Malignant peripheral nerve sheath tumour in the nasopharynx of a cow

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Abstract: This case describes the fi ndings in a Swiss Braunvieh cow with a malignant peripheral nerve sheath tumour (MPNST) in the nasopharynx. The major clinical signs were mixed dyspnoea with inspiratory and expiratory noises. Radiographic views of the head revealed an irregular mass with soft-tissue density in the nasopharynx originating from the dorsal pharynx and occupying and restricting the pharyngeal cavity. Endoscopic examination showed a lobulated mass obstructing almost the entire lumen of the aboral nasal passages and nasopharynx. Postmortem examination revealed a lobulated mass in the choanae with a broad attachment to the dorsal pharynx and histologically a soft tissue sarcoma with tumour cells positive for the S-100 and p75NTR (neurotrophin receptor) proteins and negative for CNPase. Electron microscopic examination showed few structures that indicated that the tumour originated from Schwann cells. Der vorliegende Fall beschreibt die Befunde einer Kuh mit einem malignen peripheren Nervenscheidentumor im Nasopharynx. Das klinische Hauptsymptom war eine gemischte Dyspnoe mit inspiratorischen und exspiratorischen Stenosegeräuschen. Die röntgenologische Untersuchung des Kopfes stellte eine irregulär geformte weichteildichte Masse im Nasopharynx dar, die vom Pharynxdach ausging und das Lumen des Pharynx massiv einengte. Die Endoskopie bestätigte, dass eine lobulierte Masse das Lumen des Nasopharynx nahezu verschloss. Die Sektion zeigte makroskopisch eine lobulierte Masse, die bis in die Choanen reichte und grossfl ächig dem Pharynxdach entsprang. Histologisch handelte es sich um ein Weichteilsarkom mit mmunhistochemisch S-100 und p75NTR (Neurotrophin Rezeptor) positiven, aber CNPase negativen Tumorzellen. Eine elektronenmikroskopische Untersuchung konnte nur bei vereinzelten Zellen Strukturen finden, die auf Schwann-Zell Ursprung der entarteten Zellen hinwiesen.

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Summary

This case describes the findings in a Swiss Braunvieh cow with a malignant peripheral nerve sheath tumour (MPNST) in the nasopharynx. The major clinical signs were mixed dyspnoea with inspiratory and expiratory noises. Radiographic views of the head revealed an irregular mass with soft-tissue density in the nasopharynx originating in the dorsal pharynx and occupying and restricting the pharyngeal cavity. Endoscopic examination showed a lobulated mass obstructing almost the entire lumen of the aboral nasal passages and nasopharynx. Postmortem examination revealed a lobulated mass in the choanae with a broad attachment to the dorsal pharynx and histologically a soft tissue sarcoma with tumour cells positive for the S-100 and $p75^{NTR}$ (neurotrophin receptor) proteins and negative for CNPase. Electron microscopic examination showed only few structures that indicated that the tumour originated from Schwann cells.

Key words: bovine, tumour, nasopharynx, MPNST, immunohistochemistry

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Der vorliegende Fall beschreibt die Befunde einer Kuh mit einem malignen peripheren Nervenscheidentumor im Nasopharynx. Das klinische Hauptsymptom war eine gemischte Dyspnoe mit inspiratorischen und exspiratorischen Stenosegeräuschen. Die röntgenologische Untersuchung des Kopfes stellte eine irregulär geformte weichteildichte Masse im Nasopharynx dar, die vom Pharynxdach ausging und das Lumen des Pharynx massiv einengte. Die

Endoskopie bestätigte, dass eine lobulierte Masse das Lumen des Nasopharynx nahezu verschloss. Die Sektion zeigte makroskopisch eine lobulierte Masse, die bis in die Choanen reichte und grossflächig dem Pharynxdach entsprang. Histologisch handelte es sich um ein Weichteilsarkom mit immunhistochemisch S-100 und $p75^{NTR}$ (Neurotrophin Rezeptor) positiven, aber CNPase negativen Tumorzellen. Eine elektronenmikroskopische Untersuchung konnte nur bei vereinzelten Zellen Strukturen finden, die auf Schwann-Zell Ursprung der entarteten Zellen hinwiesen.

Schlüsselworte: Rind, Neoplasie, Nasopharynx, MPNST, Immunhistochemie

Introduction

Diseases involving the nasal cavity, conchae, ethmoid and nasopharynx are not uncommon in cattle. Aetiologies of these diseases include conditions that affect the nasal mucosa such as rhinitis, papillomatosis, actinobacillosis and granulomas (due to mycosis, parasites or allergy), foreign bodies, conchal cysts and tumours (Stöber, 2002; Radostits et al., 2007). Endemic ethmoidal tumours are malignant and among the most important tumours of the nasal cavity; they originate from the olfactory mucosa of the ethmoid and invade the nasal cavity and paranasal sinuses (Stöber, 2002; Caswell and Williams, 2007; Radostits et al., 2007). Becker and co-workers (1972) published a review of the German veterinary literature on nasal tumours in cattle from 1893 to 1973. This review included 21 case reports and a study of 20 ethmoidal tumours and was supplemented by the description of one case each of anaplastic carcinoma, osteoma, osteosarcoma and osteochondroma seen by the authors. Since then there have been only sporadic reports of bovine nasal tumours including squamous cell carcinoma (Pycock et al., 1984), osteosarcoma (Fischer and Roming, 1989), nasal chondrosarcoma (Beytut et al., 2006), lymphosarcoma (Crocker and Rings, 1998), malignant schwannoma (Mandrioli et al., 2005), liposarcoma (Shive et al., 2006) and osteoma (Wuersch et al., 2009). Dyspnoea and stertorous breathing sounds originating from the nasal cavity were clinical signs that were common to all the published cases. The

purpose of this report was to describe a cow with dyspnoea and stertor caused by a soft-tissue sarcoma, which could not be conclusively classified histologically. However, the results of immunohistochemical and electron microscopic examinations were consistent with the diagnosis of a poorly differentiated malignant peripheral nerve sheath tumour (MPNST).

History, clinical signs and laboratory findings

A four-year-old Swiss Braunvieh cow was referred to the Department of Farm Animals, University of Zurich, because of noisy breathing and dyspnoea, which was first noticed ten days previously. The cow weighed 550 kg, had a body condition score of 2/5 and was listless. The head and neck were kept in a stretched position and there was mixed dyspnoea with inspiratory and expiratory stenotic sounds and occasional mouth breathing. An abdominal breathing pattern was observed, and auscultation of the lungs revealed normal breath sounds. Skin turgor was reduced but the rectal temperature (38.8°C), the heart (76 beats per minute) and respiratory rates (24 breaths per minute) were normal. Ruminal and intestinal motility was normal and foreign body tests and simultaneous swinging and percussion auscultation were negative. Faecal analysis and urinalysis (Combur^{9}-Test, Roche, Basel) were normal. Abnormal laboratory findings included increased haematocrit (38%, normal range 30 to 33%), increased concentrations of total solids (102 g/litre, normal range 60 to 80 g/litre), fibrinogen (8 g/litre, normal range 3 to 5 g/litre) and calcium (3.02 mmol/litre, normal range 2.30 to 2.60 mmol/litre) and low-normal inorganic phosphorus concentration (1.38 mmol/litre, normal range 1.30 to 2.40 mmol/litre).

Radiographic, endoscopic and bioptic findings

Laterolateral radiographic views of the head and lungs revealed an irregular, space-occupying, soft-tissue density mass in the nasopharynx. The mass originated in the dorsal pharynx and caused severe restriction of the pharyngeal cavity (Fig. 1). Osteolysis was not seen. Endoscopic examination via the right nasal passage showed a lobulated mass with superficial necrotic areas in the nasopharynx. The lumen of the aboral nasal passages and nasopharynx was nearly completely obstructed by the mass (Fig. 2). The endoscope could not be advanced beyond this mass. Examination of a tissue sample of the mass obtained

using a biopsy instrument (FB-25K-1 round cup biopsy forceps, Olympus Switzerland, Volketswil) revealed chronic purulent inflammation.

Treatment, disease course and pathological findings

Although the information provided by biopsy specimens from the surface of a mass is of limited diagnostic value, the cow was treated with cefquinome (2.5 mg/kg s.c, once daily; Cobactan® 7.5 %, Veterinaria, Pfäffikon) for 8 days, flunixin (1.1 mg/kg i.v, once daily; Fluniximin®, Graeub AG, Bern) for 3 days and dexamethasone (0.03 mg/kg i.m.; Dexadreson®, Veterinaria) once. The cow also received 30 litres of NaCl-glucose solution i.v. over 3 days. Despite treatment, there was deterioration of the patient's condition and worsening of the dyspnoea, which necessitated euthanasia. Postmortem examination showed that the choanae contained a lobulated pliable mass, which was up to 10 cm thick and had a broad attachment to the dorsal pharynx (Fig. 3). The dorsal mucous membrane of the caudal third of the nasopharyngeal duct was thickened and contained multiple, small, nodular and confluent nodules. Histological examination of the neoplasm revealed a non-capsulated mass that infiltrated the nasopharyngeal mucosa and consisted of loosely arranged spindle-shaped to stellate cells (Fig. 4a). The neoplastic cells formed interwoven bundles, which were embedded in abundant extracellular mucinous matrix and supported by a delicate fibrovascular stroma. In some areas, the nuclei tended to be arranged in a palisading pattern. The nuclei were oval to spherical with small clumps of chromatin and one to several distinct nucleoli. There was distinct anisokaryosis and the mitotic rate was 1 to 2 cells per high-power (x400) field.

Immunohistochemistry, special stains and electron microscopy of the neoplastic tissue

Immunohistochemical examination of tissue sections stained with the mesenchymal cell marker vimentin (monoclonal mouse anti-vimentin; DakoCytomation, Code VIM 3B4) revealed strong staining of the ovoid, short spindle-shaped or stellate cytoplasm with occasional elongated cytoplasmic projections (Fig. 4b). The neoplastic cells did not react with anti-cytokeratin antibody (monoclonal mouse anti-human cytokeratin, DakoCytomation, Code M 0821), a marker for epithelial cells. Additional immunohistochemical studies

yielded the following findings: In the method used, the anti-S100 antibody (calcium-binding protein, predominantly associated with sustentacular cells of the central and peripheral nervous system; polyclonal rabbit anti-cow S100, DakoCytomation, Code N 1573) stained more than 50% of the cells including the nuclei (Fig. 4c). The anti- $p75^{NTR}$ receptor antibody (nerve growth factor receptor; monoclonal anti-mouse CD271, Mitenyi) stained almost 50% of cells (Fig. 4d). The anti-NSN antibody (neuron specific enolase; monoclonal mouse anti-human neuron specific enolase, DakoCytomation, Code M 0873) stained approximately one third of cells, and anti-glial fibrillary acidic protein (GFAP) antibodies (polyclonal rabbit anti-GFAP, DakoCytomation, Code N 1506) stained only sporadically tumour cells. Anti-actin antibody (monoclonal mouse anti-human α-smooth muscle actin; Clone 1 A4, Code N 1584, DakoCytomation) and anti-desmin antibody (monoclonal mouse anti-human Desmin, Clone D33, DakoCytomation) stained mostly fibrovascular stromal cells and only sporadic tumour cells. The anti-synaptophysin antibody (principal protein of synaptic vesicle p38; monoclonal mouse anti-synaptophysin, clone SY38, DakoCytomation, Code M 0776), anti-CNPase antibody (an enzyme almost exclusively limited to oligodendrocytes and Schwann cells (Reynoldes et al., 1989; Sprinkle, 1989); anti-CNPase, Millipore, Clone 11-5B) and anti-melan-a antibody (a transmembrane protein expressed in normal melanocytes and many melanomas; monoclonal mouse anti-human, clone A 103, DakoCytomation, Code N 1622) did not produce any staining.

The extracellular matrix stained weakly with alcian blue. Cryostat sections stained with oil red revealed that optically empty cytoplasmatic vacuoles seen in tumour cells did not contain fat.

Electron microscopic examinations were carried out on specimens cut from paraffin blocks using a small punch biopsy instrument. Tumour cells appeared as poorly differentiated, short spindle-shaped to polyhedral cells. In some sections, the cells were arranged in palisades and had a high nuclear-cytoplasmic ratio, a paucity of cell organelles and no predominant organelle type. These cells did not appear to be derived from Schwann cells (Fig. 5), but this is a common characteristic of malignant nerve sheath tumours (Dickersin, 1988). Only a few cells had thin interdigitating projections. Hints of basement membrane and narrow areas of cell-to-cell contact were seen only occasionally. Luse bodies were not

identified. There were few microfilaments, and no criteria to indicate that the tumour cells were derived from fibroblasts, chondroblasts, osteoblasts, synovial cells, fat cells, smooth or skeletal muscle cells or endothelial cells. Embedment of tumour cells in a mucoid extracellular matrix was readily apparent via electron microscopy.

Diagnosis

The following findings strongly suggested that some of the tumour cells were derived from Schwann cells: a strong positive reaction with anti-S100 and anti-$p75^{NTR}$ receptor antibodies, long interdigitating projections seen in a few cells, a moderate number of organelles and absence of a dominant organelle. Anisokaryosis, poor cell differentiation, invasive growth and the large size of the tumour indicated malignancy. Taken together, these findings suggested a diagnosis of malignant peripheral nerve sheath tumour (MPNST) arising from the dorsal pharynx. There were no lesions in other organs and no metastases in the regional lymph node examined.

Discussion

The main clinical signs in our patient were dyspnoea, open-mouth breathing and inspiratory and exspiratory stenotic breathing noises. A clinical diagnosis of upper respiratory tract disease caused by a large mass in the nasopharynx was made using radiography and endoscopy. A pharyngeal injury caused by oral calcium administration two months previously could not be ruled out (Braun et al., 2004). However, clinical signs associated with this type of injury are usually more severe and occur immediately after bolus application. The differential diagnosis of a nasopharyngeal mass should include other chronic inflammatory processes and neoplasia. Biopsy did not provide a definitive diagnosis because only superficial inflamed tissue was collected and not the deeper neoplastic tissue.

With the exception of endemic ethmoid carcinoma in cattle (Pospischil et al. 1979), case reports of bovine nasopharyngeal tumours are rare. To our knowledge, the present case is only the second report of bovine peripheral nerve sheath tumour (PNST) in the nasopharynx; a malignant nasopharyngeal schwannoma in a cow

with similar clinical and gross pathological signs was described by Mandrioli et al. (2005). The nasopharyngeal location was not mentioned in a review of PNST in cattle (Stöber 2002), however these tumours are occasionally seen, particularly along the sympathetic trunk, during meat inspection. Most of these tumours do not cause clinical signs (Summers et al., 1995), although a study from Argentina reported a cluster of cases of malignant schwannoma from 1998 to 2001 in cattle; clinical signs included ataxia, paresis and paralysis (Murcia et al. 2008). The tumours were associated with nerve roots and had histological and electron microscopic features typical of Schwann cell tumours. Electron microscopy revealed virus particles, which had the morphological features of retroviruses. The nasopharyngeal malignant schwannoma described by Mandrioli et al. (2005) was characterised by the histological arrangement of spindle-shaped tumour cells in Antoni type A and B patterns, positive immunoreactivity for S-100 protein and typical electron microscopic features for Schwann cells such as intact basement membrane, paucity of cell organelles and intercellular contact sites. With the exception of immunoreactivity for S-100 protein, the neoplasm described in this report did not have the histomorphological characteristics typical of a tumour derived from Schwann cells, and the electron microscopic characteristics were only vaguely similar to Schwann cells. Virus particles were not seen in neoplastic cells.

Tumours of the peripheral nervous system (PNST) are generally rare in animals. The nomenclature relating to PNST in animals has been adopted from human medicine and is confusing. Schwannomas (synonyms: neurilemmomas, neurinomas) are Schwann cell derived tumours, which are usually solitary, benign and encapsulated and have cells arranged in Antoni type A and Antoni type B pattern. Another histomorphological pattern is the Verocay body formation. In Antoni type A tissue, the fusiform cells are arranged in bundles or parallel to one another and the spindle-shaped nuclei often have a palisading pattern. Antoni type B tissue consists of fewer cells, which are more loosely arranged and have small dark nuclei. Malignant tumours are referred to as malignant schwannomas (Summers et al., 1995). Neurofibromas (poorly differentiated neurofibromas are referred to as neurofibrosarcomas) are also Schwann cell derived tumours but contain other nerve sheath cell types, such as endoneurial fibroblasts and probably perineurial cells, and are therefore mixed tumours (Summers et al.,

1995). In humans neurofibromatosis type 1 (also known as Morbus von Recklinghausen) is a genetic disorder.

In dogs, many of the PNST's are malignant and poorly differentiated and therefore the original cell type is difficult to identify. In such cases, the term malignant peripheral nerve sheath tumour (MPNST) is preferred (Summers et al., 1995). The World Health Organisation (WHO) lists benign and malignant forms under the heading of peripheral nerve sheath tumour. However, they also mention that no general agreement has been reached in veterinary medicine with respect to definition and differentiation from other soft tissue sarcomas, and that these tumours are not well defined immunohistochemically (Koestner et al., 1999).

Malignant (M)PNST's are spindle cell sarcomas that are poorly defined histomorphologically and difficult to differentiate from fibrosarcoma, anaplastic sarcoma, haemangiopericytoma and other sarcomas. Immunohistochemical and/or electron microscopic examinations are needed to confirm that some of the tumour cells are derived from Schwann cells (Hirose et al., 1992). The S-100 protein is the most commonly used marker in the differential diagnosis of schwannoma and neurofibroma, although its occurrence is not limited to Schwann cells (Nielsen et al., 2011). The protein is found in various cells of neuroectodermal origin. CNPase was found to be a useful marker for Schwann cells in bovine PNST's too (Nielsen et al., 2011). In human medicine, the $75p^{NTR}$ protein is also used as a marker for Schwann cells and for MPNST's, but also for various other non-neural mesenchymal tumours (Fanburrg-Smith and Mietinnen, 2001). Despite the lack of complete specificity, the $p75^{NTR}$ protein was among the markers used for the immunohistochemical diagnosis of schwannomas in the rectum and colon of human patients; all of 20 S-100-positive tumours were also positive for $p75^{RT}$ (Mietinnen et al., 2001). Taken together, the positivity of more than 50% of tumour cells for the S-100 protein and almost 50% of cells for the $p75^{NTR}$ protein strongly suggest that Schwann cell-derived cells constituted the bulk of the neoplasm described in this report, despite the CNPase-negative reaction of the cells. Furthermore, electron microscopic findings did not contradict the tumour characterisation.

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Legend to figures

Figure 1: Laterolateral radiographic view of the head of a cow with a large irregular soft-tissue sarcoma obstructing most of the nasopharynx. Rostral is to the left. 1 dorsal pharynx, 2 tumour, 3 majority of nasopharynx is obstructed.

Figure 2: Endoscopic view of the left nasal passage of a cow with a lobulated soft-tissue sarcoma obstructing almost the entire lumen of the aboral nasal passages and nasopharynx.

Figure 3: Postmortem examination of the nasopharynx of a cow with soft-tissue sarcoma; sagittal section in the median. The heterogeneous lobulated tumour (1) obstructs the entire nasopharynx and infiltrated extensively the nasopharyngeal mucous membrane (2).

Figure 4 a - d:

a) Histology of the soft-tissue sarcoma in the nasopharynx of a cow. The neoplasm consists of loosely arranged fusiform to stellate cells that form interwoven bundles embedded in abundant extracellular mucinous matrix. The cell bundles are supported by a delicate fibrovascular stroma and in some areas the nuclei tend to have a palisading pattern. Nuclei are oval to spherical with small clumps of chromatin and one to several distinct nucleoli. There is distinct anisokaryosis (H&E, 20x objective).

b) Immunohistochemical staining for vimentin. The strongly positive staining tumour cells are fusiform, ovoid or stellate and some have elongated cellular projections (20x objective).

c) Immunohistochemical staining for S-100. There is mild to strong staining of the cytoplasm and nucleus of more than 50% of the tumour cells (40x objective).

d) Immunohistochemical staining for p75NTR (‘nerve growth factor receptor’, CD 271). Slightly fewer than 50% of the tumour cells are stained. There is light staining of the cytoplasm, strong staining of the cell membranes and no nuclear staining in positive cells (40x objective).

Figure 5: Electron micrograph of a nasopharyngeal soft-tissue sarcoma from a cow. The short fusiform to polyhedral tumour cells are poorly differentiated and in some areas have a palisading pattern and a high nuclear-cytoplasmic ratio. Some cells have elongated, narrow and interdigitating cytoplasmic projections (*) shown in A2 (enlarged area from A1) and B2 (enlarged area from B1). There is a paucity of cell organelles. There were a few isolated tight cell-to-cell contacts (not shown).

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