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Cutaneous Melanoma in the Grey Horse

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Ekvina kutana melanom hos skimlar

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SAMMANFATTNING

Ekvina kutana melanom uppstår mycket oftare hos skimlar än hos hästar med annan pälsfärg. En del forskare har dragit slutsatsen att melanomen är neoplasier som kan klassificeras efter utseende och utbredning makro- och mikroskopiskt. Ju mer välavgränsade tumörerna är, desto mer benigna verkar de vara. Andra forskare menar att hudförändringarna i själva verket är resultatet av en störning i pigmenteringen som har att göra med hur hästarnas gråa pälsfärg utvecklas. Enligt denna teori är melanomen inte maligna, men har ändå förmågan att sprida sig i kroppen.

De vanligaste områden för primära kutana melanom är undersidan av svansen, det peri-anala området, olika områden på huvudet och i anslutning till könsorganen. Vanliga ställen för metastaser är bl.a. lymfknutor, blodkärl, bukhinna, mjälte, lungkapillärer, skelettmuskulatur, lever, öronspottkörtel, ben och benmärg.

DNA-studier på skimlar med och utan kutana melanom har visat en koppling mellan vissa genotyper och sannolikheten för att utveckla kutana melanom. Detta skulle kunna förklara varför skimlar drabbas i mycket större utsträckning än hästar av andra färger.

SUMMARY

Equine cutaneous melanomas arise much more often in horses with a grey coat color than in horses of other colors. Some scientists have in different studies come to the conclusion that the melanomas are neoplasms which can be classified by examining their histological and macroscopic features. The more defined and demarcated a tumor is, the more benign it tends to be. Other scientists believe that the skin lesions are the result of a pigmentation disorder that is linked to the greying of the coat color. According to this theory, the lesions are not malignant although they have the ability to metastasize.

The most common areas for primary cutaneous melanomas are underneath the tail, the peri-anal region, different sites of the head and on or close to the genitalia. Common sites for metastases are e.g. lymph nodes, blood vessels, the peritoneum, the spleen, capillaries of the lungs, skeletal muscle, the liver, the parotid salivary gland, bone and bone marrow.

Studies of the DNA from grey horses with and without cutaneous melanomas have shown a correlation between certain genotypes and the likelihood of developing cutaneous melanomas. This could explain why grey horses are much more probable to develop these tumors than horses of other colors.

INTRODUCTION

Equine cutaneous melanomas are far more commonly observed in grey horses than horses of solid colors. The melanomas have different features, both macroscopically and microscopically, which can be predictive of prognosis and malignancy. The objective of this study of literature regarding cutaneous melanomas in grey horses is to describe the nature and etiology of these lesions. Not everyone doing research in the field of cutaneous melanomas believe that these are in fact true neoplasms. I have focused on why grey horses are more likely to be affected than horses of other colors. A classification of different tumors, as well as common sites for primary lesions and metastases is also addressed.

When working as a veterinarian, these things are important to have insight in so that correct diagnosis can be made. The diagnosis is essential for the expected prognosis of a tumor, and will therefore have a great impact on any decision (e.g. concerning treatment) an owner of a melanoma-bearing horse might have to make. Also, I believe that gaining more knowledge about neoplasms in one species will only help us to understand more about cancer in other species, such as humans.

MATERIALS AND METHODS

When searching for articles I used multiple on-line databases, but mostly PubMed (20110201), Google Scholar (20110201) and Web of Science (20110215). With search terms such as (melanom* OR neoplasm* OR pigmentation disorder*) AND (horse* or equine) AND (grey OR gray) the search results were reviewable. Many articles seemed very well grounded and interesting when reading the abstracts, but unfortunately I found that they were often written in German or other languages. I could therefore not include them in my literature study, since I only understand English and Swedish.

RESULTS

Description, definition and classification

The grey horse

A grey horse is born with a dark fur coat and dark skin. With each shedding, the fur becomes lighter and lighter until finally turning white. The skin however stays dark throughout the horse's life. Dermal melanomas in grey horses are very common, with 70-80% of all grey horses affected by the age of 15 years (M'Faydean, 1933).

Cutaneous melanomas

Melanomas derive from the mature, pigment producing cells found in the skin and in other areas of the body. These cells are called melanocytes and originate from the neuroectoderm (Moulton, 2002). In a study of 53 horses with cutaneous melanoma four different tumor patterns were recognized (Valentine, 1995):

- Melanocytic nevi (29 horses)
    - Average 5 years of age, any coat color (17 greys, 12 of other colors).
    - Histologically distinguishable from other tumor patterns.
    - Benign, involving superficial dermis and/or the epidermal-dermal junction.

    Others have added more features to the description of the melanocytic nevi:

    - Common sites for these tumors are legs or trunk, whereas they are rarely found in the area of the tail, perineum or genitals (Scott & Miller 2011).
    - In some literature referred to as melanocytomas. Depending on their location within the layers of the skin they can be classified as junctional, intradermal or compound (Jones & Hunt, 1997).

- Dermal melanoma (8 horses)
    - Average 13 years of age, only grey horses.
    - Solitary demarcated, hemispheric nodules involving deep dermis.
    - Metastasizes sometimes (2 of 6 horses at follow-up).

- Dermal melanomatosis (12 horses)
    - Average 17 years of age, only grey horses.
    - Histologically similar to dermal melanoma but distinguished from this by clinical features.
    - Infiltrative plaque with or without associated nodules involving deep dermis.
    - Will often metastasize (6 of 6 horses at follow-up).

- Anaplastic malignant melanoma (2 horses)
    - Older horses of all colors.
    - Histologically distinguishable from other tumor patterns.
    - Large, locally invasive, often poorly pigmented.
    - Metastasizes quickly.

Fleury et al., (2000a) described equine cutaneous melanomas to be of mainly two different characters; either they were nodule-like or plaque-like (Table 1). The latter was mainly observed underneath the tail of the horses, and when the plaques were large they were also associated with nodules. These observations correspond well to the classifications determined by Valentine (1995) regarding dermal melanoma and dermal melanomatosis. Histologically the two tumor types were of mainly two patterns, or a mixture of these. Fleury et al., (2000a) speculated in whether all plaque-like tumors transform into nodule-like tumors. They supported their theory with the finding that tumor cells were present in the periphery of apocrine sweat glands in both tumor types. The authors also believed all the tumors they found were of a melanomatosis type described by Valentine (1995). In contradiction to Valentine (1995), Fleury et al., (2000a) thought that some of the melanomas found might originate from the apocrine sweat glands and not from melanocytes. “Ulceration of the lesions, as well as mutilation such as loss of the tail were observed in advanced cases. In very

rare instances, we observed subcutaneous masses of pigment in the parotid gland region. Observed complications of melanomas were considered to be due to mechanical scratches by feces or infection of wounds. The animals with severely developed tumors in peri-anal regions had diarrhea and transit difficulties leading to cachexy." Cited from Fleury et al., (2000a).

Table 1. Two different types of cutaneous melanoma tumors and their characteristics, as described by Fleury et al., (2000a)

	Nodule-like tumors	Plaque-like tumors
Appearance	Black or blue, hemispheric, raised from the skin surface	Infiltrative plaque, when larger associated with nodules
Size	0,5 – >10 cm in diameter 1,5 – 5 cm in height	5 – 20 cm in diameter
Macroscopic features	Tumor masses were heavily pigmented, round and extruding from epidermis, although only involving dermis.	Pigmented areas in subcutis, distant from epidermis; occasionally larger round pigmented masses. No extrusion from epidermis.
Microscopic features	Heavily pigmented areas consisted of concentric ring of cells separated by connective tissue. In association with these areas, lightly pigmented tumorous masses were found, similar to those near apocrine sweat glands in the plaque-like tumors. Apoptotic bodies were present.	Small, lightly pigmented cell masses with atypical melanocytes in association with apocrine sweat glands; as well as larger, heavily pigmented cell masses in reticular dermis. The melanocytes were large, irregular and clear with large nuclei. Melanophages were present containing a variation in the amount of granula.

In another study, 58 grey horses, mostly Spanish Pure Breed (SPB) or crossbreeds of SPB, were examined to determine whether the cutaneous melanomas of grey horses are in fact neoplasms, or if they are the result of a pigmentary disorder, as some believe. 52 of the 58 horses had what the authors, Rodríguez et al., (1997), called dark melanotic (DM). Based on the description of theses DMs, they seem to be same type of lesion that has been referred to as cutaneous melanomas by previous authors. Leukotrichia (whiteness of the fur in a circumscribed area) was seen in 6 horses. Biopsies were taken from normal looking skin, primary DM and metastatic DM. The tissues were treated with special antibodies serving as markers so that melanocytes as well as macrophages could be detected. Also, a marker indicating nuclear proliferation was used to determine the proliferative rate of the DMs. DM was found in all horses older than 10 years, and was observed in horses as young as 3 years. In 34 cases, the DMs were solitary, and in 18 cases they were multiple or a combination of multiple and solitary. The DMs were of different shapes and sizes, and black or grey except in 5 cases where they were ulcerated and/or depigmented. Necropsy was performed on 9 horses, 2 of in which no DM was found, and 1 in which DM was detected only after sectioning the

skin of the tail. Lymph nodes and parotid glands were involved in 5 cases each, and serous membranes and muscles in 4 cases each. Sites of primary tumors are summarized in Figure 1. Histologically, neither the skin lesions nor DM in internal organs showed any signs of malignancy (no junctional activity in skin lesions and almost no inflammation). Instead, the lesions were found to have an appearance reminding of human blue nevi, similar to melanotic nevi in horses. The authors suggested that DM therefore might not be a neoplasm. Intensely pigmented cells were observed in peri-follicular and peri-vascular arrangements. The DMs increased in size with age and involved the superficial and deep dermis. They consisted mainly of melanocytes and melanophages. No junctional activity was observed. The marker used to detect nuclear proliferation showed a proliferation index of 1-2 % in the skin and 1 % in metastases. This is indicative of benign or non-neoplastic processes. The authors believed this further supported the theory that DMs are the result of a pigmentation disorder and not neoplasms (Rodríguez et al., 1997).

Common sites of primary tumors

In the previously mentioned study of 264 grey Camargue-type horses by Fleury et al. (2000a), 83 were found to carry melanoma. Examination of these 83 horses showed that the most common sights for primary lesions were underneath the tail and the peri-anal region (Figure 1).

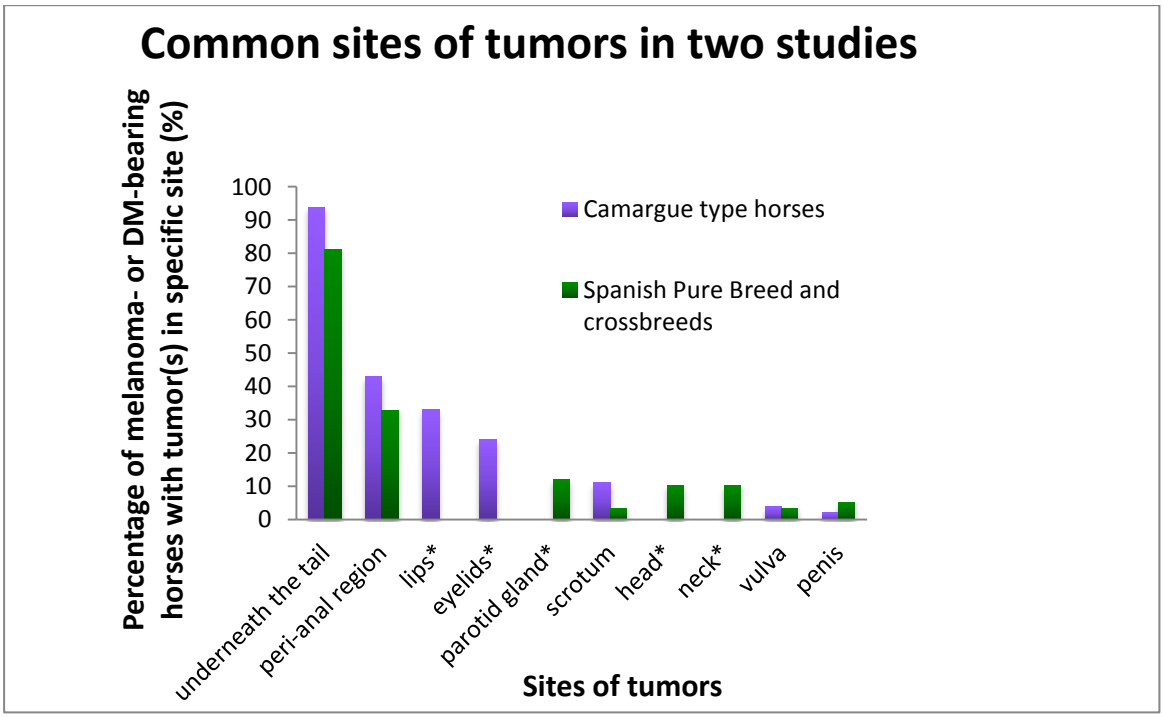

*Figure 1. Common sites of tumors in Camargue-type horses and Spanish Pure Breed and crossbreeds. *In the two studies, sites for tumors were defined in different ways. After Fleury et al., (2000a); Rodríguez et al.,(1997).*

Common sites of metastases

In the study of 83 Camargue-type horses with cutaneous melanoma, necropsy was performed on four horses. Metastasis was detected in primarily regional and distant lymph nodes, but also in the parotid gland, the linear peri-lymphatic area in the inter-costal spaces, thoracic lymphatic vessels between effected nodes, the lungs, the liver and the peritoneum. Horses affected with melanoma in this study were rarely found to suffer from loss of function of organs due to metastasis to visceral areas. Signs of dysfunction were only present when several inner organs had been affected. (Fleury et al., 2000a).

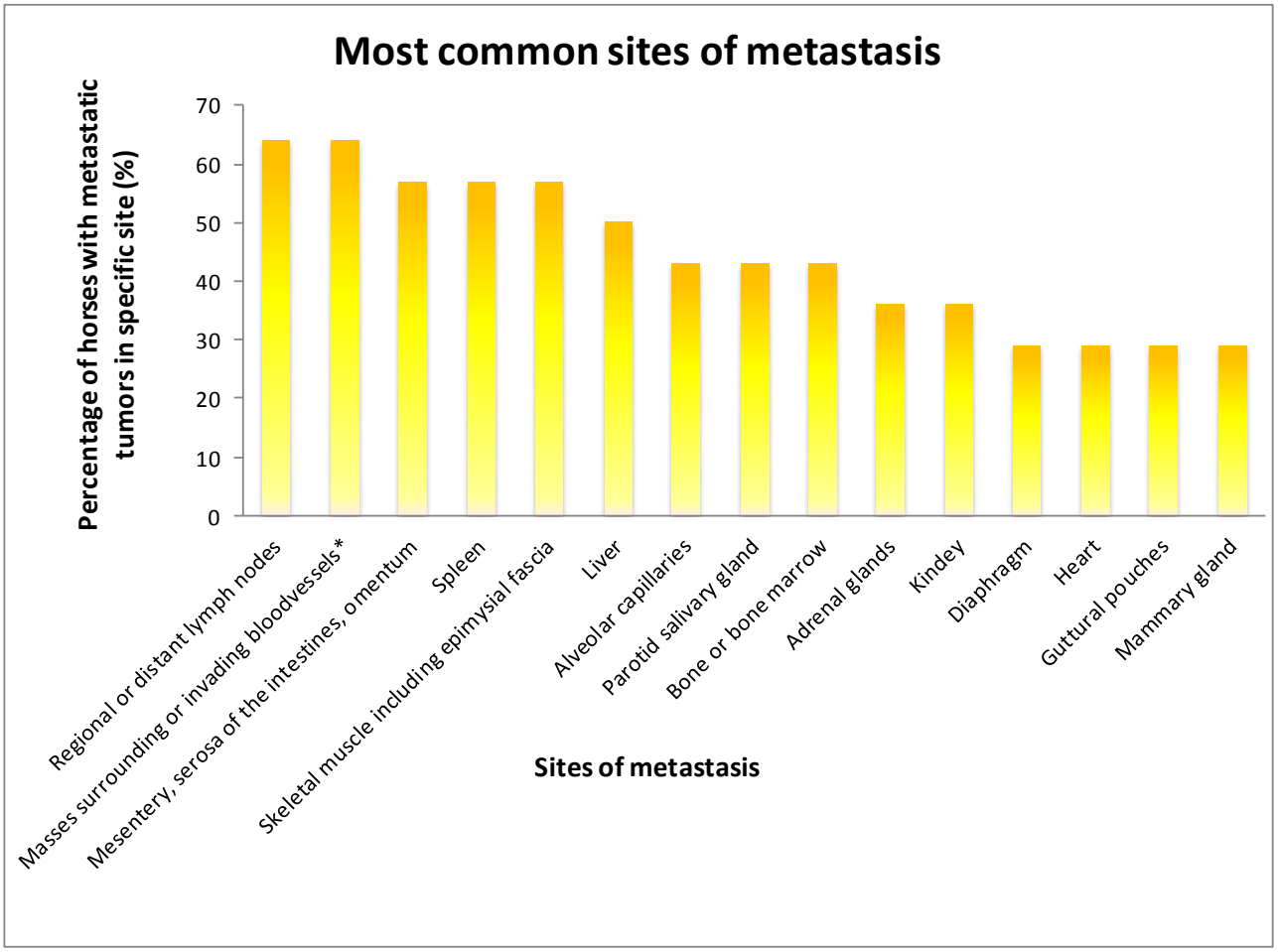

*Figure 2. *Meaning all blood vessels except alveolar capillaries. After MacGillivray et al. (2002).*

14 horses with histologically confirmed melanoma in areas other than the epidermis were studied by MacGillivray et al., (2000) at an animal hospital. All horses were older (median age was 16.5 years). Their patient records were examined and a gross post mortem examination had been performed on all horses. Even though no selection for coat color was made in the studied horses, all 14 horses that were diagnosed with metastatic melanoma at the hospital during the study period were grey. Characteristic skin tumors were present in 11 horses. In one horse with no cutaneous masses the parotid salivary gland was thought to be the area of the primary tumor. 11 horses had tumors involving the peri-anal and anal region, and 9 of these 11 horses also had tumors underneath their tail. In 7 horses, subcutaneous

masses were detected, and in 2 horses masses were found in adipose tissue. The primary complaints for which the owners of the horses had brought them to the hospital included weight loss and depression, dermal melanoma, epistaxis, ataxia, colic, peripheral edema and respiratory distress. The cause of death was metastatic melanoma in 12 of the horses (natural or euthanized). Weight loss, depression and dermal melanoma were the primary complaints in 4 cases. Before euthanization, rectal examination was performed in 2 of these horses. Intra-abdominal masses were detected in both cases. In 9 of the other 10 horses antemortem diagnostic tests were performed and metastatic melanoma was confirmed in 5 cases. 4 of the 10 horses were rectally examined and intra-abdominal masses were detected in all 4. 2 horses were presented with epistaxis, and both were found to be anemic. Upper respiratory endoscopy was performed and melanoma was found in one or both guttural pouches of both horses. The most common sites for metastasis according to the study by MacGillivray et al. (2002) are summarized in Figure 2.

Histology and Immunohistology

From studying the 14 horses with metastic melanoma, MacGillivray et al., (2000) made some histological observations. The neoplastic melanocytes were found to have large, round or ovoid and euchromatic nuclei with distinct nucleoli. They were spindloid to stellate in shape, and surrounded by melanophages of different sizes. Mitotic figures were rarely observed. In all 14 horses the histological features were similar except in one case where the cells had the appearance of an anaplastic malignant melanoma.

In 2004, Seltenhammer et al., had the objective to compare human malignant melanoma and grey horse melanoma. Focusing on the latter, 27 tissue samples from histologically confirmed cutaneous melanoma from grey horses (16 benign, 11 malignant) and four tissue samples from malignant melanoma in solid colored horses were examined. 11 non-melanomic equine tissue samples were used as controls. Melanomas were dominated by different types of cells, for example spindle cells, epithelioid cells, or a mixture of the two. Three different types of melanoma were distinguished (Seltenhammer et al., 2004):

- Benign dermal grey horse melanoma. Homogenous, often with a pseudo-capsule. Well-defined areas of heavy pigmentation, often in association with apocrine sweat glands or skin adnexa. Mixed cell type tumors were a common feature. Melanophages associated with apoptosis in the center of tumors as well as nuclear atypia was also observed. Immunostaining showed highly proliferative melanocytes in the margins of tumor areas.
- Malignant dermal grey horse melanoma. The sizes of cells and nuclei differed between cells, and mitotic figures were observed. Pleomorphic epithelioid cells and balloon cells were the most common cell types. The tumor areas were diffusely defined, and the pigmentation ranged from heavily pigmented to amelanotic areas. In some cases vascularization was increased and activated lymphocytes could be seen.
- Anaplastic malignant solid-colored horse melanoma. Mitotic activity was high and vascular as well as lymphatic invasion was seen. Tumor cells often had two nuclei and

were mainly pleomorphic polyhedral and epithelioid cells. Tumor areas were vague, and in two cases tumors were amelanotic. Also, in two cases there was junctional activity where the epidermis was ulcerated.

Melanomas were located in dermis and/or subcutis. Specific for the grey horse melanomas was that no tumor cells were seen in the dermal-epidermal junction. With the exception of two malignant tumors, none of the tumors involved epidermis (Seltenhammer et al., 2004).

The proliferation markers Ki-67 and PCNA used in immunohistology were found to help distinguish between benign and malignant tumors (Seltenhammer et al., 2004). The melanocyte differentiation antigen gp100 occurred much more frequently in malignant tumors than benign. The authors came to the conclusion that based on the immunohistological and histological features seen in these tumors, they are in fact neoplasms and not pigment storage disorders as Rodriguez et al., (1997) believed. Scott & Miller (2011) on the other hand, reported that no difference could be detected in Ki-67 or PCNA when comparing metastatic and benign melanomas, and that these markers therefore were of no use concerning predictability of tumor behavior.

The melanoma-associated antigens Melan-A, MAGE-1, MAGE-3 and PCNA were found to be expressed at higher levels in faster proliferating cells in cell cultures (Chapman et al., 2008). MAGE-genes are not expressed in melanotic nevi but are often found in malignant cutaneous melanomas of humans. Immunohistochemical staining for these genes might therefore be an important prognostic tool (Busam et al., 2000).

Etiology

Although different theories have been presented on how dermal melanomas and melanomatosis arise, the actual mechanism has not yet been determined. One theory describes that the altered metabolism of melanin is the initial cause. In greying horses, melanin is produced by melanocytes, but the melanin granula is not incorporated into the hair follicles, and therefore the hairs become unpigmented. The altered metabolism leads to hyperplasia of melanoblasts, either by new melanoblasts forming, or by increased activity of existing melanoblasts. The hyperactivity results in overproduction of melanin, and it is here that hyperplastic melanoblasts are thought to become malignant. In contrast to the development of melanomas in humans, exposure to UV-light is not considered a risk factor, since these tumors in grey horses often arise in shaded areas such as the peri-anal region (Scott & Miller, 2011).

In another article written by Fleury, et al. (2000b) it was found that the onset of the melanoma occurred most frequently between five and nine years of age in Camargue horses. Gender did not seem to be a risk factor. Because tumors were most frequently found in the shady areas of the peri-anal region and underneath the tail, sunlight was not considered to be a risk factor either. When taking in mind the areas where tumors were most frequently found, the authors suggest that it is likely that the pathogenesis for these tumors is associated with mucus-associated, hairless, thin skin.

A study was performed on 296 grey Lipizzaner horses older than four years to gain further knowledge on the impact of heritability on, and the occurrence of, melanocytic tumors. All horses had pedigrees that could be traced back up to 32 generations. Out of the 296 horses, 148 (50 %) were found to be melanoma bearing. 75.6 % of affected horses had tumors underneath their tail, but only 4.3 % of the tumors were located in the peri-anal and anal region. In 50 % of the peri-anal and anal tumors, depigmentation similar to vitiligo was observed in the same area. This was mostly seen in older horses. A 0-5 scale was used to evaluate the severeness of the tumors (0 being free of melanoma, 5 being ulcerated tumors, spread to internal organs and causing secondary symptoms such as fever and diarrhea). Although several horses were found to be stage 4 cases ("Extensive confluent melanoma, covered with skin, signs of destruction (necrosis, ulceration) and metastasis", cited from Seltenhammer et al., (2010)), none of them showed any clinical illness. This supported the authors' theory that cutaneous melanocytic tumors in grey horses show less malignancy than in horses of solid colors. Comparing the prevalence of melanomas in horses from different studfarms, the authors found that hereditability had a statistically significant effect on the development of the tumors (Seltenhammer, M.H. et al., 2010).

Genotype and melanoma

The grey phenotype has been shown to be caused by a duplication in an intron (STX17), resulting in a *cis*-acting regulatory mutation (Rosengren Pielberg et al., 2008). A *cis*-regulatory is an area of the DNA-molecule that will regulate the expression of a gene on the same chromosome. It was also shown that the gene carrying the mutated intron, along with a neighboring gene, were both overexpressed in melanomas found in grey horses. The prevalence of melanoma was found to be higher in horses carrying a loss-of-function mutation in ASIP (agouti signaling protein). Four genes were found in the region of the mutation causing the grey color, and all these four genes were expressed in melanomas. 694 grey Lipizzaners were genotyped for the duplication in STX17. It was found that horses with a homozygote duplication shifted from dark grey to white much faster than heterozygote Lipizzaners. The prevalence for melanoma and vitiligo was much lower in the heterozygote horses. The authors believed that the duplication acts predisposing for the development of melanoma by stimulating the dermal melanocytes to proliferate. (Rosengren Pielberg et al., 2008).

Using Northern blotting analysis, the expression of two melanocyte-specific antigens was found to differ between grey and solid-colored horses. In solid-colored horses normal levels of mRNA were found, in grey unaffected horses none was detected but in tumor samples from grey horses expression was high. The fact that there was no expression in the grey, healthy horses was thought by the authors to indicate a roll in the depigmentation of greying horses. Pedigrees and phenotypic information for 71 different Camargue horses was analyzed using 6 different heritability models. The results supported the theory that prevalence for melanoma in grey horses is strongly correlated to the horses' genetic heritage. Therefore, the authors

believed that selection for melanoma-free horses would have an important impact on health for grey horses. Although they are far more susceptible to the cutaneous melanoma, malignancy is much rarer than in solid-colored horses. The authors of this article suggested a possible hypothesis regarding this. Because of the reduced activity of melanocytes in greying, aging horses, tumor growth and metastasis might progress in a much slower way. This allows the grey horse to stay healthy and unaffected for a much longer time than a solid-colored horse with the diagnosis of cutaneous melanoma (Rieder, S. et al., 2000).

DISCUSSION

As the study by Valentine (1995) was the first one I read when researching cutaneous melanomas in grey horses, the patterns she described for different melanomas became a benchmark when reading other articles. I find that tumors studied by other scientists for the most part fit well into the classifications melanocytic nevi (being benign), dermal melanoma (which will metastasize sometimes), dermal melanomatosis (which will often metastasize) or anaplastic malignant melanoma (which will metastasize quickly). Dermal melanoma seems to be the same type of tumor as Fleury, C. et al. (2000a) describe as nodule-like tumors, in the same way that dermal melanomatosis is what Fleury, C. et al. (2000a) call plaque-like tumors. However, it should be stressed that the melanocytic nevi, although occurring in horses of all colors, seem to be more frequently occurring in grey horses. Drawing any definite conclusions regarding color of the fur from studying 29 horses with melanocytic nevi is questionable. Nor should any conclusions be drawn about fur color when only 2 horses with anaplastic malignant melanoma were included. I would propose a study on a larger number of horses before agreeing with Valentine (1995) on this matter.

When reading different articles about the cutaneous melanomas in grey horses, I find that the results from some studies lead to different conclusions about this disease. Several different studies support the theory that these lesions so commonly seen in grey horses are neoplasms (Valentine, 1995; Fleury et al., 2000a; MacGillivray 2002; Seltenhammer 2004, 2010). Rodríguez et al. (1997) on the other hand, found that no signs of malignancy could be observed when the lesions were studied histologically. Also, the proliferation index was low, indicating that the lesions were not neoplastic. Instead, Rodriguez et al. (1997) believed the alternated areas of skin to be the result of a pigmentation disorder. I consider the nature of the lesions being neoplastic is far more likely and well-grounded than arising from a pigmentation disorder. I say this because different study groups in various countries have reached the same results and found the same patterns in the different types of cutaneous melanoma. The histological patterns that were described by Rodríguez et al. (1997) seem like the melanocytic nevi described by Valentine (1995). If all horses studied by Rodríguez et al. (1997) had melanocytic nevi and not dermal melanoma, dermal melanomatosis or anaplastic malignant melanoma, it is natural that no malignancy was found. This might explain why his results differ from the work of others.

How common different sites are for primary tumors of the dermal melanoma and dermal melanomatosis types differ slightly between studies. Underneath the tail and the peri-anal

region are by far the most frequently affected areas, followed by various sites of the head such as lips, eyelids and parotid gland; and genitalia (Rodríguez et al., 1997; Fleury et al. 2000a). At times, I find that the statistics are presented in a misleading way. For example, the prevalence of cutaneous melanocytic tumors in female genitalia is reported to be 3.44% (Rodríguez et al., 1997). When looking closer at the facts, one finds that these 3.44 % actually account for 2 cases out of 2 females which have been compared to the whole group (2 females and 56 males). In my opinion, results regarding gender should be divided into different groups when considering gender specific aspects of the tumors. Therefore, a more correct description of the prevalence of tumors in female genitalia in this case would be 100% (2 cases in 2 females). Fleury, et al., (2000a) found the prevalence of tumors in female genitalia to be 3.8 %. Nowhere in the article do the authors specify how many of the horses were females. This is also to be considered as a serious shortcoming. Naturally, the same reasoning should be applied to the prevalence of tumors in the male genitalia presented in both articles.

Metastasis of the equine cutaneous melanomas can occur in many different places throughout the body. In the study performed by MacGillivray et al., (2002), lymph nodes and blood vessels were the most common sites of metastases (64 %). This indicates that the neoplasms spread both with blood and lymph. The peritoneum and associated structures, the spleen, skeletal muscles, alveolar capillaries, the parotid salivary glands and bone or bone marrow are other common sites of metastases.

It is interesting to read about the possibility to stain for MAGE-genes in humans. If the method also could be applied to cutaneous melanomas of horses, it might help predict the malignancy of a tumor. This would probably have a great impact on both prognostics and decisions concerning what therapy, if any, would be best. For example, one would quickly try to surgically remove a tumor with a high expression of MAGE-genes to avoid or minimize metastasis. More research is needed to see if this could be predictive of malignancy in the equine tumors.

Rosengren Pielberg et al. (2008) found that horses with a homozygote duplication in the STX17 intron were at much higher risk of developing melanoma than heterozygote horses. I believe this is a trustworthy explanation to the high prevalence of cutaneous melanomas seen in grey horses compared to solid-colored horses. The duplication is linked to the grey fur phenotype and is only observed in grey horses. These results correspond well to the findings of Rieder et al., (2000). I agree with the ideas of Rieder et al., (2000), that when selecting horses for breeding who are likely to produce offspring of the grey phenotype, the genotype should be considered. By doing this one can probably reduce the incidence of cutaneous melanoma. More research on this area is needed. It would be of great value if for example a relatively cheap blood test indicating if a certain horse is homozygote or heterozygote for the STX17 intron duplication could be developed.

LITTERATURE

Busam, K.J., Iversen, K., Berwick, M., Spagnoli, G.C., Old. L.J. & Jungbluth, A.A. (2000). Immunoreactivity with the Anti-MAGE Antibody 57B in Malignant Melanoma: Frequency of Expression and Correlation with Prognostic Parameters. *Modern Pathology*, 13, 459-465.

Chapman, S.W.K., Metzger, N., Grest, P., Feige, K., von Rechenberg, B., Auer, J.A. & Hottiger, M.O. (2008). Isolation, establishment, and characterization of ex vivo equine melanoma cell cultures. *In Vitro Cellular & Developmental Biology – Animal*, 45, 152-162.

Fleury, C., Be'rard, F., Balme, B. & Thomas, L. (2000a). The Study of Cutaneous Melanomas in Camargue-Type Gray-Skinned Horses (1): Clinical-Pathological Characterization. *Pigment Cell Research*, 13, 39-46.

Fleury, C., Be'rard, F., Leblond, A., Faure, C., Ganem, N., & Thomas, L. et al., (2000b). The Study of Cutaneous Melanomas in Camargue-Type Gray-Skinned Horses (2): Epidemiological Survey. *Pigment Cell Research*, 13, 47-51.

Jones, T.C. & Hunt, R.D. (1997). *Veterinary Pathology*. 6. ed. Philadelphia: Lea & Febiger. Pp. 857-858.

MacGillivray, K.C., Sweeney, R.W. & Del Piero, F. (2002). Metastatic Melanoma in Horses. *Journal of Veterinary Internal Medicine*, 16, 452-456.

M'Faydean, J. (1933). Equine Melanomatosis. *Journal of Comparative Pathology and Therapeutics*, 46, 186-204.

Moulton, J.E. (2002). *Tumors in Domestic Animals*. 4. ed. Berkeley: University of California Press. Pp. 78-82.

Rieder, S., Stricker, C., Joerg, H., Dummer, R. & Stranzinger,G. (2000). A comparative genetic approach for the investigation of ageing grey horse melanoma. *Journal of Animal Breeding and Genetics*, 117, 73-82.

Rodríguez, M., García-Barona, V., Peña, L., Castaño, M. & Rodríguez, A. (1997). Grey horse melanotic condition: A pigmentary disorder. *Journal of Equine Veterinary Science*, 17, 677-681.

Rosengren Pielberg, G., Golovko, A., Sundstrom, E., Curik, I., Lennartsson, J., Seltenhammer, M.H., Druml, T., Binns, M., Fitzsimmons, C., Lindgren, G., Sandberg, K., Baumung, R., Vetterlein, M., Stromberg, S., Grabherr, M., Wade, C., Lindblad-Toh, K., Ponten, F., Heldin, C-H., Solkner, J. & Andersson, L. (2008). A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nature Genetic*, 40, 1004-1009.

Seltenhammer, M.H., Heere-Ress, E., Brandt, S., Druml, T., Jansen, B., Pehamberger, H. & Niebauer, G.H. (2004). Comparative Histopathology of Grey-Horse-Melanoma and Human Malignant Melanoma. *Pigment Cell Research*, 17, 674-681.

Seltenhammer, M.H., Simhofer, H., Scherzer, S., Zechner, P., Curik, I., Sölkner, J., Brandt, S.M., Jansen, B., Pehamberger, H., & Eisenmenger, E. (2010). Equine melanoma in a population of 296 grey Lipizzaner horses. *Equine Veterinary Journal*, 35, 153-157.

Scott, D.W., & Miller, H.M. Jr. (2011). *Equine Dermatology*. 2. ed. Missouri: Elsevier Saunders. Pp. 504-508.

Valentine, B.A. (1995). Equine Melanocytic Tumors: A Retrospective Study of 53 horses (1988 to 1991). *Journal of Veterinary Internal Medicine*, 9, 291-297.