Review Article

Melanoma in horses: Current perspectives

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Keywords: horse; equine neoplasia; equine melanoma; dermal melanomatosis; melanocytic nevus; visceral melanoma

Summary

Debate surrounding the nature of equine melanoma has resulted in an underestimation of its life-threatening potential. Contrary to popular dogma, the variable, often slow, rate of growth commonly associated with equine melanoma does not warrant benign classification. Equine melanoma is a malignant neoplasm with the capacity for local invasion and metastasis. A classification scheme was proposed in 1995, but this does not address the progressive nature of equine malignant melanoma (EMM). Additionally, frustration with conflicting therapeutic recommendations has led many practitioners to incorrectly advocate benign neglect. This article addresses the need for a clinically applicable, standardised classification system, provides a review of current therapies and recommendations for equine practitioners, and comments on the future directions of EMM research.

Introduction

Melanoma in horses is a common, variably pigmented (grey/brown/black), infiltrative neoplasm that often presents in advanced stages as a multicentric malignancy. It is most commonly seen in grey- and white-coated horses and, according to one widely-cited reference, reaches approximately 80% prevalence in aged populations (McFadyean 1933). McFadyean (1933) also predicted that virtually all grey horses will develop ‘melanocytosis’ if they live long enough.

The clinical and pathological nature of equine melanoma has been debated for over 200 years. Early authors suggested that equine melanoma was either non-neoplastic (i.e. a mole/pigmented nevus), benign pigment cell dysplasia or a pigment storage disorder (Dick 1832; Leblanc et al. 1952; Law 1916; Levene 1979; 1980; Rodríguez et al. 1997). Others reported melanoma as a true neoplasm; however, debate over classification of melanoma as a benign or malignant neoplasm continued throughout the 20th century (Jaeger 1909; Runnells and Benbrook 1941; Valentine 1995; Robertson 1996). More recently, the concept of equine melanoma as a form of malignant neoplasia - or neoplasia with malignant potential - has become more accepted, with some sources claiming that at least 66% of equine melanocytic tumours eventually become malignant (Scott 1988).

The purpose of this article is to review the current state of knowledge on equine malignant melanoma (EMM), present a new system for staging EMM, discuss current therapies and comment on the future direction of EMM research.

Gross and microscopic pathology

Valentine (1995) wrote an extensive review of the pathological characteristics of equine melanoma based on a retrospective study of 53 cases. She proposed that there are at least 4 manifestations of equine melanotic disease: melanocytic nevus, discrete dermal melanoma, dermal melanomatosis, and anaplastic malignant melanoma. Discrete dermal melanoma and dermal melanomatosis, collectively referred to as dermal melanoma, represent the large majority of melanoma diagnoses in grey horses.

Discrete dermal melanomas are seen in grey horses and generally exist as single masses in typical or atypical locations. Afflicted horses are mature, but not aged, with an average age of 13 years. Discrete dermal melanomas are further differentiated into benign and malignant forms, with surgical excision appearing to be curative in most circumstances; this finding was supported by studies of Rowe and Sullins (2004).

Dermal melanomatosis is a condition seen in grey horses involving multiple cutaneous masses, with at least one of the masses presenting in a ‘typical’ location. These typical sites include the undersurface of the tail, anal, perianal and genital regions, perineum, and lip commissures (Seltenhammer et al. 2004). Like discrete dermal melanoma, dermal melanomatosis is most frequently seen in mature horses. However, dermal melanomatosis is associated with a slightly older average age of 17 years, a figure at variance with our studies in which the mean age was <10 years (Robertson 1996). According to Valentine (1995), these masses are not amenable to surgical resection and are very likely to be associated with visceral metastasis. This condition is considered to be potentially fatal.

Discrete dermal melanomas and dermal melanomatosis are generally histologically indistinguishable, presenting as indistinct, heavily-pigmented tumour cells in the deep dermis (Fig 1). Whilst Valentine (1995) described these manifestations of melanotic disease as distinctly separate, it seems equally probable that these diagnoses exist as a continuum.

Discrete dermal melanoma may represent a malignancy in situ - a ‘condition in which one or several cells have acquired the ability to invade and metastasise, but have not yet
Melanomas in horses, multiple nodules may be seen at once. Nodules commonly occur on the perineum, tail base, sheath, commissures of the lips, in the parotid/jugular furrow and subauricular lymph nodes (Fleury et al. 2000; Seltenhammer et al. 2003) [Fig 2].

During stages of the disease in which there is little growth, lesions may exist for many years and cause no clinical problems for either horse or owner. However, this changes when lesions enlarge and coalesce. In some cases, tumour bulk is so substantial in the area of the throat latch that affected horses are unable to flex at the poll, turn their heads from side to side, or eat and drink in comfort [Fig 3]. Similarly, coalescing perianal tumours frequently become large enough to limit defaecation and result in faecal impaction. Lesions may become quite large and develop necrotic cores capable of ulceration, leading to secondary bacterial infection. Surgical debulking of advanced lesions is difficult and very often unrewarding.

Expansile tumour growth deeply infiltrates musculature and may compromise blood supply, leading to the development of fissures penetrating deeply into soft tissue and, at times, to bone. Figure 4 is an example of such a large, complex mass on the perineum of a 13-year-old Thoroughbred gelding. Tumour growth around the anus was extensive and the horse experienced intermittent discomfort associated with rectal faecal impaction. This was somewhat controlled through dietary modification, but was a persistent problem. At the junction of the tail and the perineum, a fissure developed within the mass that terminated at the adjacent vertebral body. Attempts to debride this fissure and promote healing by granulation were unproductive and the horse was subjected to euthanasia due to pain and incipient osteomyelitis.

Almost invariably, tumour nodules that are expanding are quite firm, strongly suggesting the activation of fibroplasia by tumour cells or in response to tumour cell growth. Likewise, we have seen many tumours that are well-vascularised and this may reflect enhanced angiogenesis as a result of tumour cell proliferation.

There is currently no consensus on whether multiple lesions on a single horse are metastatic or arise spontaneously as multicentric, separate neoplasms. However, there are many references that provide evidence of implantation, lymphatic and haematogenous metastasis (Kirker-Head et al. 1985; Pulley and Stannard 1990; Schott et al. 1990; Murray et al. 1997; Rodriguez et al. 1998; Patterson-Kane et al. 2001; MacGillivray et al. 2002; Covington et al. 2004) [Fig 5].

There are also reports of primary visceral masses. Primary visceral melanomas have been documented in the gastrointestinal tract, vertebral canal, skeletal muscles, guttural pouches and salivary glands of grey horses (Traver et al. 1977; Baptiste et al. 1996; MacGillivray et al. 2002; Caston and Fales-Williams 2010). Tarrant et al. (2001) report one such case in which a 22-year-old, grey, Arabian gelding presented for acute colic and was found to have melanotic masses in the liver, spleen, bone marrow, and superficial cervical and tracheobronchial lymph nodes, but not the skin. The pattern of organ infiltration was consistent with haematogenous, lymphatic and transcocloemomic metastasis and the masses were found to be compatible with dermal melanomatosis, but a primary dermal tumour was not located. Although the authors admit that a primary cutaneous mass may have been overlooked, they also present 2 proposals for the origin of primary visceral melanotic disease. The first of these speculations relies upon the embryological rest theory, which suggests that melanocytes become retained in an embryological rest and experience malignant transformation. Alternatively, these authors propose that abnormal cell migration could result in...
melanocytes in atypical sites. There is little evidence to support or refute either hypothesis, and further studies are necessary if the origin of visceral melanotic disease is to be identified.

Additionally, there are 3 reports of congenital melanoma. All 3 cases involved masses believed to be of dermal origin. The first of these cases involved a 30 x 20 x 10 cm mass on the dorsal midline of a 6-month-old chestnut Arabian colt. This case resulted in humane euthanasia due to the expansile, infiltrative and metastatic nature of the mass (Hamilton and Byerly 1974). The remaining 2 cases involved a 3-month-old grey Quarter Horse filly with a 1 cm ulcerated perineal mass and a 2-week-old (grey at maturity) Appaloosa colt with an 18 x 7 mm mass in the skin of the left lateral thorax. Both were successfully treated with surgical excision (Cox et al. 1989).

Based on clinical observations and a review of published case reports, we agree with the aforementioned suggestion that equine melanoma possesses the potential for malignant behaviour. In particular, dermal melanoma demonstrates a proclivity for local invasion and metastasis. As such, we propose that all manifestations of dermal melanoma in grey horses should be referred to as EMM. In addition, we propose that EMM is best classified into stages of increasing expression of malignant behaviour (i.e. rapid growth, local invasion, metastasis etc.). As the majority of cases seen by us have presented as more advanced manifestations of disease (i.e. not amenable to surgical excision), we recognise the importance of regular, standardised evaluations for detection of significant changes in tumour behaviour. Consequently we have proposed a clinical classification system in which EMM of grey horses occurs in 5 stages (Table 2).

Current therapies for equine malignant melanoma
While many therapies have been tested, there is no widely accepted treatment for EMM. A limited number of early stage malignancies located on the tail or in the perineal region have been surgically excised with apparent success (Rowe and Sullins 2004). However, surgical excision is not a realistic option in many advanced cases, particularly those with significant local invasion of tumours in areas, such as the parotid salivary gland, that pose a more complicated surgical approach. It is also important to note that localised excision does not prevent further progression of disease. Nearly half of the horses in the study performed by Rowe and Sullins (2004) experienced either an increase in pre-existing tumour size or number of tumours at distant or local sites following localised surgical excision.

Cimetidine, a histamine type II receptor antagonist, has been used as a therapeutic agent for melanoma. A study by Goetz et al. (1990) showed cimetidine to be effective at reduction of tumour size and number in 3 adult horses with multifocal melanoma. Diagnosis was confirmed in 2 of the 3
horses via histopathology, while the third was diagnosed based on clinical examination alone. Additionally, while all 3 horses were noted to have multiple masses in multiple anatomic locations, the maximum diameter measured prior to initiation of cimetidine therapy did not exceed 2 cm. Treatment consisted of oral administration of 2.5 mg/kg bwt of cimetidine every 8 h for a period of 4–12 months. All 3 horses were reported to have a 50–90% decrease in size and number of tumours (Goetz et al. 1990). However, a more recent study by Laus et al. (2010) found cimetidine therapy to be ineffective at reducing tumour size or number in horses with dermal melanomatosis, with only one out of 10 treated horses showing a reduction in tumour volume. Histopathology and cytology were used to confirm the diagnosis of melanoma in all of the horses that participated in this study. The trial involved a total of 15 horses evenly divided into a control group and 2 treatment groups, one in which the horses were administered 3.5 mg/kg bwt of cimetidine orally every 12 h for 60 days and a second in which the horses were administered 7.5 mg/kg bwt of cimetidine orally daily for 60 days. The horses were evaluated weekly during treatment and monthly following treatment for a total of 12 examinations. The only horse to show a slight, localised, reduction in tumour size belonged to the second treatment group (7.5 mg/kg bwt per os s.i.d.) (Laus et al. 2010). The findings of Laus et al. (2010) are in congruence with similar studies by Bowers et al. (1994) and Warnick et al. (1995).

Various mechanisms of action for cimetidine have been proposed, including selective inactivation of suppressor T cells, enhanced natural killer cell activity, antagonism of histamine type II receptors and anti-inflammatory effects, but their significance with regard to treatment of EMM has yet to be established (Eisenthal et al. 1986; Sahasrabudhe et al. 1987; Whitehead et al. 1988; Wamnick et al. 1995). Inconsistent results have made cimetidine an unreliable treatment option for horses with EMM. Some propose that these inconsistencies may be related to the variability within EMM itself, suggesting that variations in cellular metabolism, differentiation or receptor activation may be responsible (Laus et al. 2010), but there is no evidence to substantiate this theory.

Treatment of EMM using toremifene, a triphenylethylene derivative, has also been attempted. This orally administered compound has primarily been used in the treatment of human patients with metastatic breast cancer (Ebbs et al. 1987; Valavaara et al. 1988), but has been shown to exhibit antioestrogenic and nonantioestrogenic inhibitory effects on melanocyte lines in vitro (Mäenpää et al. 1993). Toremifene was administered transdermally in a topical gel preparation to one horse with malignant melanoma. It was found to provide high local concentrations at the site of administration and a slight reduction in tumour volume, but results were never verified with histopathology and further studies were not performed (Soe et al. 1997).

Recent reports indicate that cisplatin might be useful in the treatment of equine dermal melanoma. In one such study (Theon et al. 2007), 13 horses with 16 subcutaneous and

**Fig 2:** Multiple, small, distinct melanoma nodules on the perineum and ventral tail of an 11-year-old Thoroughbred gelding (photo courtesy of Ms Karen Witter).

**Fig 3:** Multiple coalescing nodular and plaque-like melanomas in the area of the parotid salivary glands and subauricular lymph nodes of an 11-year-old Thoroughbred gelding.
Dermal melanotic tumours were treated with a sesame oil-based cisplatin formulation administered via intratumoral injections. Epinephrine was added to the cisplatin formulation to optimise drug localisation and prevent bleeding. Treatment consisted of a series of 4 intratumoral administrations at 2 week intervals, resulting in an 81% success rate. Therapy was less effective in larger, more advanced tumours and in tumours that had previously been treated by other methods, especially cryotherapy. In addition, new tumours continued to develop outside of the treated area during the follow-up period, which ranged from 2 to 6 years.

Similarly, a study testing the efficacy of implanting cisplatin-containing biodegradable beads in various equine cutaneous tumours, including melanoma, has also been performed. Each 3 mm bead contained approximately 1.6 mg of cisplatin in a commercially available calcium sulphate and dextran sulphate matrix material. Two out of 13 cases of cutaneous melanoma were treated with beads alone, while the remaining 11 cases received a combination of conventional debulking or CO₂ laser debulking in addition to bead implantation. Researchers found that treatment resulted in ‘successful resolution of the tumour for at least 2 years after treatment’ (Hewes and Sullins 2006).

A preliminary study to evaluate the therapeutic potential of an autochthonous vaccine has also been performed (Jeglum 1997). The vaccine contained whole, autogenous tumour cells combined with adjuvant and was injected subcutaneously over regional lymph nodes. In the study, the vaccine was administered every other week for 6 weeks and then every 6 weeks for an undisclosed period of time. The author reported tumour regression in 11 out of 12 horses; however, corroborative data have yet to be presented (Jeglum 1997). In addition, a study examining the efficacy of local suicide gene therapy in combination with a systemic anti-cancer vaccine was evaluated in a single horse with melanoma (Finocchiaro et al. 2009). In this study, superficial tumours were removed under general anaesthesia and used, in combination with live irradiated xenogenic Chinese hamster ovary cells, to prepare a vaccine that was later injected subcutaneously into the flanks. Vaccine administration occurred weekly for 5 weeks, every 2 weeks until Day 105 and then every 28 days until Day 245. In addition, the surgical margins were infiltrated with lipoplexes carrying the HSVtk gene and ganciclovir directly following surgery. Weekly doses of HSVtk lipoplexes and ganciclovir were injected into the remaining subcutaneous tumours. After 5 localised treatments of 6 masses, 4 ‘developed draining fistulae that healed and dissolved completely’ and 2 decreased in size by approximately 50%. Three untreated lesions decreased in size until they disappeared completely. Follow-up continued for 33 months and the horse was reported to have no local relapses of melanoma in the areas that had been treated surgically, but follow-up regarding other tumour sites was not mentioned (Finocchiaro et al. 2009).

An alternate immunotherapeutic approach that has shown some promise in treating EMM is interleukin therapy. In 2001, a study aimed at evaluating the effects of intratumoral IL-12 injection on grey horses with metastatic melanoma was performed (Heinzerling et al. 2001). Twelve masses in a total of 7 grey horses received intratumoral injections of IL-12 encoding plasmid DNA in a series of 1–4 cycles. Each cycle consisted of 3 injections administered every 2 days. Mean tumour size, as evaluated by caliper measurements, was reduced to 41% of baseline after a single treatment cycle (Heinzerling et al. 2001). The results of a follow-up study evaluating the effects of intratumoral injection of IL-18 and IL-12 encoding plasmid DNA on 26 mature grey horses with metastatic melanoma have recently been published (Müller et al. 2011). Treatment consisted of 2 cycles, spaced 2 weeks apart, of 3 intratumoral injections of approximately 250 μg of plasmid DNA administered every 2 days. Changes in tumour

Fig 4: Large confluence of nodular and plaque-like melanomas on the ventral tail and perineum with ulceration and fissure formation at the junction of the tail and perineum in a 13-year-old Thoroughbred gelding.

Fig 5: Multiple coalescing tumour nodules in the mesentery of a horse subjected to euthanasia for progressive weight loss and colic.
size were primarily evaluated by caliper measurements, but ultrasound evaluation was also performed when possible. Sixty-four days after initiation of treatment, tumours injected with IL-12 encoding plasmid DNA demonstrated an average decrease in tumour volume to approximately 80% of baseline caliper measurements. IL-18 was somewhat less effective, with final tumour volumes averaging approximately 90% of baseline measurements. Control masses, injected with empty plasmid DNA, demonstrated a slight increase in tumour volume, averaging approximately 110% of baseline by Day 64 (Müller et al., 2011). Both studies showed the therapy to be well-tolerated, with occasional, mild, peritumoural swelling as the only noted adverse effect (Heinzerling et al., 2001; Müller et al., 2011).

Genetics and genomics
A connection between grey coat colour and EMM has long been recognised. A fully dominant, autosomal trait controlled by a cis-acting regulatory mutation, more specifically a 4.6 kb duplication in intron 6 of syntaxin-17, has been proposed as the probable genetic link between grey coat colour and EMM. The mutation is associated with 4 genes: NR4A3 (nuclear receptor subfamily 4, group A, member 3), STX17 (syntaxin 17), TXNDC4 (thioredoxin domain-contain-4') and INVS (inversin), with notably higher levels of STX17 and NR4A3 present in melanomas. Horses that were homozygous for the mutation were found to grey more rapidly and were also more homogeneously white with less speckling than heterozygous horses. Homozygotes were also found to exhibit a significantly higher incidence of melanoma (Piebberg et al., 2008).

Further studies are needed to evaluate the association between mutations and EMM to determine whether manifestations of the disease are related to one or a combination of these genes or perhaps others that have yet to be identified. A complete study of the equine genome and further study of the physiological mechanisms associated with the implicated genetic mutation(s) are necessary. We believe that there must be common mutations leading to the high incidence of EMM, that the acquisition of multiple mutations may account for the progression of clinical stages, and that identification of mutations will be the first step in developing effective targeted therapies.

Summary and clinical implications
Based on uncertainties and controversy in the veterinary literature, many veterinarians counsel horse owners that most melanomas will follow a benign, lengthy clinical course. Unfortunately, these ‘benign’ tumours may eventually lead to the humane destruction of affected horses, when tumour bulk, location or infiltrative growth compromises various functions, including eating, drinking, defaecation, urination or breeding.

Many veterinarians do not biopsy suspect lesions, feeling that gross appearance of darkly pigmented nodules, alone, is sufficient for diagnosis. These veterinarians may also feel that biopsies are unnecessary, since they consider lesions to be benign. Paradoxically, some equine specialists appear reluctant to either surgically biopsy or resect melanomas for fear of inciting aggressive tumour growth or promoting the development of tumour metastases. This reluctance is not supported by data in published studies, which show that localised lesions can be effectively removed (Rowe and Sullins, 2004).

Practitioners who are presented with a mass they suspect to be EMM are encouraged to perform an excisional biopsy, when possible, and submit the sample for histopathology. Although EMM has a fairly characteristic gross appearance and distribution, it is critically important to get histological confirmation of tumour identity. Proper confirmation and documentation of diagnosis is not only important for guiding treatment, but is also essential if relationships between histopathological characteristics, therapeutic efficacy and prognosis are to be identified.

All owners of grey horses, regardless of the horse’s current disease stage, should be educated regarding the probability of this disease occurring and encouraged to perform regular examinations to identify new masses and/or evaluate tumour growth rate. Confirmed cases of EMM should be treated as malignant, regardless of histopathological classification. Surgical excision should be encouraged in cases with masses in Stages 1 or 2 in locations that allow for complete surgical excision. Horses in advanced stages of disease are unlikely to benefit from surgical intervention alone. Cisplatin may be used alone to decrease tumour bulk in large tumours or as an adjunct to surgical debulking. Additionally, various trials for experimental therapies are ongoing and may represent the best treatment options for horses in Stage 4.

Understanding of the factors involved in the development and progression of EMM is limited, especially in contrast to the knowledge base associated with human and canine malignant melanoma. Multiple therapies have been developed and studied, but there are no successful treatments for advanced stages of EMM. Much further study is necessary if we are to understand the genetics and pathophysiology of this disease and ultimately produce successful therapeutic and preventative options.

Authors’ declaration of interests
No conflicts of interest have been declared.

References