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Case Report

Cutaneous Schwannoma in a Cow

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Abstract

Schwannoma is a peripheral nerve sheath tumor (PNST), commonly found as a spindle cell tumor of autonomic nerves and rarely involving the skin of cattle. The present report describes the histopathology and immunohistochemistry features of a localized (solitary) benign PNST with final diagnosis of cutaneous schwannoma in a 4-year-old female Holstein bovine. The dome-shaped, well circumscribed, firm, non-smooth surfaced mass was composed of spindle-shaped cells arranged predominantly in interlacing fascicles or streams with a moderate to strong intervening collagenous stroma. Histopathologic changes included typical hypocellular areas with pale scant eosinophilic cytoplasm (Antoni B pattern) similar to myxomatous tissue, and hypercellular areas with deeply eosinophilic cytoplasm (non-typical Antoni A pattern) without nuclear palisading or Verocay bodies formation. Immunohistochemical reactions for S-100 protein, a Schwann cell marker, and vimentin were strong in the neoplastic cells. Other markers as desmin, neuron specific enolase (NSE), CD34, and p53 were all negative. It was concluded that concurrent evaluation of both histological and immunohistochemical features are required for the final diagnosis of schwannomas in domestic animals.

Key Words: Schwannoma, histopathology, immunohistochemistry (IHC), skin, cattle

Introduction

Benign tumors arising from the peripheral nerve sheath include schwannomas (neurilemmoma) and neurofibromas (10). The only cell of origin in schwannomas is the Schwann cell, whereas neurofibromas are composed of a mixture of Schwann cells, perineural cells and endoneural fibroblasts (14, 24). In humans, schwannomas display Antoni type A or B pattern and Verocay bodies. Malignant forms of this tumor, classified as malignant peripheral nerve sheath tumors (MPNST) rarely present those patterns and may be difficult to diagnose in the absence of complementary exams (4, 11).

In recent years most of the authors in veterinary medicine and veterinary pathology have indicated the term "benign peripheral nerve sheath tumors" (BPNSTs) instead of schwannomas and neurofibromas because of their similar clinical behavior. Yet, it is necessary to establish distinct histological criteria for the classification of these tumors (8, 10) since the veterinary literature is somewhat inconsistent and confusing in classifying these neoplasms (22). Additionally, unlike in humans, the existence of true neurofibromas in domestic animals is questionable (11).

There is a subset of these tumors that arise from small peripheral nerves of the skin and subcutis (7). Spindle cell tumors of the skin are common in dogs and cats, sporadic in horses, and uncommon to rare in other domestic species (13). In cattle, schwannomas occur as a spindle cell tumor and are usually found in autonomic nerves including the epicardial plexus, the mediastinal nerve plexus, thoracic and cervical sympathetic ganglia

(11) and rarely involve the skin (13, 28). To our knowledge there is only one published report as yet of a cutaneous schwannoma in cattle in the veterinary literature (21). The present report describes the histopathological and immunohistochemical features of a solitary BPNST with final diagnosis of schwannoma in the skin of a cow.

Case report

A 4-year-old female Holstein bovine was referred with a raised nodular lesion at the medial aspect of the right hock joint. On physical examination the vital signs as pulse rate, respiratory rate and rectal temperature were normal. The packed cell volume (PCV) was 34% and other hematological reference values such as RBC, WBC and the percentage of cell blood count were within normal ranges. Further clinical examination revealed no significant abnormalities in other locations. Investigation for enzootic bovine leukosis by ELISA was negative. Complete excision of the tumor was curative and no evidence of recurrence was detected within the last18 months.

Grossly the mass (10 mm x 8 mm in size) was dome-shaped, well circumscribed, firm, non-smooth surfaced, red to pink, and alopecic without any pigmentation, ulceration or hemorrhages (Fig. 1). Surgical resection was carried out under local anesthesia. One cm thick slices of the tissue were fixed in 10% neutral buffered formalin (pH=7.3), processed routinely and embedded in paraffin wax. Four µm thick tissue sections were stained with hematoxylin and eosin and Masson´s trichrome stains. Additional 4 µm sections mounted on poly-L lysine-coated slides were deparaffinized and hydrated before antigen retrieval in a microwaves oven with citrate buffer (pH 6.0) for IHC procedures. Briefly, endogenous peroxidase activity was blocked for 5 minutes with 3% hydrogen peroxide (Novocastra Lab. Newcastle, UK). All ready-to-use primary monoclonal anti-human antibodies such as S-100 protein (S1/61/69), vimentin (V9), desmin (DE-R-11), neuron specific enolase (NSE; 5E2), CD34 (QBEND/10) and p53 (IMX25) were provided from Novocastra Laboratories, Newcastle, UK, and used according to the manufacturer´s instructions. The sections were exposed to each of the above antibodies for 30-60 minutes with 3.3´diaminobenzidine (DAB) as the chromogen, and the sections were counterstained with Harris´s hematoxylin. For the negative controls, the primary antibody was replaced by non-immune serum. Appropriate external and internal controls were also used.

Microscopically the mass revealed a non-encapsulated tumor extending from the superficial dermis into the deep dermis and subcutaneous tissue with areas of varying cellularity (Fig. 2). The mass was composed of spindle-shaped cells characterized by oval, spindle-shaped or wavy nuclei. The neoplastic cells were arranged predominantly as interlacing fascicles or streams and contained a moderate to strong intervening collagenous stroma. In addition, the neoplasm revealed typical hypocellular areas with scant pale eosinophilic cytoplasm (Antony B pattern) similar to myxomatous tissue (Fig. 3) and hypercellular areas with deeply eosinofilic cytoplasm (non-typical Antony A pattern) without nuclear palisading or Verocay bodies formation. No mitotic activity, cellular pleomorphism, necrosis or nerve fibers were identified. The histological components of the epidermis looked normal whereas dermal adnexa were not detected within the tumor mass. Masson´s trichrome staining demonstrated small to moderate amounts of collagen fibers among the neoplastic cells forming an irregular, loose stroma. Immunostaining disclosed a strong and diffuse reactivity for S-100 protein as a Schwann cell marker (Fig. 4), and vimentin was observed within the neoplastic cells cytoplasm. All other markers were negative. Based on the histopathology and IHC results the neoplasm was diagnosed as bovine cutaneous schwannoma.

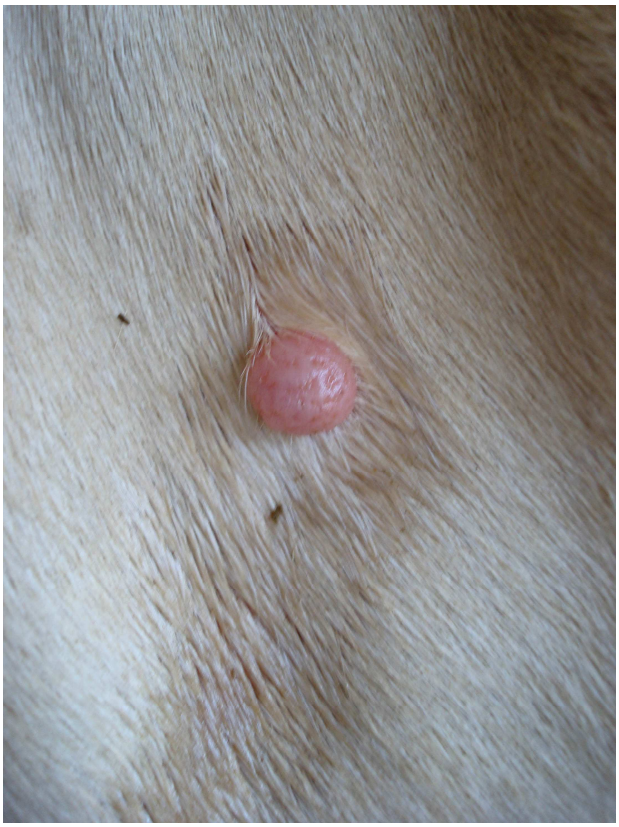

Figure 1. Schwannoma, skin, in the medial aspect of the right hock joint of a cow. The neoplasm has a red to pink and dome-shaped protruding appearance. It is well circumscribed with a non-smooth and alopecic surface and has no specific pigmentation, ulceration or hemorrhages.

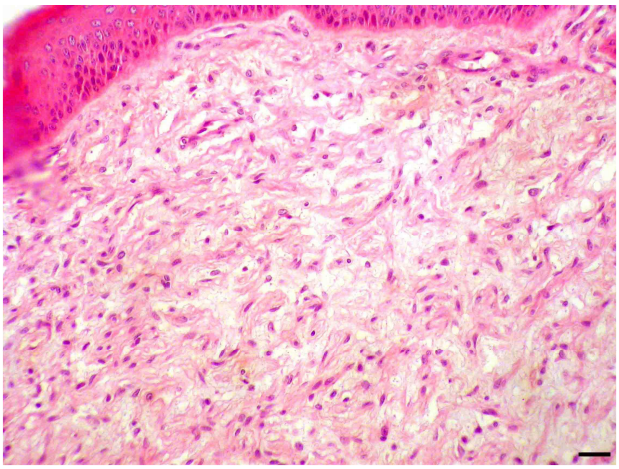

Figure 2. Schwannoma, skin, bovine. Microscopic examination of the mass revealed a non-encapsulated tumor composed of spindle-shaped cells characterized by oval, spindle-shape, or wavy nuclei predominantly arranged as interlacing fascicles or streams and containing a moderate to strong intervening collagenous stroma (HE, scale bar=55 µm).

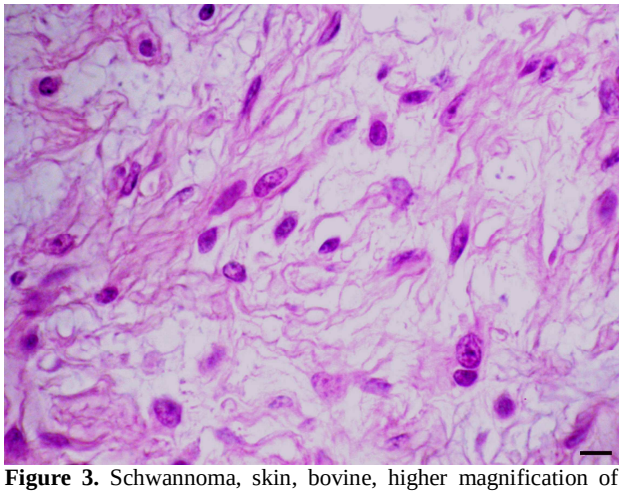

Figure 3. Schwannoma, skin, bovine, higher magnification of Fig. 2. The neoplastic mass shows typical hypocellular and myxoid areas with poorly eosinophilic cytoplasm (Antoni B pattern) (HE, scale bar=14 µm).

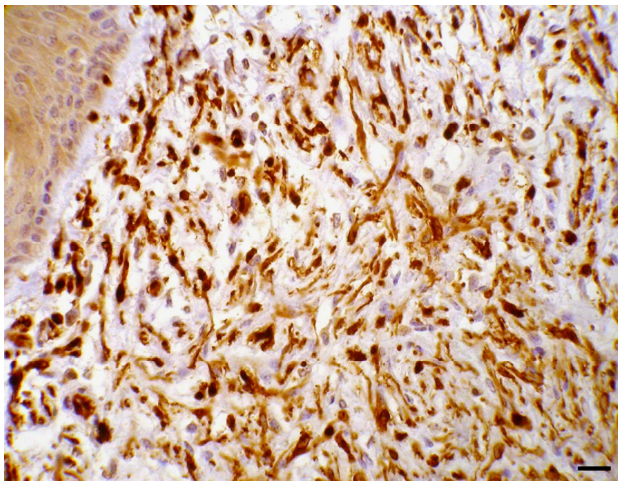

Figure 4. Schwannoma, skin, bovine. The neoplastic cells of the mass express a strong and diffuse reactivity for S-100 protein as a Schwann cell marker. (IHC, scale bar=14µm).

Discussion

Schwannomas and neurofibromas, as the most common BPNSTs, are derived from nerve sheath cells of the peripheral nerve such as Schwann cells, perineural cells and endoneural fibroblasts in varied composition and proportion (5, 25). Some authors believe that the differentiation between these tumors can be made solely based on certain histopathological features supported by other ancillary tests such as IHC and electron microscopy (7, 24). Some of the previous studies on the classification of PNSTs have shown that no clear distinction can be made between schwannomas and neurofibromas in animals due to unreliable IHC markers (10, 28). Yet, the use of these markers alone or in combination may be inadequate because of the considerable cytomorphologic and IHC overlap between them and the standard light microscopy (6, 24).

The tumor described herein was diagnosed as cutaneous schwannoma based on the morphologic features revealed by the routine histological staining (HE) and was further supported by IHC markers. Histopathologically, the features of schwannoma vary within and among the tumors based on the presence or absence of anaplastic changes and the extent of hemorrhage, degeneration, fibrosis and mineralization (13). In human medicine, schwannomas are composed of two patterns including Antoni A and Antoni B. The classic Antoni A arrangements displays an organized and highly cellular area in the collagenous stroma (6, 24). Other features are nuclear palisading, formation of Verocay bodies, and hyalinized microvessels. Nerve fibers are absent within the tumor but are often present at the tumor margin (7, 20, 24). The present neoplasm revealed non-typical Antoni A pattern without nuclear palisading or Verocay bodies formation. It has been stated that nuclear palisading is not always seen in schwannomas and due to the lack of nuclear palisading some of this type of schwannomas are potentially difficult to be separated from cellular neurofibromas (6). Unlike in humans, it appears that this microscopic pattern with Verocay bodies as the hallmark of schwannomas could be rare in domestic animals (7). However, lack of some histopathologic features of schwannoma in the HE stained sections of the present tumor and the definite diagnosis by IHC confirms the rarity of these features in tumors of domestic animals.

Antoni B pattern was predominant in the HE stained sections of the present tumor. Microscopically, this pattern of schwannomas is characterized by a loose, myxomatous and poorly cellular area that may be predominant in some sections (13). Schwannomas consisting exclusively of Antoni B areas might mimic the histologic appearance of neurofibromas (6, 9). The microscopical features of neurofibromas include spindled-

shaped and elongated neoplastic Schwann cells admixed with perineural-like cells and endoneural fibroblasts with numerous small nerve fibers (28). Characteristic buckled and/or tapering wavy nuclei are found in a fibromyxoid or thin and wire-like collagenous stroma (14, 20, 24, 30). Nielsen *et al*. (16) reported a case series of peripheral nerve sheaths hybrid tumor which had histological and IHC features of neurofibroma and schwannoma. The ultrastructural studies have shown that schwannomas are mainly composed of Schwann cells (5). However, application of complementary diagnostic aids such as transmission electron microscopy, reliable IHC markers and special staining methods can be somewhat beneficial for tumor differentiation in these cases.

Unlike bovine neurofibromas, schwannomas are true neoplasms (1). Tanimoto and Ohtsuki (26) reported a solitary schwannoma in the submucosa of the cecum of a Holstein-Friesian cow with unusual morphologic features. A plexiform schwannoma in the subcutis of a 6-month-old pig has also been reported by Tanimoto and Ohtsuki (27). Currently, Schoniger *et al*. (23) reported cutaneous schwannoma in 22 horses that had microscopic features like those of human schwannoma and had benign clinical behavior. In a previous report, Sasani and Bazargani (21) diagnosed multiple subcutaneous nodules as a cutaneous schwannoma in a 9-year-old Holstein-Friesian cross cow. However, there have been a limited number of case reports describing cutaneous BPNSTs in the veterinary literature (25), especially bovine schwannoma.

The histopathological features of the present case were supported by a diffuse strong immunoreactivity to S-100 protein. S-100 protein immunoreactivity is restricted to a subpopulation of the tumor cells, because this marker only labels the neoplastic Schwann cells but not the remaining cells (14, 20, 24, 30). IHC findings on formalin-fixed, wax-embedded bovine tissue samples by Stoica *et al*. (25) showed that schwannomas expressed both vimentin and S-100 with varying labeling intensities. By IHC, PNSTs are generally positive for S-100 protein and vimentin (10). Therefore, the diffusely positive S-100 protein and vimentin immunolabeling, together with the negative results of desmin and CD34 markers confirmed the present neoplasm as a cutaneous schwannoma.

In the present case, CD34, NSE and p53 were negative. Although CD34 or the human hematopoietic progenitor cell antigen is known to be expressed on vascular tumors, it is also a marker of nerve sheath cells. It is now believed that the nature of CD34-positive cells corresponds to that of endoneural fibroblasts (3, 9). The results obtained by Park *et al*. (19) revealed that CD34 might be a useful sensitive marker for neurofibromas rather than schwannomas. Unlike neurofibromas, schwannomas demonstrate Schwann cells as the only cell of origin without significant subpopulation of endoneural fibroblasts. Therefore, CD34 could be a useful marker for the differential diagnosis of the two tumors. For this reason, the CD34 negativity in the present tumor strongly confirmed the definitive diagnosis of schwannoma. NSE marker, as a glycolytic isoenzyme, can be used to identify peripheral nerves, neural and neuroendocrine tumors and non-neuronal tumors (15) but the results obtained by some authors revealed negativity for this marker in bovine schwannomas (12, 15, 18). The p53-negative cells within the present neoplastic mass confirmed a low mitotic rate and hence the benign nature of the so far described bovine schwannomas.

Tumors originated from Schwann cells, smooth muscle cells, fibroblasts, or pericytes may have similar microscopical features and, therefore, are often difficult to classify precisely on the basis of morphologic findings alone (26). Histologically, schwannomas of the skin should also be differentiated from other spindle cell tumors such as neurofibroma, fibroma and hemangiopericytoma (28). Despite some inconsistency, the Holmes's silver stain for nerve fibers has been shown to be especially useful and beneficial for histological classification of PNSTs and differentiation between neurofibromas and schwannomas because the schwannomas have few if any axons while the neurofibromatous tissue contains numerous axons (1). S100 protein labeling is irregular in neurofibromas and it is also restricted only to areas where nerve fibers are present (22). In the present case, absence of nerve fibers in the consecutive HE stained sections facilitated the final diagnosis of cutaneous schwannoma.

Other differential diagnosis of soft tissue tumors with myxoid stroma could include fibrosarcoma, myxosarcoma, embryonal rhabdomyosarcoma and myxoid liposarcoma. All these tumors similar to the PNSTs are vimentin-positive and can also express S-100 antigen with the exception of fibrosarcoma and myxosarcoma (2, 8). Histologically, presence of cytoplasmic lipid vacuoles shows an adipocytic origin (8) that was not found in the present tumor. Absence of desmin expression and sarcomere-like structures excluded a muscular origin as well. Lack of p53 positivity further ruled out the malignant nature of the present neoplasm. S100 protein marker is widely used for opportune diagnosis of melanocytic neoplasms. In humans, it is demonstrated that this antibody is expressed in almost primary and metastatic malignant melanoma including amelanotic and spindle-shaped variants (17, 29). However, it is necessary to make a differential diagnosis with amelanotic melanomas, which also may have a spindle cell appearance.

Conclusion

The present study revealed that establishing a diagnosis of schwannomas or neurofibromas by the routine histopathological staining method (HE) may be difficult and because of similar cytomorphologic features the definitive distinction between these tumors is still problematic. Application of IHC procedures with specific markers could be very useful and beneficial for diagnosing

bovine schwannomas, especially when classical morphological patterns are atypical. It seems that concurrent evaluation of both histopathological and IHC features is required for the final and definitive diagnosis of BPNSTs in domestic animals.

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