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Histocytic-like Atypical Mast Cell Tumours in Horses

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Summary

This report describes a series of four equine mast cell tumours (MCTs) with atypical morphological features. The tumours were 1–2 cm in diameter and mostly localized to the eyes (one eyelid, two conjunctiva). Histologically, they were composed of very large (up to 35 μm) round pleomorphic cells with a large central to paracentral nucleus and abundant granular cytoplasm. A large number of viable mature eosinophils were detected intermingled with the large round cells. Histochemical staining (toluidine blue and Perls' Prussian blue) and immunohistochemistry (KIT, mast cell tryptase, lysozyme and proliferating cell nuclear antigen) confirmed the mast cell origin of the atypical cells and identified an aberrant KIT protein expression in three cases. Based on morphological and immunohistochemical features, we propose to call the lesions equine histiocytic-like atypical MCTs.

Keywords: atypical; histiocytic-like; horse; mast cell tumour

Mast cell tumours (MCTs) are uncommon in horses and the majority are benign neoplasms. They are predominantly found in the skin (Valentine, 2006; Scott and Miller, 2011), but have also been described in other locations such as the conjunctiva (Hum and Bowers, 1989). The neoplastic nature of equine MCTs (EMCTs) is still under debate, but there is recent evidence that at least a proportion are truly neoplastic, as they exhibit a high proliferation rate and cellular atypia, in combination with aberrant KIT protein expression (Clarke *et al.*, 2014; Ressel *et al.*, 2015). EMCTs present as nodular masses composed of variable proportions of neoplastic mast cells and mature eosinophils, typically associated with well-circumscribed areas of collagenolysis ('flame figures') that are surrounded by eosinophilic granuloma-like infiltrates (Scott and Miller, 2011; Kiupel, 2017). Cases with low mast cell numbers can present a diagnostic challenge since their morphological features overlap with those of equine eosinophilic granulomas (EEGs) (Kiupel, 2017). On the other hand, in their poorly differentiated form, EMCTs may be difficult to differentiate from other round cell neoplasms (Ressel *et al.*, 2015). The present report describes a series of four EMCTs with morphological and immunohistochemical features similar to those described for atypical histiocytic-like MCTs in cats (Sabattini and Bettini, 2010). We therefore propose to introduce a similar subtype in horses.

A retrospective comparative re-examination of 58 EMCTs and 129 EEGs from the diagnostic archive (years 2005–2015) of the Department of Veterinary Pathology and Public Health, Institute of Veterinary Science, University of Liverpool, identified four lesions with atypical, but similar, features. All four had originally been diagnosed as EEG-like lesions;

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however, a closer inspection confirmed that they did not exhibit classical features of the latter. Special stains and immunohistochemistry (IHC) had not been performed at the time of the initial diagnosis. Clinically, the lesions had presented as small nodular masses in the haired skin or the conjunctiva (Table 1). Follow-up information was not available.

For re-examination of the four cases, consecutive sections (3–5 μm) were prepared and stained with haematoxylin and eosin (HE), toluidine blue (TB; demonstration of metachromatic mast cell granules) and Perl's Prussian blue (demonstration of haemosiderin). Briefly, for IHC, sections were dewaxed and subjected to antigen retrieval in Dako PT buffer high/low pH (Agilent Technologies Ltd., Stockport, UK) using a computer controlled antigen retrieval workstation (PT Link; Agilent Technologies Ltd.) for 20 min at 98°C. Sections were then labelled in an automated immunostainer (Link 48; Agilent Technologies Ltd.), using primary antibodies against mast cell tryptase (mouse anti-human MCT, clone AA1, Agilent Technologies Ltd.; 1 in 500 dilution), KIT protein (rabbit anti-human CD117 [c-Kit], Agilent Technologies Ltd. [A4502]; 1 in 500 dilution), lysozyme (rabbit anti-human lysozyme, Agilent Technologies Ltd. [A0099]), Iba-1 anti-AIF1/IBA1 antibody (Source Bioscience, Nottingham, UK [LS-B2402]; 1 in 500 dilution) and proliferating cell nuclear antigen (PCNA; mouse anti-PCNA, clonePC10, Dako, Glostrup, Denmark, [M0879]; 1 in 100 dilution) all diluted in EnVisionTM FLEX Antibody Diluent (K8006, Agilent Technologies Ltd.) and tested to cross-react with equine tissues (Ressel *et al.*, 2015) in a 1 h incubation at room temperature (RT). This was followed by a 30 min incubation at RT with the secondary antibody and polymer peroxidase-based detection system (anti-mouse/rabbit Envision Flex+, Agilent Technologies Ltd.). The reaction was 'visualized' with 3, 3′ diaminobenzidine (Agilent Technologies Ltd.). Equine skin with normal mast cells and a lymph node served as positive controls for KIT and MCT as well as Iba-1, lysozyme and PCNA, respectively. Consecutive sections incubated with non-immune rabbit serum or a murine subclass-matched unrelated monoclonal antibody served as negative controls. The positive reaction was represented by a distinct brown cytoplasmic (KIT, Iba-1, MCT, lysozyme, PCNA in mitotic cells), membranous (KIT) or nuclear (PCNA) reaction. The KIT expression pattern was determined according to previously described parameters (Ressel *et al.*, 2015), where a membranous labelling reaction is considered as normal, while cytoplasmic, focally stippled or diffuse labelling is classified as aberrant.

Table 1
Clinical information and gross features of four cases of atypical histiocytic-like mast cell tumour in horses

Case	*Breed*	*Age (years)*	*Tumour location*	*Tumour size (cm)*
1	Thoroughbred	Unknown	Conjunctiva	1.5 × 1.5 × 1
2	Thoroughbred	11	Haired skin (eyelid)	1.5 × 1 × 0.5
3	Thoroughbred	9	Conjunctiva	2 × 1.5 × 0.5
4	Warmblood	4	Haired skin (not specified)	1.5 × 1 × 0.5

Histologically, all four lesions presented as completely excised, well delineated and expansile, densely cellular subepithelial masses. The infiltrates were dominated by viable mature eosinophils, intermingled with individual large (up to 35 μm diameter) pleomorphic round cells with a central to paracentral nucleus, moderate anisokaryosis and anisocytosis and abundant finely granular cytoplasm (Fig. 1). The granular material was strongly metachromatic in the toluidine blue-stained sections (Fig. 1, inset), suggesting their mast cell nature. This was further supported by IHC: the cells showed strong MCT expression (Fig. 2). To obtain further evidence of the neoplastic nature of the large atypical mast cells, the lesions were labelled for KIT and PCNA. In all cases the large cells were KIT positive. Indeed, in 3/4 cases, the KIT expression pattern was that reported for EMCTs suggested to be truly neoplastic (Ressel *et al.*, 2015), representing the aberrant, focally stippled cytoplasmic expression (Fig. 3). They were also mostly PCNA positive (Supplementary Fig. 1), confirming that they were proliferating; however, mitotic figures were rare. In order to fully rule out the initial diagnosis of EEG and considering the size and low nuclear-to-cytoplasmic ratio (both unusual for mast cells), IHC for macrophage markers was performed. This showed that the atypical cells were indeed negative for lysozyme and Iba-1; however, the infiltrates generally contained a moderate number of lysozyme- and Iba-1-positive macrophages (Supplementary Figs. 2 and 3, respectively). Based on their atypical morphology, mast cell tryptase and KIT expression and their proliferative nature, we classified the large cells as neoplastic mast cells and we suggest that the lesions represent equine atypical histiocytic-like mast cell tumours. Supplementary Fig. 4 schematically represents the variability of the neoplastic mast cells in the different EMCT subtypes described so far.

Our case series shares many features with a feline MCT subtype, so-called 'atypical' or 'histiocytic-like' MCTs (Sabattini and Bettini, 2010; Kiupel, 2017). These have been described in cats <4 years of age and are generally small and located within