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CASE REPORT

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Concomitant melanoma and keratoma affecting the equine digit: clinical, pathological, and long-term follow-up findings

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Abstract

Background This case report details a long-term follow-up of a hoof melanoma with dermo-epidermal activity (resembling Spreading Superficial Melanoma (SSM)) in a bay horse with a history of a right front hoof keratoma. Melanomas involving the horse's foot are seldom reported and usually diagnosed as anaplastic melanomas based on signalment and post-mortem examination. The clinical-pathological characteristics of the foot melanoma in this bay horse are consistent with SSM-like described in humans, which is considered an intermediate malignant tumour attending their biological behaviour. However, a definitive diagnosis is limited by the single case and the lack of references in horses.

Case presentation A 12-year-old bay Andalusian gelding underwent keratoma removal on the lateral aspect of the hoof wall. A partial resection of the hoof wall was performed for this purpose. Additionally, a plaque-like, hyperkeratotic pigmented lesion, 2 × 2X0,4 cm in size, was observed at the lateral aspect of the coronary band and was also resected for histopathological examination. Microscopically, a melanocytic tumour, characterised by small nests of large polygonal or epithelioid cells infiltrating the basal and suprabasal epidermis, the dermo – epidermal junction, and the superficial dermis, was observed. The neoplastic cells exhibited large euchromatic nuclei, prominent nucleoli, moderate pleomorphism and 4 mitotic figures per 2,37mm^2; variable amounts of dark granules (melanin) were present in the cytoplasm, as well as in numerous peritumoral macrophages. The immunophenotype of the tumour cells was PNL2 + + +, S100 + +, AE1/AE3-. A diagnosis of melanoma with dermo-epidermal junction and marked intraepidermal activity (consistent with superficial spreading melanoma) was made.

A magnetic resonance imaging (MRI) performed, revealed no further invasion into surrounding structures. Treatment was based on surgical resection and multiple local chemotherapy sessions with cisplatin were applied. The biopsies obtained after treatment showed partial regression of the tumour and different stages of healing. After 26 months of follow-up, there was no signs of malignant spreading into surrounding structures including the pedal bone and distal metastasis but a dark – coloured area persists over the lateral aspect of the coronary band.

Conclusions This case presents a concomitant keratoma and melanoma with dermo – epidermal activity, resembling a spreading superficial melanoma. After a follow – up of 26 months the horse remains healthy and sound providing new information for clinicians and pathologists. Despite the poor prognosis associated with foot malignant melanocytic tumours, it is important that an early and accurate diagnosis is reached through different diagnostic

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modalities such as advanced imaging techniques and histopathology. Additionally, these findings demonstrate that the current classification and prognosis for equine foot melanomas are insufficient.

Keywords Dermo-epidermal junction melanoma, Foot, Horse, Keratoma, MRI, Melanocytic Tumour

Background

Melanocytic tumours represent among the 3.8–18.7% of all equine neoplasms [1, 2]. Most studies are focused on old grey or diluted coat horses because of their high prevalence, clinical and financial significance [3–9]. In non-grey horses, melanocytic tumours are considered uncommon with a greater likelihood of being malignant [6]. The foot is an unusual site of presentation. Only seven single reports involving the coronary band and hoof wall have been described and all were diagnosed as infiltrative malignant melanoma with poor prognosis [9–15]. Furthermore, thorough diagnosis and treatment are often not pursued due to the grave outcome. Therefore, documented follow – up of tumour progression is rare [11, 15].

Keratomas are space-occupying masses in the hoof wall or sole produced by hyperplasia of keratinocytes at the coronary band or in the secondary epidermal laminae [16]. The prognosis for keratoma carries a success rate of 83% for surgical treatment compared with 43% for conservative management [17]. Keratoma can overlap with other primary hoof lesions, such as malignant neoplasia. Moreover, secondary inflammatory processes are commonly associated to keratomas, highlighting the importance of multiple diagnostic tests to reach the accurate diagnosis of hoof lesions [15, 16, 18].

This report describes the clinical history, pathological findings, treatment, and long-term outcome of a keratoma and a non-invasive, non-disseminated melanoma with dermo-epidermal activity, involving the lateral aspect of the coronary band and lamellae of the right forelimb in a horse.

Case presentation

A 12-year-old bay Andalusian gelding was presented at the Teaching Veterinary Hospital of the University of Córdoba with a mild right forelimb lameness of 2/5 according to the American Association of Equine Practitioners (AAEP) Grading of lameness for 10 months. The horse had a history of recurrent abscess formation at the lateral aspect of the sole of the foot, which had been treated by its farrier prior to referral to the hospital. The hoof wall had developed a defect over the lateral aspect, distal to the coronary band (Fig. 1a). The horse was referred for further investigation and treatment.

On arrival, the horse was bright, alert, and responsive. The lateral aspect of the hoof wall was partially resected by the previous treatment of hoof abscesses. There was a mild pain response elicited by application of hoof testers. There was no heat around the foot nor near the coronary band. At physical examination, a hyperkeratotic pigmented lesion, 2×2x0.5 cm in size, plaque was found close to the lateral aspect of the coronary band (Fig. 1b). On radiological examination, a focal, circular radiolucent and well-defined area over the lateral aspect of the solar margin of the pedal bone, consistent with pedal osteitis was identified (Fig. 2).

Based on gross and radiological findings, a diagnosis of keratoma was made. Partial hoof wall resection and curettage of the affected bone was performed. During the surgical procedure, a mass measuring 0.9×5x1 cm in size was identified. It exhibited characteristic columnar proliferation and thickening of keratin, consistent with a keratoma, and was therefore removed. Once the hoof wall was resected, the pigmented plaque located close to the lateral aspect of the coronary band was resected and submitted for histopathology (Fig. 1c).

A fixation plate was placed in the distal region of the lateral hoof sidewall to support the hoof and covered by a fiberglass cast up to the pastern. The horse recovered uneventfully from general anaesthesia, and it was fully weight bearing after the procedure. It was discharged two days following surgery, on strict box rest. The histopathological diagnosis was a melanoma with dermo-epidermal junction activity.

At re-examination, the fiberglass cast was removed, and the fixation plate remained in place. The surgical wound had a mild exuberant granulation tissue formation. At the dorso-lateral aspect of the coronary band, over the granulation tissue, a bright black circumferential mass of 1×1,5 cm in size was found (Fig. 1d), suggestive of tumour recurrence. Therefore, a biopsy was taken and submitted for histopathology. The diagnosis of a reoccurring melanoma with dermo – epidermal junction activity was confirmed.

Further diagnostic imaging techniques such as magnetic resonance imaging (MRI) of the hoof was performed to better evaluate the extension of the neoplasm. The dorsolateral aspect of the coronary band appeared irregularly outlined and moderately thickened, with ill-defined margins. The most superficial portion of that region showed high signal intensity on all sequences. In contrast, the internal layer was isointense on T1W images, whereas on T2 FSE and STIR images there was

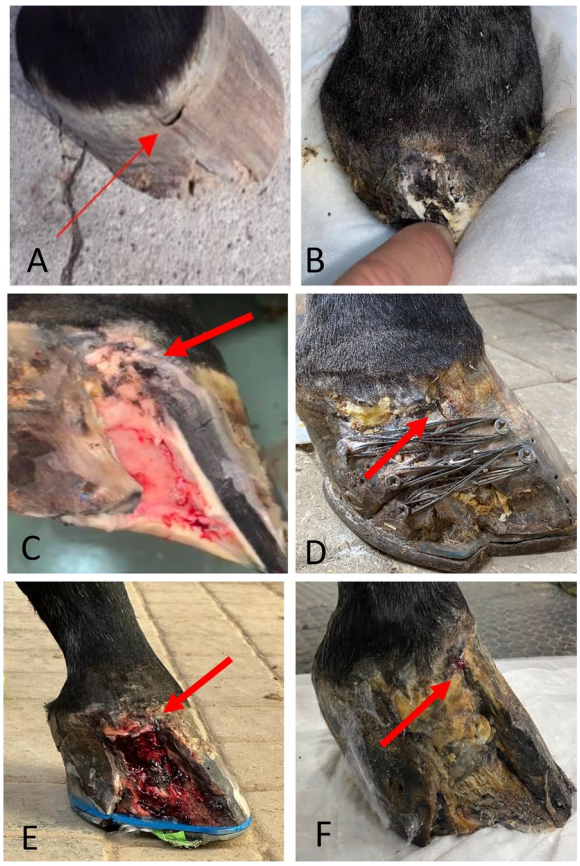

Fig. 1 Horse. Distal right forelimb. **a** Detail of the right foot prior referral. There is a hoof wall defect over the lateral aspect, distal to the coronary band. **b** Detail of the right foot on arrival. Appearance of the flattened, hyperkeratotic, crusted, and pigmented lesion at the lateral aspect of the hoof wall. **c** Intraoperative photograph of the lateral aspect of the foot with part of the plaque–like pigmented lesion involving the coronary band and stratum lamellatum (arrow). **d** Appearance on re-examination at 6 weeks after surgery. There is mild exuberant granulation tissue formation and a bright black circumferential mass at the lateral aspect of the coronary band (arrow). **e** Appearance of the treated lesion 6 months after the first chemotherapy session. The defect over the lateral hoof wall was stabilised with metal bridges. A dark–coloured mass around the lateral aspect of the coronary band can be observed (arrow). **f** Appearance of the defect 26 months after the initial presentation. There are signs of reepithelization, and the lateral hoof wall defect is covered by granulation tissue

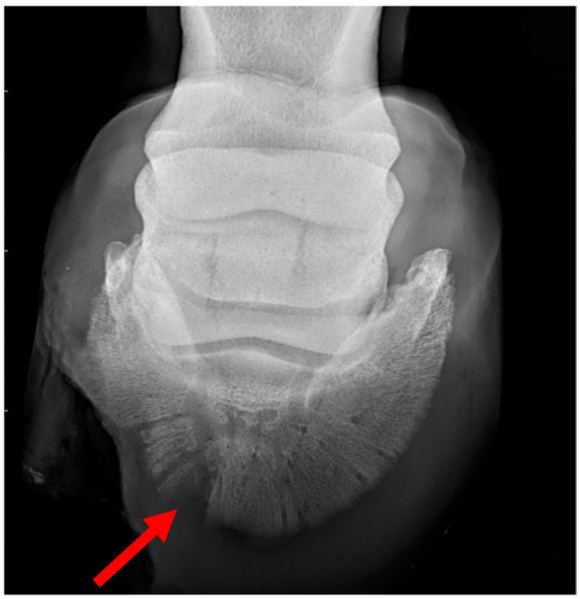

Fig. 2 Dorso65ºproximal-palmarodistal oblique (DP-PaDO) radiographic projection of the distal right forelimb. There is a focal, circular, well defined radiolucent area over the lateral aspect of the solar margin of the pedal bone (arrow)

an ill-defined marked high signal intensity (Fig. 3). The findings at the lateral aspect of the coronary band and the dorsal hoof wall were consistent with the neoplastic process and confirmed no invasion of the surrounding structures.

Therefore, a second surgical resection was performed under general anaesthesia to remove all possible malignant tissue. Post-operatively, the area infiltrated by the tumour was treated with 1 g/cm^2 of cisplatin (Pfizer®) in a sesame oil – based slow – release carrier. A shoe with a lateral notch was placed under the affected limb at the surgical wound to facilitate hoof growth. A protective bandage was applied to cover the defect. Following the second surgical procedure, six chemotherapy infiltration sessions were performed, at a regular 2–week intervals. The chemotherapy agent cisplatin was administered intralesional via a 21G needle at a concentration of 1 g/cm2 in a sesame oil-based slow-release carrier. The defect over the lateral hoof wall was treated as a quarter crack and stabilised with metal bridges along the defect. Regular farriery helped to maintain and restore the foot balance.

On re-examination by the referring veterinarian, six months after the last chemotherapy session, a new dark-coloured mass 1×1x0,4 cm in size, over the previous region was discovered. An incisional biopsy confirmed the persistence of the tumour that exhibited the same dermo-epidermal growth pattern, and long side an active wound healing was observed (Fig. 1e). Further chemotherapy sessions with cisplatin were advised on a 2–week interval, as performed previously. Throughout the treatment, control biopsies confirmed the persistence of tumour nests.

Eleven months later, the lateral hoof wall defect was covered by granulation tissue with a bar shoe applied to the foot. The horse has been comfortable at walk throughout this period. Currently, there are no signs of

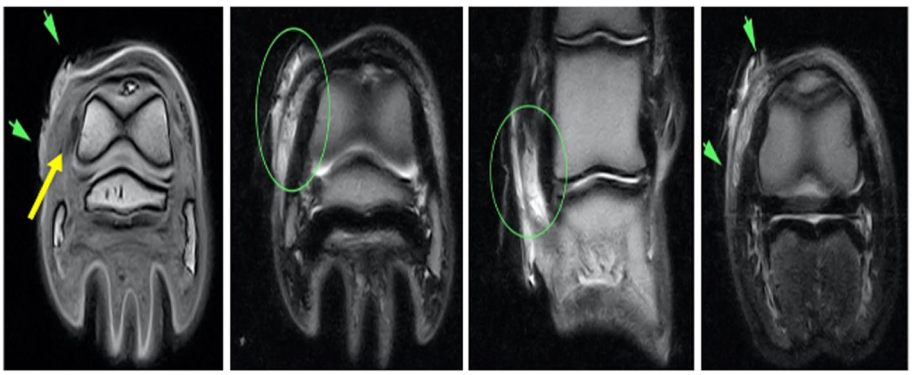

Fig. 3 Magnetic resonance imaging (MRI) examination sequences of the right forelimb. The dorsolateral aspect of the coronary band is irregularly outlined and moderately thickened, with ill-defined margins. The most superficial portion of this region shows high signal intensity on all sequences (green arrows). The most internal portion is isointense on T1W images (highlighted by yellow arrow), whereas on T2W FSE there is ill–defined marked high signal intensity (green circles)

malignant spreading into surrounding structures including the pedal bone and distal metastasis (Fig. 1f). It is notable that a dark – coloured area persists over the lateral aspect of the coronary band. The area exhibits the same consistency as the adjacent hoof horn, namely a lack of strength and fragility.

For the histopathological examination, all tumour samples were fixed in 10% neutral-buffered formalin, processed routinely in wax – paraffin, sectioned, and stained with haematoxylin and eosin (HE), PAS and Giemsa for microscopic examination. The first excised tumour and all consecutive biopsies revealed a malignant melanoma infiltrating the basal and suprabasal epidermis, dermo-epidermal junction, as well as the most superficial dermis (Fig. 4a-c). Neoplastic cells were arranged in small nests of large polygonal to epithelioid melanocytes that proliferate blurring the dermo-epidermal junction (Fig. 4b, 4c). Isolated neoplastic cells infiltrated the stratum spinosum and corneum (Fig. 4c). Tumour cells exhibited single, large, round-to-oval, central-to-paracentral euchromatic nuclei, and 1 or 2 prominent magenta nucleoli. The mitotic count was 4 mitotic figures per 2,37mm^2. The cytoplasm amount was moderate to abundant and contained variable quantity of dark granules (melanin) often located at the periphery near the cytoplasmic membrane (Fig. 4c, d). Numerous melanin-laden macrophages (melanomacrophages) were found in the stroma near the tumour (Fig. 4a, 4b). The affected epidermis showed marked hyperkeratosis and dyskeratosis as well as foci of bacterial overgrowth at the superficial stratum corneum. Moderate inflammatory cell infiltrate composed by numerous neutrophils, scarce macrophages and lymphocytes was present within the epidermis and the stroma adjacent to the tumour.

An immunohistochemical study was performed to further characterize the neoplasia. Monoclonal antibody anti-PNL2 (a sensitive melanocyte marker of equine melanocytic tumours) [18] (Sta. Cruz Biotechnology, SC59306); polyclonal anti-S100 protein (a marker of numerous types of cells such as cells originated in the crest neural (Schwann cells melanocytes); S100 is a good marker of melanocytic tumours of horses but less specific that PNL2) [18] (Dako, Z0311); monoclonal antibody against pan-cytokeratin (Dako, clone AE1-AE3); and monoclonal antibody against Ki67 (a nuclear marker of cellular proliferation) [20] (Dako, clone MIB-1) were used. These antibodies have demonstrated cross-reactivity with horse tissues using formalin-fixed and paraffin-embedded samples [19–21]. All neoplastic cells showed strong, granular, or diffuse, cytoplasmic immunolabelling for PNL2 (Fig. 4e, 4f); S100 protein reacted with most tumour cells but the staining was weak. Epidermal keratinocytes showed strong immunolabelling for AE1-AE3, whereas neoplastic cells didn't for this antibody (Fig. 4g). That allowed us to demonstrate the complementary immunolabelling of the tumour nests within the keratinocytes. Ki67 antibody showed strong and diffuse nuclear immunolabelling of numerous neoplastic cells as well as germinal keratinocytes (Fig. 4h). The Ki67 proliferation index was of 18,8% (calculated at 400X magnification fields within areas of heaviest Ki67, 94 positives out of 500 neoplastic cells) [20].

Based in the histopathological and immunohistochemical findings a diagnosis of melanoma with dermo-epidermal junction activity (regarding Superficial Spreading Melanoma) was made.

Discussion and conclusion

Foot neoplasia is a rare condition observed in horses [7, 8, 12, 14]. Although melanoma is one of the most common neoplasms in grey horses, there is paucity of

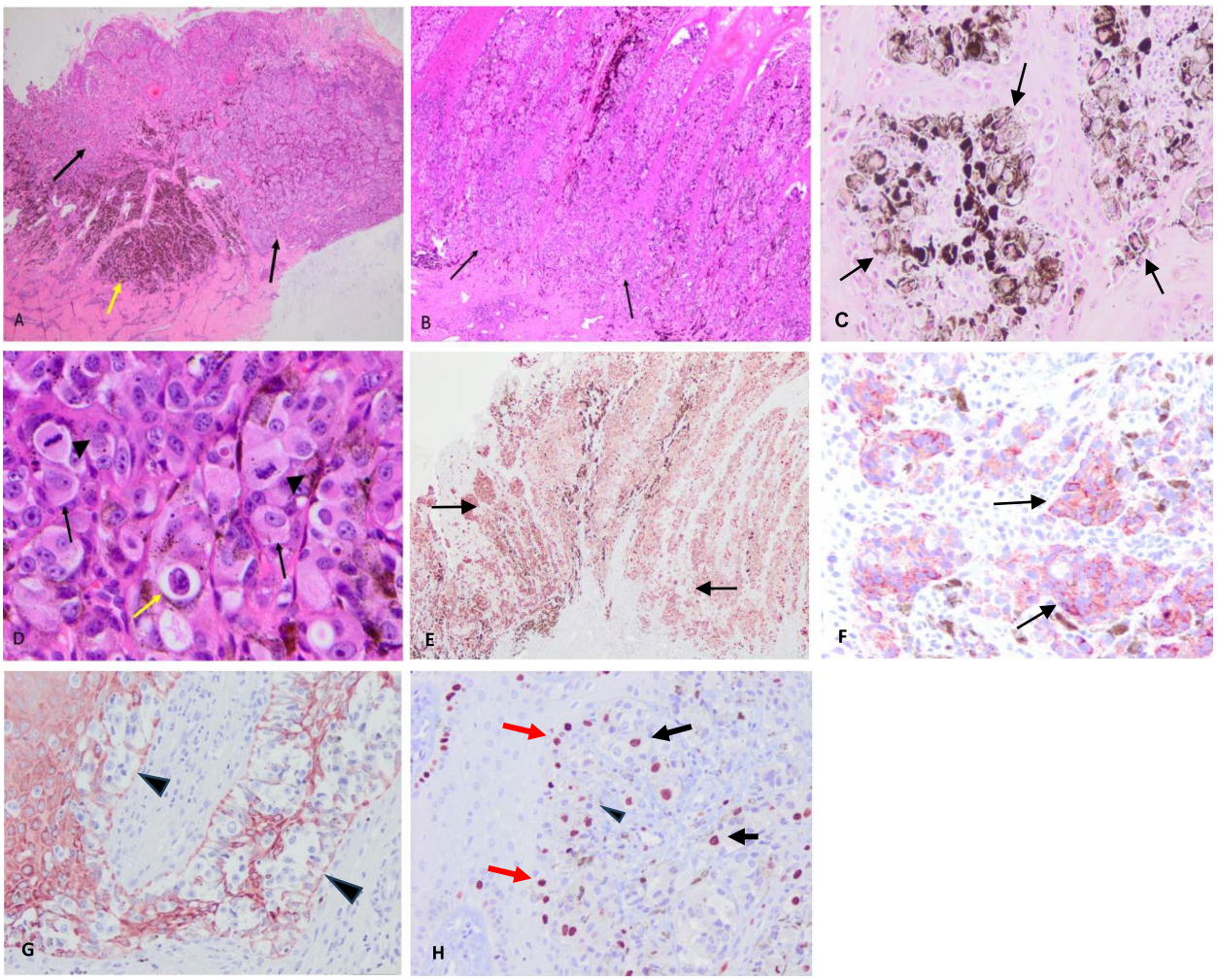

Fig. 4 Horse. Primary tumour at the coronary band. **a**. Low-magnification view of the tumour. Melanoma with dermo-epidermal junction activity. The basal layer, dermo-epidermal junction and superficial dermis is infiltrated and effaced by nests of neoplastic cells containing variable quantity of dark pigment (black arrows). In the adjacent stroma are numerous melanin-laden macrophages (yellow arrow). HE (**b**) Higher-magnification at the margin of the lesion shows numerous neoplastic nests infiltrating the basal stratum of the epidermis and dermo-epidermal junction (black arrows).HE (**c**) High magnification at dermo-epidermal junction. Nests of neoplastic melanocytes bearing black pigment (melanin) in the cytoplasm (black arrows) obscuring the basement membrane. HE (**d**) Detail of tumour nests with large polygonal neoplastic melanocytes (arrows). Mitosis (arrowhead) and apoptosis (yellow arrow). HE (**e**) PNL2 IHC. Strong and diffuse cytoplasmic immunolabeling of tumour cells located along the dermo-epidermal junction (arrows). **f** PNL2 IHC. Cross section at dermo-epidermal junction showing the immunoreaction of neoplastic melanocytic cells (arrows). **g** Immunolabelling of basal keratinocytes with AE1/AE3 antibody (arrows). Note that nests of neoplastic melanocytes within them are negative (head arrow). **h** Ki 67 IHC. Intense and diffuse nuclear immunolabeling of many neoplastic melanocytes is observed (black arrows), as well as the neighbouring basal keratinocytes (red arrows)

literature of both, foot melanomas and other melanomas in bay horses [12, 13]. To the best of the author's knowledge, a search of Pubmed, Google Scholar and Web of Science revealed no previous cases of foot melanoma with dermo-epidermal junction activity (resembling human SSM) at the foot of a bay horse with a documented long-term follow-up. Human SSM are histologically characterized by prominent epithelioid or pagetoid growth pattern of neoplastic melanocytes; presence of single melanocytes in the upper reaches of the epidermis; sharp demarcation; normal or hyperplastic epidermis and dermal elastosis. These characteristics were observed in this foot melanoma, except dermal elastosis which could be explained by the anatomical location (low sun exposure) and particularities of the species. In addition, human SSM seems to have less aggressiveness than nodular melanomas [22].

Interestingly this case presents two different overlapping pathologies that share some similarities with previous studies where foot neoplasia was not considered as a

primary differential diagnosis [14, 15]. Thus, keratoma is a common cause of lameness in the horse is often associated with localised inflammation after trauma at the transition between new horn production at the coronary band and the hoof wall [23]. Studies suggest that chronic inflammation in the hoof, often associated with keratoma growth, may predispose to the development of various neoplastic lesions, although a direct connection is not yet established [14, 15, 24].

The main clinical sign was lameness with chronic, focal, suppurative inflammation that was associated with a keratoma. During physical examination and resection of the hoof wall to remove the keratoma, the pigmented proliferative lesion was clearly demonstrated. The histopathological diagnosis of melanoma and keratoma led us to focus on this underlying lesion. We presumed that either the disruption of normal horn growth at the coronary band that generated the keratoma or the chronic inflammation and keratoma, facilitated tumour development. In any case, there is a controversy about the role of abnormal hoof growth and chronic hoof inflammation in the development of hoof neoplasia [15].

A recent study describes *Treponema spp*. and *keratinopathogenic fungi* in keratomas of donkeys that could play a role in the development of aberrant keratin masses [25]. In this case, initial samples revealed focal bacterial proliferation (coccobacilli), however, infection with *Treponema spp*. or keratinopathogenic fungi were not observed in routine histopathologic or special stains (Giemsa) examination.

Advanced diagnostic imaging procedures like MRI and computed tomography (CT) have been used to diagnose hoof neoplasms and surgery planning [26, 27]. The study carried out by Nagel was the first report highlighting the use of advanced imaging modalities for diagnostic purposes. However, advanced imaging cannot solely be used to provide a definitive diagnosis of an aggressive neoplasia [18]. The MRI scan performed in this case revealed that the dorsolateral aspect of the coronary band was irregularly outlined and moderately thickened, with ill-defined margins. The signal intensity pattern identified did not correspond with the normal appearance of melanoma, which would have shown low signal intensity on T2W images and high signal intensity on T1W images [28, 29]. The hoof horn could have potentially influenced the image acquisition of the tumour and disturb the normal appearance of melanoma.

Given the neoplastic nature and location of the lesion, an extensive resection was the recommended course of action. However, this was not a viable option. A complete resection of the hoof wall at the level of the coronary band, had a potential risk of long convalescence period due to surface infection and hoof wall instability [23]. Based on the physical examination, advanced imaging results, and the fact that the patient had not deteriorated, local chemotherapy treatment was considered the best option to benefit the animal.

Cisplatin is supported by evidence in the literature for reducing tumour size, particularly in cases with small discrete nodules [30, 31]. Intralesional treatment with cisplatin has shown a success rate of 81% in horses with dermal melanocytic tumours [30, 31]. Therapy was less effective in larger, more advanced tumours and in tumours that had previously been treated by other methods [31].

In the presented case, the use of 1 g/cm^2 cisplatin helped to treat the recent appearance of new melanocytic tissue, as the latest histopathological report showed neoplastic cell necrosis, apoptosis, an inflammatory response around the tumour cells, and advanced re-epithelialisation, suggesting a phase of melanoma regression. After sixteen months post treatment, the horse has remained comfortable and has been taken for daily walks. The shoeing has been done every 5–6 weeks with a hospital plate and fiberglass cast around the defect. Although the last control biopsy showed evidence that there is no complete remission of the tumour, there are no signs of further invasion or metastasis.

In horses there is an accepted classification for melanocytic tumours [3]. In a retrospective study based on 53 cases, Valentine subclassified equine melanocytic tumours as: melanocytic naevi or melanocytomas (observed both in grey and non-grey horses), dermal melanoma and melanocytosis (observed exclusively in grey-horses) and anaplastic malignant melanoma (only observed in non-grey horses) [3]. Different studies over the years have discussed these four categories but, exceptionally new histopathological variants of melanocytic tumours have been proposed [6].

Schöniger and Summers (2009) in a retrospective study based in 20 melanocytic tumours described three new variants of equine melanocytic naevi resembling the human melanocytic naevi counterpart [6]. These variants include intradermal common melanocytic naevi, cellular blue naevi and combined cellular blue naevi. The authors found differences in cytomorphological features, location and frequency between these variants and the commonly recognized dermal melanomas of grey horses [6]. This study contributes to better understanding different types of benign melanocytic neoplasia regardless the coat and local invasion. In 2013, a review evaluated the histopathological features of dermal melanomas described in previous classification and aimed to establish a new standardized classification system which would display better therapeutic and preventive options [30]. However, few new studies proposed to enhance the information from Valentine´s different categories [6, 8, 30].

The histopathological features observed in all tumour samples of this foot melanoma demonstrated a marked superficial growth pattern involving the epidermis and dermo-epidermal junction (radial growth); in addition, there was no evidence of deep structures invasion (vertical growth), both in biopsies and clinical exploration made during the follow-up. The immunohistochemical study for melanocytes (PNL2 antibody) and keratinocytes (AE1/AE3 antibody) immunolabeling, allows us to better characterize this unusual intraepidermal and dermo-epidermal foot melanoma. Clinical and histopathological data differ from those of anaplastic malignant melanoma previously described in the foot of horses such as infiltrative and metastatic behaviour [10–15]. Regarding the single case of combined cellular blue naevi-like neoplasm described by Schöniger and Summers [6], our case share some morphological features, i.e. basal epidermis and dermo-epidermal involvement by epithelioid neoplastic melanocytes arranged in small nests. Nonetheless, herein the tumour was poorly demarcated, neoplastic melanocytes displayed cellular atypia and pleomorphism, moderate to high mitotic count and moderate proliferation index (assessed by Ki67 nuclear protein expression) which is consistent with a melanoma [21]. However, human-SSM display similar histologically features [22]. In addition, human SSM seems to have less aggressiveness than skin nodular melanomas [22]. Thus, the features of the case report here are consistent with human SSM subtype and would explain its evolution over the time with low aggressiveness.

Current classification of human melanocytic tumours encompasses histological, clinical, epidemiologic, and genetic characteristics [32]. Interestingly, SSM subtype is considered "intermediate malignant tumour" attending their biological behaviour and suggests us that the clinicopathological presentation of the foot melanoma in this bay horse could be explained with this classification [32]. The main limitation for a definitive diagnosis is the single case described here and the lack of references in horses.

In conclusion, this case of comorbidity, keratoma and melanoma with dermo – epidermal activity (resembling SSM), with a follow – up of 26 months, provides applicable new information for clinicians and pathologists. Our aim is to emphasise that, despite of the poor prognosis of most malignant melanomas of the foot, early accurate diagnosis using advanced imaging techniques and histopathology is necessary. Furthermore, these results highlight that the current classification and prognostic criteria of equine melanoma are incomplete. Further clinical and pathological studies are needed to better characterise equine melanocytic skin neoplasms and understand the role of keratoma and chronic inflammation in the development of hoof neoplasia.

Abbreviation

SSM Superficial Malignant Melanoma

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Ethical guidelines

Not applicable.

Authors' contributions

F. B. and E. M. contributed to the study design and data analysis interpretation. F. B., K. G, D. A., A. B. and E. H. contributed to the clinical study, treatment, and follow-up. E. M. and I.M. R contributed to the histopathological study and immunohistochemical study. All authors contributed to the preparation of the manuscript and gave their final approval.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Data availability

Data are available upon reasonable request at the corresponding author.

Declarations

Ethics approval and consent to participate

No ethical review required – retrospective case report of clinical case.

Consent for publication

This study is a case report, identification information was excluded. The images published in the manuscript are provided by the authors.

Competing interests

The authors declare no competing interests.

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