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Diagnosis of equine penile and preputial masses: A clinical and pathological perspective

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Introduction

Masses of the prepuce and penis are common clinical presentations for the equine veterinarian, encompassing a number of differential diagnoses. In such cases, it is important to evaluate the external genitalia thoroughly and devise a methodical diagnostic and treatment plan to allow for accurate prognostication and optimal survival rates. Masses of the prepuce and penis include tumours of epithelial, mesenchymal or round cell origin, such as squamous cell carcinoma (SCC), papillomas, melanocytic tumours, lymphoma, sarcoids, fibrosarcomas (Van Den Top *et al.* 2010) and fibromas; the latter as described by De Meyer *et al.* in this issue (De Meyer *et al.* 2015). Non-neoplastic conditions that can result in masses or enlargement of the external genitalia in male horses include infection or inflammation (primary, or secondary to neoplasia) leading to balanitis or balanoposthitis. Such diseases frequently lead to chronic irritation and discomfort. Some neoplastic conditions may metastasise to cause more severe sequelae.

Diagnosis

Given that tumours comprise the majority of genital masses diagnosed in male horses, adoption of a thorough, standardised, approach to clinical evaluation is required. Van den Top et al. (2010 and 2011) have proposed a systematic assessment and classification tool for evaluating tumour type and behaviour, treatment protocol and prognosis. Visual inspection and palpation of the mass (or tumour) should occur with thorough evaluation of the external genitalia under standing sedation using an alpha-2 agonist and acepromazine to facilitate safe examination. Some clinicians advocate caution with use of acepromazine in stallions due to the possible risk of paraphimosis, priapism and penile paralysis. The mass should be assessed for size, location, mobility, and degree of infiltration / involvement of the corpus cavernosum and corpus spongiosum. Palpation of enlarged superficial and deep inguinal lymph nodes can

provide information about possible metastases. The superficial inguinal lymph nodes lie dorsolateral to the penis, and the deep inguinal lymph nodes are located just outside the pelvis adjacent to the internal inguinal ring. The medial iliac lymph nodes (adjacent to the external iliac arteries) should also be evaluated per rectum. Lymph node palpation and fine needle aspirate biopsies can result in false positive and false negative results for metastases (Van Den Top *et al.* 2010). It should be considered that although regional lymphadenopathy may be an indicator of metastases, enlargement can also be due to "reactive" lymph nodes, secondary to inflammation or infection associated with the tumour. Distant metastases to the thoracic cavity can be evaluated by radiographic assessment, however most tumours affecting the equine penis and prepuce metastasise locally via the regional lymph nodes, with pulmonary and skeletal metastases only in advanced disease (Cramer *et al.* 2011; Nelson *et al.* 2015). Routine use of radiography is not warranted for the majority of cases.

Ultrasonography of the primary penile tumour is commonly used in humans, and can provide information about the gross extent of the tumour and tissue invasion or involvement of various structures (Hyland and Church 1995). To the authors' knowledge, there are scarce descriptions of the use of ultrasonography for evaluation of genital tumours in male horses, but this modality may be a useful diagnostic adjunct.

The TNM (tumour, node, metastasis) classification system is widely used in human oncology to aid with appropriate choices of treatment and prognosis. Recent work within the equine literature has also highlighted the importance of histological grading in penile and preputial tumours. A positive correlation between high grade SCCs and metastases has been demonstrated and it has also been shown that tumour grading is an important prognosticator for survival in horses (van den Top *et al.* 2008; van den Top *et al.* 2015). This information, in

conjunction with a published classification system (Van den Top *et al.* 2011) requires a representative biopsy to be taken such that tumour grading can guide treatment protocol and provide information on prognosis. A full thickness punch or excisional biopsy is required to assess tumour architecture and depth of invasion.

Expression of cell proliferation markers, such as Ki67, and tumour suppressor genes, such as p53, may also be evaluated using immunohistochemical staining of histopathological sections (van den Top *et al.* 2015). Such markers are increasingly used in assessment of numerous types of small animal neoplasms such canine mast cell tumours (Webster *et al.* 2007) and feline mammary tumours (Zappulli *et al.* 2015), amongst many others, but their use in equine diagnostic pathology is considerably less frequent. Whilst these and similar molecular markers may provide further prognostic information for different equine penile and preputial tumour types in the future, studies to date have yet to show compelling prognostic potential (van den Top *et al.* 2015).

The diagnostic evaluations described (Ensink 2015; Van Den Top *et al.* 2010; Van den Top *et al.* 2011) represent a "gold standard" approach to penile and preputial masses, but it should also be considered that many cases have attendant financial constraints, other limitations in resources, differing owner priorities or present with additional clinical challenges such tumour accessibility. Acknowledging these factors is critical when formulating decisions regarding treatment protocol and surgical approach. For such cases, histopathology of the tumour is frequently only performed *after* treatment-based surgery has been undertaken if gross appearance of the mass is consistent with common tumour types. Whilst the concern of metastasis is important to both veterinarian and owner, the possibility of local tumour recurrence is also a critical question. Histopathological assessment of surgeon cut edges /

margins of surgically removed masses may be informative if histopathological analysis is undertaken post surgery. Consideration of all of these factors may lead to an individual diagnostic approach to equine genital masses in many cases.

Squamous Cell Carcinomas (SCC)

Squamous cell carcinoma is the most common neoplasm of equine external genitalia, with Brinsko and van den Top *et al*. (Brinsko 1998; van den Top *et al.* 2008) reporting incidences of 49 - 82.5%. Male genital SCC predominantly affects older horses and most studies document an average age range of 17.4 - 19.8 years (Howarth *et al.* 1991; Mair *et al.* 2000; Strafuss 1976; van den Top *et al.* 2008). The veterinary literature does not conclusively support any specific breed predilection for genital SCC, but ponies have been frequently highly represented (Howarth *et al.* 1991; Mair *et al.* 2000; van den Top *et al.* 2008). It has also been proposed that breeds with non-pigmented genitalia may be at higher risk for development of SCCs (Schumacher 2006). Papillomaviruses in man have long been implicated in the aetiology of SCC development, and recent work has identified a number of variants of *Equus caballus* papillomavirus 2 (EcPV2) within the tissue of equine penile papillomas, penile intraepithelial neoplasia (PIN) and SCCs (Bogaert *et al.* 2012; Lange *et al.* 2013; Newkirk *et al.* 2014; Scase *et al.* 2010; van den Top *et al.* 2015; Zhu *et al.* 2015). Histological evaluation of equine penile tumours frequently shows papillomas undergoing a spectrum of changes as part of a continuum of transition to SCC (Van den Top *et al.* 2011).

Genital SCCs in horses can have a number of different gross appearances depending on the stage of disease. Early lesions include depigmented plaques (Figure 1), irregularities of the penile or preputial surface and non-healing erosions with or without accompanying granulation tissue (Van den Top *et al.* 2011). More advanced lesions can appear as solid

masses and may have a typical cauliflower-like appearance or contain necrotic areas. Owners often notice SCCs incidentally during micturition, but associated clinical signs can include dysuria, preputial oedema, or sanguineous / purulent discharge secondary to infection or tissue necrosis. Other reported abnormalities are wide-based stance, frequent protrusion of the penis, excoriation of the genital integument and changes in gait (Van den Top *et al.* 2011). SCCs are malignant tumours, but tend to be slow to metastasise, although it should be noted that pulmonary or skeletal metastases may occur in advanced cases (Cramer *et al.* 2011; Nelson *et al.* 2015).

Histologically, squamous cell carcinomas, including those of the penis and prepuce, tend to have a very characteristic appearance, with clusters of neoplastic cells exhibiting varying degrees of keratinization, prominent nuclei often with conspicuous nucleoli, and frequently prominent mitotic figures (Cramer *et al.* 2011) (Figure 2). Equine SCCs are frequently infiltrated by CD3+ T lymphocytes, CD79+ B lymphocytes, IgG+ plasma cells and macrophages (Perez *et al.* 1999).

Squamous papillomas

Squamous papillomas (warts) tend to occur on the nose, distal limbs and external genitalia. They are the most common tumours in young horses, age 1 – 3 years (Scott 2003). Papillomas on the external genitalia of male horses tend to affect older horses however, and the published mean age range is 16.2 – 18 years (Gardiner *et al.* 2008; Howarth *et al.* 1991; van den Top *et al.* 2008). Both congenital and acquired papillomas have been reported (Scott 2003; White *et al.* 2004). Papillomas begin as small, approximately 1 mm diameter, raised, smooth, shiny grey to white papules (Van Den Top *et al.* 2010) and can be present over the whole penis, although most appear over the glans, the urethral process and preputial fold (van

den Top *et al.* 2008). Equine genital papillomas have been reported to progress to SCCs, but there is also a report of widespread penile papillomatosis associated with EcPV-2 that remained clinically and histologically unchanged over a 2 year period (Knight *et al.* 2011).

Fully developed papillomas are approximately 2 – 20 mm in diameter, and 5 mm in height, broad based to pedunculated, grey, pink or white masses with hyperkeratotic frond-like projections (Scott 2003). Some examples show clear histological evidence of viral infection, namely cytopathic change and the presence of koilocytes, cells which are undergoing ballooning degeneration, which have eccentrically placed pyknotic nuclei. As a corollary of this observation, equine papillomavirus type 2 DNA has been amplified from equine penile papillomas (Knight *et al.* 2011; Lange *et al.* 2013).

Melanocytic tumours

Melanomas have been reported in horses aged 2 – 29 years, with an average of 13 years (Gardiner *et al.* 2008; Howarth *et al.* 1991; van den Top *et al.* 2008). They typically affect all parts of the prepuce and penis other than the glans, with the prepuce listed as the third most common site of occurrence for equine melanocytic neoplasms (Ramos-Vara *et al.* 2014). Melanocytic tumours are nodular in appearance (Figure 3) and firm on palpation. They may be solitary or multiple and can be positioned dermally or subdermally over intact or ulcerated skin (Phillips and Lembcke 2013).

Several histopathological sub-types of equine cutaneous melanocytic proliferative lesions have been described, but with the exception of the melanocytic naevus, all should be considered to have malignant potential. Diagnosis of the majority of melanocytic tumours is frequently straightforward, due to the heavy pigmentation characteristic of this tumour type

(Figure 4). However, diagnosis of poorly pigmented or amelanotic examples may be challenging, particularly as these tumours may have a range of gross morphologies, which historically led to human amelanotic melanoma being dubbed "the great masquerader" (Koch and Lange 2000). These tumours may also exhibit a variable microscopic appearance. Consequently immunohistochemical staining may be required for increased histopathological diagnostic confidence, and in this regard it is notable that PNL2 has been suggested to be a sensitive immunohistochemical marker of equine melanocytic neoplasms that is more specific than S100 protein or PGP 9.5, both of which are used in the diagnosis of melanocytic neoplasms in humans and other veterinary species (Ramos-Vara *et al.* 2014).

Other types of equine genital tumours

Sarcoids are tumours of fibroblastic origin, usually with an overlying hyperplastic epidermal component. Bovine papillomaviruses (BPV) 1 and 2 have long been implicated in the development of equine sarcoids and BPV nucleic acid has recently been visualised in these tumours using in situ hybridization, adding to the weight of evidence suggesting a causative association (Gaynor *et al.* 2015). Although sarcoids may occur at sites all over the body, many different types are described in the preputial and paragenital regions.

Fibrosarcomas, a malignant proliferation of fibroblasts, may also arise in the penile and preputial regions and are usually firm to fleshy infiltrative masses (Scott 2003). They are invasive and capable of metastasis. Van den Top *et al.* (2008) have reported a fibrosarcoma within the prepuce of a horse. Other tumour types are uncommon findings on the penis and prepuce of horses, but reports include, lymphomas, lipomas, neurofibromas, adenocarcinomas, basal cell carcinomas and haemangiosarcomas (Van Den Top *et al.* 2010). The case report by De Meyer *et al.* (2015) describes the rare finding of a preputial fibroma in

an 11 year old gelding. Other than the large size of this mass, there were no additional clinical signs in this horse. In their paper, the authors describe their diagnostic approach and the histological evaluation of this tumour.

Balanitis or balanoposthitis

Infection or inflammation of the penis and prepuce can occur as a primary finding, due to equine herpesvirus 3, *Trypanosoma equiperdum*, *Habronema spp*., *Halicephalobus gingivalis* (Muller *et al.* 2008), *Pythium spp.*, and numerous bacterial species. As mentioned by De Meyer *et al*. (2015), consideration of geographical prevalence of certain infectious causes of penile and preputial inflammatory lesions is an important component of clinical evaluation. Balanitis or balanoposthitis can also accompany tumours of the penis or prepuce due to ulceration or necrosis of tissues secondary to the neoplastic process. It is important to differentiate the two aetiologies during thorough examination of the external genitalia. Histopathological assessment of tissue sections can be a useful aid in diagnostic assessment, particularly when an underlying neoplasm with secondary inflammation is suspected.

Conclusion

Diseases of the equine penis and prepuce encompass a wide variety of neoplastic and non-neoplastic lesions, the full scope of which is beyond the remit of this Clinical Commentary. In all cases, a thorough and methodical approach to clinical evaluation is required. Whilst diagnosis of some lesions may be clinically straightforward, others may present an excellent opportunity for close dialogue between the clinician and the diagnostic pathology laboratory. Advances in molecular pathology have led to a much better understanding of the pathogenesis of many equine penile and preputial lesions, such as virally associated squamous papillomas, squamous cell carcinomas and sarcoids. Equally, in the field of

diagnostic pathology, additional diagnostic modalities such as immunohistochemistry may aid in the diagnosis of specific lesions such as poorly pigmented or amelanotic melanocytic neoplasms, and in such cases application of equine-specific clinical research is particularly valuable.

Figure Legends

Figure 1: Squamous cell carcinoma. Arrow indicates the raised plaque-like appearance of the lesion.

Figure 2: Photomicrograph of an equine squamous cell carcinoma. Arrow indicates a focus of keratinization within a cluster of neoplastic cells. Scale bar indicates 100 microns. Haematoxylin and eosin stain.

Figure 3: Melanocytic tumour with a nodular macroscopic appearance.

Figure 4: Photomicrograph of an equine melanocytic tumour. In this example, the neoplastic cells are heavily pigmented, obscuring nuclear detail. Scale bar indicates 300 microns. Haematoxylin and eosin stain.

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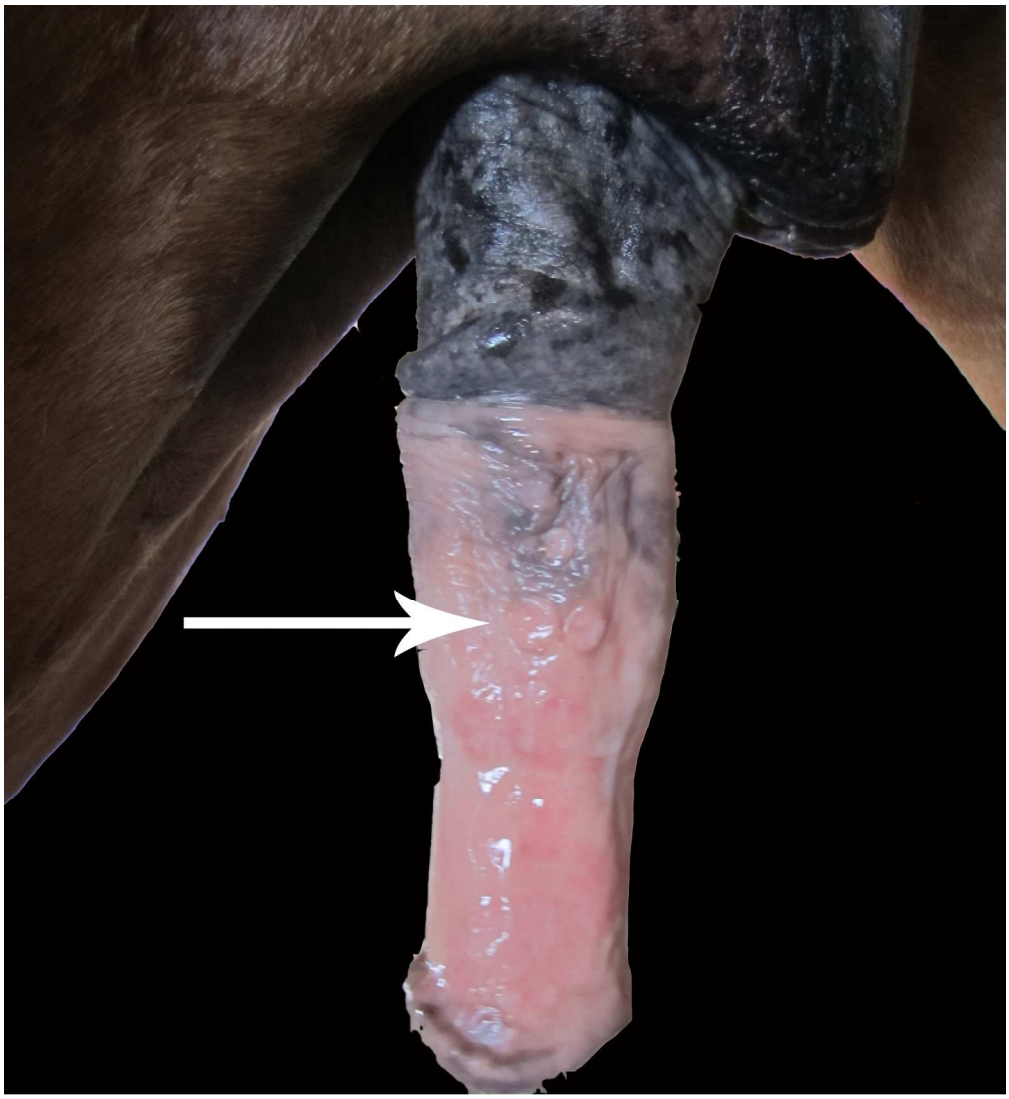

Figure 1: Squamous cell carcinoma. Arrow indicates raised plaque-like appearance of the lesion.
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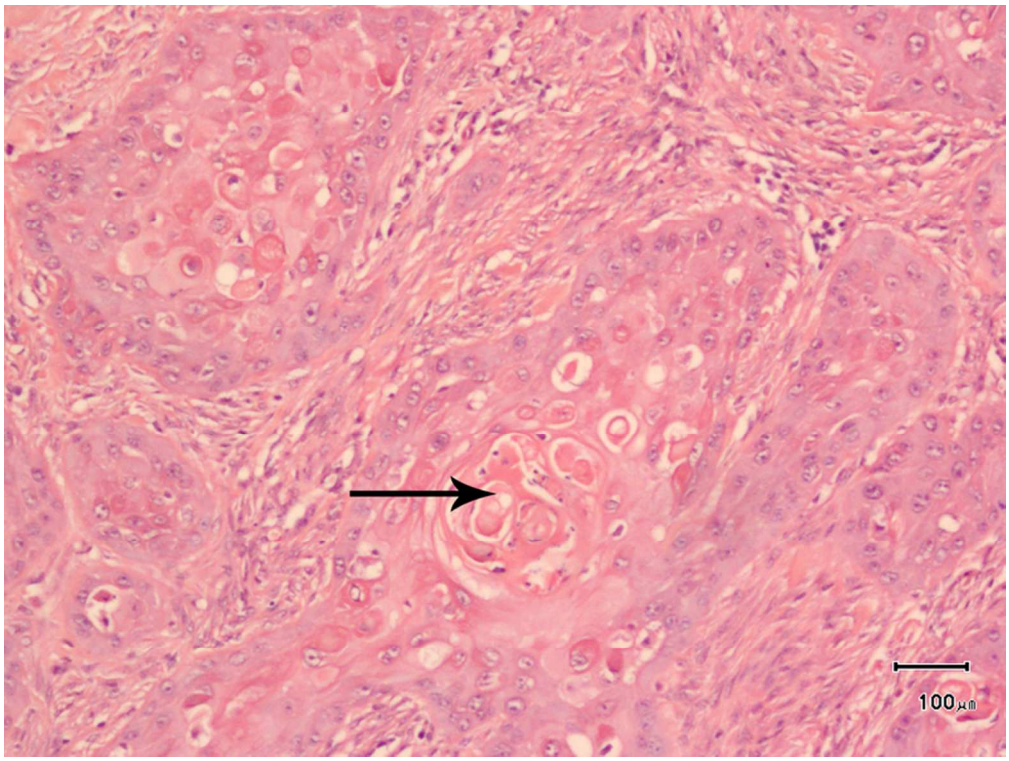

Figure 2: Photomicrograph of an equine squamous cell carcinoma. Arrow indicates a focus of keratinization within a cluster of neoplastic cells. Scale bar indicates 100 microns. Haematoxylin and eosin stain.
75x56mm (300 x 300 DPI)

Figure 3: Melanocytic tumour with a nodular macroscopic appearance.
98x96mm (600 x 600 DPI)

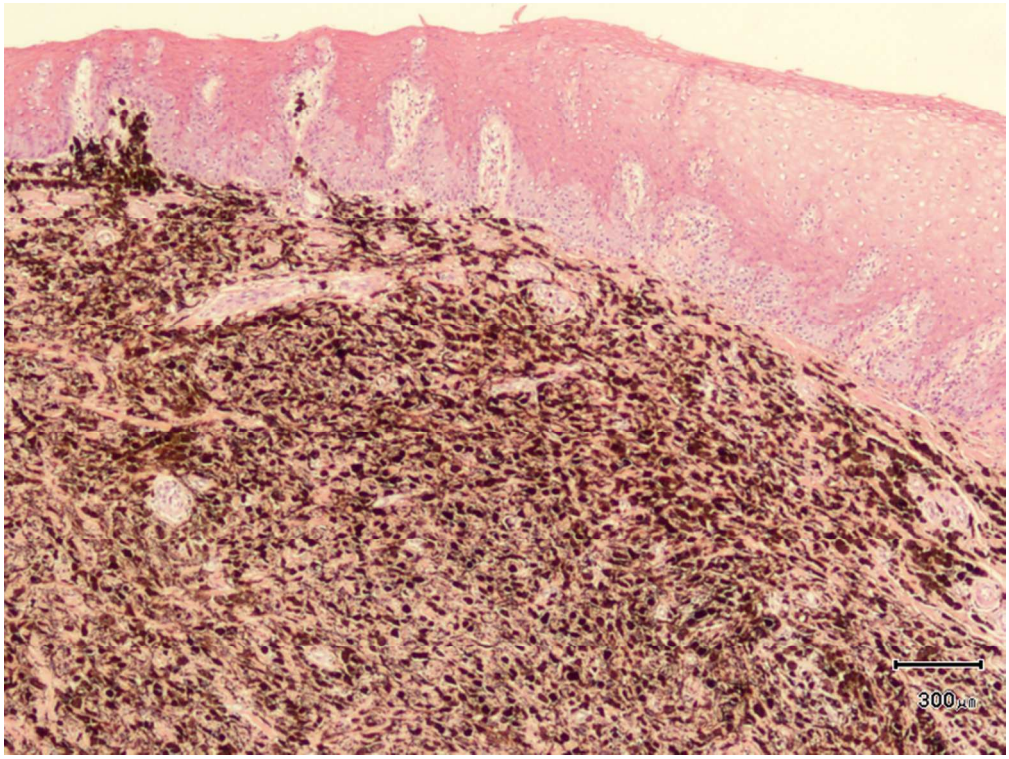

Figure 4: Photomicrograph of an equine melanocytic tumour. In this example, the neoplastic cells are heavily pigmented, obscuring nuclear detail. Scale bar indicates 300 microns. Haematoxylin and eosin stain.
75x56mm (300 x 300 DPI)