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Case Report

Concurrent thoracic mesothelioma and thyroid C-cell adenoma with amyloid deposition in an aged horse

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Abstract

A 21-year-old American Saddlebred mare died with a history of weight loss and breathing difficulties of 1 month duration. Post-mortem examination revealed a copious pleural effusion with multifocal to coalescing numerous white to grey nodular masses on the serosal surface of the pericardium, lungs and thoracic cavity. In addition, the left thyroid gland was markedly enlarged. A thoracic mesothelioma and C-cell adenoma with amyloid deposits of the left thyroid gland were diagnosed by histopathology and confirmed by immunohistochemistry employing antibodies against cytokeratin (CK), vimentin and calcitonin. Amyloid deposits in the thyroid tumour were confirmed by Congo red staining with apple-green birefringence under polarized light. Mesothelioma remains an uncommon neoplasm encountered in aged horses. Discussion includes the diagnostic challenge of differentiating carcinomatosis from mesothelioma by histology and differentiating reactive and neoplastic mesothelial cells by cytology.

Keywords: Aged horse, Amyloid, C-cell adenoma, Equine, Mesothelioma, Thyroid adenoma.

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Introduction

In equine medicine, neoplasia occurs less frequently than in cats and dogs. In a necropsy survey in horses from the University of Kentucky Veterinary Diagnostic Laboratory, neoplasia occurred in 8% of the 15–19 year-old horses, increasing up to 17% in adults, age 30 and older (Williams 2000). The most frequently diagnosed tumours in aged horses are pituitary adenoma, melanoma, squamous cell carcinoma, thyroid adenoma, abdominal lipoma and lymphosarcoma (Junge *et al.* 1984; Sweeney & Gillette 1989; Mair & Brown 1993; Edwards & Proudman 1994; Williams 2000; Freeman & Schaeffer 2001; Brosnahan & Paradis 2003; Ueki *et al.* 2004; Garcia-Seco *et al.* 2005).

Multiple neoplasms in an individual horse are rare except for multiple endocrine tumours which are periodically encountered during post-mortem examination involving hyperplasia and neoplasia in the thyroid, pituitary and adrenal glands, bearing similarity with the human multiple endocrine neoplasia (MEN) syndrome (De Cock & MacLachlan 1999). Independent of the equine variant of the MEN syndrome described above, a single equine case of concurrent malignant neoplasms has been reported consisting of a mediastinal squamous cell carcinoma and a mixed compact cellular and follicular thyroid carcinoma (Hovda *et al.* 1990). Herein, a case of multiple neoplasms, not part of the equine variant of the MEN syndrome is reported, specifically the macroscopic, histological and immunohistochemical attributes of a concurrent mesothelioma in the pleural cavity and a thyroid C-cell adenoma with amyloid deposits in an aged horse.

Case history

A 21-year-old American Saddlebred mare was found dead with a history of decreased food intake, weight loss, breathing difficulties and fatigue of 1 month duration. The horse had been initially diagnosed with

recurrent airway obstruction ('heaves') and treated by oral corticosteroid administration. The daily diet consisted of commercial senior feed and hay. The vaccination status was up-to-date. The horse had contact with other horses and an alpaca at the farm.

Necropsy findings

On November 2016, the body of a 546 kg, 21-year-old American Saddlebred mare was brought to the Veterinary Medical Diagnostic Laboratory, University of Missouri (Columbia, MO, USA) for post-mortem examination. There was approximately 10 litres of yellow tinged serosanguineous fluid in the thoracic cavity. The serosal surfaces of the pericardium, lung lobes and the rib cage were covered by numerous multifocal to coalescing, white-grey nodular masses up to 2 cm in diameter, often arranged in grape-like clusters (Fig. 1). The serosal surface of the lung was thickened. The cardiac musculature was grossly normal in appearance. The left thyroid gland was enlarged and measured 7.0 × 5.5 × 5.0 cm. The left thyroid gland was replaced by a well-demarcated firm white mass with compression of the residual thyroid parenchyma against the capsule (Fig. 1). The right thyroid was grossly normal and measured 4.0 × 3.0 × 3.0 cm. No gross lesions were noted in the pituitary or adrenal glands. The most significant gross pathological findings were extensive neoplastic masses in the thoracic cavity and pleural effusion. At this point, the differential diagnosis considered for the thoracic masses included mesothelioma and carcinomatosis. The thyroid mass suggested the presence of a thyroid follicular adenoma or C-cell adenoma.

Histopathological findings

Representative sections of thoracic masses

The serosal surface was infiltrated and replaced by an extensive proliferation of polygonal cells forming nests, cords and ducts of variable size and shape without encapsulation (Fig. 2**)**. Neoplastic cells had indistinct cell borders, moderate amounts of cytoplasm, oval nuclei with stippled chromatin and variably distinct nucleoli. Moderate anisocytosis and anisokaryosis were noted. Multinucleated cells were few in number. The number of mitotic figures was 1 to 2 per ten high power (400X) fields. The serosal surface of the sections examined was lined by attenuated or plump mesothelial cells often multilayered and occasionally interspersed with neoplastic cells. Immunohistochemistry analyses employing monoclonal antibodies against pan-cytokeratin and vimentin revealed that neoplastic cells strongly expressed pan-cytokeratin (epithelial cell marker) and vimentin (mesenchymal cell marker) (Fig. 3). The positive controls for pan-cytokeratin and vimentin were canine small intestinal sections. The epithelial cells and myofibroblasts stained positively for pan-cytokeratin and vimentin, respectively. Negative controls were the first or second antibody alone and the background staining was minimal.

Representative sections of the left thyroid gland

The left thyroid gland parenchyma was extensively replaced by a moderately cellular neoplasm composed of clusters of polyhedral cells resembling C-cells (parafollicular cell) that were often separated by an abundant pale eosinophilic amorphous and homogeneous amyloid-like material (Fig. 2). Neoplastic cells typically had distinct cell borders, moderate to large amounts of vacuolated to pale eosinophilic finely granular cytoplasm and round to oval nuclei with euchromatic to stippled chromatin, variably distinct nucleoli (1 to 2) and with moderate anisocytosis and anisokaryosis. There were typically 2 mitoses observed in ten high power (400X) fields. Neoplastic cells were confined within the pre-existent thyroid capsule and the mass of neoplastic cells markedly compressed the residual thyroid parenchyma. Tumour emboli were not seen in the thyroid lymphatic vessels. The neoplastic cell cytoplasm stained positively for calcitonin using immunohistochemistry (Fig. 4). The homogenous eosinophilic material stained positive with Congo red and had apple-green birefringence when subjected to polarized light, specific for amyloid deposition (Fig. 4). Although C-cell adenoma was considered the diagnosis, to exclude the presence of carcinomatosis or

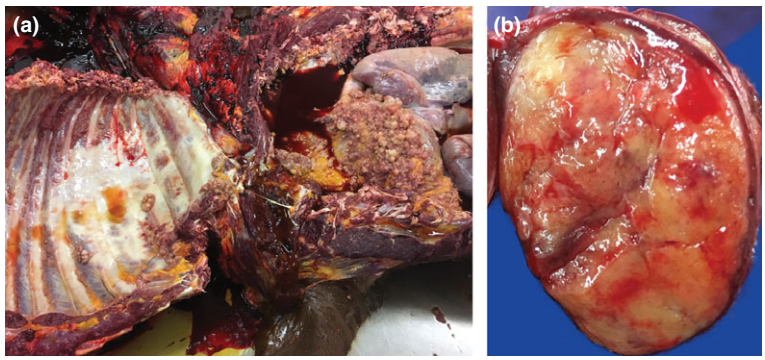

Fig. 1. Pathological aspects of the thoracic mesothelioma and C-cell adenoma in an aged horse. (a) Multifocal to coalescing masses are easily identified on the pleural surfaces and thoracic wall. (b) The left thyroid gland is replaced by an expansile neoplasm contained within the pre-existing thyroid capsule.

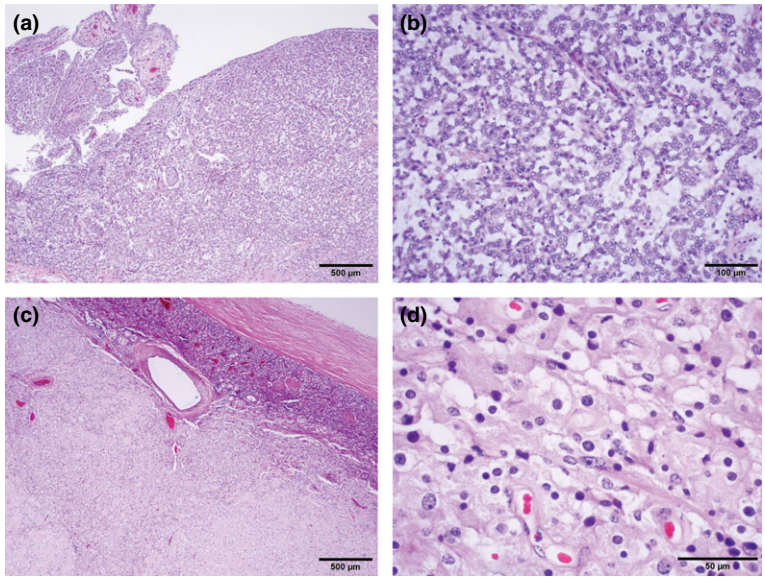

Fig. 2. Photomicrographs of the mesothelioma (a, b) C-cell adenoma (c, d). (a) The serosal surface is infiltrated and replaced by an extensive proliferation of polygonal cells forming nests, cords and ducts of variable size and shape. (b) At higher magnification, neoplastic cells have indistinct cell borders, moderate amounts of cytoplasm, oval nuclei with stippled chromatin and variably distinct nucleoli. (c) The left thyroid mass consists of compact sheets (or solid clusters) of polyhedral cells and compresses the remaining parenchyma at the periphery. (d) At higher magnification, neoplastic cells have moderate amount of eosinophilic faintly granular cytoplasm and round pleomorphic nuclei with euchromatic to stippled chromatin and variably distinct nucleoli.

metastasis of the thyroid neoplasm to the thoracic cavity, immunohistochemistry for calcitonin (C-cell marker) on sections of the pleural neoplasm was utilized. Neoplastic cells within the thoracic masses were negative for this protein.

Sections examined from the liver, spleen, kidneys, pituitary gland and adrenal glands were unremarkable. The pathological evaluations were summarized as a mesothelioma with pleural effusion and thyroid C-cell adenoma with amyloid deposits. The intrathoracic, mediastinal and pleural masses most likely caused the pleural effusion secondary to obstruction of lymphatic drainage and compression of the cranial vena cava. It can be concluded that the

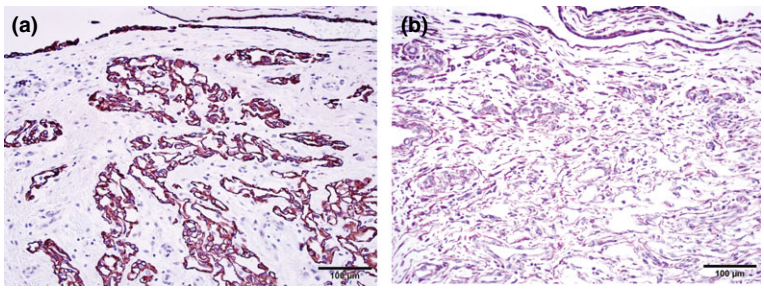

Fig. 3. Immunohistochemical characterization of the mesothelioma. Neoplastic cells express pan-cytokeratin (a) and vimentin (b).

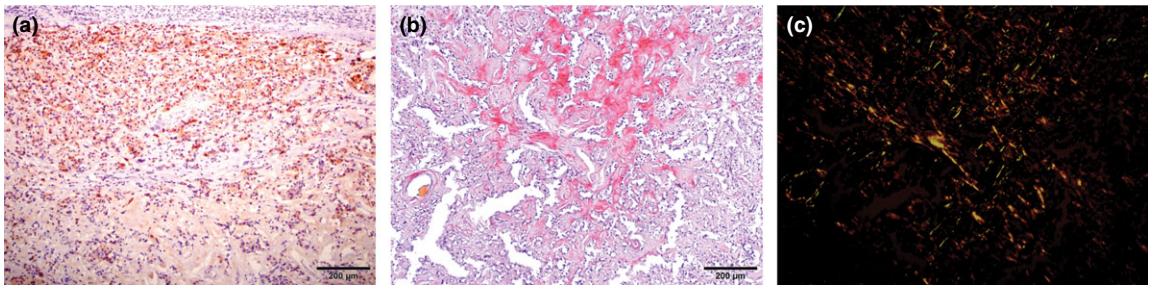

Fig. 4. Immunohistochemical characterization (a) and Congo red staining (b, c) of the left thyroid mass. (a) Positive cytoplasmic labelling for calcitonin in C cells of the thyroid tumour. (b) The hyalinized stroma of the C-cell adenoma is positive for Congo red stain. (c) The congophilic material exhibits characteristic apple-green birefringence under polarized light, specific for amyloid.

animal succumbed to cardiorespiratory failure caused by compression of the copious effusion and widespread distribution of the intrathoracic mesothelioma.

Discussion

In horses with progressive weight loss, common conditions to consider are malnutrition, malocclusion, parasitism and systemic diseases. The differential diagnoses for chronic respiratory disease in the equine species include inflammatory airway disease similar to chronic obstructive pulmonary disease (COPD), granulomatous pleuritis/pneumonia, widespread carcinomatosis and lymphosarcoma. These particular diseases were considered as possible causes of the clinical symptoms of this American Saddlebred mare prior to necropsy. Based on gross examination, the most likely differential diagnoses of thoracic masses included mesothelioma, carcinoma, carcinomatosis or metastatic neoplasms.

On histopathological examination, mesotheliomas exhibit a variety of histological subtypes, broadly differentiated into three categories: epithelioid, sarcomatoid and mixed (or biphasic). Epithelioid mesotheliomas are comprised of polygonal, oval or cuboidal cells that are often challenging to distinguish from non-neoplastic reactive mesothelial cells (Husain *et al.* 2013). Sarcomatoid mesotheliomas are often comprised of spindle cells but may contain lymphohistiocytoid cells and/or heterologous rhabdomyosarcomatous, osteosarcomatous or chondrosarcomatous elements (Husain *et al.* 2013). Mixed mesotheliomas exhibit both epithelioid and sarcomatoid components (Husain *et al.* 2013). In general, the histopathological diagnostic challenge encountered in any case of mesothelioma is defined by the specific histological subtypes of mesothelioma that maybe similar to another neoplastic category. Carcinomas are a differential diagnosis for epithelioid mesothelioma. Sarcomas and other spindle cell neoplasms are a differential diagnosis for sarcomatoid mesothelioma. Synovial sarcoma and metastatic pleomorphic carcinoma of lung are a differential diagnosis for mixed mesothelioma (Husain *et al.* 2013). In addition, desmoplastic

mesotheliomas may resemble fibrous pleuritis (Husain *et al.* 2013).

In the present case report, the neoplastic cells were characteristic of an epithelioid subtype. The main differential diagnoses for the multiple nodular pleural masses seen in this horse thus consisted of carcinomas or carcinomatosis. Interestingly, epithelioid mesotheliomas are further classified in human pathology and some of these histological classes have been reported in dogs (papillary, tubular, solid, sclerosing, cystic, deciduoid) (Munday *et al.* 2017). By applying this further subclassification, the predominant pattern in this case was tubular. Immunohistochemical stains are useful to distinguish mesothelioma from epithelial neoplasms. Neoplastic mesothelial cells typically are immunoreactive for both cytokeratin and vimentin. Generally, carcinomas are cytokeratin positive and vimentin negative. In the present case, the pleural neoplastic cells in histological sections were positive for both cytokeratin and vimentin, helpful for confirmation of the mesenchymal origin of the neoplastic cells.

Fine needle aspiration biopsy was not attempted prior to necropsy. The main difficulty in cytology evaluation would have been to differentiate a reactive mesothelial cell from a neoplastic mesothelial cell. Cytological examination of solid masses or, more often, cavitary effusions related to mesothelioma can be challenging. The presence of an effusion itself in a body cavity may lead to mesothelial hyperplasia and atypia. The cytomorphology of reactive mesothelial cells can include pronounced anisocytosis, anisokaryosis, binucleation and multinucleation, sometimes making the distinction between a reactive and neoplastic process impossible with cytological evaluation alone (Thompson & Rebar 2016). In cases with strong criteria of malignancy, to confirm the specific malignant population of cells, the distinction between mesothelioma, carcinoma and adenocarcinoma generally requires additional data such as immunocytochemical staining and/or ultrastructural evaluation (Thompson & Rebar 2016; Schappa *et al.* 2017). If immunocytochemical staining is not available, effusion samples can be converted into a cell block and processed for immunohistochemical staining (Marcos *et al.* 2017).

Over the past decades, mesotheliomas have been described in horses arising within the pleural cavity, peritoneal cavity, pericardium, tunica vaginalis or affecting multiple sites. Several cases have been reported previously (Straub *et al.* 1974; Kramer *et al.* 1976; Ricketts & Peace 1976; Carnine *et al.* 1977; Colbourne *et al.* 1992; Mair & Brown 1993; Harps *et al.* 1996). In the horse, mesotheliomas are not known to be associated with asbestos exposure. The clinical signs induced depend on the location of the tumour. Treatment generally entails provision of supportive care to alleviate the clinical signs, if possible. Spreading of mesothelioma most likely occurs by infiltration and implantation, but rarely metastasis to distant organs. No metastases to the lymph nodes and internal organs were observed in the case reported herein.

A concurrent neoplasm involving the left thyroid gland occurred in this American Saddlebred mare. The histomorphological feature of the neoplasm was consistent with a C-cell adenoma and confirmed via immunoreactivity with calcitonin antibody. C-cell adenomas are comprised of parafollicular C cells, which are derived from the neural crest. Microscopically, neoplastic cells are polygonal and contain amphophilic granules with a round, uniform, medium size nucleus. They are arranged in compact sheets subdivided by a fine fibrovascular stroma without capsular invasion (Ueki *et al.* 2004). C-cell carcinomas are typically more densely cellular versus their benign counterparts and the neoplastic cells are more pleomorphic (de Lima *et al.* 2001). The C-cell tumour in this case did not display features of malignancy, such as local tissue invasion, lymphovascular invasion and metastasis to distant sites. Thyroid follicular adenomas are common findings during postmortem examination of aged horses (Schlotthauer 1931; Dimock *et al.* 1944; Cotchin & Baker-Smith 1975). They generally present as a unilateral nodule composed of non-functional follicular cells. C-cell adenomas are uncommonly reported in the U.S., but may be under-diagnosed or misdiagnosed as follicular adenomas in older horses. A survey of thyroid cancer in horses over 7 years of age from the Department of Veterinary pathology (Kitasato University, Aomori) and Equine Research Institute (Japan

Racing Association) reported 12 horses with non-functional C-cell adenomas among 38 horses suggesting that C-cell adenomas would be found more frequently than thyroid follicular adenomas (Ueki *et al.* 2004). Malignant neoplasms occur less frequently than their benign counterpart in the thyroid (Dalefield & Palmer 1994; Elce *et al.* 2003). C-cell carcinomas are most frequently reported in bulls (Capen & Black 1974). In aged bulls and horses, C-cell tumours may be associated with other endocrine neoplasms such as pheochromocytoma and pituitary adenomas (Capen & Black 1974; De Cock & MacLachlan 1999). However, the adrenal and pituitary glands were unremarkable in the present case. Surgical resection of thyroid neoplasia is warranted when serum thyroid hormone concentrations are abnormal and/or the enlarged mass compromises breathing or eating (Breuhaus 2011). Total surgical removal of C-cell adenomas and carcinomas has been reported with positive outcome with up to 3 years follow-up (Lucke & Lane 1984; van der Velden & Meulenaar 1986; Kuwamura *et al.* 1998).

Amyloid deposits were confirmed in the stroma of the C-cell adenoma reported herein. Historically, previous descriptions of C-cell carcinoma mentioned amorphous eosinophilic material, predominantly extracellular (Albores-Saavedra *et al.* 1964; Meyer 1968). Investigation with electron microscopy and *in vitro* culture of C-cell carcinoma cells has confirmed the production of amyloid by the tumour cells (Albores-Saavedra *et al.* 1964). Amyloid deposits present in C-cell adenoma or carcinoma are derived from procalcitonin produced by C-cells leading to ACal fibril formation and contain the P component as found with other forms of amyloid (Sletten *et al.* 1976; Sipe *et al.* 2014; Rosol & Meuten 2017). In a recent study, both calcitonin and katacalcin have been detected in all samples of C-cell carcinoma in humans (Erickson *et al.* 2015). The peptide sequence of the calcitonin precursor is composed of the signalling peptide (amino acids 1-25), propeptide (amino acids 25-82), calcitonin protein (amino acids 85-116) and katacalcin protein (amino acids 121-141) (Erickson *et al.* 2015).

Amyloid deposition is a characteristic feature of thyroid C-cell neoplasms. Amyloid deposits are routinely confirmed during histopathological examination with use of special stain such as Congo red or thioflavin T. Several sections might be necessary to find interstitial amyloid deposits in thyroid C-cell tumours because focal deposits of amyloid may not be detected if only a few sections are examined. Thus, potential amyloid deposits might have been overlooked in a survey that described 12 non-functional C-cell adenomas in aged horses (Ueki *et al.* 2004) and in a previously reported case involving a 12-year-old Dutch Warmblood mare with C-cell carcinoma; neither of which reported amyloid deposition (van der Velden & Meulenaar 1986). In general, endocrine amyloid deposits are considered to be localized to the thyroid and derived from hormonal polypeptides that are hypothetically hypersecreted. However, a case report of nephrotic syndrome involving glomerular deposition of amyloid derived from calcitonin produced by a C-cell carcinoma in a woman indicates that endocrine amyloidosis may not always be confined within the affected gland (Koopman *et al.* 2017). Amyloid deposits did not occur in the mesangial subendothelium of the kidneys in the case reported herein.

In conclusion, a unique case of concurrent neoplasms, a mesothelioma and a C-cell adenoma, occurred in an aged horse. Amyloid deposits were detected in the interstitium of the C-cell adenoma. The case described herein highlights diagnostic challenges that might be encountered by a clinician and pathologist when aspirated or fixed tumour samples are examined.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. No ethical approval was required as this is a case report with no original research data.

Contributions

JSF drafted the manuscript and KK carried out the macroscopic and histopathological examination. ABR drafted the clinical pathology section of the manuscript. All authors read, critically edited and approved the final manuscript.

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