin (polyclonal Dakko, dilution 1: 1500). obj. 40X **C.** D- Reaction to a tick bite. Note marked hyperkeratosis. Inset: Perivascular eosinophilic inflammatory infiltrate. HE, obj. 20x.



Discussion

The diagnosis of PNST was established based on cytological, histopathological and histochemical findings, and confirmed by immunohistochemistry. The post-granuloma vaccine was diagnosed by cytology and the eosinophilic dermatitis by histopathological examination.

Peripheral nerve sheath tumors are neoplasms, which likely originate from Schwann cells or endoneural and epineural fibroblasts. They are commonly classified as Schwannomas or neurofibromas. In general, they appear mainly in the skin and subcutaneous tissue as a single mass of different proportions, though there are reports of occurrence in the eyes, tongue, intestine and peripheral nerves (SCHÖNIGER; SUMMERS, 2009) of different species.

In humans, this neoplasm is a clinical condition called neurofibromatosis, characterized by the formation of nodules in variable numbers, mainly in the skin. In the present case, the location and morphology of the nodule are consistent with reports in the literature, which suggests tumors are most common in cutaneous-subcutaneous regions because of their neuroectodermal origin.

No predisposition for PNST has been observed based on age, race or sex, but the occurrence of such neoplasms is more common in adult or elderly animals. In a study of 12 dogs with PNST, there was considerable variability regarding breeds, as well as age affected (ranging from 2 months to 15 years), and both males and females had the disease (SCHÖNIGER; SUMMERS, 2009).

The sex and breed of the animal did not stand out in a study on tumors in cattle in the south of the country. During a 45-year interval, only four fibroids were reported, affecting adult cattle from four to eight years of age (LUCENA et al., 2011). Similar to other species with single nodules (BABOVIC-VUKSANOVIC et al., 2004), skin localization is usually non-invasive, and surgical excision is curative with a low recurrence rate.

The cytological analysis of the neoplasm complemented the examination findings revealing a benign mesenchymal neoplasm, which could not be differentiated as a possible PNST. This can be attributed to low cellularity and cellular morphology, similar to that observed in other neoplasms of mesenchymal origin. The histopathological evaluation confirmed a neurofibroma, with a main differential diagnosis of Schwannoma. In cattle, Schwannoma is uncommon with reports in the skin (AHMADI et al., 2012) and in the multicentric form (CANFIELD, 1978). Another interesting feature is the formation of concentric complexes, suggestive of nerve fibers, observed in cases of neurofibroma, which strengthened the diagnosis in this case.

In Brazilian cattle, vaccination against foot-and-mouth disease is mandatory and often stimulates the formation of granulomas at the site of application, especially on the neck. This made vaccine-associated granuloma a likely cause for the bilateral masses in the neck of the patient in the present report. The chronic inflammatory reaction results from adjuvant components of oily vaccines (SPICKLER, 2003), which potentiate the infiltration of cells, resulting in the formation of granulomas or abscesses around the substance.

Vaccine reactions are characterized by an inflammatory process that may or may not be associated with the vaccine substance, which may vary depending on the type of vaccine. In a study using the Rabies vaccine in cats, granulomas which formed after vaccination showed Morin positive blue cells, suggesting that the same metal adjuvants identified in the vaccine stimulated inflammation in the skin (SCRUGGS; LEBLANCK, 2015). This means there may be a variation in the vaccine substrate due to the type of vaccine. In the present report, the vaccine substance was found inside and outside the cytoplasm of macrophages, as an amorphous and strongly basophilic granular material.

The inflammatory responses were classified as granulomatous or pyogranulomatous. This discrete difference may reflect the time of vaccination, since the material aspirated from within the pyogranulomatous areas was serous, similar to the administered content. However, there was no information about the vaccination practices in the present case. Cytological evaluation of vaccine reactions in pigs (WIEDMEYER et al., 2014) showed inflammation ranging from granulomatous or lymphocytic, occasionally with hemorrhage, to mixed or abscessed tissue, depending on the cellularity of the nodule. This highlights the wide variation in the inflammatory response that can be observed in vaccine reactions, such as was seen in the cow in this work.

In this study, cytology was imperative for identifying vaccine-associated nodules without performing a biopsy so as to avoid further tissue reaction at the site of administration. In this context, evaluation of cellularity and lesion extent are important in the identification of the reaction process as well as differentiating cutaneous lesions in cattle, and in this case, the hygienic conditions of vaccine management. Although fine needle aspiration is rarely used in production animals, it is believed that the cytological examination should be more widely used in order to define the morphological pattern of

lesions (WIEDMEYER et al., 2014), without the use of invasive techniques.

Tick infestations are common in bovines and are closely linked to large economic losses due to damage to the leather and poor weight gain (GASHAW; MERSHA, 2013), as well as participation in the epidemiological chain of Cattle Tick Fever. The Ixodidae family is the most significant species in domestic animals, and host skin damage results from the transfixation of chelicerae and the action of salivary secretions of these parasites (MAULDIN; PETERS-KENNEDY, 2016). In this case, the presence of ticks was usually found in association with lesions. The presence of ectoparasites was diffuse, but most pronounced along the medial aspect of the limbs, tail and back of the animal. In areas where the parasite was not found, the skin was erythematous, alopecic, tumefied and mildly ulcerated.

The pattern observed in this work is consistent with previous literature, which characterizes cutaneous lesions secondary to tick bites including papules, rounded or nodular areas that may have crusts, erosions, ulcerations and alopecia. The lesions are especially common on the ears, head, neck, armpit, groin, and legs.

Pain and pruritus are quite variable with tick bites (MAULDIN; PETERS-KENNEDY, 2016). In this case, no pruritus was reported, but discomfort was observed during the clinical evaluation, especially in areas of the lower limb. It is important to highlight that wounds are a gateway for secondary bacterial infections as well as myiasis (GASHAW; MERSHA, 2013).

The histopathological findings in the skin reflect the continuous exposure to anticoagulant, anti-inflammatory factors, and complement inhibitors, among other substances present in the saliva of these parasites. Epidermal hyperplasia, necrosis, folliculitis, furunculosis, as well as eosinophilic or neutrophilic perivascular and dermal inflammation are described. There can also be collagen degeneration and occasional lymphocyte infiltration in addition to the formation of granulomas (MAULDIN; PETERS-KENNEDY, 2016).

In this case, there was a predominance of intradermal, perivascular, and perifollicular eosinophils, a cellularity commonly seen in parasitic infections. In addition, moderate acanthosis and hyperkeratosis were found, forming papillary projections in the epithelium. Cytological harvesting by scarification was performed in this region, but may not have been the best diagnostic choice since it captured keratin alone, which comprises only one of the findings of parasite reaction.

Conclusion

The use of different techniques was fundamental for the examination and diagnosis of cutaneous lesions in this bovine. Peripheral nerve sheath tumor, post-vaccine granulomas and eosinophilic dermatitis are important dermatopathies that can be found in cattle and should be inserted as a differential diagnoses of cutaneous neoplasms and idiopathic dermatitis for the species. Cytological examination may be useful in the diagnosis of these conditions, but when the cytology does not allow for conclusive interpretation, histopathology, and occasionally immunohistochemistry, should be performed for an accurate diagnosis of the lesion.

References

AHMADI, N.; ORYAN, A.; GHANE, M.; DANESHBOD, Y. Cutaneous schwannoma in a cow. *Brazilian Journal Veterinary Pathology,* São Paulo, v. 5, n. 2, p. 81-85, 2012.

BABOVIC-VUKSANOVIC, D.; PETROVIC, L.; KNUDSEN, B. E.; PLUMMER, T. B.; PARISI, J. E.; BABOVIC, S.; PLATT, J. L. Survival of human neurofibroma in immunodeficient mice and initial results of therapy with pirfenidone. *Journal of Biomedicine and Biotechnology,* Bethesda, v. 2004, n. 2, p. 79-85, 2004. DOI: 10.1155 / S1110724304308107

CANFIELD, P. The ultrastructure of bovine peripheral nerves heath tumours. *Veterinary Pathology,* California, v. 15, ed. 3, p. 292-300, 1978. DOI: 10.1177/030098587801500303

GASHAW, B. A.; MERSHA, C. K. Pathology of tick bite lesions in naturally infested skin and hides of ruminants: A Review. *Acta Parasitologica Globalis,* Dubai, v. 4, n. 2, p. 59-63, 2013. DOI: 10.20372/1547201119.01

LUCENA, R. B.; RISSI, D. R.; KOMMERS, G. D.; PIEREZAN, F.; OLIVEIRA-FILHO, J. C.; MACÊDO, J. T.; FLORES, M. T.; BARROS, C. S. A retrospective study of 586 tumours in Brazilian cattle. *Journal of Comparative Pathology,* England, v. 145, n. 1, p. 20-24, 2011. DOI: 10.1016 / j.jcpa.2010.11.002

SCHÖNIGER, S.; SUMMERS, B. A. Localized, plexiform, diffuse, and other variants of neurofibroma in 12 dogs, 2 horses, and a chicken. *Journal Veterinary Pathology,* São Paulo, v. 46, n. 5, p. 904-915, 2009. DOI: 10.1354 / vp.08-VP-0322-S-FL

SCRUGGS, J. L.; LEBLANC, C. J. Identification of blue staining vaccine-derived material in inflammatory lesions using cultured canine macrophages. *Veterinary Clinical Pathology,* New Jersey, v. 44, n. 1, p. 152-156, 2015. DOI: 10.1111 / vcp.12228

SPICKLER, A. R.; ROTH, J. A. Adjuvants in veterinary vaccines: modes of action and adverse effects. *Journal of Veterinary Internal Medicine,* United States, v. 17, n. 3, p. 273-281, 2003. DOI: 10.1111 / j.1939-1676.2003.tb02448.x

MAULDIN, E. A.; PETERS-KENNEDY, J. Integumentary system. In: MAXIE, M. G. (Ed.). *Jubb, Kennedy, and Palmer's pathology of domestic animals*. 6. ed. St. Louis: Elsevier, 2016. p. 511-580.

WHITE, S. D.; EVANS, A. G. Alterações na pele. In: SMITH, B. P. *Medicina interna de grandes animais.* 3. ed. São Paulo: Manole, 2006. p. 182-206.

WIEDMEYER, C. E.; FANGMAN, T. J.; SCHWARTZ, K.; PAYNE, B. Fine-needle aspiration and cytology as an antemortem method for evaluating in jection-site lesions. *Journal Swine Health and Production*, Iowa, v. 22, n. 4, p. 244-247, 2014.