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DISEASE IN WILDLIFE OR EXOTIC SPECIES

T-lymphocyte-rich Thymoma and Myasthenia Gravis in a Siberian Tiger (*Panthera tigris altaica*)[☆]

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Summary

A 10-year-old captive male Siberian tiger (*Panthera tigris altaica*) presented with acute onset collapse, vomiting and dyspnoea, preceded by a 6-month period of progressive muscle wasting. Following humane destruction, post-mortem examination revealed a large multilobulated mass in the cranial mediastinum, which was diagnosed as a T-lymphocyte-rich thymoma with the aid of immunohistochemistry. Retrospective serology for acetylcholine receptor antibodies (titre 3.90 nmol/l) confirmed a diagnosis of thymoma-associated myasthenia gravis. Thymomas are reported rarely in wild carnivores, but when detected they appear to be similar in morphology to those seen in domestic carnivores and may also be accompanied by paraneoplastic syndromes. The clinical signs of myasthenia gravis in the tiger were consistent with those reported in cats and dogs and the condition is proposed as an important differential diagnosis for generalized weakness in captive Felidae.

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Thymoma is an uncommon neoplasm of the cranial mediastinum, composed of neoplastic thymic epithelium usually accompanied by various degrees of lymphocytic infiltration (Jacobs *et al.*, 2008). In people, thymomas are organised into five categories (A, AB, B1, B2 and B3) using a World Health Organization (WHO) classification system that aims to predict the clinical behaviour and prognosis of this neoplasm (Travis *et al.*, 2004; Suster and Moran, 2006). In animals thymomas are classified on the basis of the predominant cell population within the mass and may be lymphocyte predominant, epithelial cell predominant or of an intermediate lympho-epithelial subtype. Immunohistochemistry (IHC) is often required to confirm the presence of neoplastic thymic epithelial cells in lymphocyte-rich thymomas

and to differentiate thymomas from mediastinal lymphomas, which occur more commonly in most animals (Jacobs *et al.*, 2008). Spontaneously-occurring thymomas have been reported in a range of domestic animals including dogs and cats (Day, 2008), cattle (Ecco *et al.*, 2006), rabbits (Kunzel *et al.*, 2012) and goats (Hadlow, 1978) as well as in various laboratory primates and rodents (Walsh and Poteracki, 1994; Brandes *et al.*, 2004; Schwartz *et al.*, 2011). However, in wildlife species reports of thymomas are scarce. Here we describe the morphological and paraneoplastic features of a thymoma diagnosed at post-mortem examination in a captive Siberian tiger (*Panthera tigris altaica*).

A 10-year-old, male neutered Siberian tiger, born and housed at the Zoological Society of London (ZSL) Whipsnade collection, presented on 27th October 2011 to resident veterinary staff in sternal recumbency with acute onset vomiting, depression and a right-sided head tilt. The tiger had been treated for progressive muscle wasting and suspected renal insufficiency with oral benazepril hydrochloride (0.5 mg/

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kg q24h; Fortekor Flavour 20 mg for dogs; Novartis, Camberley, Surrey, UK) for 6 months prior to acute presentation. Following collapse, general anaesthesia was induced to facilitate clinical examination using 480 mg ketamine (ketamine 1 g powder for reconstitution; Kyron Laboratories, Benrose, Johannesburg, South Africa) and 6 mg medetomidine (Zalopine 10 mg/ml; Orion Pharma, Newbury, Berkshire, UK) administered by remote intramuscular injection. Endotracheal intubation was performed and anaesthesia was maintained with oxygen and isoflurane (Isoflurane-Vet 100% w/w inhalation vapour; Merial Animal Health, Harlow, Essex, UK). On physical examination, the tiger was tachycardic with poor peripheral circulation. An abnormal respiratory pattern, characterized by inspiratory stridor accompanied by irregular periods of apnoea, was observed and intermittent positive pressure ventilation was initiated. Venous blood samples were obtained, but standard haematological and biochemical parameters were within published reference values for this species (ISIS, 2002). On welfare grounds, the tiger was humanely destroyed and submitted for pathological examination.

Post-mortem examination revealed a large, 1.5 kg, 18 × 15 × 10 cm, well-demarcated, multilobulated, mediastinal mass within the cranial thorax (Fig. 1). The pleural and peritoneal cavities both contained small amounts of serosanguineous fluid. There was generalized depletion of subcutaneous fat stores in addition to moderate atrophy of skeletal muscle over the hindquarters.

Representative tissue samples were collected and fixed in 10% neutral buffered formalin and submitted



Fig. 1. Intrathoracic mass after reflection of the ventral thorax and sternum. A well-demarcated, multilobulated mass expands the cranial mediastinum. Bar, 3 cm.

to Abbey Veterinary Services, Newton Abbott, UK, for examination. Tissue samples were processed routinely and sections (4 µm) were stained with haematoxylin and eosin (HE). Subsequently, samples from the mediastinal mass were transported to the University of Glasgow Veterinary Diagnostic Services for IHC. Sections were labelled with a panel of primary antibodies including mouse anti-vimentin (Clone V9, Dako, Ely, UK; dilution 1 in 50), which did not require antigen retrieval, and mouse anti-human cytokeratin (Clone MNF116, Dako; dilution 1 in 100), which required enzymatic antigen retrieval with proteinase K. Heat-induced epitope retrieval using sodium citrate buffer (pH 6.0) was required for the following antibodies: mouse anti-human CD79αcy (Clone HM57, Dako; dilution 1 in 100); mouse anti-human B cell-specific activator protein (Clone DAK-Pax 5, Dako; dilution 1 in 100) and rabbit anti-human CD3 (Dako, dilution 1 in 100). IHC was performed using a Dako Autostainer. Tissue sections were also stained with Astra blue (Sigma-Aldrich, Gillingham, UK) as previously described (Blaies and Williams, 1981).

Microscopically, the mass was a large, multilobulated, densely cellular and poorly demarcated tumour, composed of abundant, densely packed and well-differentiated small to medium sized lymphocytes arranged in sheets that frequently obscured a less numerous background population of polygonal to stellate neoplastic cells. The neoplastic cells were arranged in variably packed cords and supported by a well-vascularized collagenous and adipose connective tissue stroma. The neoplastic cells were large (15–20 µm in diameter) and poorly differentiated with moderate amounts of finely granular eosinophilic cytoplasm and indistinct cell borders (Fig. 2). The nuclei were oval, centrally located, often with a single prominent magenta nucleolus and peripheralized chromatin. There was moderate anisocytosis and anisokaryosis, but mitoses were rare, with <1 mitotic figure per 10 high-power (×400) fields. Throughout the tumour were numerous, multifocal aggregates of eosinophils admixed with smaller numbers of plasma cells and mast cells.

IHC demonstrated strong labelling of stellate neoplastic cells with cytokeratin-specific antibodies (Fig. 3). The predominant population of lymphocytes was CD3⁺ (Fig. 4), but showed little evidence of labelling with either CD79 or Pax-5 antibodies. Rare scattered mast cells were identified with Astra blue staining. There was no evidence of immunolabelling of any cells with vimentin-specific antibodies.

Following microscopic examination of tissue samples, serum was submitted to the Comparative Neuromuscular Laboratory (Department of Pathology,

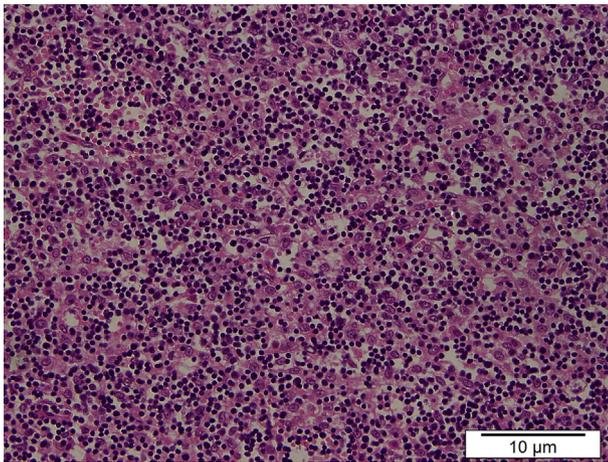


Fig. 2. The mass is composed of sheets of small to medium sized lymphocytes that obscure a background population of polygonal to stellate neoplastic cells. HE.

University of California, San Diego, La Jolla, California, USA) for detection of acetylcholine receptor antibodies (AChR). A titre of 3.90 nmol/l was measured by immunoprecipitation radioassay (normal reference levels; dog <0.6 nmol/l; cat <0.3 nmol/l). Retrospective serological assays for feline immunodeficiency virus and feline leukaemia virus were negative.

On the basis of the histological appearance and observed pattern of immunoreactivity, the tumour was diagnosed as a T-lymphocyte predominant thymoma. Although CD3⁺ T lymphocytes formed a large component of the tissue, the epithelial characteristics of the neoplastic cells confirmed a thymic origin. Despite good labelling with most of the IHC antibody panel, as well as previous reports in the literature of successful vimentin-immunolabelling of tiger

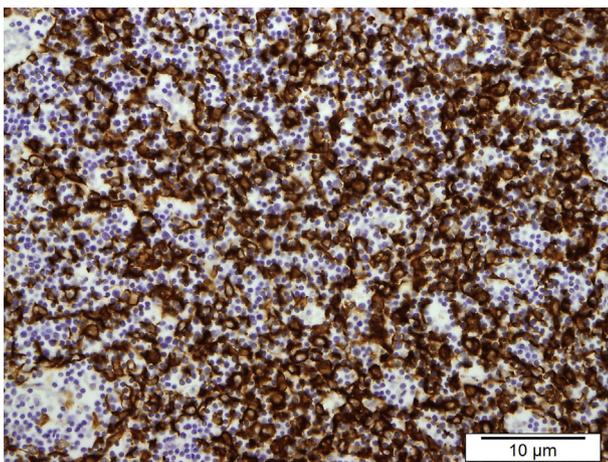


Fig. 3. Stellate neoplastic cells demonstrate strong labelling with mouse anti-human cytokeratin antibodies. IHC.

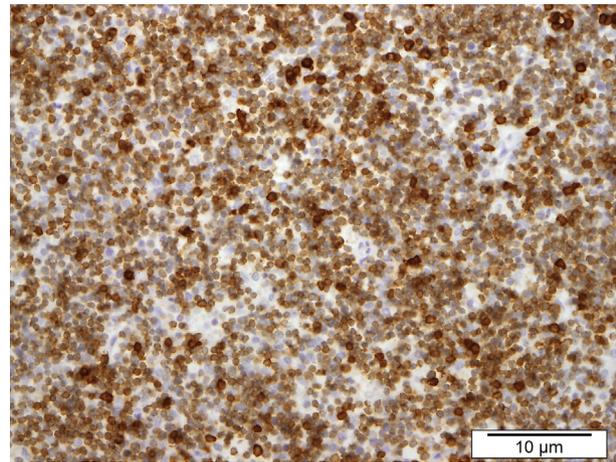


Fig. 4. Lymphocytes demonstrate ubiquitous immunoreactivity with rabbit anti-human CD3 antibodies. IHC.

tissues (Scudamore and Meredith, 2001; Kang *et al.*, 2006), anti-vimentin antibodies failed to react with any mesenchymal cells in these sections. Modification of the labelling methodology, such as extending incubation times or increasing antibody concentrations, may be required to further optimize the technique (Ramos-Vara *et al.*, 2008).

To the authors' knowledge, this is the first report describing the morphological features of a thymoma in a Siberian tiger. In the veterinary literature, there is one report of a thymic mass in an aged female tiger with myasthenia gravis, but the morphology of this mass was not described (Wallace and Teare, 1994). Reports of thymoma in other captive wildlife species are limited to an asymptomatic thymoma in an African spot-necked otter (*Lutra maculicollis*), which was detected at routine health examination and subsequently removed, and a single report of thymoma-associated myasthenia gravis in an adult female polar bear (*Ursus maritimus*) (Kenny *et al.*, 2004; Pye *et al.*, 2010). In these two animals, the lesions were predominately composed of spindle-shaped epithelial cell populations with varying degrees of lymphocytic infiltration.

In animals, thymic epithelial tumours show variable tendencies to cause clinical disease in different livestock and companion animal species. Clinically significant lymphocyte-rich thymomas are most frequently reported in the dog, but in small ruminant species, such as the goat and sheep, lymphoid thymomas are typically incidental findings at necropsy examination (Hadlow, 1978; Valli and Gentry, 2007). Although the signs of a space-occupying mediastinal mass, such as dyspnoea, cardiovascular impairment and exophthalmos, may occur in any species (Valli and Gentry, 2007; Day, 2008; Kunzel *et al.*, 2012), the most severe clinical signs in people and domestic

carnivores are usually associated with the presence of thymoma-associated paraneoplastic disease, most commonly myasthenia gravis.

Myasthenia gravis is an autoimmune disorder that causes focal or generalized muscle weakness as a consequence of autoantibody-mediated destruction of acetylcholine receptors within neuromuscular synapses (Shelton, 2002). In people, myasthenia gravis is diagnosed in 30–44% of cases of thymoma and its occurrence is usually associated with widespread infiltration of the thymoma with numerous T lymphocytes in various stages of maturation (thymopoiesis) (Marx *et al.*, 2010). In domestic animals, the syndrome is less well described, but paraneoplastic myasthenia gravis can be seen at a similar frequency in dogs with thymoma (Aronsohn *et al.*, 1984; Jacobs *et al.*, 2008) and the presence of follicle-like aggregates of lymphocytes within canine and feline thymomas is thought to be positively associated with its occurrence (Valli and Gentry, 2007).

In human patients, myasthenia gravis is characterized by abnormal limb muscle fatigue and weakness, which worsens with exercise. Localized symptoms, such as oculomotor weakness or difficulties chewing and swallowing, may also be seen (Allen and Lueck, 2002). Clinical signs in affected dogs also range from localized deficits, such as dysphagia and regurgitation, to generalized muscle weakness or acute, fulminating disease with rapid onset tetraparesis and respiratory failure (Dewey *et al.*, 1997; Shelton *et al.*, 2000; Shelton, 2002). In cats, generalized muscle weakness is the most common presentation (Shelton *et al.*, 2000).

In exotic Felidae and other species, diagnosis of myasthenia gravis relies on demonstrating circulating AChR antibodies with cross-reactivity to fetal canine and feline muscle antigen (Shelton, 2002; Kenny *et al.*, 2004). Demonstration of AChR antibodies using the immunoprecipitation radioassay has previously been used to diagnose myasthenia gravis in a captive Siberian tiger from Milwaukee Country Zoo, using antibody titres from three healthy tigers (<0.1 nmol/l) to provide a baseline value for this species (Wallace and Teare, 1994). Cross-reacting AChR antibodies were substantially elevated in our case in comparison to this baseline, as well as to reference values used for domestic carnivores. Clinical signs described in Siberian tigers with elevated AChR antibody levels are consistent with those reported in dogs and cats with myasthenia gravis. In particular, similarities were noted between the severe fulminating form of myasthenia gravis in dogs and the acute and severe nature of the end-stage disease presentation in this case (Dewey *et al.*, 1997; King and Vite, 1998; Kenny *et al.*, 2004).

In conclusion, this paper presents the first morphological description of a T-lymphocyte-rich thymoma in a Siberian tiger, as well as the second laboratory-confirmed case report of myasthenia gravis in this species. The histological and paraneoplastic features of this tumour are consistent with thymomas in domestic carnivores and good cross-reactivity of tiger AChR antibodies with feline and canine antigens is demonstrated. Thymoma-associated myasthenia gravis should be considered as an important differential in captive tigers and other carnivores presenting with generalized or focal muscle weakness, dysphagia and regurgitation or with acute collapse and respiratory compromise.

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Conflict of interest statement

The authors report no conflict of interest.

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