Sarcoids

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KEYWORDS

• Equine • Sarcoid • Skin tumor • Bovine papillomavirus type 1
• Bovine papillomavirus type 2

KEY POINTS

- Sarcoids are the most common equine skin tumors worldwide.
- Sarcoid pathogenesis is multifactorial, and the tumor is associated with bovine papillomavirus types 1 and 2.
- Clinical presentation varies and includes occult, verrucous, nodular, fibroblastic, mixed, and malignant (malevolent) types. The tumor is nonmetastasizing but can become very aggressive locally. Multiple tumors are common; all clinical types can be present in the same horse.
- No treatment protocol is universally effective. The tumor has a high risk of recurrence. Recurrent and large tumors carry a worse prognosis.

INTRODUCTION

Sarcoids were reported as being the most common type of equine skin neoplasm as early as 1936 and this continues to be the case to date, on a worldwide basis.[1–4] Approximately 1% to 11.5% of all horses have sarcoids,[5,6] and this type of tumor is reported to account for up to 90% (35%–90%) of all skin neoplasms in horses.[7–14] The tumor has also been reported in other equids, including zebras, donkeys, and mules,[14–17] as well as other mammals such as giraffes and sable antelopes.[18] An incidence of 0.6 cases per 100 animal-years was reported in a population of donkeys.[15] Sarcoids are nonmetastatic, fibroblastic neoplasms that rarely regress spontaneously; they can remain static or become very aggressive locally. Although the condition is not lethal in itself, size and distribution of the tumor or tumors can severely compromise the use and value of the horse, and lead to a decision of euthanasia.

PATHOGENESIS

Equine sarcoids have a multifactorial etiology (**Box 1**). An association with infectious agents in the form of bovine papillomavirus (BPV) types 1 and 2, as well as genetic risk factors, has been documented.

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Box 1
Pathogenesis: factors needed for sarcoid development

- Equid (horse, donkey, mule, zebra) with genetic susceptibility
- Bovine papillomavirus (BPV) types 1 or 2
- Delivery of BPV-1 or BPV-2 into the skin (vector, direct contact, skin lesion, skin inflammation)

Bovine Papillomavirus and Association with Sarcoid Formation

The BPV types 1 and 2 of the family Papillomaviridae have been implicated as major factors in the pathogenesis of sarcoids. Viral genomes, but not intact virions, have been consistently demonstrated in sarcoid lesions but not in other equine skin tumors or equine papillomas.[19–24] The presence of BPV DNA in sarcoids has varied between 73% and 100%, with lowest rates in studies based on formaldehyde fixed tissue stored for long periods.[21,23,25] BPV represents the only papillomavirus noted to cause infection across species. BPV has also been demonstrated to induce fibroblastic tumors in mice and malignant fibroblastic tumors in hamsters.[26,27] Papillomavirus DNA is often detected in macroscopically normal skin surrounding sarcoid lesions, and local recurrence has been correlated with DNA-positive surgical margins.[21] The levels of viral DNA were also reported to be significantly higher in and around more aggressive, rapidly growing, and multiple tumors in comparison with single or mild-type lesions.[21,22] Previously BPV was considered to reside in the dermis, but later studies have shown that the infection also involves the keratinocytes in the epidermis.[28,29]

Voss[30] was able to induce sarcoid lesions in healthy horses by inoculation of sarcoid tissue or cell-free supernatant to scarified skin. These lesions were indistinguishable from naturally occurring tumors. However, in other studies the injection of sarcoid tissue extract did not always result in tumor formation at other sites of the affected horse or on donors. In these studies, BPV-1 and BPV-2 inoculated into horse skin did cause fibroblastic proliferation, but lesions did not look like sarcoids histologically (they were lacking epidermal component and fibroblast activation at the dermoepidermal junction), and what did form spontaneously regressed, which rarely happens in naturally occurring sarcoids.[31,32] These horses with induced lesions also developed BPV antibodies, a feature not seen in horses with sarcoid tumors.

The BPV types 1 and 2 belong to the subgroup deltapapillomavirus or fibropapillomavirus, formerly called subgroup A, which has the capability to infect keratinocytes (KC) and induce proliferation of both KC and fibroblasts. Conversely, BPV of the subgroup xipapillomavirus or epitheliotropic virus, subgroup B, infects KC but can only induce proliferation of the epithelium.[33]

The genomes of all BPV are divided into early and late regions. Early regions encode 3 oncogenic proteins, E5, E6, and E7, and also nonstructural proteins. The late regions encode structural proteins L1 and L2.[34] BPV infection of equine fibroblasts appears to be mainly nonproductive with respect to producing complete viruses. If only the early genes, which encode for the oncogenic proteins in sarcoid tissue, are transcribed and the late genes are not, viral capsids are not formed. Likewise, expression for L1 was demonstrated in tissue,[35] but the expression of structural proteins or capsid formation needed for virion production was lacking.[22,35] The lack of virions in equine sarcoids has further been demonstrated when equine lesion extract was inoculated into cattle and the formation of papilloma was not induced.[32] One proposed reason for the inability to produce virions is that host-specific, well-differentiated KC constitute a required cellular environment that is necessary for expression of viral capsid proteins.[36]

However, one study was able to demonstrate that apart from BPV-1 DNA being present in all sarcoid samples tested, the detection of E5 and E7 was demonstrated in sarcoid epidermis. One lesion also had the late region L1 capsomer present in the squamous layer, indicating that the virus could be productive.[29]

It has been shown that BPV-1 genomes can transform equine fibroblasts, and that the tendency of fibroblasts to proliferate faster, survive longer, and grow independently of substrate was more pronounced with more viral E5, E6, and E7 gene expression.[37] In one study, all 23 sarcoids were positive for the presence of E5 by Western blot analysis, whereas all nonsarcoid tissues examined were negative.[38] Fibroblast transformation was further associated with E5 and E6 upregulating the expression of the mitogen-activated protein kinase (MAPK) p38 in BPV-1–expressing fibroblasts. Inhibition of p38 reduced proliferation and inhibited cellular invasiveness.[39] The oncoproteins E6 and E7 are activated via the activator protein 1, and by E5 upregulating equine matrix metalloproteinase 1, which contributes to the invasion capability of fibroblasts.[39] Furthermore, E5 was shown to be able to inhibit transcription of major histocompatibility complex (MHC)-I heavy chain and to prevent MHC-I complex from reaching the cell surface by retention in the Golgi apparatus in fetal equine fibroblasts. Thus, E5-induced downregulation of MHC-I might lead to tumor cells escaping immune responses.[40] In addition, in one study an altered DNA methylation status and redox milieu was demonstrated in BPV-1–infected fibroblasts derived from equine sarcoids. The investigators concluded that this might contribute to tumor formation.[41]

It has been proposed that BPV in equine and donkey sarcoid have genetic differences in comparison with BPV in cattle, in particular within the E5 sequence.[15,42] This aspect is potentially important in the pathogenesis of sarcoid formation. However, another report suggested that the BPV E5 sequences in sarcoids and the published BPV sequences were identical.[38] Later studies showed that BPV-1 RNA from equine sarcoids had a unique deletion within the L2 protein. For this reason, no late protein is detected and BPV does not produce infective virions in the epidermis. This finding suggested that the virus differed from BPV-1 in cattle.[43]

Bovine Papillomavirus Transmission

Sarcoids usually appear where the skin is thin or at sites of previous trauma.[11,12,22,44–46] Transmission of the virus to the skin is supposed to be via fomite, rubbing, and insect vectors. BPV-1 DNA has been detected in both biting and nonbiting flies (*Musca autumnalis, Fannia carnicularis*, and *Stomoxys calcitrans*) in the close environment of sarcoid-affected horses and donkeys. Furthermore, identical viral DNA was detected in sarcoids of animals from which these flies were collected.[45,47,48] Transmission between contact animals was suggested in a population of donkeys, but in this study, genetic factors (pedigrees were not included) might have been important.[15]

BPV DNA has been demonstrated in normal skin of sarcoid and healthy horses and also in horses with inflammatory skin conditions.[49,50] In nonsarcoid skin, the BPV DNA is found in the epidermis. In one study, more than 70% of normal skin with BPV DNA was from sarcoid-affected horses or horses living in contact with cattle. Fifty percent of healthy horses living together with sarcoid horses had BPV DNA, compared with 30% in control horses.[49] It has also been demonstrated that, in contrast to sarcoid lesions, nonsarcoid equine skin is more likely to have viral DNA in the epidermis and inflamed epidermis. The presence of BPV in inflamed skin was suggested to be a predisposing factor to the disease.[51] Brandt and colleagues[29] showed that BPV DNA was found not only in the dermis but also in the epidermis in all sarcoids examined, with more DNA copies in occult sarcoid epidermis than in the epidermis of fibroblast tumors.

One study also detected BPV DNA gene E5 in peripheral blood in sarcoid-bearing horses but not in healthy controls, suggesting a possible role for peripheral mononuclear cells as a reservoir for virus. The same group has also detected BPV DNA in lesions of hoof cancer.[52] However, Nasir and colleagues[53] were unable to detect the presence of BPV DNA in 34 diseased donkeys, and concluded that latent virus in circulating peripheral blood cells does not play a role in the pathogenesis and epidemiology of the equine sarcoid.

Genetic Factors

In addition to the viral association, genetic factors also seem to be important for the development of sarcoid lesions. In horses, a breed predilection for appaloosas, Arabians, quarter horses, and thoroughbreds has been documented, whereas standardbreds and Lippizaner horses are at a decreased risk. Mules, donkeys, and zebras also can be affected. In thoroughbreds and warmbloods, the increased risk has been associated with MHC-I A3 and W13 alleles, whereas in the standardbred and Lippizaner a decreased risk is associated with decreased W13 allele and a lack of W13, respectively. Furthermore, MHC-I A3 occurred more frequently in sarcoid-affected French horses.[54–58] In zebras, the prevalence in the Cape Mountain zebras (an inbred type of zebra, descendants from only 30 animals) is 25% to 53%, compared with 1.9% in outbred zebras.[17,59] On the other hand, a study of Swiss warmblood horses did not record a higher prevalence in offspring from stallions with known sarcoids in comparison with those with sires without a known history of sarcoids.[5]

CLINICAL PRESENTATION

Sarcoids are most commonly first noticed in horses 1 to 7 years of age.[14,60] The onset of the disease at an older age is rarely reported. In horses, there is no reported color or gender predisposition.[60] In donkeys, however, young males are reported to develop sarcoids more frequently than older males or females.[14]

The macroscopic presentation of sarcoids can be highly variable. A clinically based classification of sarcoids has been suggested by Knottenbelt,[6] which includes occult, verrucous, nodular, fibroblastic, mixed, and malignant/malevolent types of tumors. A high proportion of affected horses (14%–84%) have multiple tumors, and all types of sarcoids can be present in the same horse.[6,14] Sarcoids often develop at the site of previous trauma or where the skin is thin. Exposure to trauma can induce a worsening of the lesion and a more aggressive development of the tumor.[6,22,46,61] An early age of onset, time, and number of lesions are associated with larger tumor size.

Clinical Classification of Sarcoid Type

Occult sarcoids are focal areas with alopecia, scaling, skin thickening, hyperkeratosis, and hyperpigmentation.[6] Common locations include the neck, face, sheath, medial thigh, and shoulder. Differentials are mainly infectious folliculitis (bacterial folliculitis, dermatophytosis) and alopecia areata.

The verrucous type has a rough, alopecic, raised surface, which can be verrucous and irregular. This type is usually found on the head and neck and in the axillae and groin. The main differentials are papillomas or hamartomas.

Nodular sarcoids can be divided into types A and B whereby type A are spherical subcutaneous masses and type B have dermal involvement, which precludes the independent movement of the overlying skin. The overlying skin is often haired, but can become alopecic and ulcerated. These types are often seen in the eyelid region and

groin, and on the prepuce. Differentials include infectious, reactive inflammatory lesions (eosinophilic granuloma, foreign body) or other neoplastic processes.

Fibroblastic lesions are fleshy, ulcerated masses; type 1 is pedunculated and type 2 has a broad, locally invasive base. Common locations include the axillae, groin, legs, and the periocular region. Tumors can resemble granulation tissue or infectious processes, such as habronemiasis. Another differential is squamous cell carcinoma. Nonfibroblastic tumors can become fibroblastic after being traumatized.

Mixed forms (2 or more types) of tumors are common.

Malignant/malevolent tumors are aggressive and locally invasive. These tumors extend widely into adjacent skin and subcutis, are invasive, and infiltrate lymphatic vessels.

The occult and verrucous type can remain static for years if not traumatized. Nodular tumors can also remain unchanged, although less often. Any type of sarcoid lesion can develop into an aggressive fibroblastic or malignant/malevolent tumor if traumatized.

DIAGNOSIS

Sarcoids are diagnosed based on clinical signs and histopathology, as other conditions can macroscopically be difficult to differentiate from sarcoids (**Box 2**). For example, alopecia areata, bacterial folliculitis, and dermatophytosis can mimic occult sarcoids. Moreover, all types of nodular conditions (eosinophilic granulomas, melanomas, schwannomas, and so forth) can be mistaken for nodular sarcoids. One important differential diagnosis for fibroblastic sarcoids is exuberant granulation tissue. A biopsy should include deep dermal tissue and be at least 6 mm in diameter if a biopsy punch is used to harvest the sample. For thick, crusted lesions, a biopsy punch does not always reach deep enough into the tissue to reach the tumor, therefore a double-punch technique may be used whereby deep samples are obtained by a 6-mm punch biopsy introduced into a superficial opening created by an 8-mm punch biopsy.

Before verifying the diagnosis by biopsy, serious consideration should be given to the fact that traumatized sarcoids can potentially transform into a more aggressive type of tumor. Traumatic intervention should not be performed on a presumed sarcoid unless a treatment plan has been established after the diagnosis is confirmed.

Fine-needle aspiration (FNA) for cytology is usually unrewarding and carries the same risk of exacerbating tumor growth. FNA is not recommended for the diagnosis of sarcoids.

Histopathology typically reveals fibroblast proliferation. These spindle cells are often arranged in bundles, and have oval nuclei and small nucleoli. Mitotic figures are usually present in low numbers, but this can vary. Fibroblasts and collagen fibers have a whorled, tangled, crisscross, or linear or mixed pattern. Fibroblasts at the dermoepidermal junction can be arranged perpendicular to the basement membrane (picket-fence

Box 2
Diagnosis of sarcoid

- Differentials depend on the type of sarcoid
- Lesions clinically compatible with sarcoid and polymerase chain reaction positive for BPV-1 or BPV-2 DNA
- Histopathology: biopsy only if there is an intention to treat, should the diagnosis be verified

pattern), but this finding is not always present. The neoplastic cells are typically present immediately beneath the basement membrane, and extend downwards. A marked hyperplastic epidermis with deep rete ridges is present in approximately half of the cases. As sarcoids can be ulcerated, granulation tissue can be present along the surface. Occult and verrucous sarcoids have areas of marked hyperplastic epidermis with orthokeratotic hyperkeratosis. From the dermal-epidermal junction, the proliferating, spindle-shaped cells form a plaque-like infiltration. Hair follicles can still be unaffected and produce hairs.[62,63]

Schwannomas (nerve sheath tumors) can histologically be difficult to distinguish from sarcoids. Differentiation can be made with the help of immunohistochemistry staining for S-100 protein: schwannomas typically express S-100, whereas sarcoids stain negatively.[64]

Yet another diagnostic option is to demonstrate the presence of BPV-1 or BPV-2 DNA by polymerase chain reaction via superficial swabs and scrapings. A positive result is highly suggestive of the diagnosis. In one study, BPV DNA was demonstrated in 88% and 93% of sarcoid lesion swabs and scrapings, respectively, whereas all control lesions were negative.[20]

TREATMENT

Sarcoids are not metastatic but often show aggressive, infiltrative growth, and are notorious for a high recurrence rate after surgery. Furthermore, recurrent lesions are more refractory to treatment and carry a poorer prognosis. Spontaneous regression is rare.[60] If a horse exhibits lesions compatible with possible sarcoids, this tumor type has to be thoroughly discussed with the owner. As no treatment protocol has been universally effective, an assurance that the lesion will stay harmless or can be successfully treated can never be made.

Benign neglect is an option, especially for static occult and verrucous lesions and small tumors located where they are not at risk of being traumatized. In a study of periocular sarcoids, 15 lesions were left untreated because of their small size or uncertain diagnosis. In all these, cases treatment was later required (from 16 weeks to 15 years).[61]

Ligation of the base of sarcoids that have a stalk has been anecdotally reported to be successful. This treatment induces ischemia and necrosis of the tumor, and can only be attempted if the neck of the tumor is thin.

Surgical Intervention

If complete surgical removal with wide margins is possible, it may be an optimal choice for therapy. The presence of BPV DNA has been demonstrated up to 16 mm outside the macroscopic lesion, and local recurrence has been correlated to DNA-positive surgical margins.[65] The probability of local recurrence after sharp surgery or carbon dioxide laser was significantly higher for large sarcoids and sarcoids that had previously failed to respond to treatment.[65]

Sharp surgery has been reported to result in 50% to 64% recurrence of the tumor within 6 months. With nontouch techniques that avoid autoinoculation and wide surgical margins, the result improved to 82% (18 of 22) complete remission without recurrence.[65] In a retrospective study of 28 periorbital verrucous and nodular sarcoids without extensive involvement of the eyelid, 23 had recurrence (82%) and 39% grew additional tumors after surgery.[61] Furthermore, recurrence was associated with a more aggressive behavior. Combination of sharp surgery and other therapy, for example, cisplatin injection, has been reported to improve success rates. In one

case report, surgery was combined with photodynamic treatment in a horse with multiple lesions. Some, but not all sarcoids went into remission.[66]

Carbon dioxide laser treatment was reported by Carstanjen and colleagues[67] to result in remission without recurrence in 62% of sarcoids from 60 equids, of which 45 were horses. Follow-up was longer than 6 months. Animals with multiple sarcoids had a higher risk of recurrence. In another study, 20 of 28 sarcoids (71%) resolved after laser treatment.[65] Advantages over conventional surgery are reduced risks of postoperative swelling, pain, and hypergranulation.

Cryotherapy has achieved up to 42% to 100% success with no recurrence, with an average healing time of 2 to 4 months.[46,62] A successful outcome was obtained in 11 of 14 (79%) horses treated by cryosurgery.[65] However, when small (<2 cm^2), occult, or verrucous periocular sarcoids were treated with liquid nitrogen, 91% had aggressive, rapid-growing relapses within 12 weeks of treatment.[61] Side effects reported included posttreatment alopecia or regrowth of white hair and, if used on the face or periocular area, facial nerve paralysis and loss of the upper eyelid function. Caution is also warranted when lesions are located close to joints, as septic arthritis has been reported.

Hyperthermia through heating the sarcoid with a thermo-probe has been used alone or in combination with chemotherapy, radiotherapy, or immune modulation. Only a small number of cases resolved and stayed in remission for 6 months to 1 year. In a study of periocular sarcoids, 2 lesions were treated but neither case responded successfully.[61]

Radiotherapy, also known as interstitial brachytherapy (implants of, eg, iridium-192, cobalt-60, radium-226, radon-222, or gold-198) was effective in 50% to 100% of cases, with a low (5%) recurrence rate. By use of ionizing-radiation brachytherapy (iridium-192), 8 of 8 and 13 of 15 periocular and nonocular sarcoids, respectively, went into remission.[68,69] In another study, 66 nodular, fibroblastic, and mixed tumors were treated with brachytherapy γ-radiation with platinum-sheathed iridium. Of the 53 cases that were followed up, 98% resolved.[61]

Chemotherapy can be effective, either by intralesional injections (cisplatin powder emulsion in sesame oil or almond oil, or aqueous formulation of fluorouracil) or topical application (fluorouracil, thiouracil). Mixing cisplatin with oil increases the local concentration and prolongs the retention time of the drug, and reduces the risk of cisplatin-induced nephrotoxicity and hepatotoxicity. Cisplatin is usually recommended at a dose of 1 mg/cm^3 and is deposited intralesionally using sedation and analgesia 4 times at 2-week intervals. The treatment can be combined with debulking surgery. In a large study of 409 sarcoid lesions in horses, mules, and donkeys, 96.3% went into complete remission. Large lesions, gross postoperative residual disease, and a history of having previous treatments were negative prognostic factors. Local reactions were noticed after the third and fourth treatments in some cases.[70] In another study including 21 sarcoids, relapse-free survival rate was 92% at 1 year and 77% at 4 years.[71] Complete regression was recorded in 53%. Eighty-seven percent did not have recurrence after 12 months and another 27% decreased in size.[72] In 18 periocular, nodular, or fibroblastic sarcoids, cisplatin 1 mg/mL solution in almond oil led to resolution in 6 (33%) limited-size lesions. Injectable material was noted to ooze from the lesions after injection, indicating a risk of environmental contamination.[61] In another study, the efficacy of intralesional cisplatin in combination with intratumoral interleukin (IL)-2 led to complete regression in 53%, compared with only 14% when IL-2 was used alone.[73]

The intralesional administration of 5-fluorouracil into 13 sarcoids at the dose of 50 mg/cm^3 given every 2 weeks for up to 7 treatments resulted in complete regression in 61.5% of cases. Follow-up time was 3 years. Lesions larger than 13.5 cm^3

responded less favourably.[74] Topical 5% 5-fluorouracil was used twice daily for 5 days then once daily for 5 days, followed by 5 applications on an every-other-day schedule in 9 periocular, occult, and verrucous lesions. Six sarcoids resolved, and 3 improved but later developed into fibroblastic tumors.[61]

AW4(5)-LUDES, a combination of topical fluorouracil, thiouracil, and heavy metals, has been reported to result in 35% to 80% complete regression.[61] When used in 159 periocular small occult or verrucous sarcoids, 35% resolved. Significant scarring and a detrimental effect on eyelid function were recorded in 6 cases.[61] Because of the risk of side effects, this treatment is not recommended for periocular regions, distal limbs, and coronary bands.

With all chemotherapy protocols, consideration must be given to the risk of exposing health care personnel, owners, and others who handle the treated horses. Despite the implementation of safety precautions (eg, double gloves, mask, safety bench), surface contamination with antineoplastic drugs including 5-fluorouracil was detected in 65% to 75% of samples taken from 6 cancer treatment centers in the United States and Canada.[75] Furthermore, one study detected cytotoxic drugs (CD) or metabolites in urine samples from nurses not directly involved in drug preparation or administration.[76] Urine samples were positive for CD or drug metabolites, including platinum in 4.8% to 29% of health care personnel who were handling or administering these drugs in Italian hospitals.[77] In another study, urine samples from family members of treated patients were examined. In all samples, the antineoplastic drugs (5-fluorouracil and cyclophosphamide) or metabolites of the drugs were demonstrated. Primary DNA damage was significantly increased in leukocytes of nurses exposed to CD in comparison with controls.[78] These findings stress the importance of establishing strict safety precautions for both health care workers and owners who handle the horses.[79]

Antiviral Treatments

Acyclovir, used in human herpesvirus infections, is metabolized to the active form acyclovir triphosphate in virally infected cells. It inhibits viral DNA replication, but is not known to eradicate latent virus. Topical daily application of acyclovir 5% was used in 47 sarcoids, in a few of which after surgical debulking. Thirty-two of 47 (68%) lesions went into remission, whereas incomplete resolution was observed in the remaining 15 (32%). Tumor thickness was associated with a less favorable response.[80]

Cidofovir, used for the treatment of human cytomegalovirus and human papillomavirus, selectively inhibits viral DNA synthesis and interferes with caspase-3 activity.[81,82] Cidofovir and sucralfate gel was used topically to treat 1 occult, 1 fibroblastic, and 1 mixed fibroblastic/verrucous sarcoid. All had previously had surgery but relapsed. The occult and fibroblastic sarcoids resolved.[83]

Xanthates inhibit replication and transcription of DNA and RNA viruses. Subcutaneous injections of the xanthate tricyclodecan-9-yl-xanthogenate and potassium salt of lauric acid, given at 3-week intervals, were used to treat 15 sarcoids. In some cases the xanthate was given in combination with human tumor necrosis factor (TNF)-α. Complete resolution was recorded in more than 50% of cases at an 18-month follow-up.[25]

Immune-Modulating Treatments

Bacillus Calmette-Guérin (BCG) from *Mycobacterium bovis* is an immune modulator that stimulates host lymphocytes and natural-killer cells. With this therapy, only sarcoid cells undergo necrosis, as has been shown histologically. Inflammatory

reactions to BCG frequently require nonsteroidal anti-inflammatory (eg, flunixin) and/or corticosteroid treatment. The best results have been seen with periocular sarcoids. In one study, 18 of 27 (67%) lesions treated with BCG vaccination resolved. The recurrence rate was 16.5%.[65] In another study, 26 occult or verrucous and 283 nodular or fibroblastic periocular sarcoids received BCG treatment. Perilesional injections were not effective. Of 300 tumors treated intralesionally, 69% resolved, 25% remained unchanged, and 7% worsened. Treatment with 1.1 mg/kg flunixin and 0.2 mg/kg dexamethasone was given after the third injection to prevent anaphylaxis, a potential side effect of repeated BCG injections. With this protocol, 1 horse experienced an anaphylactic reaction.[61] BCG injections can be combined with debulking surgery.

Imiquimod is an immune-modulating agent used to treat human genital warts, actinic keratosis, and superficial basaliomas. Imiquimod has potent antiviral and antitumoral activity. It stimulates both the innate and acquired immune system via toll-like receptor 7, thereby inducing a T-helper–1 cytokine response (IL-2, IL-12, interferon [IFN]-α and -γ) as well as an increase of TNF-α, IL-1, IL-6, and IL-8. A 5% cream (Aldara) was used in one study, applied topically 3 times weekly for up to 32 weeks. Fifty-six percent of treated sarcoids went into complete remission and 20% had partial remission. Overall, 80% had a greater than 75% reduction in tumor size.[84] Side effects were pain, erythema, exudation, and erosion of the area to which Aldara was applied. In an open pilot study, 46 sarcoids were treated. Eighty-two percent went into complete remission after a mean treatment time of 3.7 months (up to 10.5 months). Three sarcoids relapsed within the follow-up time (mean 29.8 months).[85] Imiquimod was applied to healthy horse skin in 2 horses, 3 times weekly for 3 consecutive weeks. Both horses developed alopecia, pain, and thick crust formation at the application site. The lesions extended beyond the application area. Histologically the treated skin showed crusting, orthokeratotic hyperkeratosis, and epidermal hyperplasia. There was a moderate, subepidermal diffuse lymphoplasmacytic infiltration. In the deeper dermis, there was edema of the tunica adventitia and a perivascular infiltration of plasma cells.[86]

Baypamun P, an inactivated parapoxvirus used as a nonspecific immune stimulator, was shown to be ineffective in the treatment of equine sarcoids.[87]

Vaccination with chimeric virus-like particles (viral antigens BPV-1 L1-E7, without viral DNA) was well tolerated in 12 sarcoid-affected horses and led to an antibody response. This response, however, did not correlate with tumoral response to the therapy. Only 2 of 12 regressed and stayed in remission (follow-up was 63 days). Others either regressed and relapsed, regressed but developed new tumors, did not improve, or deteriorated.[88] In another study, neutralizing antibody levels remained high 2 years after the third injection. This factor could potentially be protective in horses susceptible to BPV-induced transformation of fibroblasts, based on either genetic risk factors or previous sarcoid history.[89]

A single report claimed good results using autogenous vaccine.[90] However, exacerbation or spreading of the disease has been reported after vaccination.[61] Inoculation with either sarcoid tissue or cell-free supernatant from minced tumors onto the scarified skin of sarcoid-free horses resulted in the appearance of tumors at the inoculation site. These sarcoids were morphologically indistinguishable from naturally occurring sarcoids.[30]

Miscellaneous

XXterra is an ointment containing zinc chloride and extract from bloodroot (*Sanguinaria canadensis*), which is rich in alkaloids, especially sanguinarine, chelerythrine, and protopine. XXterra has been used over many years to treat sarcoids or sarcoid-like

lesions. It is claimed to be immunomodulating and cytotoxic to cancer cells. Alkaloids and zinc chloride have escharotic and caustic properties. Sanguinarine has been shown to induce apoptosis, inhibit angiogenesis, and cause cell necrosis.[91–97] In an open pilot study, XXterra was used once daily for 4 to 6 days, then every fourth day, on 16 sarcoids. The treated area was covered with a bandage when possible. Total regression was seen in 10 (62%) after a mean treatment time of 2.5 months (maximum treatment period 6 months). The mean follow-up time was 34.8 months. One sarcoid relapsed within the follow-up period.[85] XXterra was applied to healthy horse skin in 2 horses, being used once daily for 5 days followed by twice-weekly application. The total treatment time was 3 weeks. There was no cover bandage. Alopecia, crusting, oozing, and erosions were seen at the application site. Histologically the treated area showed moderate orthokeratotic hyperkeratosis and epidermal hyperplasia. In the superficial dermis, there was evidence of fibroblast proliferation and disorganization of collagen fibers. There was also a mild, diffuse infiltration of lymphocytes. A perivascular infiltration of plasma cells was seen in the deeper dermis, where collagen fibers were still organized. Adnexa were absent or sparse.[86] Destruction of tissue including nose and ear cartilage, as well as deep tissue necrosis, has anecdotally been reported to occur.

Viscum album (European mistletoe) extract was injected intralesionally into 32 clinically suspected sarcoids, 3 times weekly for 105 days, in a placebo-controlled study.[98] Lesion remission was seen in 38% of treated sarcoids, compared with 13% in the placebo (NaCl) treatment group. European mistletoe contains triterpene, which has been shown to induce apoptosis in cancer cell lines in vitro. It also contains viscotoxins, alkaloids, and mistletoe lectin (ML-1), which have cytotoxic and immune-stimulating properties.

In conclusion, conflicting data exist regarding the efficacy of most of these cancer treatments, with many of the early publications being inconclusive for reasons of study bias.[99]

Factors indicating a less favorable prognosis

- Trauma to the tumor without treatment initiation
- Large tumor size
- Lack of response to, or relapse after, previous treatment intervention
- Viral DNA in surgical margins
- Treatment termination before eradication of tumor tissue

REFERENCES

1. Jackson C. The incidence and pathology of tumours of domesticated animals in South Africa. Onderstepoort J Vet Sci Animal Indust 1936;6:378–85.
2. Pascoe RR, Summers PM. Clinical survey of tumours and tumour-like lesions in horses in south east Queensland. Equine Vet J 1981;13(4):235–9.
3. Ragland KW, Keown GF. Equine sarcoid. Equine Vet J 1970;2:2–11.
4. Shaffer PA, Wobeser B, Martin LE, et al. Cutaneous neoplastic lesions of equids in the central United States and Canada: 3,351 biopsy specimens from 3,272 equids (2000-2010). J Am Vet Med Assoc 2013;242(1):99–104.

5. Studer S, Gerber V, Straub R, et al. Prevalence of hereditary diseases in three-year-old Swiss Warmblood horses. Schweiz Arch Tierheilkd 2007;149(4):161–71.
6. Knottenbelt DC. A suggested clinical classification of the equine sarcoid. Clin Tech Equine Pract 2005;4:278–95.
7. Buechner-Maxwell V. Skin tumors. In: Robinson NE, Sprayberry KA, editors. Current therapy in equine medicine. 6th edition. St Louis (MO): Saunders Elsevier; 2009. p. 692.
8. Meuten DJ, editor. Tumors in domestic animals. 4th edition. Ames (IA): Iowa State Press; 2002.
9. Quinn G. Skin tumours in the horse: clinical presentation and management. In Pract 2003;25:476.
10. Valentine BA. Survey of equine cutaneous neoplasia in the Pacific Northwest. J Vet Diagn Invest 2006;18(1):123–6.
11. White SD. Diseases of the skin. In: Smith BP, editor. Large animal internal medicine. 4th edition. St Louis (MO): Mosby Elsevier; 2009. p. 1306.
12. Foy JM, Rashmir-Raven AM, Brashier MK. Common equine skin tumours. Comp of Contin Vet Practice 2002;24:242–52.
13. Marti Lazary S, Antczak DF, Gerber H. Report of the first international workshop on equine sarcoid. Equine Vet J 1993;25:397–407.
14. Goodrich I, Gerber H, Marti E, et al. Equine sarcoids. Vet Clin North Am Equine Pract 1998;14:607–9.
15. Reid SW, Gettinby G, Fowler JN, et al. Epidemiological observations on sarcoids in a population of donkeys (*Equus asinus*). Vet Rec 1994;134(9):207–11.
16. Marais HJ, Nel P, Bertschinger HJ, et al. Prevalence and body distribution of sarcoids in South African Cape mountain zebra (*Equus zebra zebra*). J S Afr Vet Assoc 2007;78(3):145–8.
17. Marais HJ, Page PC. Treatment of equine sarcoid in seven Cape mountain zebra (*Equus zebra zebra*). J Wildl Dis 2011;47(4):917–24.
18. van Dyk E, Oosthuizen MC, Bosman AM, et al. Detection of bovine papillomavirus DNA in sarcoid-affected and healthy free-roaming zebra (*Equus zebra*) populations in South Africa. J Virol Methods 2009;158(1–2):141–51.
19. Angelos JA, Marti E, Lazary S, et al. Characterization of BPV-like DNA in equine sarcoids. Arch Virol 1991;119(1–2):95–109.
20. Martens A, De Moor A, Ducatelle R. PCR detection of bovine papilloma virus in superficial swabs and scrapings from equine sarcoids. Vet J 2001;161(3):224–6.
21. Martens A, De Moor A, Demeulemeester J, et al. Polymerase chain reaction analysis of the surgical margins of equine sarcoids for bovine papilloma virus DNA. Vet Surg 2001;30(5):460–7.
22. Chambers G, Ellsmore VA, O'Brien PM, et al. Association of bovine papillomavirus with the equine sarcoid. J Gen Virol 2003;84:1055–62.
23. Carr EA, Théon AP, Madewell BR, et al. Bovine papillomavirus DNA in neoplastic and non-neoplastic tissues obtained from horses with and without sarcoids in the western United States. Am J Vet Res 2001;62(5):741–4.
24. Nasir L, Campo MS. Bovine papillomaviruses: their role in the aetiology of cutaneous tumours of bovids and equids. Vet Dermatol 2008;19(5):243–54.
25. Otten N, Marti E, Söderström C, et al. Experimental treatment of equine sarcoid using a xanthate compound and recombinant human tumour necrosis factor alpha. Zentralbl Veterinarmed A 1994;41(10):757–65.
26. Boiron M, Levy JP, Thomas M, et al. Some properties of bovine papilloma virus. Nature 1964;201:423–4.

27. Haralambus R, Burgstaller J, Klukowska-Rötzler J, et al. Intralesional bovine papillomavirus DNA loads reflect severity of equine sarcoid disease. Equine Vet J 2010;42(4):327–31.
28. Bogaert L, Martens A, Kast WM, et al. Bovine papillomavirus DNA can be detected in keratinocytes of equine sarcoid tumors. Vet Microbiol 2010;146(3–4): 269–75.
29. Brandt S, Tober R, Corteggio A, et al. BPV-1 infection is not confined to the dermis but also involves the epidermis of equine sarcoids. Vet Microbiol 2011;150(1–2):35–40.
30. Voss JL. Transmission of equine sarcoid. Am J Vet Res 1969;30(2):183–91.
31. Olson C, Cook RH. Cutaneous sarcoma-like lesions of the horse caused by the agent of bovine papilloma. Proc Soc Exp Biol Med 1951;77:281.
32. Ragland WL, Spencer GR. Attempts to relate bovine papilloma virus to the cause of equine sarcoid: equidae inoculated intradermally with bovine papilloma virus. Am J Vet Res 1969;30(5):743–52.
33. Campo MS. Bovine papillomavirus: old system, new lessons?. In: Campo MS, editor. Papillomavirus research: from natural history to vaccine and beyond. Wymondham, England: Caister Academic Press; 2006.
34. Shah KV, Howley PM. Papillomaviruses. In: Fields BN, Knipe DM, Howley PM, et al, editors. Fields virology. 3rd edition. Philadelphia: Lippincott-Raven Publishers; 1996. p. 2077–101.
35. Nasir L, Reid SW. Bovine papilloma viral gene expression in equine sarcoid tumours. Virus Res 1999;61(2):171–5.
36. Yuan Z, Gallagher A, Gault EA, et al. Bovine papillomavirus infection in equine sarcoids and in bovine bladder cancers. Vet J 2007;174(3):599–604.
37. Sousa R, Dostatni N, Yaniv M. Control of papillomavirus gene expression. Biochim Biophys Acta 1990;1032(1):19–37.
38. Yuan ZQ, Gault EA, Gobeil P, et al. Establishment and characterisation of equine fibroblast cell lines transformed in vivo and in vitro by BPV-1: model systems for equine sarcoids. Virology 2008;373(2):352–61.
39. Carr EA, Théon AP, Madewell BR, et al. Expression of a transforming gene (E5) of bovine papilloma virus in sarcoids obtained from horses. Am J Vet Res 2001; 62(8):1212–7.
40. Yuan Z, Gault EA, Campo MS, et al. p38 mitogen-activated protein kinase is crucial for bovine papillomavirus type-1 transformation of equine fibroblasts. J Gen Virol 2011;92(Pt 8):1778–86.
41. Marchetti B, Gault EA, Cortese MS, et al. Bovine papillomavirus type 1 oncoprotein E5 inhibits equine MHC class I and interacts with equine MHC I heavy chain. J Gen Virol 2009;90(Pt 12):2865–70.
42. Altamura G, Strazzullo M, Corteggio A, et al. O(6)-methylguanine-DNA methyltransferase in equine sarcoids: molecular and epigenetic analysis. BMC Vet Res 2012;8:218.
43. Chambers G, Ellsmore VA, O'Brien PM, et al. Sequence variants of bovine papillomavirus E5 detected in equine sarcoids. Virus Res 2003;96(1–2): 141–5.
44. Wilson AD, Armstrong EL, Gofton RG, et al. Characterisation of early and late bovine papillomavirus protein expression in equine sarcoids. Vet Microbiol 2013;162(2–4):369–80.
45. Bogaert L, Martens A, De Baere C, et al. Detection of bovine papillomavirus DNA on the normal skin and in the habitual surroundings of horses with and without equine sarcoids. Res Vet Sci 2005;79(3):253–8.

46. Carr EA. New developments in diagnosis and treatment of equine sarcoid. In: Robinson NE, Sprayberry KA, editors. Current therapy in equine medicine. 6th edition. St Louis (MO): Saunders Elsevier; 2009. p. 698.
47. Kemp-Symonds JG. The detection and sequencing of bovine papillomavirus type 1 and 2 DNA from *Musca autumnalis* (Diptera: Muscidae) face flies infesting sarcoid-affected horses [MSc thesis]. London: Royal Veterinary College; 2000.
48. Finlay M, Yuan Z, Burden F, et al. The detection of bovine papillomavirus type 1 DNA in flies. Virus Res 2009;144(1–2):315–7.
49. Bogaert L, Martens A, Van Poucke M, et al. High prevalence of bovine papillomaviral DNA in the normal skin of equine sarcoid-affected and healthy horses. Vet Microbiol 2008;129(1–2):58–68.
50. Yuan Z, Philbey AW, Gault EA, et al. Detection of bovine papillomavirus type 1 genomes and viral gene expression in equine inflammatory skin conditions. Virus Res 2007;124(1–2):245–9.
51. Wobeser BK, Hill JE, Jackson ML, et al. Localization of bovine papillomavirus in equine sarcoids and inflammatory skin conditions of horses using laser microdissection and two forms of DNA amplification. J Vet Diagn Invest 2012;24(1):32–41.
52. Brandt S, Schoster A, Tober R, et al. Consistent detection of bovine papillomavirus in lesions, intact skin and peripheral blood mononuclear cells of horses affected by hoof canker. Equine Vet J 2011;43(2):202–9.
53. Nasir L, McFarlane ST, Torrontegui BO, et al. Screening for bovine papillomavirus in peripheral blood cells of donkeys with and without sarcoids. Res Vet Sci 1997;63(3):289–90.
54. James VS. A familial tendency to equine sarcoids. Southwest Veterinarian 1968;21:235–6.
55. Lazary S, Gerber H, Glatt PA, et al. Equine leukocyte antigens in sarcoid affected horses. Equine Vet J 1985;17:283–6.
56. Meredith D, Elser AH, Wolf B, et al. Equine leukocyte antigens: relationships with sarcoid tumors and laminitis in two pure breeds. Immunogenetics 1986;23(4):221–5.
57. Angelos J, Oppenheim Y, Rebhun W, et al. Evaluation of breed as a risk factor for sarcoid and uveitis in horses. Anim Genet 1988;19(4):417–25.
58. Brostrom H, Fahlbrink E, Dubath ML, et al. Association between equine leukocyte antigens (ELA) and equine sarcoid tumours in the population of Swedish halfbreds and some of their families. Vet Immunol Immunopathol 1988;19:215–23.
59. Nel PJ, Bertschinger H, Williams J, et al. Descriptive study of an outbreak of equine sarcoid in a population of Cape mountain zebra (*Equus zebra zebra*) in the Gariep Nature Reserve. J S Afr Vet Assoc 2006;77(4):184–90.
60. Broström H. Equine sarcoids. A clinical and epidemiological study in relation to equine leucocyte antigens (ELA). Acta Vet Scand 1995;36(2):223–36.
61. Knottenbelt DC, Kelly DF. The diagnosis and treatment of periorbital sarcoid in the horse: 445 cases 1974 to 1999. Vet Ophthalmol 2000;3:169–91.
62. Scott DW, Miller WH, editors. Equine dermatology. 2nd edition. St Louis (MO): Saunders Elsevier; 2011. p. 479.
63. Martens A, De Moor A, Demeulemeester J, et al. Histopathological characteristics of five clinical types of equine sarcoid. Res Vet Sci 2000;69(3):295–300.
64. Bogaert L, Heerden MV, Cock HE, et al. Molecular and immunohistochemical distinction of equine sarcoid from schwannoma. Vet Pathol 2011;48(3):737–41.

65. Martens A, De Moor A, Vlaminck L, et al. Evaluation of excision, cryosurgery and local BCG vaccination for treatment of equine sarcoids. Vet Rec 2001;145: 665–9.
66. Reschke C. Successful treatment of an equine sarcoid. Case report on a combined surgical and photodynamic therapy. Tierarztl Prax Ausg G Grosstiere Nutztiere 2012;40(5):309–13.
67. Carstanjen B, Jordan P, Lepage OM. Carbon dioxide laser as a surgical instrument for sarcoid therapy—a retrospective study on 60 cases. Can Vet J 1997; 38(12):773–6.
68. Théon AP, Pascoe JR. Iridium-192 interstitial brachytherapy for equine periocular tumours: treatment results and prognostic factors in 115 horses. Equine Vet J 1995;27(2):117–21.
69. Byam-Cook KL, Henson FM, Slater JD. Treatment of periocular and non-ocular sarcoids in 18 horses by interstitial brachytherapy with iridium-192. Vet Rec 2006;159(11):337–41.
70. Théon AP, Wilson WD, Magdesian KG, et al. Long-term outcome associated with intratumoral chemotherapy with cisplatin for cutaneous tumors in equidae: 573 cases (1995-2004). J Am Vet Med Assoc 2007;230(10):1506–13.
71. Théon AP, Pascoe JR, Galuppo LD, et al. Comparison of perioperative versus postoperative intratumoral administration of cisplatin for treatment of cutaneous sarcoids and squamous cell carcinomas in horses. J Am Vet Med Assoc 1999; 215(11):1655–60.
72. Théon AP, Pascoe JR, Carlson GP, et al. Intratumoral chemotherapy with cisplatin in oily emulsion in horses. J Am Vet Med Assoc 1993;202(2):261–7.
73. Spoormakers TJ, Klein WR, Jacobs JJ, et al. Comparison of the efficacy of local treatment of equine sarcoids with Il-2 or cisplatin/Il-2. Cancer Immunol Immunother 2003;52(3):179–84.
74. Stewart AA, Rush B, Davis E. The efficacy of intratumoral 5-flourouracil for the treatment of equine sarcoids. Aust Vet J 2006;84(3):101–6.
75. Connor TH, Anderson RW, Sessink PJ, et al. Surface contamination with antineoplastic agents in six cancer treatment centers in Canada and the United States. Am J Health Syst Pharm 1999;56(14):1427–32.
76. Sessink PJ, Boer KA, Scheefhals AP, et al. Occupational exposure to antineoplastic agents at several departments in a hospital. Environmental contamination and excretion of cyclophosphamide and ifosfamide in urine of exposed workers. Int Arch Occup Environ Health 1992;64(2):105–12.
77. Turci R, Sottani C, Ronchi A. Biological monitoring of hospital personnel occupationally exposed to antineoplastic agents. Toxicol Lett 2002;134(1–3):57–64.
78. Villarini M, Dominici L, Piccinini R, et al. Assessment of primary, oxidative and excision repaired DNA damage in hospital personnel handling antineoplastic drugs. Mutagenesis 2011;26(3):359–69.
79. Yuki M, Sekine S, Takase K, et al. Exposure of family members to antineoplastic drugs via excreta of treated cancer patients. J Oncol Pharm Pract 2013;19(3): 208–17.
80. Stadler S, Kainzbauer C, Haralambus R, et al. Successful treatment of equine sarcoids by topical acyclovir application. Vet Rec 2011;168(7):187.
81. Bonatti H, Aigner F, De Clercq E, et al. Local administration of cidofovir for human papilloma virus associated skin lesions in transplant recipients. Transpl Int 2007;20(3):238–46.
82. Andrei G, Snoeck R. Cidofovir activity against poxvirus infections. Viruses 2010; 2(12):2803–30.

83. Scagliarini A, Bettini G, Savini F, et al. Treatment of equine sarcoids. Vet Rec 2012;171:330.
84. Nogeira SA, Torres SM, Malone E, et al. Efficacy of imiquimod 5% cream in the treatment of equine sarcoids: a pilot study. Vet Dermatol 2006;17:259–65.
85. Pettersson C, Bergvall K, Humblot P, et al. Topical treatment of equine sarcoids: a clinical pilot study comparing Aldara and Xxterra. Vet Dermatol 2011;22:466.
86. Bergvall K, Broström H, Grandon R. Clinical and histological response to topical application of Xxterra and Aldara on healthy horse skin. Vet Dermatol 2012; 23(Suppl 1):50.
87. Studer U, Marti E, Stornetta D, et al. The therapy of equine sarcoid with a non-specific immunostimulator—the epidemiology and spontaneous regression of sarcoids. Schweiz Arch Tierheilkd 1997;139(9):385–91.
88. Mattil-Fritz S, Scharner D, Piuko K, et al. Immunotherapy of equine sarcoid: dose-escalation trial for the use of chimeric papillomavirus-like particles. J Gen Virol 2008;89(Pt1):138–47.
89. Hainisch EK, Brandt S, Shafti-Keramat S, et al. Safety and immunogenicity of BPV-1 L1 virus-like particles in a dose-escalation vaccination trial in horses. Equine Vet J 2012;44(1):107–11.
90. Kinnunen RE. Equine sarcoid tumour treated by autogenous tumour vaccine. Anticancer Res 1999;19(4C):3367–74.
91. Adhami VM, Aziz MH, Reagan-Shaw SR, et al. Sanguinarine causes cell cycle blockade and apoptosis of human prostate carcinoma cells via modulation of cyclin kinase inhibitor-cyclin-cyclin-dependent kinase machinery. Mol Cancer Ther 2004;3(8):933–40.
92. Adhami VM, Aziz MH, Mukhtar H, et al. Activation of prodeath Bcl-2 family proteins and mitochondrial apoptosis pathway by sanguinarine in immortalized human HaCaT keratinocytes. Clin Cancer Res 2003;9(8):3176–82.
93. Kaminskyy V, Lin KW, Filyak Y, et al. Differential effect of sanguinarine, chelerythrine and chelidonine on DNA damage and cell viability in primary mouse spleen cells and mouse leukemic cells. Cell Biol Int 2008;32(2):271–7.
94. Hussain AR, Al-Jomah NA, Siraj AK, et al. Sanguinarine-dependent induction of apoptosis in primary effusion lymphoma cells. Cancer Res 2007;67(8):3888–97.
95. Basini G, Bussolati S, Santini SE, et al. Sanguinarine inhibits VEGF-induced angiogenesis in a fibrin gel matrix. Biofactors 2007;29(1):11–8.
96. Basini G. The plant alkaloid sanguinarine is a potential inhibitor of follicular angiogenesis. J Reprod Dev 2007;53(3):573–9.
97. Kim S, Lee TJ, Leem J, et al. Sanguinarine-induced apoptosis: generation of ROS, down-regulation of Bcl-2, c-FLIP, and synergy with TRAIL. J Cell Biochem 2008;104(3):895–907.
98. Christen-Clottu O, Klocke P, Burger D, et al. Treatment of clinically diagnosed equine sarcoid with a mistletoe extract (*Viscum album austriacus*). J Vet Intern Med 2010;24(6):1483–9.
99. Kienle GS, Berrino F, Büssing A, et al. Mistletoe in cancer—a systematic review on controlled clinical trials. Eur J Med Res 2003;8(3):109–19.