



A Review on Equine Sarcoid: Current Techniques Employed in Sciences for Diagnosis, Prevention and Control

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ABSTRACT

Equine sarcoids are the most common equine skin tumors distributed world-wide which affects horses, donkeys and mules. Bovine papillomavirus (BPV) is causally associated with equine sarcoid with genetic, fly vectors and skin trauma identified as potential predisposing factors for development of the disease. The methods of transmission are contamination of the environment with subsequent trauma; infection of the skin direct or indirect transmission from affected animals; biting, rubbing, fomites or management practices and face flies. Dermatophytis, chronic skin rubbing, equine papillomatosis, squamous cell carcinoma, fibroma, fibrosarcoma, melanoma and granulation tissue are the common differential diagnoses of equine sarcoids. Clinical examination, histopathology, detection of BPV DNA by PCR, electron microscope and immunohistochemistry are the diagnostic technique of equine sarcoid. The histopathological characteristics of sarcoids, found that the classic epithelial changes of hyperkeratosis, hyperplasia, elongated rete pegs and picket fence. Treatment of equine sarcoids are challenging for the veterinarians due to the variable clinical presentation of the lesions and the frequent local recurrences. Both surgical and non-surgical techniques are used for the treatment of equine sarcoids with variable success rates. None of the current treatment techniques for equine sarcoid have been proven to be 100% successful. Awareness enhancement to make equine owners, prevention of fly contact, use of breeding from those individuals that are 'immune', high genetic resistance, early diagnosis and treatment of any confirmed lesions are the most valuable prevention methods for equine sarcoids. The objectives of this paper is to review on equine sarcoid and to assess current techniques employed for diagnosis and prevention of equine sarcoid

Keywords: Diagnosis, Equine sarcoid, Papillomavirus, Prevention

The equine sarcoid, a locally aggressive, fibroblastic skin tumour, is the most common dermatological neoplasm of equines which found in worldwide. Bovine papilloma virus (BPV) types 1 and 2 are causally associated with the development and pathogenesis of equine sarcoids which represent the only known cross-species papillomavirus infection which occurs in horses, donkeys and mules. Sarcoids vary greatly in their size and appearance, the nature in which they grow, potentially spread and the way respond for treatment (Goldschmidt and Hendrick, 2002).

Diagnosis of equine sarcoid can be done clinical examination by, histopathology and detection of BPV DNA by PCR techniques and others. The choices of appropriate treatment for each clinically recognizable

types of equine sarcoids are determined by different factors (Pascoe, 2005). Prevention of equine sarcoid may be facilitated by future development of vaccines against bovine papilloma virus. There is much reduced chance of a sarcoid developing at the site when fly repellents and petroleum jelly are applied immediately after wounding. Prevention of fly contact seems to be a major issue and so these measures apply more to seasons when flies are a problem (Humphreys *et al.*, 2004). Equine sarcoid affects equids, that may interfere with the use of the horse, causing considerable loss to the equine, but also represents a therapeutic challenge to the veterinarian practitioner since there is no current 100% effective treatment for this disease, and recurrency is often reported (Scott and Miller, 2003). Understanding what causes of equine sarcoid,



diagnosis and prevention in equine, not only reduces the economic loss but also enhance different services provide by equine. Present paper is focused on the present status, new diagnostic approaches and therapeutic strategies of equine sarcoid.

Etiology

The name “sarcoid” means “flesh-like” and should suggest the proliferative sarcomatous appearance of lesions. Sarcoids with a characteristic gross appearance which are not painful and also non-pruritic. Bovine papillomavirus (BPV) types 1 and 2 are causally associated with the development and pathogenesis of equine sarcoids (Nasir and Campo, 2008). Bovine papillomavirus (BPV) belong to the Papillomaviridae family which consists on non-enveloped DNA virus that infect humans, many domestic and wild species of animals BPV very resistant virus that can survive several months in the environment. Bovine papillomavirus DNA has been detected in up to 100% of examined sarcoid tumours. In addition, viral oncogenes and capsid gene transcripts have also been identified in sarcoids, providing evidence for direct involvement of BPV in the pathogenesis of sarcoids (Chambers *et al.*, 2003).

Pathogenesis

When equine fibroblast gets infected by BPV, a number of cellular changes can happen resulting in cell transformation and uncontrolled growth. Initially the MHC involved in immune regulation plays a main role. Expression of the major papillomavirus oncoprotein, E5, mediates numerous aspects of the viral pathogenesis both in bovine and equine tumours. This protein disturbs the cell cytoskeleton and the Golgi apparatus, activates numerous protein kinases, including growth factor receptor kinases, thus interfering with proper cell-cycle control and signal transduction cascades, and down-regulates MHC class I. Therefore, this protein is responsible for both cell proliferation and evasion of the host immune response (Ashrafi *et al.*, 2002).

Predisposing Factors

Age

Sarcoids are generally observed in young adult horses, between three and six years of age, also have been reported

in young and old age but most sarcoids are commonly seen in young horses (Hartl *et al.*, 2011).

Gender

Sex has one of the factors for sarcoid development and high risk among male donkeys, with sarcoids occurring most commonly on the paragenital region. Where a gender predisposition has been reported, geldings have been at an increased risk compared to stallions and mares (Reid *et al.*, 1994).

Breed

Breeds such as Quarter Horses, Arabians and Appaloosas are roughly higher risk to develop sarcoids as compared to thoroughbreds. But thoroughbreds are roughly twice as likely to develop sarcoids as standardbred. The reason for this predisposition suggested that Quarter Horses and Appaloosas are more likely to be in contact with cattle and thus more likely to be in contact with BPV. Alternatively, it may be that these breeds are more likely to work on rough surfaces and thus more likely to develop wounds to the legs. In addition to lifestyle factors, differences in the genetic makeup of these various breeds may contribute to the differences in susceptibility to development of Sarcoid. The thinner-skinned breeds such as Arabian are particularly predisposed to the disease. Lipizzaner horses appear to have some resistance towards the condition, and it has already been identified the genetic basis of this state. However, no breed is free from developing sarcoids (Hartl *et al.*, 2011).

Diagnosis

Diagnosis of equine sarcoid can be done by clinical examination, histopathology and detection of BPV DNA by PCR, immunohistochemistry. An individual lesion on a horse are difficult to diagnose but a clinical examination and anamnesis based on duration of the problem, localization of the lesion, evolution, age and breed of the horse combined with clinician experience should be sufficient in most cases. Diagnosis based on clinical appearance might, in one hand, mean that the treatment can be instituted immediately leading to a better prognosis but, on the other hand, can result in misdiagnosis and inappropriate recommendations or treatments followed

by higher recurrent rates that are harder to resolve with subsequent treatment. In those cases where some diagnostic confusion is present because of atypical tumor characteristics or lack of clinical experience, lab-assisted diagnosis might be required (Martens *et al.*, 2000).

Clinical presentation

A presumptive diagnosis is based on clinical appearance. Different types of sarcoid tend to exhibit different growth rates and some are more locally aggressive than others. The equine periorbital sarcoid is more clinically challenging (Knottenbelt and Matthews, 2001). The notorious difficulties with treatment of sarcoids in particular make the prognosis for periorbital sarcoid very guarded. The function of the eyelid is critical to the health of the eye and the thin elastic nature of the eyelid skin introduces further difficulties. Complications can arise directly or indirectly from the treatment but failure to treat the tumours at this site can also have severe consequences for vision and use for riding. Inadequate tissue removal results in recurrence and complete removal can result in unacceptable cicatrisation and loss of function (Scott and Miller, 2003).

Occult sarcoid: An area of altered, slightly thickened skin with thin hair coat and slight changes in hair pigment may be encountered. The lesions are characteristically roughly circular, slow growing; they may progress to “warty” verrucose growths or if injured may develop rapidly into fibroblastic lesions (Charles, 2005). The predilection sites include the skin around the mouth and eyes, the neck and other relatively hairless areas of the body (McCauley *et al.*, 2002).

Verrucose (Warty) sarcoid: Lesions exhibit a typical warty like appearance with variable degrees of hyperkeratotic roughness, thickness, flaking and scaling above the fibroblastic part of the tumor. This type of sarcoids tends to present itself as a slow growing and not very aggressive pattern over limited or wide areas until insulted. Individual lesions can be flat or pedunculated giving a true wart-like appearance. Sites commonly affected comprise face and ear base, axillae and groin/sheath areas (Scott and Miller, 2003).

Nodular sarcoid: Nodular sarcoids are subcutaneous, easily moveable nodules, often but not always spherical,

covered by intact, apparently normal skins which are divided in type A and B nodules. In type A nodules there are no strong attachments between covering skin and underlying tissues to the nodules. Type B nodules bare obvious dermal involvement the overlying skin cannot be moved independently (Hartl *et al.*, 2011).

Fibroblastic sarcoid

Fibroblastic sarcoids are large fibrous masses with an ulcerated surface. This is the most aggressive sarcoid type and can evolve from any other type after accidental or iatrogenic manipulation, including biopsy. Skin wounds, especially at the distal limbs, are also at risk for the development of fibroblastic sarcoids. Fibroblastic sarcoids are liable to trauma, haemorrhage and local infection with bacteria or maggots. Fibroblastic sarcoids can be sessile with an invasive character or pedunculated with a small base (Scott and Miller, 2003).

Mixed sarcoid: Mixed (occult, verrucose, Nodular and Fibroblastic) sarcoids are a combination of two or more of these types can be represent a progressive or transient state between the verrucose or occult types and the fibroblastic/nodular types (Hartl *et al.*, 2011).

Malignant sarcoid: Malignant or malevolent sarcoid is particularly invasive type of tumor which can infiltrate into lymphatic vessels resulting in multiple tumor masses along the vessels and local lymph nodes. Malignant type is most commonly occurred in the skin of the jaw and face, elbow and medial thigh areas (Knottenbelt, 2005).

Histopathological examination

The histological properties seem to be mainly dependent of the clinical type. Typical histopathological signs of sarcoids are dermal proliferation of fusiform or spindle-shaped fibroblasts; epidermal hyperplasia and hyperkeratosis; rete peg formation and a picket fence formation at the dermal-epidermal junction (Mohamed *et al.*, 2012).

In verrucose sarcoids, the epithelial component is much more important than the dermal, sometimes only lying as a small band of active fibroblasts against the epidermis. In the fibroblastic type there is always partial or total ulceration of the epidermis with infiltration of polymorphonuclear cells (Chambers *et al.*, 2003).



Polymerase Chain Reaction (PCR)

A more recent diagnostic approach is the detection of BPV DNA by PCR. Polymerase chain reaction detection of BPV DNA has many advantages being a non-invasive technique, having easy sampling and minimal trauma to the tumor. PCR aims to amplify BPV DNA from skin tissue samples from 98-100%. Unsuitability for diagnosing occult sarcoids, the lower sensitivity compared to clinical diagnosis and the low specificity due to high prevalence of BPV DNA in the normal skin of horses are the disadvantages of PCR. Real-time PCR is an established tool for DNA quantification that measures the accumulation of DNA product after each round of PCR amplification. It has engendered wider acceptance of the PCR due to its improved rapidity sensitivity, reproducibility and the reduced risk of carry-over contamination (Nasir and Campo, 2008).

Electron microscopy

Transmission electron microscopy can be a useful procedure for tumor identification in selected cases. In all cases, the diagnostician should have specific features in mind when undertaking ultrastructural examination. A microscopically verified diagnosis is often necessary for optimizing patient management and, in the field of cancer at least, many purely clinical diagnoses are re-assigned by histopathological microscopical investigation. In tumour diagnosis, successful characterization of a given tumour depends on finding its distinctive cell and/or matrix structures (distinctive or specific ultrastructure). There are a few general ultrastructural features that distinguish neoplastic cells from normal cells. These features are altered size and often segmented shapes of the nuclei, increased numbers, increased size, and variations in the shape of nucleoli (Eyden, 2001).

Immunohistochemistry

Immunohistochemistry is an important ancillary diagnostic aid for tumor identification. The advent of a broad variety of antibodies has facilitated the identification of tumors through the use of antibodies that bind to cell specific proteins. Immunohistochemical stains use monoclonal or polyclonal antibodies raised against the pathogen to target areas in tissue where a pathogen is present, coupled with

a technique for flagging the pathogen either a fluorescent dye or a chemical chromogen. A method for localizing specific antigens in tissues and cells based on antigen-antibody recognition (McCauley *et al.*, 2002).

Differential diagnosis

A definitive diagnosis of equine sarcoid confirm from the distinctive histopathologic features and the differential diagnoses of the equine sarcoids depend on the sarcoid type. Dermatophytosis, chronic skin rubbing, equine papillomatosis (warts), hyperkeratosis, squamous cell carcinoma, fibroma, fibrosarcoma, melanoma and granulation tissue are the common differential diagnoses of equine sarcoids which are not specific for the type of sarcoid (Knottenbelt and Matthews, 2001).

TREATMENT

Treatment of equine sarcoids has always been challenging, due to the variable clinical presentation of the disease and its high recurrence rate after treatment. Several treatment methods have been described throughout the years, with variable success rates, but there is no current treatment known to be 100% effective (Von Felbert *et al.*, 2005). The factors that should be considered when choosing the appropriate treatment for each case of equine sarcoids and that might affect the prognosis include the value of the equines, the cost of treatment, the type, behaviour, location and number or extent of the tumors, time between tumor development and presentation, history and the risk of therapy both for the patient and owner (Pascoe, 2005).

Surgical treatments

Laser removal

A CO₂ laser is a surgical instrument that cuts and vaporises soft tissue with minimal intraoperative haemorrhage and less post-operative oedema and pain compared to scalpel blades. Equipment is expensive and is not commonly available. Failure is possible as with any surgical technique that does not remove all sarcoid cells from the site. A success rate of 60–80% has been reported when the laser is used to cut and vaporise sarcoid tumours (McCauley *et al.*, 2002).

Cryotherapy

Cryotherapy involves application of liquid nitrogen at -196°C , either by spray or probe, to destroy tumour cells through the formation of intracellular ice and subsequent rupture of cell membranes. Thermocouples should always be used to monitor the temperature and depth of the freeze (Hewes and Sullins, 2009). Overall, a success rate of approximately 70–80% has been previously reported for cryotherapy following surgical debulking of sarcoids (Martens *et al.*, 2001c).

Conventional excision

Surgical excision of equine sarcoids has been applied for decades with variable success with high recurrence rates of 40–72%. This can be attributed to the infiltrative nature of the tumour and auto-transplantation of tumour cells during the surgical procedure. The detection of BPV DNA at the surgical margin of an excised sarcoid correlates with an increased risk of recurrence. When to perform excision at least 12 mm wide demarcations from normal skin margins were required ((Brostrom, 1995).

Non-surgical treatment

Chemotherapy

Administration of cytotoxic drugs results in tumour cell death and can be performed in systemically and locally. The rationale for local chemotherapy is based on achieving a high drug concentration over time in the tumour while sparing normal tissue. Due to its cutaneous localisation, sarcoids are most convenient for local chemotherapy. The most widely used drug in this manner is cisplatin with success rate of treatment approximately 90%. Side effects of treatment are strictly local inflammation, swelling and focal ulceration (Stewart *et al.*, 2006).

Radiofrequency hyperthermia

Radiofrequency current-induced hyperthermia is a technique that raises temperature to 50°C for 30 seconds, affecting tumor growth. Only small areas of about 1 cm in diameter are affected, which limits the area treated per application, requiring multiple applications for anything larger. On the other hand, this has the benefit of limiting

the collateral heating of normal tissue and so side effects appear to be minimal which makes the method best suitable for periocular sarcoids (Hewes and Sullins, 2009).

Radiotherapy

Radiotherapy is the use of ionizing radiation to selectively destroy tissue. It is considered the gold standard treatment for equine sarcoid. Cell death occurs by injuring DNA to prevent replication and by injuring critical proteins to prevent cell function. Radiation using gamma radiation in interstitial sources of Iridium 192 or radio-gold pellets (Au^{198}) or topical radiation using Strontium 90 (beta radiation) may be used. Teletherapy can be used but again the facilities are not widely available to horses. It is usually used for smaller lesions in areas for which no other method is suitable for periocular sarcoids. In reality this treatment method is best regarded as a last resort to be used when there is no alternative (Byam-Cook *et al.*, 2006).

Immunotherapies/Immunological methods

Immunotherapies have been widely applied and are thought to alter the immunologic relationship between the host and the tumor cells, and therefore stimulate or augment the patient's cell-mediated immunity leading to increased recognition of tumor cells antigens as foreign (Knottenbelt and Matthews, 2001).

Ligation

A ligature of nylon thread, a rubber elastic band or even a tail hair can be used around the base of the lesion to cut off its blood supply, killing the tumor cells by subsequent necrosis. Nodular lesions in the groin area have particular satisfying results. Ligation is not amenable for flat or sessile lesions, extensive lesions and those where the margins of the tumor can't be accurately defined (Knottenbelt and Matthews, 2001).

Photodynamic therapy

Photodynamic therapy is a therapeutic modality based on administration of a photosensitizer drug that will be accumulated in the tumor cells, and appropriate activation of the photosensitizer by visible light. Following



activation, free radicals and the highly reactive singlet oxygen are formed, resulting in tumor cells destruction (Tamzali *et al.*, 2012).

The adverse side effects of photodynamic therapy are local edema, ulceration, the development of a darkened scab of necrotic tissue on the surface of treated tumor, possible damage to blood vessels, and systemic toxicity especially if the photosensitizer is administered systemically. Some photosensitizers can also cause long term photosensitization which requires restrictions of exposure to direct sunlight. This technique seems less suitable for occult sarcoids, if the photosensitizer is applied topically (Byam-Cook *et al.*, 2006).

CONTROL AND PREVENTION

Bovine papillomavirus plays a role in the development of equine sarcoid and it seems local that prevention of BPV infection would result in a decreased incidence of sarcoids. The overall prevalence of less than in horse makes it unlikely that development of a preventative vaccine would be economically feasible, despite the fact that such treatment would be potentially successful as a preventive if given before exposure to BPV. A therapeutic vaccine designed to treat equine sarcoid would be ideal, but there are many viral and species factors that make design of a vaccine difficult. Genetic predisposition can also affect an individual horse ability to recognise and destroy virally infected tumor cells (Chambers *et al.*, 2003).

Awareness enhancement should be undertaken to make equine owners realise the consequences of faulty interference in the treatment of sarcoids. Since flies carry the virus, taking care to keep flies away from punctures, cuts, scrapes and keeping injured areas to be clean (Humphreys *et al.*, 2004).

Pre-emptive measures taken at the time of wounding have a profound limiting effect on the tendency toward sarcoid transformation with the wound bed. Following veterinary treatment of wound on a horse with a sarcoid, or where the wounded horse is in contact with other sarcoid infected subjects, the wound should remain protected until it has clearly healed and flies will not be attracted to the site. Fly repellents and petroleum jelly can be used to restrict fly contact. However, the longer wounds are left open and the higher the fly population, the greater the risk of sarcoid

transformation. Owners of horses with sarcoids should be given specific instructions concerning the immediate measures that should be applied in the events that the horse sustains an injury (Knottenbelt, 2005).

Long-term eradication of sarcoids could involve breeding from those individuals that are 'immune' or have a high genetic resistance to developing the condition. Current management of sarcoids is based on good fly control, early diagnosis and treatment of any confirmed lesions (Ashrafi, 2008; Mattil-Fritz *et al.*, 2008).

CONCLUSION AND RECOMMENDATIONS

Equine Sarcoid is a type of skin tumour found quite commonly in equine species which as a unique locally invasive, benign neoplastic-like tumour of the skin lesions. There are different types of sarcoid and they can vary quite widely in appearance. The predisposing factors for equine sarcoid are formation exposure to Bovine papillomavirus, genetic predisposition and skin infection/open wounds. Diagnosis of equine sarcoid can be accessed in clinical examination, biopsy followed by histopathology and PCR techniques. The best possible diagnostic method should be used for each individual lesion taking into account the type, anatomical location, duration, and previous treatment history and owners resources. The best choice of treatment method is critical. Currently, there is no uniformly effective therapy for equine sarcoid.

Therefore, based on the above conclusion the following recommendations are forwarded:

- ❑ Understanding the causes and predisposing factors about equine sarcoids and implementing current diagnostic and treatment techniques reduce economic losses and enhance use of equines.
- ❑ Local chemotherapy combined with surgery and local immunotherapy should be applied which induce rather effective tumour regression.
- ❑ Conduct effective control strategies for face fly to reduce its abundance and use of fly repellent, insecticide properly.
- ❑ Economically feasible and potentially successful preventive measures should be developed for equine sarcoid include minimizing complications of equine wound and dermatologic surgery.

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