**SHORT COMMUNICATION**

**Histocytic-like atypical mast cell tumours in horses**

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**Summary**

This report describes a series of four equine mast cell tumours with atypical morphological features. Tumours were 1 to 2 cm in diameter and mostly localised to the eyes (1 eyelid, 2 conjunctiva). Histologically, they were composed of very large (up to 35 m) round pleomorphic cells with a large central to paracentral nucleus and abundant granular cytoplasm. Large number of viable mature eosinophils were detected intermingled with the large round cells. Histochemical stain (toluidine blue, Perls’ prussian blue) and immunohistochemistry (KIT, mast cell tryptase, lysozyme, PCNA) 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: equine; mast cell tumour; eye; histiocytic-like; atypical

Mast cell tumours (MCTs) are uncommon, in the majority benign neoplasms in horses. 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 is truly neoplastic, as they exhibit a high proliferation rate and cellular atypia, in combination with aberrant KIT protein expression (Clarke, 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 (EEG) (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 coherent 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; 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 routinely stained with haematoxylin-eosin (HE), toluidine blue (TB; demonstration of metachromatic mast cell granules) and Perl’s Prussian blue (demonstration of haemosiderin), and underwent IHC. 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 stained 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; 1:500), KIT protein (Rabbit antihuman CD117(c-Kit), Agilent Technologies Ltd (A4502); 1:500), lysozyme (Rabbit antihuman lysozyme, Agilent Technologies Ltd (A0099)), Iba-1 (Anti-AIF1 / IBA1 Antibody (Source Bioscience, (LS-B2402); 1:500) and proliferating cell nuclear antigen (PCNA) (Mouse anti PCNA, clonePC10, Dako (M0879)),1 in 100) 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 visualised with 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 staining reaction is considered as normal, while a cytoplasmic, focally stippled or diffuse staining is classified as aberrant.

Histologically, all four lesions presented as completely excised, well delineated and expansile, densely cellular sub-epithelial masses. The infiltrates were dominated by viable mature eosinophils, intermingled with individual large (up to 35 µm in diameter) pleomorphic round cells with 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 section (Fig. 1, inset), suggesting their mast cell nature. This was further supported by immunohistochemistry: 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 stained 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 in the majority 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), immunohistochemistry 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 Fig. 2, 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 Figure 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 less than four years of age and were generally small and located within the deep dermis or subcutis (Henry and Herrera, 2013). Histologically, this MCT subtype is characterised by large, polygonal cells with abundant light pink cytoplasm and large nuclei that are found alongside large numbers of viable eosinophils (Sabattini and Bettini, 2010). Due to their histological appearance, feline histiocytic-like mast cell tumours are often misdiagnosed as granulomatous inflammation (Goldschmit and Hendrick, 2002; Kiupel, 2017). As they are rare, there is insufficient data to assess whether their behaviour differs from that of well differentiated MCTs in the cat (Kiupel, 2017).

In our case series, the neoplastic processes were generally small and exhibited features indicative of benign behaviour (well-demarcated, non-invasive, very low mitotic rate). However, all cases were lost for any follow-up, so this interpretation cannot be further substantiated; this represents a clear limitation of our study. The processes were predominantly localised around the eye (conjunctiva and eyelid), both in mucosa and haired skin, which raises the question whether a specific mast cell subtype is responsible for their particular atypical morphology, or whether the local environment might play a role in the pathogenesis.

In conclusion, the present report describes a morphological variant of EMCTs that has so far not been described. Lesions were histologically different from all previously reported EMCTs and mainly localised to the eyes. Based on their similarity to a subtype of feline MCTs, we propose to call the lesions equine histiocytic-like atypical MCTs.

Conﬂict of interest statement

None of the authors of this paper has a ﬁnancial or personal relationship with other people or organisations that could inappropriately inﬂuence or bias the content of the paper.

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**Figure Legends**

**Figure 1**.Case 1. A. The infiltrate is dominated by viable, mature eosinophils with scattered individual large atypical cells... Inset: Atypical cells exhibit abundant granular cytoplasm and round central nuclei with distinct nucleoli. HE stain, B. The large cells exhibit abundant metachromatic cytoplasmic granules. Toluidine Blue stain. Scale bars = 50 m.

**Figure 2.** Case 2. The large atypical cells exhibit strong mast cell tryptase expression. Scale bar = 50 m.

**Figure 3.** Case 2. The large atypical cells exhibit a focal stippled cytoplasmic staining for KIT. Scale bar = 50 m.

**Supplementary Figure 1.** Case 1**.** Both the large atypical cells (arrow) as well as surrounding cells, morphologically consistent with macrophages (arrowhead), are found to proliferate, based on their expression of PCNA. Scale bar = 50 m.

**Supplementary Figure 2.** Case 1. The large atypical cells are negative for Iba-1 (arrow), but a large number of positive macrophages (arrowhead) is found disseminated in the infiltrate. Scale bar = 50 m.

**Supplementary Figure 3.** Case 1. The large atypical cells are negative for lysozyme (arrow), whereas scattered infiltrating macrophages are positive (arrowhead). Immunoperoxidase method, Papanicolaou’s haematoxylin counterstain. Scale bar = 50 m.

**Supplementary Figure 4.** Schematic size comparison between cells of different subtypes of equine mast cell tumours. (A) Neoplastic mast cells in a well differentiated equine mast cell tumour. (B). Neoplastic mast cells in a poorly differentiated equine mast cell tumour. (C). Atypical histiocytic-like mast cells in case no. 1 of the present case series. HE stain. Scale bar = 10 m.