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Chapter

Equine Sarcoid

Beatrice Funiciello and Paola Roccabianca

Abstract

The equine sarcoid is the most common skin neoplasia in the horse. It has a worldwide distribution and can also affect other equids such as donkeys, zebras, and mules. All breeds can develop the disease at any age, with no sex predilection, although geldings seem to be overrepresented. This fibroblastic neoplasm has several clinical presentations and microscopic features and has a nonmetastatic behavior but can be severely locally invasive. In many cases, multiple sarcoids may develop simultaneously or sequentially during their life and spontaneous remission is rarely reported. The etiology is multifactorial and involves bovine papillomaviruses, genetic, and environmental factors. Treatment options include different modalities depending on multiple factors: lesion type, location and extent, individual patient, facilities, owner, and financial issues.

Keywords: sarcoid, neoplasia, tumor, skin, horse, equids, donkey, mule, zebra

1. Introduction

The equine sarcoid is the most common skin neoplasia in the horse. This fibroblastic neoplasm has a multifactorial etiology and is nonmetastatic but can be severely locally invasive. First described in 1936, it has a worldwide distribution and can also affect other equids such as donkeys, zebras, and mules as well as other mammals [1–3]. Prevalence of sarcoid varies among published studies; however, many reports include cases from referral clinics that may not exactly reflect the entire equine population. Reported percentages of sarcoid among skin diseases and skin neoplasms vary from 13% to 90% and 8% to 38% when considering ocular neoplasms. There are also some geographical variations that may correlate with variations of risk factors, including the presence of cattle and vectors near horses [1, 4–6]. Horses of all breeds and colors can develop the disease at any age, most presenting a first lesion between 2 and 9 years of age. There is no demonstrated sex predilection, although geldings seem to be overrepresented [3, 5, 7]. Data about the incidence of sarcoids in the population are available only for donkeys and not for horses [8].

Affected animals can never be considered free of the disease even after successful treatment and presence or history of sarcoid can lower the likely sale value of the animal [2].

2. Etiopathogenesis of the equine sarcoid

2.1 Bovine papillomavirus infection

To date, it is widely recognized that sarcoids are associated with the presence of bovine papillomaviruses (BPV), typically BPV-1 and/or BPV-2. In two Brazilian

studies, newly proposed BPV 'BsR-UEL-4' and BPV-13 were found in some equine sarcoids, suggesting the need for further research regarding BPV serotype involvement in the development of these tumors [9–12].

The prevalence of BPV-1 and BPV-2 types seem to vary among geographical areas. In Europe and Australia, BPV-1 is most detected. In eastern USA, an almost equal proportion of both virus types was found, whereas in Canada and Western USA, BPV-2 was demonstrated in most of the samples [12–17].

The bovine papillomavirus genome comprises early and late coding regions. The early (E) genes encode nonstructural proteins involved in viral replication, maintenance of the episomal state, and activation of cell proliferation. The late (L) genes encode structural proteins (viral capsid) produced only in the life cycle of keratinocytes in natural hosts. A non-coding long control region is also present playing a role in viral replication and transcription [18, 19]. The main factors identified in sarcoid oncogenesis are the E2, E5, E6, E7, and p53 proteins. The E2 protein has regulatory effects on viral transcription and on the expression of matrix metalloproteinases (MMPs) that may be implicated in neoplastic cell invasiveness. The E5 protein exerts its function by binding to platelet-derived growth factor-β receptor (PDGFβ-R) thus activating p38 mitogen-activated protein kinase (MAPK) to induce fibroblastic transformation in sarcoids and down-regulate the major histocompatibility complex (MHC) I to facilitate the evasion of the immune system. The E6 protein can interfere in the activity of the p53 protein and has anti-apoptotic activity. The E7 protein cooperates in evading innate immunity [19–27]. The oncogenesis of equine sarcoids also involves loss of expression of the Fragile Histidine Triad (FHIT) and of the O^6-methylguanine-DNA methyltransferase (MGMT) tumor suppressor proteins [28, 29]. Recent studies have evaluated the role of small non-coding RNAs that regulate gene expression (microRNAs) in the development of sarcoids, and the role of aberrant methylation (S100A14 gene) is under research [30–34].

It seems that BPV infection in horses starts in the epidermis, where it can remain latent, with a subsequent presence of viral material within sub-epidermal fibroblasts where full transformation takes place [35–37]. Latency seems to take place also in peripheral blood mononuclear cells (PBMC) [38]. The infection in horses is abortive, the virus is present episomally but intact virions have never been detected. Furthermore, intralesional viral load seems to be correlated to disease severity [39, 40].

2.2 BPV transmission

Viral transmission between animals has not been completely elucidated yet. Direct contact with cattle, contaminated surfaces, and flies are presumably the most common routes of transmission [35, 36, 41, 42]. Infected equids may possibly spread BPV infection to horses and donkeys through contact. Appropriate fly protection and hygiene should be basic control measures in the presence of cattle and sarcoid-affected animals [35, 36].

2.3 Genetic risk factors

Bovine papillomavirus infection alone is not sufficient to promote normal cells transformation into sarcoid tumors, the presence of genetic factors and trauma are associated with the disease [43]. All breeds can be affected but Quarter Horses, Appaloosas, and Arabian horses are reported to be at greater risk than Thoroughbreds. Standardbreds have an even lower risk of developing sarcoids [5, 44, 45]. Certain equine families have an increased prevalence of sarcoid lesions and an association between the disease and equine leukocyte antigen (ELA) alleles has been observed in several breeds. The ELA W13 allele associated with the MHC

Figure 1.
Sarcoid development on the jugular groove, possibly triggered by injection micro-trauma.

II has been linked with sarcoid susceptibility in studies involving different breeds such as Swiss, Irish, French, and Swedish Warmbloods, and Thoroughbreds. The ELA W13 allele is not expressed in Standardbreds, a breed at lower risk of developing lesions [43, 46, 47]. Other MCH-encoded antigens are reported to play a role in sarcoid development: W3, B1, A3, A5, A16, A20, W5, W11, and W21 [1, 48–50]. A breed specific antigen, the Abe108, has been associated with sarcoids in Freiberger horses that lack A3, A5, and W13 antigens [50].

2.4 Trauma

Skin trauma is involved in sarcoid initiation, progression, and possibly recurrence. Micro-trauma due to injections (**Figure 1**) or even insect bites can be followed by sarcoid development, even long after apparent healing. Furthermore, sarcoids are a well-recognized possible complication and cause for delayed healing in both traumatic and surgical wounds in horses [2, 5, 51].

3. The biological behavior of the equine sarcoid

An individual animal may present only with one sarcoid, but most commonly, affected horses develop multiple sarcoids during their lives. These neoplasms may remain static for months or years and then, slowly or suddenly become aggressive and progress in type and/or extension without apparent reason (**Figure 2A** and **B**). Sarcoids tend to be locally invasive, sometimes extending into subcutaneous and
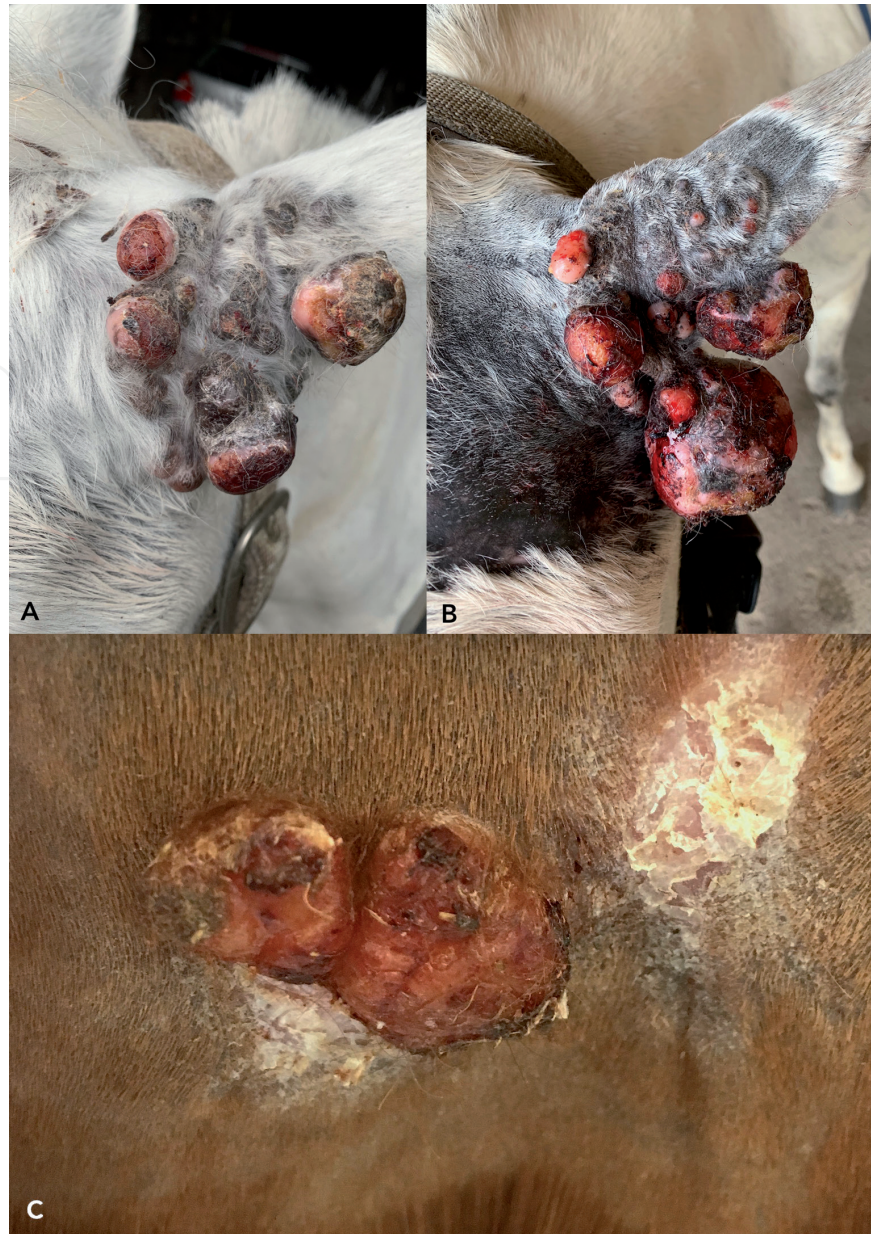
Figure 2.
(A) Ear sarcoid slowly grown over years. (B) Same horse (hair clipped) few weeks after, the sarcoid underwent rapid growth at the beginning of the fly season. (C) Fibroblastic sarcoid development on a recently treated occult sarcoid. Note the "healthy" scar on the right where a similar occult sarcoid was successfully treated simultaneously.

muscular planes, especially periocular lesions. They do not metastasize, however, with the exception of the malignant form that can spread to lymphatics and cause the formation of multiple masses along the lymphatic vessels and at remote sites such as lymph nodes [52].

Spontaneous regression is rarely reported and usually these horses do not develop new sarcoid tumors. Only in one recent study on a population of Franches-Montagnes horses in Switzerland has a high proportion of spontaneous remission been observed [53]. The mechanisms for spontaneous regression are not clear and antibodies have been detected only in donkeys [5].

The equine sarcoid has high frequency of recurrence after treatment (**Figure 2C**), especially following surgical excision. Recurrent tumors are usually more aggressive than the initial lesion and tend to grow rapidly and be more invasive. Recurring sarcoids can appear within a few days or weeks to months or years. The recurrence is often due to incomplete removal or spread of sarcoid cells during the procedure [1, 2, 52].

4. Clinical presentations of the equine sarcoid

Sarcoids have been classified into six different types depending on their macroscopic appearance (**Table 1**). This clinical classification is important because different types require different therapeutic approaches and have differing prognoses. One subject may carry more than one type of sarcoid and commonly, though unpredictably, milder forms can progress to more severe types [52, 54, 55].

4.1 Occult sarcoid

The occult sarcoid presents as an area of hairless skin, generally roughly circular. The skin may be thinned and/or have variably hyperkeratotic or roughened areas and contain one or more nodules, usually about 2–5 mm in diameter (**Figure 3A** and **B**). Occult sarcoids may involve extensive surfaces and individual horses may carry several lesions (**Figure 3C**). In some cases, only partial alopecia with thin hair and mild changes in skin and/or hair pigmentation (darker or paler) can be detected. Pruritus and pain are not present. These sarcoids have a slow progression toward verrucose growth.

Occult lesions can develop at any site but with predilection for the skin around mouth, eyes, the neck, and areas with less hair such as the medial thighs (**Figure 3D**) and forearms. They rarely affect the limbs.

Differential diagnoses for occult sarcoids are: idiopathic hypotrichosis/alopecia, dermatophytosis, alopecia areata, rub marks, chronic rubbing and scarring, bullous conditions (pemphigus foliaceous, and vasculitis), and burns.

Type	Subtype	Features
Occult	—	Roughly circular, hairless thinned and/or hyperkeratotic skin, may contain nodules
Verrucose	—	Warty, hyperkeratotic area, may have nodules and/or occult halo
Nodular		Subcutaneous spherical masses
A—no cutaneous involvement	A1	Deeper tissues are not involved, loose capsule and defined margins
	A2	Deep tissue involvement with poorly defined margins and invasive 'bound-down' nature
B—cutaneous involvement	B1	Deeper tissues are not involved, loose capsule and defined margins
	B2	Deep tissue involvement with poorly defined margins and invasive 'bound-down' nature
Fibroblastic		Fleshy, ulcerated appearance, fibrocelullar scab
1—pedunculated	1a	Distinct pedicle without palpable or histological presence of tumor extensions
	1b	Distinct pedicle with palpable root, poorly defined margins, invasive 'bound-down' nature
2—sessile/broad-based		Poorly defined margins, invasive 'bound-down'
Mixed	—	Verrucose, nodular and fibroblastic features present in variable proportions within the same lesion
Malignant	—	Multiple, locally invasive nodular and fibroblastic sarcoids with subcutaneous connections, may spread to lymphatics

Table 1.
Summary of the clinical classification of sarcoid types and features.

Figure 3.
(A) Occult sarcoid in the pectoral region: circular roughened hairless area with one small nodule within.
(B) Large occult sarcoid: note mild hair loss, alterations in skin pigmentation, and presence of nodules.
(C) Multiple occult sarcoids, the central one has a verrucose central area with occult halo. (D) Early occult
sarcoid (blue circle) on the medial thigh. Sparsely haired shining skin with mild pigmentary changes.

4.2 Verrucose sarcoid

The verrucose form has a characteristic "wart-like" appearance, which is the main reason for calling sarcoids "warts". These lesions are alopecic and neither pruritic nor painful unless secondarily infected (**Figure 4A**). Some may ulcerate and bleed (**Figure 4B**). Thickness and size vary, small nodules may develop in the hyperkeratotic area and some lesions may present a pathognomonic occult margin/halo (**Figure 4C**). They usually grow slowly but progression to a more aggressive form is possible, especially with trauma. As occult sarcoids, the verrucose ones can coalesce and cover large body areas (**Figure 5**).

Verrucose sarcoids can develop in any region with predilection sites being face (periorbital), axillae, groin, body, and sheath. Limbs are rarely affected.

Differential diagnoses for verrucose sarcoids are: papillomatosis (warts), linear keratosis/epidermal nevus, dermatophytosis, chronic blistering.

4.3 Nodular sarcoid

Nodular sarcoids are firm and well-defined subcutaneous masses, usually spherical with variable diameters from few mm to 7 cm. In many cases, the nodules may be multiple and coalescing. Pain and pruritus are not typical features.

Figure 4.
(A) Verrucose sarcoid in the axillary region (hair has been clipped), note another one in the sternal region. (B) Verrucose sarcoid with fissures and mild bleeding. (C) Verrucose sarcoid with occult halo.

Figure 5.
(A) Large verrucose sarcoid with nodular formations on the side of the neck. (B) Same horse after hair clipping: note extended hyperkeratotic and occult areas that were not previously visible because of coverage by hair.

Similar to other forms, they very rarely develop on the limbs and the predilection sites are the groin, sheath, and eyelids.

A further classification has been suggested for these sarcoids based on skin and deep tissues involvement.

- Type A nodules do not involve skin that is not altered and can be freely moved over the nodule. Two subtypes exist:

  ○ Type A1: the nodule can be moved from both the skin and the underlying tissues, usually has a fibrocelullar capsule. In some lesions, a skin pedicle is palpable.

  ○ Type A2: no skin involvement but the nodule cannot be moved independently from the underlying tissues, it has a 'bound-down' nature. Very common around the eye.

- Type B nodules are characterized by visible and/or palpable alterations of the skin. They cannot be freely moved from the overlying skin that may look normal or be alopecic, thinned, hyperkeratotic, or ulcerated. Some may have adjacent occult changes. Also, two subtypes are recognized:

  ○ Type B1: no involvement of the deeper structures (**Figure 6A**).

  ○ Type B2: locally invasive with 'bound-down' nature and no separation from deeper layers (**Figure 6B**).

Differential diagnoses for nodular sarcoids are: fibroma/fibrosarcoma, neurofibroma, eosinophilic/collagenolytic granuloma, melanoma, equine cutaneous mastocytosis/malignant cutaneous mastocytosis/congenital mastocytoma, lymphosarcoma/lymphoma/cutaneous histiocytic lymphoma, dermoid cyst, and *Hypoderma* spp./foreign body cyst.

4.4 Fibroblastic sarcoid

Fibroblastic sarcoids are a more aggressive form with fleshy and ulcerated appearance, often covered by a fibrocellular scab and possibly secondary infection.
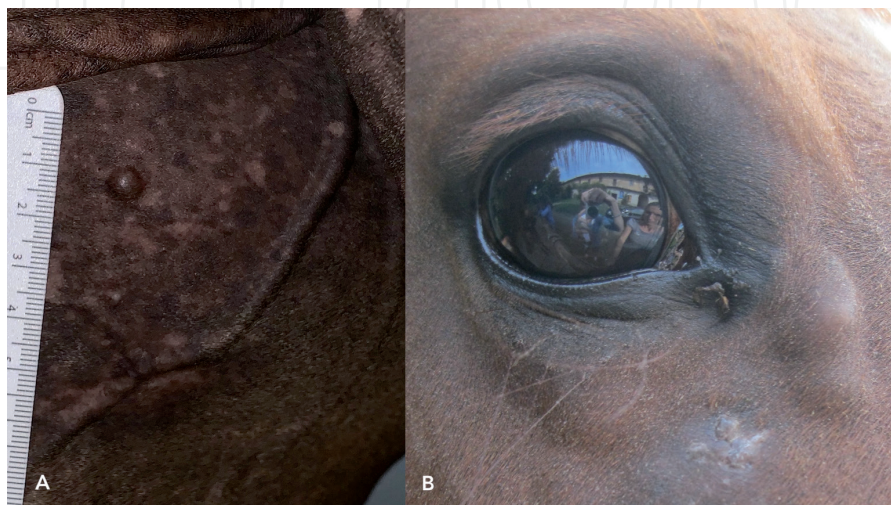

Figure 6.
(A) Type B1 nodular sarcoid on the medial thigh. (B) Three type B2 nodular sarcoids around the eye, an occult area is also present.

Bleeding and serum exudation are common and can be heavy with trauma. These surface characteristics attract flies that may contribute to irritation and self-trauma. Fibroblastic sarcoids commonly develop at wound sites (both traumatic and surgical), on the site of other sarcoids, especially if treatment attempts have been unsuccessful (**Figure 2C**) and they are usually more difficult to manage. Excessive granulation tissue may develop especially at wound sites thus complicating the diagnosis of sarcoid. Pruritus and pain rarely characterize these lesions. Predilection sites for fibroblastic sarcoids are groin, eyelid, wounds, coronets, and distal limbs. At some of these sites they carry a very poor prognosis. The classification of this form includes:

- Type 1 pedunculated fibroblastic sarcoids: characterized by a narrow pedicle with apparently normal skin and a fleshy crown. Subtypes are:

  ○ Type 1a: pedunculated with no palpable tumor and thickening at the base, no extensions detected on histology (**Figure 7A**).

  ○ Type 1b: this is pedunculated and rooted, where palpable alterations are detected beneath the pedicle, sometimes alteration are also visible (**Figure 7B**).

- Type 2 sessile fibroblastic sarcoid: the lesion is broad-based with invariably ill-defined margins and extensive invasion of the lateral and deeper tissues (**Figure 7C**).

Differential diagnoses for fibroblastic sarcoids are: exuberant granulation tissue, habronemiasis, pythiosis, botryomycosis/pyogranuloma/pseudomycetoma,
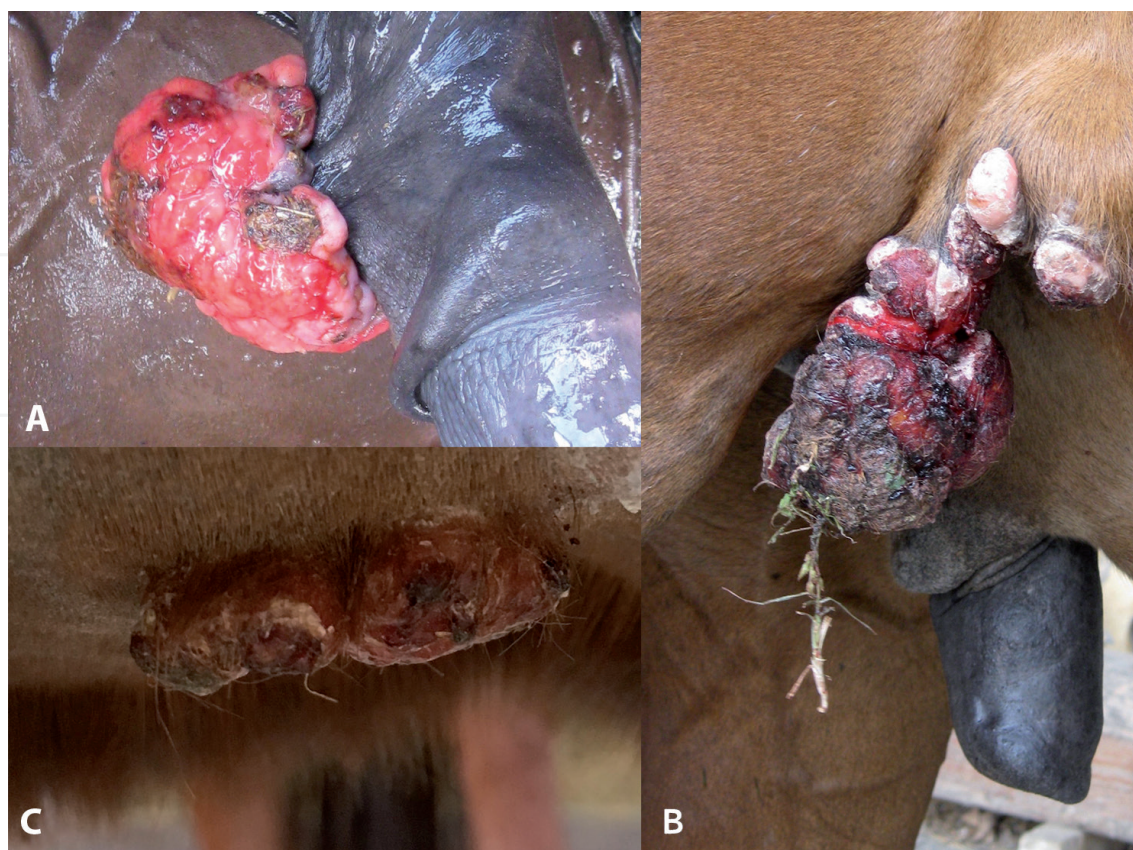

Figure 7.
(A) Type 1a fibroblastic sarcoid on the penis. (B) Type 1b fibroblastic sarcoid with clear tumor involvement of the pedicle. (C) Type 2 sessile fibroblastic sarcoid.

Figure 8.
Mixed sarcoid: a type B2 nodule with a small fibroblastic sarcoid within an occult area.

hemangioma/hemangiosarcoma, cavernous hemangioma/vascular hamartoma, neurofibroma/neurofibrosarcoma (ulcerated), fibrosarcoma, squamous cell carcinoma, sweat gland tumor, giant cell sarcoma, and mycosis fungoides.

4.5 Mixed sarcoid

Most sarcoids could be classified as mixed since different types (verrucose, nodular, and fibroblastic) are often present in variable proportions within the same lesion. Nevertheless, the definition of mixed sarcoid is usually reserved for those where a specific sarcoid type is not considered predominant. These cases may probably represent the transition/progression phase between one clinical type into the other. The combinations and extents of the various types are multiple, and they usually tend to become more aggressive, especially as the fibroblastic type grows (**Figure 8**).

Predilection sites for mixed sarcoids are the face, eyelids, groin, and medial thigh but mixed sarcoids can appear everywhere.

Differential diagnoses for mixed sarcoids are mixtures of granulation tissue within verrucose or fibroblastic lesions, habronemiasis, pemphigus complex.

4.6 Malignant sarcoid

The most recently described form of sarcoid tumor is the malignant type. It is usually, but not always, characterized by a history of repeated trauma or interference (also with inappropriate treatments) with another type of sarcoid. The particular behavior of the malignant type is the development of multiple, locally invasive nodular and fibroblastic sarcoids. Often cords of nodules and ulcerated lesions are visible and/or palpable, when these connections are subcutaneous the classification should be that of malignant sarcoid. They can be localized or spread through the lymphatic vessels invading local tissues with possible associated lymph node enlargement. No disseminated metastasis has been reported even for this form. A rare particular and dangerous form presents with a ring of nodules surrounding a verrucose or occult central area, especially on the neck/jugular and buttock regions. Predilection sites include jaw, face (**Figure 9**), elbow, and medial thigh.

Differential diagnoses for malignant sarcoids are squamous cell carcinoma, lymphoma/lymphosarcoma, subcutaneous mycosis, lymphangitis, glanders, epizootic lymphangitis/histoplasmosis, and hypertrophic scarring/cheloid.

Figure 9.
Malignant sarcoid on the face: 'bound-down' invasive nodules with a central area with occult to verrucose changes, ulceration, and a fibroblastic component.

5. Clinical examination and diagnostic procedures

The clinical examination should include signalment and a full thorough history with details on lesion development, especially about the behavior and progression. The clinical presentation of sarcoid lesions and their features are usually clearly recognizable, especially if multiple tumors of different types are present on the same horse. The confirmation of the diagnosis is not always straightforward and possible differential diagnoses and concurrent conditions should be considered [55, 56].

Depending on the sarcoid type, the full list of differential diagnoses should be considered when choosing the diagnostic procedures. The diagnosis of sarcoid is confirmed by histopathology, thus a biopsy sample is needed. Partial or excisional biopsy should provide sufficient information but a risk of exacerbation due to the surgical trauma should always be taken into account. If possible, a total excisional biopsy is preferable, the owner should be carefully advised about the implicit risks and a proper therapeutic plan should be prepared when taking the biopsy to avoid any exacerbation triggered by the procedure. If benign neglect is the plan, the opportunity of taking a biopsy should be carefully evaluated [2, 55].

5.1 Equine sarcoid pathology

Histopathology is deemed necessary to confirm the diagnosis of many equine sarcoids [57]. It is important to stress that due to the variable microscopic features of equine sarcoids, small biopsies may not provide enough tissue to differentiate sarcoids from other lesions such as granulation tissue, fibromas, or fibrosarcomas.

This is especially true if samples are obtained from ulcerated areas of the tumors [58]. Notably, trauma and reparative processes (wound healing) may activate cell growth and facilitate the development or heighten the progression of equine sarcoids [57–59], particularly for verrucose, occult and small nodular sarcoids [60]. Thus, excisional biopsies with wide margins should be favored for clinical reasons and because they provide with the most diagnostic material [46, 58]. If a non-excisional biopsy must be performed, sites within the mass must be carefully chosen to minimize the confounding factors of surrounding inflammation and granulation and to include intact epidermis [46].

Sarcoids derive from the proliferation of two components: the dermal fibroblasts and epidermal keratinocytes. They are regarded as biphasic tumors. Histopathology is heterogeneous and microscopic aspects and number of components varies according to the type of sarcoid [61].

Microscopic features of the epidermis may include orthokeratotic to compact hyperkeratosis, parakeratosis, irregular hyperplasia with epithelial proliferations producing long and pointed branches, termed rete pegs or rete ridges, extending deep into the dermal proliferation (**Figure 10A**) [46]. Epidermal ulceration is variable but frequent in nodular and fibroblastic sarcoids.

The amount of epithelial cell proliferation varies according with the type of sarcoid and ranges from severe hyperplasia to epidermal atrophy [1, 61]. Overall up to 46% of sarcoids lack epidermal hyperplasia and 54% lack rete peg formation [61]. Epidermal changes are maximal in verrucous sarcoids [58] and can be minimal to absent in nodular and occult sarcoids. Epidermal ulceration is common especially in nodular sarcoids [58].

All sarcoids are characterized by variable substitution of normal dermal components by neoplastic fibroblasts embedded in variable amounts of collagen. Histopathological findings consist of poorly demarcated, unencapsulated, variably infiltrative proliferation of large spindle to stellate, bland to highly atypical fibroblasts with plump, oval, nuclei with granular chromatin and variable hyperchromasia and with prominent nucleoli. Cellular atypia is low to absent and increases with time, number of excisions, ulceration, inflammation and type of sarcoid, being higher in malignant and mixed sarcoids. Number of mitoses is generally low (0–1 per HPF) if excluding malignant sarcoids. Density of neoplastic fibroblast is oftentimes higher in the superficial dermis [61]. At the dermal-epidermal junction, fibroblasts may be oriented perpendicularly to the basement membrane in the so-called "picket fence" arrangement (**Figure 10B** and **C**) [61–63]. This feature is considered highly diagnostic but is missing in up to 52% of sarcoids [61]. Additional patterns that can been seen at all levels of the dermis are whorling (**Figure 11A**), present in over 86% of tumors [64], parallel to interlacing short bundles (**Figure 11B**), storiform, herringbone, tangles or fibroblasts may be haphazardly arranged, this latter arrangement occurring more often in flat sarcoids (**Figure 11C**) [46, 62]. Amount of collagen matrix varies from minimal to abundant and can be dense, edematous, or myxoid (**Figure 11D**). Adnexal structures are variably reduced in density or obscured by the neoplasm [46].

Of all types of equine sarcoids, flat/occult sarcoids at initial stages can be easily overlooked at histopathology [63]. For this type of sarcoid, the only histopathologic finding may be an increased density of subepidermal neoplastic fibroblasts infiltrating between a reduced number of hair follicles and sweat glands [61]. The density of dermal fibroblasts is lower compared with the other types of sarcoids [58].

Immunohistochemistry can assist in the diagnosis of sarcoids although protein expression patterns are not considered highly specific. Fibroblasts in sarcoid express vimentin, the intermediate filament identifying mesodermal origin, and may be variably positive for laminin, smooth muscle actin, and type IV
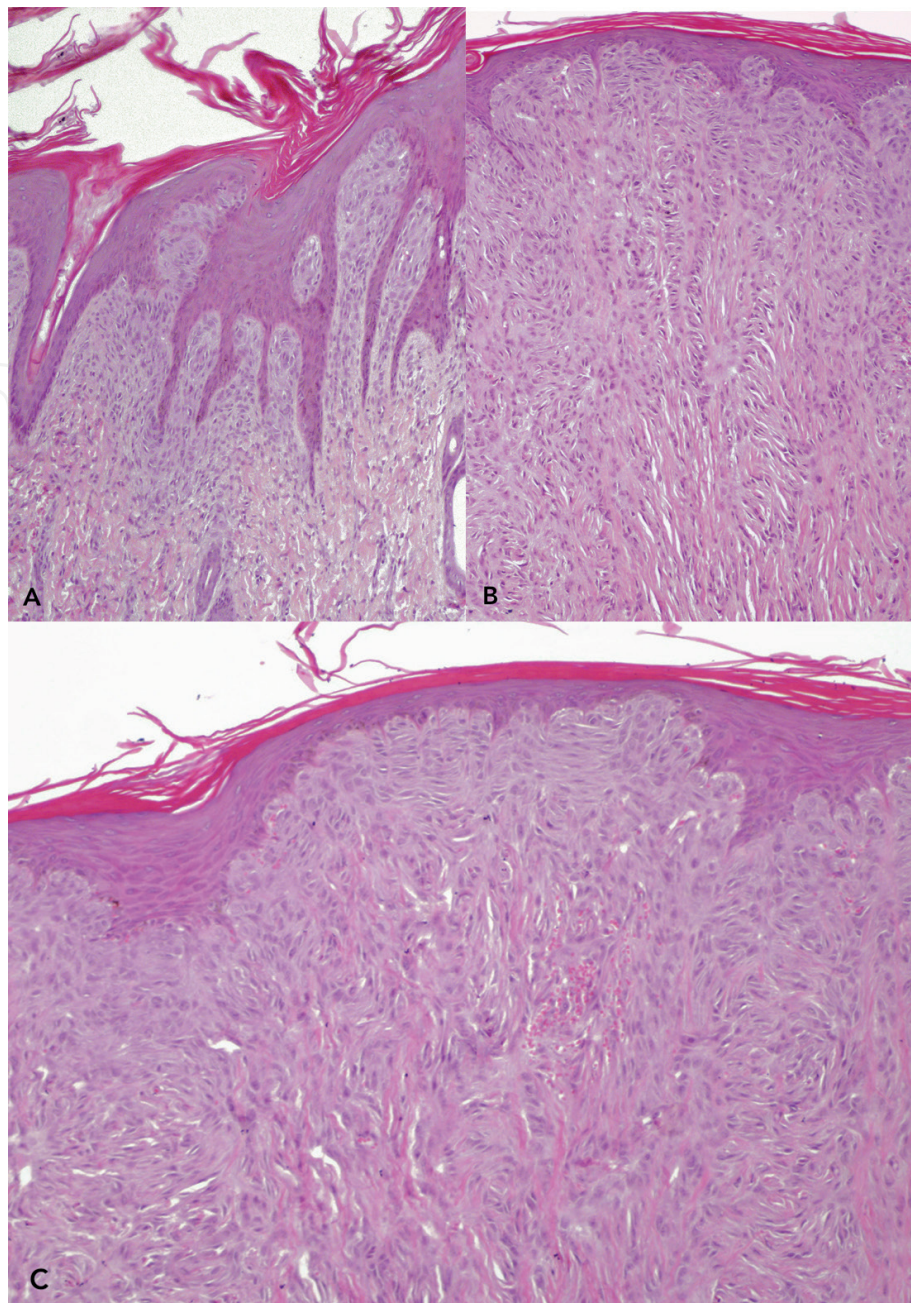
Figure 10.
(A) Moderate hyperkeratosis and severe epidermal irregular hyperplasia with rete peg formation. In the superficial dermis higher density of neoplastic fibroblasts compared to mid dermis is evident. Hematoxylin and eosin, 200×. (B) Moderate compact hyperkeratosis with mild epidermal hyperplasia and mild rete peg formation. In the superficial and mid dermis, typical picket fence arrangement of fibroblasts is present. The picket fence pattern is considered a highly diagnostic pattern but is observed in less than 50% of equine sarcoids. Hematoxylin and eosin, 20×. (C) Moderate compact hyperkeratosis with mild epidermal hyperplasia and rete peg formation. In the superficial dermis, high cellularity and typical picket fence arrangement of fibroblasts are present. The picket fence pattern is considered a highly diagnostic pattern but is observed in less than 50% of equine sarcoids. Hematoxylin and eosin, 100×.

collagen [59, 65, 66]. Sarcoids are generally S100 negative [65], however, S100 focal expression has been observed [66]. Bovine papillomavirus is involved in the pathogenesis of equine sarcoids, however, BPV infection of fibroblasts is mainly nonproductive [10]. Therefore immunohistochemistry against BPV is mostly negative [62].

BPV DNA can be detected by in situ hybridization and PCR on formalin fixed and paraffin embedded tissue sections of biopsy samples [36, 65, 67] or by PCR fresh cytological specimens obtained by swabbing or scraping of equine sarcoid tissue in non-healing wounds and recurrent cases and following recurrence after surgery [9].
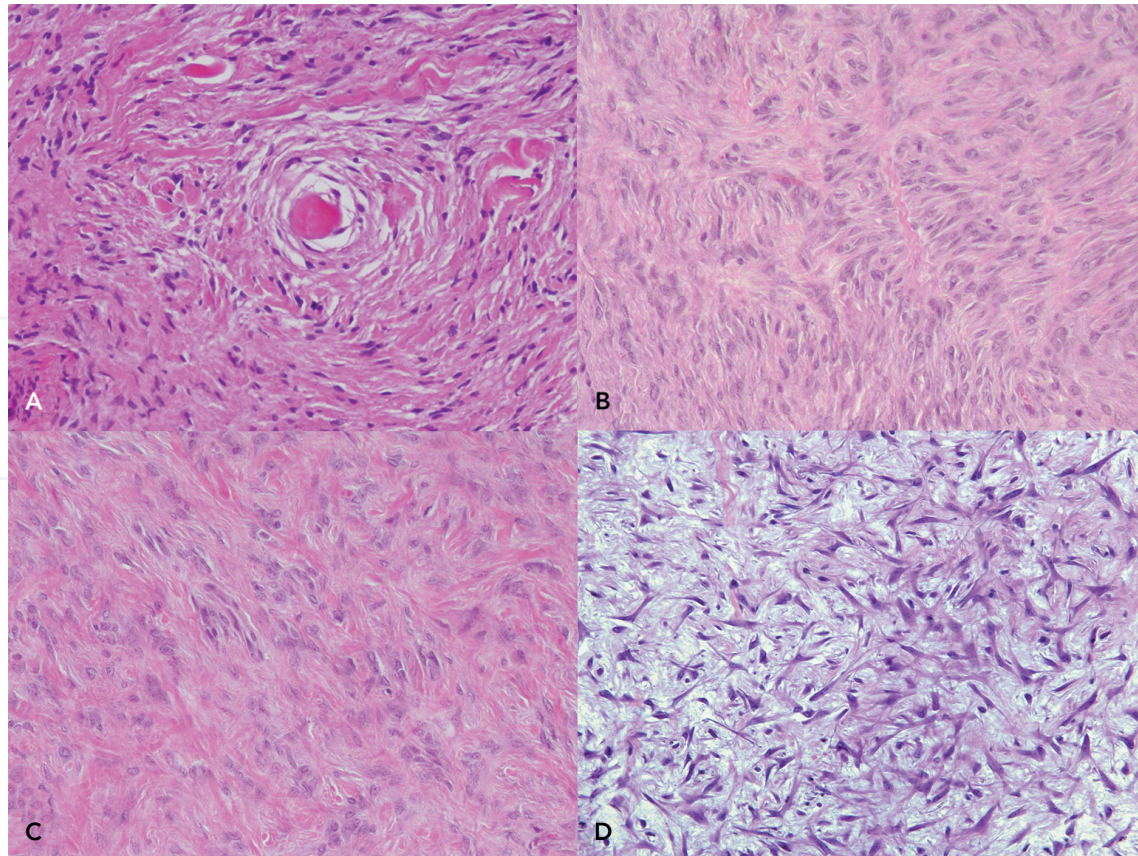
Figure 11.
(A) Bland neoplastic fibroblasts whorling around a thick collagen bundle. Whorling is considered a highly diagnostic pattern described in over 85% of equine sarcoids. Hematoxylin and eosin, 200×. (B) Area of high cellularity with plump neoplastic fibroblasts embedded in finely fibrillar to dense collagen and organized in parallel and perpendicular rows. Moderate anisocytosis and anisokaryosis are evident. Hematoxylin and eosin, 400×. (C) Area of moderate cellularity with parallel to haphazardly arranged plump fibroblasts embedded in abundant finely fibrillar to dense collagen. Moderate anisokaryosis is evident. Hematoxylin and eosin, 400×. (D) Area of high cellularity with haphazardly arranged highly atypical fibroblasts with spindle to stellate morphology embedded in finely fibrillar to myxoid stroma observed in a recurrent sarcoid. Hematoxylin and eosin, 200×.

DNA from BPV 1, 2 [14, 67] and 13 [12] is detected in up to 90% of equine sarcoids by in situ hybridization in the nuclei of fibroblasts and keratinocytes [37, 67]. Disadvantages of DNA detection are the unsuitability for diagnosing occult sarcoids, the lower sensitivity compared to clinical diagnosis, and the low specificity due to high prevalence of BPV DNA positivity in normal equine skin samples [35], cutaneous inflammation [68], and in other skin-associated spindle cell soft tissue tumors such as peripheral nerve sheath tumors (PNSTs), fibrosarcomas, myxosarcomas, and fibromas [66].

In summary, the most diagnostic histopathologic features, when present, are the epidermal changes of hyperkeratosis, hyperplasia with elongated rete pegs and "picket fence" aspect in conjunction by proliferation of fibroblasts [9, 35, 62]. However, common to most sarcoids are the fibroblastic dermal proliferation and presence of BPV DNA [10, 35, 67].

Microscopic features of sarcoids can overlap with other lesions. Differential diagnosis may be challenging because of the variable histological configuration of the dermal proliferation especially in cases with extensive ulceration or lack of distinctive epidermal lesions. Major histopathological differentials include granulation tissue (proud flesh), fibroma, fibrosarcoma, and peripheral nerve sheath tumors (e.g. schwannoma and neurofibroma) [57, 63]. Granulation tissue is characterized by fibrous tissue oriented at right angles to newly formed capillaries and is often associated with edema

and a prominent inflammatory component. When fibroblastic sarcoids are ulcerated, it may not be possible to differentiate them from granulation tissue and clinical follow-up becomes necessary. Fibromas can be differentiated morphologically as well-circumscribed, expansile, sparsely cellular tumors composed of a monomorphic population of mature fibroblasts with no epidermal proliferation. Fibrosarcoma is more pleomorphic with higher cytological atypia but multiple patterns are rarely observed, and the epidermal component is absent. Peripheral nerve sheath tumors (PNST)/Schwannoma are characterized by variable presence of highly cellular often palisading areas (Antoni A pattern) and low cellular myxoid areas (Antoni B pattern). These areas are associated with the presence of typical Verocay bodies composed of acellular areas between areas of nuclear palisading. Immunohistochemical staining for S-100 protein may be useful in differentiating PNSTs from sarcoids; however, focal S100 positivity has been reported in sarcoids [62, 66].

6. Management of the equine sarcoid

A treatment should be prompted as soon as possible following diagnosis, and in some cases, suspicious lesions could be treated immediately after biopsy [5].

Several treatment modalities for the management of equine sarcoids are historically 'known' and anecdotal reports and retrospective studies on more or less effective therapies exist, but valuable prospective double-blinded trials are lacking in the literature [3].

6.1 General considerations

Before choosing a proper therapeutic plan, some general considerations must be made [3, 55]:

- The prognosis is usually very guarded and owners must be thoroughly informed about possible complications associated with the condition.

- Sarcoid-affected animals can never be considered free of the disease, even after successful treatment.

- Each lesion can require a specific treatment and can react in a different way compared to other sarcoids even on the same horse.

- The extent and location of the tumor greatly affect the decisional process. Periorbital sarcoids (**Figure 12**) tend to penetrate the underlying musculature. Function of the upper eyelid must be preserved and any possible deformation in the healing process must be avoided. Sarcoids over tendons, joints or the facial nerve can have severe complications. The worst sites are the elbow and the face, where sarcoids much more tend to local invasion and progression to the malignant form.

- The duration of the lesion is important as early intervention usually requires less aggressive treatments. It is also easier to treat small lesions that extensive ones that may also be under transformation from one type to the other.

- Previous therapies and/or interferences influence the response to a new treatment course and possibly a different approach may be indicated.

Figure 12.
Periorbital sarcoid, eyelid function must be preserved when treating these lesions.

Wrong interference is a major cause of exacerbation and the prognosis significantly reduces with each treatment failure.

- Planning combined, prolonged or repeated treatments can be necessary for many sarcoids.

- Costs and logistics can have a great influence on the choice of the therapeutic modality.

- Professional skills and experience of the veterinarian can also affect the rate of success and the same treatment used by different clinicians can result in different outcomes.

- Animal and owner compliance for the best treatment: some are very painful, some sites (e.g. ear) are more sensitive, general anesthesia may be necessary in certain cases.

- Careful fly protection, wound management and regular checks must be part of the long-term management of any sarcoid-affected horse.

- Spontaneous remission is reported but rare, the decision to delay treatment based on a possible spontaneous remission is discouraged.

6.2 Benign neglect

As previously discussed, a proper treatment should follow the diagnosis of equine sarcoid, but in some cases benign neglect may be an option. Horses may present with such extensive lesions that any treatment method would be impractical. In other patients, the sarcoids may be small enough to render the procedure too expensive. Clinicians should opt for benign neglect with caution, both patient welfare and the lesions should be strictly monitored as sarcoids can progress. Furthermore, their presence may contribute to spread to other sites and horses [3, 55].

6.3 Surgical methods

- *Sharp surgical excision*: this technique is often appealing to practitioners and, in some cases, easy and successful but carries rates of recurrence as high as 70%, with recurrences occurring mostly within few months or even during the healing process and being much more aggressive (commonly fibroblastic) than the original sarcoid [2, 3, 46]. Wide excision is necessary to reduce the risk of recurrence, but it is not always practical or feasible and a safe margin is impossible to define. The principle of smart surgery should be applied to minimize cell contamination during surgery. Protecting the tumor with adhesive dressings before surgery reduces contamination and in case of recurrence another therapeutic method or combined treatments are indicated [3, 55]. Occult and verrucose sarcoids can be effectively removed with wide margins, nodules in the eyelids are invasive thus very dangerous, whereas other nodular lesions may respond better. However, the prognosis is usually very guarded when using surgery alone [55].

- *Cryosurgery*: this method causes tumor necrosis and is commonly used but has the same limitations as surgical excision. It can be used successfully on superficial lesions but restriction of blood flow, a defined safety margin and adjuvant chemotherapy (intralesional or topical) during the procedure can improve outcome. It can be repeated if necessary until the tumor is completely removed but the ability of the patient to resist the cold can be a limitation [3, 55, 69].

- *Hyperthermia/radiofrequency hyperthermia*: the tumor, being more sensitive to temperature than normal tissue, is heated for 30 s to 50°C weekly for up to 5 weeks. Very few cases are reported using this technique that is not generally recognized in equine practice [55].

- *Surgical electrocautery*: this method was recently reported with a high rate of success, its advantages are the minimal bleeding into the wound site with a reduced risk of tumor cell contamination and usually limited scarring. Electrocautery is one of the few options for sarcoids on the ear pinna [3, 55, 70].

- *Laser surgery*: surgical ablation with CO_2-YAG laser or diode laser devices is reported with success rates as high as more than 80%. When accurately used, this method is associated with the ability to sterilize the wound, no bleeding and avoids seeding tumor cells during the procedure. CO_2 lasers cause less thermal injury than diode ones. Primary closure may be possible, but a high rate of wound dehiscence and slow healing are disadvantages. Careful selection of the lesion is important: recurrence is most likely in verrucose sarcoids with poorly defined margins, whereas localized pinnal sarcoids and fibroblastic type 1a tumors around the eye may respond well [3, 55, 71, 72].

- *Ligation*: this method can be used only on pedunculated sarcoids where no tumor extensions are present in the pedicle below the ligature. This means that it is suitable for nodular type A1 and B1 or fibroblastic type 1a sarcoids, or any sarcoid where an artificial tumor-free pedicle can be created. The pedicle is ligated with castration/elastration bands, it works better if several bands can be placed and if adjunctive intralesional or chemotherapy are combined. The use of plastic ties or suture material that cut the lesion and partial ligation should be avoided as it carries a poorer prognosis and is associated with exacerbation or recurrence [3, 55].

6.4 Chemotherapy

Different chemotherapeutic agents and compounds can be used to treat sarcoids, usually they are topically or intralesionally administered with little or no systemic effects [55]. Systemic doxorubicin was used only in one study, but limitations and constraints to its use reserve this treatment only to very extensive or wide-spread lesions referred to specialist centers [3, 73].

- *Topical and intralesional 5-fluorouracil*: this cytotoxic and antimitotic agent can be topically applied as 5% ointment with a twice daily protocol over a few weeks. It is usually successful on small occult and verrucose lesions, or to control large areas that cannot be treated with other modalities. During treatment an inflammatory reaction can be marked but usually minimal scarring follows. It can also be combined with surgery [3, 55, 74]. The intralesional injection of 5-fluorouracil at the dose of 50 mg/cm^3 every 2 weeks for up to 7 weeks is reported with a successful rate of 61.5%, sarcoids larger than 13.5 cm^3 had a poorer prognosis compared with smaller lesions [75].

- *Topical imiquimod*: this agent is an immune response modifier with potent antiviral and antitumor activity and is used to treat human genital warts. The reported protocol for equine sarcoid is to apply the cream three times a week for 16–32 weeks. The treatment is usually associated with inflammation, alopecia and depigmentation. Administration of oral phenylbutazone can be helpful in some cases to control the discomfort [55, 70, 76].

- *Topical AW5*: it is a cream containing heavy metal salts, fluorouracil, thiouracil and steroid. Its use is restricted to veterinarians only, protocols include repeated applications but it can be contraindicated in some cases such as around the eye or other structures (facial nerve) that can be damaged. The reported success rate is around 74% depending on lesion and previous treatment history [3, 52, 55].

- *Topical acyclovir*: topical 5% acyclovir cream has been used to treat sarcoids with some benefits reported in one study [77]. A subsequent retrospective case-series and a double-blinded placebo-controlled trial resulted in no advantages from this agent compared to other treatments or placebo [70, 78]. The cream is used without prescription for human herpes virus infection and this may be attractive for owners that desire to treat horses without looking for veterinary advice with deleterious effects [3].

- *Silver nitrate*: silver nitrate caustic pencil is an old-fashioned treatment that can be applied to very localized small lesions [55].

- *Intralesional cisplatin*: this chemotherapeutic agent has been used in several studies in the form of injectable solution, emulsion and of biodegradable beads. Resolution rates are up to 93% in sarcoids less than 5 cm in diameter, larger lesions can be cured combining surgical debulking and intralesional cisplatin [3, 55]. A general protocol includes repeated injections of cisplatin oily emulsion at the dose of 1 mg/cm^3 every 2 weeks for four times but intervals may change upon patient needs. The material does not diffuse more than 5 mm in tissues so several injections every 6 mm–1 cm of tumor and margin of normal tissue are necessary. The aqueous solution has a clearance of minutes whereas the medical-grade sesame seed oil emulsion has the advantage of a lower

concentration and a slower release. Due to the high toxicity, self-protection measures must be strictly respected when handling cisplatin [3, 55, 79]. The use of biodegradable beads containing cisplatin is also reported with or without surgical debulking, the latter is usually necessary in tumors larger than 1.5 cm in diameter. Beads are placed at 1–1.5 cm intervals along the wound or tumor margins [3, 55, 70, 80].

- *Bleomycin*: bleomycin is a glycopeptide antibiotic with antineoplastic activity, it has been used to treat sarcoids intralesionally and with the use of electro-chemotherapy. Recently the topical use on occult and verrucose sarcoids of an ultradeformable liposomal preparation of bleomycin, alone or following 5-fluorouracil or tazarotene application, has shown good efficacy with the absence of pain and inflammation as an advantage [3, 81].

- *Electrochemotherapy*: electrochemotherapy is based on the use of electrically induced increases in cell membrane permeability to increase the effects of cytotoxic agents such as cisplatin, carboplatin, and bleomycin. It requires repeated general anesthesia, up to 8 treatments, and specialist equipment [3, 79, 82, 83].

6.5 Photodynamic therapy

This method is based on photosensitization of tumor cells with a topical or intralesional photosensitizer (e.g. hypericin or 5-aminolevulinic acid and derivatives) followed by the application of a specific light wavelength emitted by a proper light source, for a defined time (minutes). The mechanism is complex and takes advantage of the production of reactive oxygen species that kill sensitized cells, so it is very localized. The literature on its use on sarcoids is limited but significant benefits are reported, with or without surgical debulking [3, 84–88].

6.6 Immunotherapy

Since the involvement of BPV infection, much research is being focused on immunologic methods but without practical results so far. Moreover, horses do not seroconvert for BPV and vaccination does not prevent sarcoid development [3].

- *Spontaneous remission*: it is generally reported as rare, however, a recent study just reported a high proportion of spontaneous remission in a population of Franches-Montagnes horses in Switzerland [53]. The mechanism is not clear yet and antibodies have been detected only in donkeys [5]. Long-term immunity appear to occur in horses with sarcoids that undergo spontaneous remission [3].

- *Immunomodulation*: the use of intralesional injection of the bacillus Calmette-Guérin (BCG) is reported in different studies with high success rates, especially around the eye. Sarcoids on the distal limbs respond less or may even exacerbate [3, 69, 89, 90]. This method gives best results on nodular and fibroblastic sarcoids but may be associated with anaphylaxis, especially when repeated injections are performed [55, 90].

- *Vaccines*: attempts to stimulate sarcoid regression or potential preventive effects through autogenous and BPV-1 L1virus-like particles vaccines have been made but further studies are needed [3, 91].

- *Autoinoculation/autografting*: the inoculation of sarcoid tissue is reported in two studies, but doubts are raised about this method due to the risk of complications and the fact that it is not described for other cancers in any species [3, 92].

- *Hemotherapy*: no literature is available describing the effectiveness of this method. However, it is widely used in Central America and consists of withdrawal of venous blood and its intramuscular injection with anecdotal success [3].

6.7 Gene therapy

Mediator-governed therapy and genetic manipulation are under research but no practical treatment for the equine sarcoid has been reported yet [3, 27].

6.8 Radiotherapy

Facilities and special equipment are required for radiation therapy, which contribute to its high costs and limited availability. Different techniques exist:

- *Teletherapy*: it is expected to be effective, but few reports exist [3, 93].

- *Brachytherapy*: using radioactive radon, iridium and gold isotopes, it has become the gold standard for sarcoids, especially periorbital lesions [3, 93–96].

- *Plesiotherapy*: this surface brachytherapy method uses beta radiation from strontium^{90} and is reported on small superficial sarcoids [3, 93].

6.9 Adjunctive therapy

To remove secondary epidermal changes in sarcoid tumors, tazarotene can be used as adjunctive treatment. It is a retinoid 0.1% gel commonly used in human medicine for the management of keratinization disorders [3].

6.10 Phytotherapy

- *Viscus album austriacus*: the use of the injectable extract of the white mistletoe plant is reported to have immunomodulating effects in humans and was used in one double-blinded placebo-controlled trial in horses with sarcoids. Repeated subcutaneous injections for 15 weeks provided a positive outcome compared to placebo [3, 97].

- *Sanguinaria canadensis/zinc chloride*: commercially available compounds containing bloodroot (*S. canadensis*) and zinc chloride are anecdotally used for the treatment of equine sarcoids. Although high rates of success are reported on the internet, the use of this material by owners without veterinary advice carries risks. The use of this product on horses is not supported by scientific literature and dangerous toxicity in humans is reported [3].

6.11 Other remedies

Several 'natural' or herbal or homeopathic remedies are often used to treat sarcoids, usually with a delay in proper treatment and a risk of interference causing exacerbation of the tumor. Caution should be used considering the use of any material suggested to treat every condition in every species [3].

7. Sarcoids in other equids

Sarcoid tumors are reported also in animals other than horses. Donkeys, mules, and zebras can be affected, but reports of sarcoid tumors associated to BPV infection exist also in cats, giraffes, sable antelopes, and captive tapirs [98–104].

As far as equids are concerned, the reported prevalence of sarcoids in zebras is 25–53%, whereas incidence in UK donkeys is 0.6 per 100 animal years with apparent increased risk for young males [5, 105, 106]. The equine sarcoid is reported as the most common tumor in donkeys and presence of sarcoids among these equids and zebras is sometimes reported as outbreaks [105–109].

Diagnostic and treatment methods are the same as for horses, one study reports the use of surgical excision, intralesional 5-fluorouracil, allogenous vaccine or 5-fluorouracil in combination with autogenous vaccine in zebras [98, 110].

8. Conclusions

The equine sarcoid is a locally invasive skin neoplasm commonly encountered in practice. It has different clinical presentations, and early diagnosis with prompt treatment can improve the prognosis, but their importance is often underestimated. Several treatment options are available with variability in lesion and patient response. Spontaneous regression is rare, recurrence is common, and exacerbation is a possible complication, especially when a wrong therapy is attempted. Sarcoid-affected animals can never be considered free of the disease and horse owners must be correctly informed about the features and behavior of this tumor.

Author details

Beatrice Funiciello[1*] and Paola Roccabianca[2]

1 Private Practitioner, Torre Boldone (BG), Italy

2 DIMEVET Università degli Studi di Milano—La Statale, Lodi, Italy

*Address all correspondence to: beatrice.funiciello@bemavet.com

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