UNIVERSIDADE TÉCNICA DE LISBOA

Faculdade de Medicina Veterinária

EQUINE SARCOIDS: A THERAPEUTIC CHALLENGE

DORA GODINHO LOPES DE MELO GOMES

JURY

PRESIDENT

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LISBON

Dedication

To my parents.

"(...)
Ministrar tratamento aos animais
Isentos do sentido da razão...
É com franqueza ainda muito mais
Do que melindrosíssima missão
(...)"

Vândalo
(Manuel Reis Melo)

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E acima de tudo, à minha Família.

TITLE: EQUINE SARCOIDS: A THERAPEUTIC CHALLENGE

ABSTRACT

Equine Sarcoid is the most common skin tumor in horses and other *equidea*, where bovine papilomavirus (BPV) plays a major role in its etiopathogenesis. Sarcoids are characterized as unique locally invasive, non-metastatic, fibroblastic skin tumors with unpredictable behavior and are considered to cause substantial economic loss to the equine industry.

Several methods have been employed in the treatment of this condition, however, no current approach was found to be 100% effective or universal for all tumor types and locations and recurrence after treatment is common. Therefore, successful management remains a challenge.

The aim of this thesis is to identify, through a literature review, the most commonly employed treatments for equine sarcoids, to assess the efficacy of recent treatments observed by the author during the practice period in two different European countries, relating them with other methods and to perform an opinion study through an on-line enquire to Portuguese veterinarians in order to have an idea on how sarcoids are managed in this country.

Fifteen horses with a total of 64 tumors were enrolled, from which 29 lesions were treated using 5 different therapeutical approaches: imiquimod, bloodroot (*Sanguinaria canadensis*) extract, imiquimod and surgical excision, ligation and, laser surgery.

Cases treated with imiquimod (Aldara^{TM}), and the bloodroot extract (Xxterra^{TM}) had an excellent outcome, with 100% of success rate up to 30 months after finishing treatment, for all locations, dimensions, and types of tumors, with Xxterra being approximately 3 times faster to treat when compared to Aldara. Moreover, these 2 methods are simple, safe, non-invasive, and quite inexpensive when compared with other methods. Disadvantages include long duration of treatment, therefore if quicker results are wished, prior surgical excision followed by application of either ointment could be an alternative. Results suggest that these modalities may be a therapy of choice for sarcoids, although more detailed studies are required to corroborate these findings. No conclusion concerning treatment efficacy could be made for tumors treated by ligation or laser surgery.

The opinion study about sarcoid gave an idea of what is performed in Portugal and showed that there are no major differences between the management of this disease in Portugal when compared to other countries.

KEY WORDS: EQUINE SARCOIDS, SKIN TUMOR, BPV, ALDARA, XXTERRA, LASER SURGERY

TÍTULO: SARCOIDES EQUINO: UM DESAFIO TERAPÊUTICO

RESUMO

Sarcoides equino é o tumor cutâneo mais comum em cavalos e outros *equidea*, onde o papilomavirus bovino (BPV) desempenha um importante papel na sua etiopatogenese. Os sarcoides são caracterizados como tumores cutâneos de fibroblastos, ímpares, localmente invasivos mas não metastizaveis e de comportamento imprevisivel, capazes de causar perdas económicas significativas à indústria equina.

Vários métodos têm sido aplicados no tratamento desta condição, no entanto, não existe nenhuma abordagem terapêutica 100% eficaz ou universal para todos os possíveis tipos e localizações do tumor e a recorrência após tratamento é comum. Assim sendo, o sucesso da abordagem terapêutica permanece um desafio.

Os objectivos desta tese foram identificar, por meio de revisão bibliográfica, os tratamentos mais frequemente utilizados para o sarcoides equino, avaliar a eficácia de tratamentos recentes observados pelo autor durante o seu período de estágio em dois paises Europeus, relacionando-os com outros métodos e realizar um estudo de opinião, através de um inquérito on-line, a veterinários Portugueses de modo a ter uma noção de como os sarcoides são tratados neste país.

Neste trabalho, foram incluídos 15 cavalos com um total de 64 tumores, de entre os quais 29 foram tratados utilizando 5 abordagens terapêuticas distintas: imiquimod, extracto de sanguinária (*Sanguinaria canadensis*), imiquimod e excisão cirúrgica, laqueação e, cirurgia laser.

Os casos tratados com imiquimod (Aldara^{TM}) e com o extracto de sanguinária (Xxterra^{TM}), apresentaram um excelente resultado, com taxa de sucesso de 100% até 30 meses após o tratamento, para todas as localizações, dimensões e tipos de tumor, sendo o tempo de tratamento com Xxterra^{TM} cerca de 3 vezes mais curto do que com Aldara^{TM}.

Estes 2 métodos são simples, seguros, não-invasivos e relativamente baratos quando comparados com outras metodologias. As desvantagens incluem a longa duração do tratamento, no entanto se forem desejados resultados mais rápidos, excisão cirúrgica seguida de aplicação de um dos cremes pode ser uma alternativa. Os resultados sugerem que estas modalidades podem ser uma terapêutica de escolha para sarcoides, no entanto estudos mais detalhados são necessários para corroborar estes resultados. Nenhuma conclusão foi retirada quanto à eficácia de tratamento aplicando extracção por laqueação ou cirurgia laser.

O estudo de opinião sobre sarcoides deu uma noção sobre como esta doença é tratada em Portugal revelando não existirem diferenças significativas em relação a outros países.

PALAVRAS-CHAVE: SARCOIDES EQUINO, TUMOR CUTÂNEO, BPV, ALDARA, XXTERRA, CIRURGIA LASER

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List of Abbreviations and Symbols

5-FU – 5-Fluorouracil

ALA - Delta-aminolevulinic acid

BCG - Bacillus Calmette-Guérin (vaccine)

BPV – Bovine papillomavirus

C-myc - Cell myelocytomatosis oncogene

CVLPs - Chimeric virus-like particles

DNA - Deoxyribonucleic acid

DNA-PKcs - DNA protein kinase catalytic subunit allele

ELA – Equine leukocyte antigen

$E_{(x)}$ – Early genes

FeSarPV - Feline sarcoid-associated papillomavirus

Gy – Gray

HLA – Human leucocyte antigen

HPV – Human papillomavirus

IC/PCR – Immunocapture polymerase chain reaction

ICP - Inactivated *Corynebacterium pseudotuberculosis* solution

Ir – Iridium

$L_{(x)}$ – Late genes

LCR – Long control region

MC-1 - Cell line derived from an equine sarcoid

MHC – Major histocompatibility complex

NK – Natural killing cell

p53 - Tumour suppressor gene

PBMCs - Peripheral blood mononuclear cells

PCR – Polymerase chain reaction

PDT - Photodynamic therapy

PVf – *Papillomaviridae* family

RNA - Ribonucleic acid

RT-PCR - Reverse transcription polymerase chain reaction

SnCl4 – Stannic chloride

T-77 - Cell line derived from a tumor induced by inoculation of a combined immunodeficient foal with MC-1 cells

TLR – Toll like receptor

WBCC – White blood cell count

Introduction

In equine practice, the skin is the most common site for neoplasia and although tumors can occur in the various locations, horses do not suffer from neoplasia as frequently as other companion animals (Cotchin, 1977; Kobluk, Ames & Geon, 1995).

Sarcoids, melanomas and squamous cell carcinomas are the most common skin tumors in horses, making up over 95% of cutaneous tumors. Less frequent skin tumors include lymphosarcomas, lymphomas, basal cell carcinomas, fibromas, haemangiosarcomas and mastocytomas (Table 1) (Knottenbelt, 2008a; Kobluk *et al.*, 1995)

Table 1: Skin tumors in the horse

	Tumor	Character	Prognostic
Frequent	Sarcoid	Occult/ verrucous	Regular
		Nodular	Regular
		Fibroblastic	Reserved/bad
		Malignant	Untreatable
	Squamous cell carcinoma	Proliferative	Good
		Ulcerative	Good/bad
		Malignant	Untreatable
	Melanoma	Benign	Good
		Malignant	Very bad
Infrequent	Lipoma (cutaneous)	Benign	Good
	Mastocytoma	Benign	Good/Reserved
	Basal cell carcinoma	Benign	Good
	Neurofibroma	Malignant (locally)	Regular/bad
Rare	Lymphosarcoma	Multicentric	Incurable
	Histiocytic lymphosarcoma	Cutaneous	Regular/Reserved
	Cutaneous myxoma	Uncertain	Reserved

(adapted from Knottenbelt, 2008a)

Melanomas are the third most common skin tumors, and they arise from melanocytes. Approximately 80% of grey horses older than 15 years of age have melanotic masses. All horse breeds are susceptible to develop melanomas, but the incidence is probably higher in breeds where the grey color is very common, such as Percherons, Arabians and Lusitanos.

During aging, grey horses appear to develop a disturbance in the metabolism of melanin, leading to focal hyperplasia of the melanocytes and local over-production of dermal pigment which can predispose to malignant transformation.

Melanomas in non-grey horses tend to be far more aggressive than in grey horses (Higgins & Snyder, 2006).

Predilection sites include the perineum, vulva, the male genitalia, limbs, neck and ears.

The tumors can be hard or soft, solitary or appear in multiple nodules in the subcutaneous tissue. There are three growing patterns.

The most common is slow growth and the tumor persists for years without metastasis. The second type has a similar initial pattern but suddenly transforms to malignant characteristics and begins to spread rapidly. Finally, melanomas can have malignant characteristic from their first appearance (Higgins & Snyder, 2006).

Malignant melanomas can interfere with the horse's excretory functions, breeding and foaling, and with their performance working under saddle or in harness if located in areas such as the back of the neck.

Squamous cell carcinoma is the second most common skin tumor in horses, representing up to 20% of all diagnosed equine tumors and 6,9 to 37% of the equine skin neoplasms in many surveys (Kobluk *et al.*, 1995; Scott & Miller, 2003). These cutaneous carcinomas arise from epitelial cells in the epidermis and are often locally invasive but tend to be slow to metastasize and then only as far as local draining lymph nodes. The prevalence increases with age (mean: 12 years of age; range: 1 to 29) and in breeds with white or part-white skin, such as Appaloosa, Belgian, American Paint, Pinto, Chlydesdale and Shire. Horses that live in higher altitude and closer to the equator receive more ultraviolet radiation exposure and therefore are also at higher risk than other horses (Kobluk *et al.*, 1995; Scott & Miller, 2003).

Squamous cell carcinomas have site predilections for nonpigmented, sparsely haired areas near mucocutaneous junctions such as periorbital region (eyelids, nictitating membrane, conjunctiva, corneal limbus), genitalia (vulva, penis, prepuce), lips, nose and anus.

Squamous cell carcinoma is an invasive tumor that can either be proliferative or erosive. Proliferative lesions are more common in horses and may present as a small nodule, a cauliflowerlike growth, or an ill-defined weeping mass conforming to the tissues being invaded (Kobluk *et al.*, 1995). Erosive tumors, initially, present as small nonhealing wounds covered by a scab that readily bleeds when removed.

These tumors often arise as solitary lesions, but adjacent skin may be at risk of developing the tumor.

Equine sarcoid is the most common skin tumor in horses and other *equidea* like mules, donkeys and zebras (Jackson, 1936; Marais, 2006; Marti, Lazary, Antczak & Gerber, 1993; Ragland, Keown & Spencer, 1970), accounting for up to two thirds of all reported horse tumors (Knottenbelt, 2008b; Kobluk *et al.*, 1995; Ragland *et al.*, 1970).

Despite the similarity in terminology, sarcoids are unrelated to human sarcoidosis (Chambers *et al.*, 2003a). So far, sarcoid–like lesions have also been reported in nonequine species,

including felids, cervids, camelids and tapirs (Kidney & Berrocal, 2008; Schulman, Krafft & Janczewski, 2001).

The first known literature on sarcoids remounts to the 9th century AD, where a stable master of the Bagdad's Caliph refered to it as "warts" (Erk, 1977). One millennium later equine sarcoids were properly described by Jackson (1936) who considered it as sarcoma-like mixed tumors[1]. Jackson (1936) defined the equine sarcoid as a unique locally invasive although benign, non-metastatic, cutaneous tumor with a variable epidermal component which has a high propensity for recurrence. The term sarcoid was given to emphasize the clinical and pathological differences from papilloma, fibroma, and fibrosarcoma and to suggest the malignant sarcomatous appearance of the lesions.

Since then, various terms, some of which erroneously have been employed by clinicians to describe these tumors, such as, angleberry, equine fibrossarcoma, warts, subcutaneous fibroma and sarcoids (Mohammed, Rebhun & Antczak, 1992).

Sarcoids are generally observed in young adult horses, between three and six years of age, but have been reported in animals as young as one year and older than thirty years of age (Marti *et al.*, 1993; Ragland *et al.*, 1970; Wobeser *et al.*, 2010). Sarcoids may occur in any part of the body either as solitary or multiple lesions but the most common locations are the head, ventral abdomen and the limbs and at sites that have been previously traumatized (Jackson, 1936; Olson, 1948; Ragland *et al.*, 1970).

Macroscopically, sarcoids show a variety of appearances and have been classified in six distinct clinical forms: occult, verrucouse (warty), nodular, fibroblastic, mixed (verrucouse, nodular and fibroblastic) and malevolent (Knottenbelt, 2005).

Some animals develop only one single tumor, while others may be covered with multiple neoplasms. Although these tumors are not generally life threatening, particular locations such as eye lids or ears, can cause significant discomfort and even interfere with normal function and performance of the horse.

These tumors adversely affect the ecomic value of the horse and in some countries, such as the United Kingdom, are probably the most common cutaneous reason for euthanasia (Scott & Miller, 2003).

This is an interesting and important theme of study, not only because it is a common equine pathology that may interfere with the use of the horse, causing considerable loss to the equine industry, but also because it represents a therapeutic challenge to the veterinarian practitioner since there is no current 100% effective or universal treatment for this disease, and recurrency is often reported.

[1] The word sarcoid is actually a contraction of the word sarcoma, a tumor originating from the connective tissue, and the suffix "–oid", which means "like". So a sarcoid is a tumor "like a sarcoma". The difference is that sarcomas are generally malignant and sarcoids are usually not.

Objectives

The aim of this thesis was to identify through a literature review the most commonly employed treatments, their advantages, disadvantages indications and success rates for equine sarcoids, considering clinical type and location of the tumors.
Clinical cases of sarcoids in horses, observed by the author during the practice period in 2 European countries, were also utilized to assess the efficacy of recent treatments of this condition.
Since the practice period of the author was not performed in Portugal, an opinion study based on an on-line inquire was conducted in order to determine how equine sarcoid is managed in this country.

Training period report

The practice period of the author took place in two different European countries from the 8th of February until the 26th of June 2010, the first was in Uppsala, Sweden, at the Swedish University of Agricultural Sciences (SVA – Sveriges lantbruksuniversitet), and the second one in Newmarket, United Kingdom, at Rossdales Equine Hospital & Diagnostic Center.

Sweden

The author joined an Erasmus program of 3 months (8th of February until the 11th of May 2010) at the Horse and Ambulatory Clinics of the Swedish University of Agricultural Sciences (SVA – Sveriges lantbruksuniversitet), in Uppsala, Sweden.

Horse Clinic
During the 9 week period at the horse clinic, the author rotated between the internal medicine (stables), surgical and reception (daily clinic) departments. The case load followed by the author is summarized on Table 2.
The day would normally start at 8 am with rounds where students could present and discuss the cases they were following among each other and with teachers. Afterwards, students would be divided in groups of two and each group would follow a different veterinarian all day participating in all the veterinarian work. This included receiving the patients and assessing their clinical history, physical examination and patient checks, discussing the possible differential diagnosis with the veterinarian, as well as performing several general diagnostic and therapeutic procedures (such as placing catheters, and naso-gastric tubes, performing rectal palpations, abdominocentesis and sedations, collecting blood samples for further analyses, changing bandages and cleaning wounds, fluid therapy and drug administrations),

and assist to surgery and other complementary diagnostic methods (as X-ray, endoscopy and ultrasound).

Table 2: Cases followed at the internal medicine (stables), surgical and, reception (daily clinic) departments at the Horse Clinic

Area of intervention	Number of cases		
	Internal medicine	Daily clinic	Surgical
Gastrointestinal system	27 (31%)	7 (8%)	1 (1%)
Cardiovascular system	1 (1%)		
Musculoskeletal system	21 (24%)	3 (4%)	
Respiratory system	1 (1%)		1 (1%)
Reproductive system	1 (1%)		3 (4%)
Dermatology		13 (15%)	
Ophthalmology	3 (4%)	1 (1%)	
Neonatology	3 (4%)		

In the same period the author was also given the opportunity to train joint injections, attend to a few pathology and clinical case discussions and radiology classes and go to an endurance competition.

Ambulatory Clinic

In the 4 week practice period at the Ambulatory Clinic, the cases observed were of several species of farm animals being dairy cattle the most common.

Every day a group of 2 to 4 students would follow a different veterinarian. During this period the author was given the chance to assist as well as to perform several clinical and surgical procedures (including lameness evaluations, catheter placements, blood and milk sample collections, treatments, vaccination and castrations, among others). The cases observed are summarized on Table 3.

Table 3: Clinical cases followed at the Ambulatory Clinic

Area of intervention	**Number of cases per animal species**							
	Equine		**Bovine**		**Ovine**		**suine**	
	Non-sx	Sx	Non-sx	Sx	Non-sx	Sx	Non-sx	Sx
Gastrointestinal system	9			1				
Musculoskeletal integumentar systems	2	1	1	14	1			
Dermatology	1							
Respiratory system	1							
Reproductive system including related metabolic disorders	1	2	8+4	18	2	2		36
Ophthalmology	1							

Non-sx – non surgical cases; Sx – surgical cases

United Kingdom

The practice period performed in the United Kingdom (UK) took place in Newmarket, both at the Rossdales Ambulatory Practice and at the Rossdales Equine Hospital & Diagnostic Center from the 17th of May until the 26th of June 2010 but more on a "seeing practice" approach rather than a "hands on" one.

Ambulatory Practice

At the Ambulatory Practice, the author followed and assisted a stud veterinarian for 2 weeks. Here the day would start at 7 am with visits to the various stud farms where gestation diagnosis were performed by the veterinarian (n=10/day) followed by adequate treatments, if justified, other clinical cases were occasionally seen. The author was able to assist the veterinarian in treatments, ultrasounds, endoscopies, blood samples collection, dental checks, lameness evaluations, vetting, animal identification and microchip placements, among others.

Equine Hospital

During the 4 week practice period at the equine hospital, the author was placed as a hospital extern student, where a 24 hour a day and 7 days a week availability was expected.
The days at the hospital began at 7 am with colic checks, followed by preparation and administration of morning medication. At 8:30 am the multidisciplinary hospital personnel would gather in the morning rounds. Following rounds, students would be able to assist and scrub into the surgeries scheduled for the day and help in the pre- and post-operative patients care (urinary catheters placement, fluid therapy and other medication preparations). After surgeries students were able to help/assist the nurses, interns and other veterinarians in every kind of work, either veterinarian, as diagnostic procedures (rectal palpations, ultrasound, endoscopy, x-rays, muscular biopsies) and bandage changes among other common procedures, or non-veterinarian, that were to be performed at the hospital, giving students a general perspective about what has to be done when managing daily work in a hospital. The days would normally end at 8 or 9 pm, after colic checks and evening medications, and every other day, the author would also perform the night colic checks, either at midnight or 3 am and help with medications or other procedures. Since this placement occurred during the foaling season after hours colic emergencies where constant.
The cases observed during this period are summarized in Table 4.
In the same period, the author was also given the opportunity to assist in an endurance competition.

Table 4: Cases observed in Newmarket

Area of intervention	Non-surgical clinical cases	Surgical cases
Gastrointestinal system	9 (9%)	12 (13%)
Musculoskeletal and integumentary systems	12 (13%)	23 (24%)
Cardiovascular system	1 (1%)	
Respiratory system	2 (2%)	2 (2%)
Reproductive system	2 (2%)	6 (6%)
Dermatology	4 (4%)	5 (5%)
Neonatology	12 (13%)	5 (5%)

It has been indeed a good personal experience that allowed the student to have a special contact with two excellent and renowned veterinarian facilities well known worldwide.

The contact with different cultures and different forms of organizing the training and pre-graduate teaching also helped to transform this short stay in both countries in a very rewarding experience for the author.

Literature review

Equine sarcoids: Etiology and pathogenesis

The etiology is not fully understood, but it is believed to be multifactorial.

When Jackson (1936) first described equine sarcoids he suggested a virus origin for tumors, based on their appearance and pattern of spread. He stated that the tumor was often transferred from the leg to the lips or from the eye lid to a limb and he also noted that the tumor was possibly transferred by bridles, harness or the hands of attendants. Subsequently, more evidence of infectious etiology was provided by transmission studies. The first report is attributed to Montpellier, Dieuzeide and Badens (1939), as they demonstrated sarcoid autotransmission in a mule using fresh tumor material. Nine years later Olson (1948) showed that autotransmission was only successful when skin was scarified. In 1951, Olson and Cook inoculated bovine papilloma virus (BPV) in the skin of non-affected horses demonstrating that it was capable of producing sarcoma-like tumors. This was the first time bovine papilloma virus was postulated to play a role in sarcoid development in horses. Other researches were made in which inoculation with either sarcoid tissue or cell-free supernatant from minced tumors onto the scarified skin of sarcoid-free animals resulted in the appearance of tumors at the inoculation site (Ragland & Spencer, 1969; Tooloei, Bazargani & Sasani, 2006; Voss, 1969).

Although these sarcoids were morphologically indistinguishable from naturally occurring sarcoids (Voss, 1969), many differences were observed between experimental induced and natural occurring sarcoids. Histologically, only the dermal layers were changed in induced tumors without any involvement of the epidermis and, 'picket fence' formation[2] was not observed. Moreover, experimentally induced sarcoids regressed "spontaneously" within 45 days to 12 months after development, which is not a common feature of naturally occurring sarcoids (Lancaster, Olson & Meinke, 1977; Ragland & Spencer, 1969; Tooloei *et al.*, 2006). In addition, infected horses formed neutralizing antibodies against BPV, resulting in resistance against re-infection. Moreover, in naturally occurring sarcoids, no neutralizing antibodies are formed against BPV and horses remain susceptible to re-infection (Broström, Bredberg-Radén, England, Obel & Perlmann, 1979; Lancaster *et al.*, 1977; Ragland & Spencer, 1968), indicating that additional factors may contribute to disease progression. It has been suggested that these differences might be due to the fact that with experimental inoculation viral particles as well as bovine antigens were inserted in the organism inducing a strong immune response (Bogaert, Martens, Depoorter & Gasthuys, 2008c).

[2] perpendicular orientation of fibroblasts towards the basement membrane

Nevertheless, transmission attempts from sarcoids to cattle proved to be non-successful (Ragland *et al.*, 1970).

Relation of bovine papillomavirus to equine sarcoids

Bovine papillomavirs (BPV) belong to the *Papillomaviridae* family (PVf), which consists on non-enveloped DNA virus that infect humans as well as many domestic and wild species of animals (Borzacchiello, 2007; Campo, 2002; Lancaster & Olson, 1982; Villiers, Fauquet, Broker, Bernard & Hausen, 2004). This is a very resistant virus that can survive several months in the environment (Martens, Bogaert, Depoorter & Vanderstraeten, 2009a).

In cattle, the bovine papillomavirus natural host, there are 10 BPVs (BPV-1-10) known to cause infection. BPV-1 and -2, which belong to the δ papillomavirus genus, are commonly defined as fibropapillomavirus, as they give rise to fibropapillomas by infecting both the epithelium and underlying derma. BPV-3, BPV-4, BPV-6, BPV-9 and BPV-10, members of the ξ genus, are epitheliotropic virus, which only infect the epithelium inducing true papillomas. BPV-5 and BPV-8, belong the ε genus and seem to have dual pathology, causing both fibropapillomas and epithelial papillomas. And, BPV-7 that belongs to a novel genus but still waits for characterization (Nasir & Campo, 2008). Nevertheless, until recently, only types 1 and 2 were linked to equine sarcoids (Martens *et al.*, 2009a).

The genome of BPV is a DNA, double-stranded, closed circle of 7900 nucleotides divided in 3 regions: a long control region (LCR), a region containing early genes and, a region containing late genes. Early genes encodes for nonstructural proteins, responsible for replication and transcription (E1 and E2) and transformation (E5, E6 and E7), whereas late genes encodes the structural capsid proteins of the virus, L1 and L2. These 2 regions are separated by a stretch of non-transcribed DNA, the long control region (LCR), which contains the transcriptional promotors and enhancers, the origin of DNA replication and binding sites for numerous cellular transcription factors (Chambers *et al.*, 2003a; Nasir & Campo, 2008).

Several researchers have demonstrated the presence of BPV type 1 and, less frequently, type 2 (BPV-1, BPV-2) DNA in equine sarcoids using various molecular techniques. Lancaster *et al.* (1977) detected 80-86% of sarcoids positive to BPV DNA by DNA-DNA reassociation kinetics on fresh or frozen material. Other studies using southern blot hybridization on fresh or frozen sarcoid material, from horses and donkeys, were 86-100% positive to BPV DNA (Amtmann, Muller & Sauer, 1980; Angelos, Marti, Lazary & Carmichael, 1991; Reid, Smith & Jarrett, 1994b; Trenfield, Spradbrow & Vanselow, 1985).

More recently, BPV DNA was isolated using polymerase chain reaction (PCR) techniques. Martens, De Moor and Ducatelle (2001b) have successfully detected BPV DNA in 88% of swabs and 91% of scrapings from sarcoid-affected lesions and no BPV DNA could be detected from non-sarcoidal lesions. In the same year, Carr, Théon, Madewell, Griffey and

Hitchcock (2001a) detected 98% of BPV DNA from sarcoid lesions but, contradictory to Martens *et al.* (2001b), they also detected BPV DNA in 65% of normal skin samples obtained from sarcoid affected horses. No BPV DNA was isolated from sarcoid free horses in this study. The presence of BPV DNA in normal skin and the fact that sarcoid tumors have a high incidence of transformation to more malignant phenotypes after inadequate treatment or excision, suggests that the virus may remain in a latent phase within the fibroblasts of the dermis until some other factor, such as an injury, triggers transcriptional/viral activation, which have lead investigators to think of it as a multiple step progression (Carr *et al.*, 2001a).

Bogaert, Martens, De Baere and Gasthuys (2005) and, Bogaert *et al.* (2008d) also detected BPV DNA in sarcoid affected horses. The main difference is that they also found BPV DNA in skin swabs of 44% to 50% of sarcoid free horses living in contact with sarcoid affected horses, 73% of healthy horses living in contact with papilloma-affected cattle and in 30% of control horses. This emphasis the possibility of an infectious etiology, the presence of latent carriers and the involvement of BPV, and it also calls our attention to the possible role of other factors (genetic, immunologic), other than the viral infection, on the etiopathogenesis of sarcoids.

The presence of BPV DNA in the control group might be explained by a previous infection from neighboring cattle that stays in a latent phase for several months or years, perhaps ultimately resulting in the development of sarcoids particularly in traumatized skin of genetically predisposed horses (Bogaert *et al.*, 2005, 2008d; Broström, Fahlbrink, Dubath & Lazary, 1988).

Expression of BPV genes, including oncogenes, has been demonstrated in equine sarcoids using molecular techniques, such as RT-PCR and Western-blotting. Nasir and Reid (1999) isolated BPV type 1 DNA from equine sarcoids and demonstrated BPV-specific RNA in all samples. This was the first evidence of direct involvement of BPV in the pathogenesis of sarcoids.

Carr *et al.* (2001b) analyzed sarcoid tumors by western blotting and demonstrated the presence of the BPV E5 protein in 100% of the tumors, whereas E5 was absent in all of the non-sarcoid samples examined. In studies carried out by Bogaert *et al.* (2007), Chamber *et al.* (2003b) and Nixon *et al.* (2005) BPV E2, E5, E6 and E7 were found to be expressed in up to 100% of the sarcoid samples.

BPV has been systematically implicated in the etiology of equine sarcoids but so far, no intact viral particles have been isolated from any clinical cases. The disease is, therefore, considered to be a non-productive infection where viral DNA exists episomally[3] (Amtmann *et al.*, 1980; Lancaster, 1981). Given that no viral particles have yet been detected in equine

[3] Episome - unit of genetic material (DNA) that can either replicate independently or can be integrated into the host chromosome. Bacterial plasmids are examples of episomes. http://www.thefreedictionary.com/episomal

sarcoids, researchers tried to determine whether, like in canine transmissible venereal tumor and Tasmanian devil facial tumor disease, these tumors were induced by an infectious cell-line but concluded that they were not (Gobeil *et al.,* 2007).

Brandt *et al.* (2008b), using a very sensitive immunocapture PCR (IC/PCR) technique, demonstrated the presence of full-length BPV-1 circular genoma in a complex with L1 in 2/2 tested sarcoids which may correspond to virion precursors or intact virions. Their findings support the possibility that whole virions might occasionally be produced in this pathology. So far, DNA, RNA, proteins of the virus (Amtmann *et al.*, 1980; Carr *et al.*, 2001b; Nasir & Reid, 1999; Otten, Tscharner, Lazary, Antczak & Gerber, 1993; Teifke, Hardt & Weiss 1994; Trenfield *et al.*, 1985) and, more recently, what can possibly correspond to virion precursors or intact virions, can be isolated (Brandt *et al.*, 2008b).

Both BPV-1 and BPV-2 infections occur, but no correlation exists between BPV type and clinical presentation of sarcoids (Reid *et al.*, 1994b). Viral titre as well, does not determine clinical appearance. However, a highly significant correlation between intralesional viral load and disease severity has been reported (Haralambus *et al.*, 2010). There is strong evidence now that, in Europe, BPV-1 and less commonly BPV-2, plays an important role in the etiology and pathogenesis of equine sarcoids (Amtmann *et al*., 1980; Angelos et al, 1991; Bogaert *et al*., 2007; Lancaster *et al.*, 1977; Martens *et al.*, 2001b; Nasir & Reid, 1999).

In Australia, a BPV type different from BPV type 1 and type 2 has been found in a small number of sarcoids (Trenfield *et al.*, 1985). More recently, a published study from southern Brazil reported that a new BPV type “BR-UEL-4” was identified in a sarcoid tumor of a horse, revealing a new viral type associated with equine sarcoid (Silva *et al.*, 2010).

Relation of equine papillomavirus to sarcoids

Equine papillomavirus (EPV) also belong to the PVf. So far, there are 3 characterized types: *Equus caballus* papillomavirus type 1 (EcPV-1), type 2 (EcPV-2), and type 3 (EcPV-3) (Lange, Tobler, Ackermann & Favrot, 2010). EPV infection causes cutaneous papillomas better known as warts. These are benign growths that usually appear on 1-2 year old animals, typically persist for 1-9 months and then regress “spontaneously”, without any specific treatment. Common sites include the muzzle and lips, but it can be easily spread to other body sites, such as the lower limbs, ears, eyelids or genital area, since horses frequently rub themselves to dislodge flies. Horses develop complete immunity following infection (Ghim *et al.*, 2004).

Even though it was initially thought that an unidentified EPV could play a part in sarcoid pathogenesis, and EPV DNA has been detected in 1 out of 10 sarcoid lesions, there is not enough evidence to support that the EPV is either related to sarcoids or responsible for the subsequent development of sarcoids (Broström, 1995a; Gorman, 1985; Postey, Appleyard &

Kidney, 2007) and, some authors have even excluded it as an etiologic agent (Carr *et al.*, 2001a).

Moreover, as stated by Knottenbelt, Eduards and Daniel (1995), horses that have had viral papillomata are probably neither more nor less susceptible to the development of sarcoid later in life.

Relation of retrovirus to sarcoids

Retroviruses are enveloped RNA viruses that belong to the viral family *Retroviridae*. They replicate in a host cell via the enzyme reverse transcriptase to produce DNA from its RNA genome. The DNA is then incorporated into the host's genome by an integrase enzyme and, thereafter the virus replicates as part of the host cell's DNA.

Studies of the MC-1 cell line, derived from an equine sarcoid, and on the T-77 cell line, derived from a tumor induced by inoculation of a combined immunodeficient foal with MC-1 cells, revealed the presence of viral particles containing high molecular mass RNA genomes and reverse-transcriptase activity (Cheevers, Roberson, Brassfield, Davis & Crawford, 1982; England, Watson & Larson 1973; Fatemi-Nainie, Anderson & Cheevers, 1982). Moreover, disease related cell mediated immune responses could be demonstrated against the Mc-1 cells compared to normal allogeneic fibroblast (Broström *et al.*, 1979). However, the virus associated with MC-1 cells and their derivatives was a non-oncogenic, replication-defective virus, presumed to be an endogenous equine retrovirus, and a causative relationship between this virus and equine sarcoids was not established (Cheevers, Fatemi-Nanie & Anderson, 1986) putting aside a possible role of retrovirus in sarcoid etiology.

Genetic factors

Sarcoid susceptibility has also been associated with genetic predisposition in horses. In certain families of horses an increased occurrence of sarcoid tumors has been recognized (Angelos, Oppenheim, Rebhun, Mohammed & Antczak, 1988; Broström *et al.*, 1988; James, 1968; Meredith *et al.*, 1986). Major histocompatibility complex (MHC) genes are known to be the most important genes involved in the development of equine sarcoids, but also other genes are involved. The MHC genes code for proteins involved in the immune response as well as complement system protein components. In horses, these proteins are called equine leucocyte antigens (ELA).

There are three major classes of MHC genes, with different locations and different functions: MHC I[4], MHC II[5] and MHC III[6]. Classes I and II have been implicated in sarcoid predisposition.

MHC I codes for cell wall glycoproteins having a role in recognition and killing of virus infected cells and MHC II codes for proteins that are expressed on the cell surface of antigen presenting cells.

In breeds such as the Swiss Warmblood, the Irish Warmblood, the Selle Français, the Thoroughbred and the Swedish Halfbred, horses with haplotype ELA W13 (MHC II) are more susceptible to develop equine sarcoids (Broström *et al.*, 1988; Broström, 1995b; Lazary, Marti, Szalai, Gaillard & Gerber, 1994; Meredith *et al.*, 1986). Broström (1995b) also observed an incresed percentage of sarcoid recurrence following surgery in the presence of this allele (W13) and, that A5 haplotype was linked to early onset of equine sarcoids.

A reason for the low prevalence of equine sarcoids in Standardbreds might be the lack ELA W13 observed in this breed (Meredith *et al.*, 1986).

In the Freiberger, a Swiss draft horse, the higher sensitivity for sarcoid development is correlated with Be108, a local specificity of MHC I, and W13 is absent (Lazary *et al.*, 1994). In another breed, the Selle Français, the higher prevalence of this disease is correlated with A3 allele (MHC I), but this could be due to linkage disequilibrium with W13 (Lazary *et al.*, 1994). Indeed, ELA A3W13 has been frequently identified in families where equine sarcoid is more commonly observed (Broström *et al.*, 1988; Gerber, Dubath & Lazary, 1988; Meredith *et al.*, 1986).

Although there is a large percentage of horses with W13 allele, only a small number will develop sarcoids and this is not exclusively determinant in the pathogenesis of sarcoids. Moreover it does not mean that horses lacking W13 are not vulnerable to this pathology (Goodrich, Gerber, Marti & Antczak, 1998; Lazary *et al.*, 1994). An alternative explanation is that maybe these alleles are related to other susceptibility genes in linkage disequilibrium with the MHC, with a more direct influence on the pathogenesis (Broström *et al.*, 1988; Gerber *et al.*, 1988; Lazary *et al.*, 1994).

Nevertheless, in a study carried out by Ashrafi and coworkers (2002) they concluded that expression of papillomavirus oncoprotein E5 prevents the expression of MHC I to the cell surface causing it's retention within the cell and therefore interfering with antigen presentation and immunosurveillance of virus infected cells.

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[4] In the horse, 17 internationally accepted alleles (A1-10, A14-15, A19, W16-18, W20) and 5 regional variants (Be22, Be24-26, Be108) are distinguished serologically.

[5] Three internationally accepted alleles (W13, W22-23) and 2 local variants (BeVIII, Be200) can be determined serologically in the horse.

[6] Codes for several proteins involved in the complement system, but these are not associated with predisposition for sarcoids.

In Arabian Horses, W13 is rarely seen. In this breed the high prevalence of equine sarcoids is attributed to the presence of the heterozygotic defective DNA protein kinase catalytic subunit (DNA-PKcs) allele, responsible for severe combined immunodeficiency (Ding, Bramble, Yuzbasiyan-Gurkan, Bell & Meek, 2002).

Some researchers have identified transcriptional changes induced by BPV type 1 in equine fibroblasts (Yuan *et al.*, 2008b; Yuan, Bennett, Campo & Nasir, 2010a; Yuan, Gobeil, Campo & Nasir, 2010b). The main changes found were increases in genes that promote motility and invasion (*CXCL5*, *FRA-1* and *MMP-1*) and reduced apoptosis (*C-IAP-1*) and, decreases in genes promoting and shaping the immune response (*IL12B*) (Yuan *et al.*, 2008b). *TLR4* expression was also found to be significantly down-regulated by BPV-1 (Yuan *et al.*, 2010a).

More recently, a team composed by researchers from the Equine Clinic of Bern - Institute of Genetics and Animal Health Trust in Newmarket, England, studied two families of horses, trying to find a genetic basis for this disease. The prevalence of sarcoids within these two families were 8,6% and 16% respectively. In this study, several cromosomes reveled significant sarcoid signals, in particular, these researchers found clear positive sarcoid-related signals in chromosomes 5, 9 and 20. The identification of genes related to sarcoid tumors is one step to a better understanding of the etiology of the condition. However, the underlying mechanisms associated with genetic predisposition are currently unclear (Lesté-Lasserre, 2010).

Pathogenesis

Although papillomaviruses were initially thought to be strictly species-specific, it is now known that some can actually infect species other than the one that they are commonly associated with, resulting on a pathologic outcome different to the one observed in the natural host (Nasir *et al.*, 2007).

For example, cottontail rabbit papillomavirus causes mostly papillomas in cottontail rabbit (the natural host), but it induces skin cancer at a much higher frequency in domestic rabbits (Shope & Hurst, 1933). Similarly, BPV has been found to induce benign fibroblastic tumors in certain mice, but in hamsters it causes malignant fibroblastic tumors (Boiron, Levy, Thomas, Freidman & Bernard, 1964).

It has been recently demonstrated, by researchers from Costa Rica, the presence of BPV-1 DNA in tumors of two captive tapirs (*Tapirus bairdii*) that histologically resembled equine sarcoids (Kidney & Berrocal, 2008).

In addition, it was lately detected, for the first time, the presence of feline sarcoid-associated papillomavirus (FeSarPV) DNA sequences on bovine skin, suggesting that cattle are the reservoir host of this papillomavirus and that feline sarcoids could be the result of cross-species infection by a BPV (Munday & Knight, 2010). Moreover, cutaneous and

mucocutaneous masses from 5 captive lions were analyzed and histologically similarities were found with equine and feline sarcoids. PCR amplified DNA sequences from these lions lesions were also found similar to the previously detected in feline sarcoids and clinically normal bovine skin. All lions had been fed a diet that included bovine carcasses with cutaneous lesions and skin still attached. Since cessation of feeding lions with such diet, no additional lesions were observed within the animals, and therefore it was hypothesized that the lions were exposed to the virus by feeding on bovine carcasses that had not been skinned (Orbell, Young & Munday, 2010).

Equine sarcoids are another example of natural cross-species infection.

In malignant progression of Human papillomavirus (HPV), the viral DNA is often integrated into the host genome, which loses the regulated expression of the transforming viral genes. In contrast, BPV genes are present in a non-integrated circular episomal state during the transformation of the host cells (Amtmann *et al.*, 1980).

In the natural host, BPV infection results in the formation of complete viral particles. Distinguishly, in a non-permissive host, such as horses or other equids, BPV infection seem to result in a non-productive cycle, where only early genes, responsible for genome maintenance, regulation of cell growth and cell transformation, are transcribed (Sousa Dostatni & Yaniv, 1990). Even though BPV DNA (Nasir & Reid, 1999), and recently, what might correspond to the presence of virion precursors or intact virions (Brandt *et al.*, 2008b) has been shown in equine sarcoid lesions, there is still little evidence for expression of the BPV structural proteins, virus capsid formation, or the actual presence of intact virus particles (Carr *et al.*, 2001b; Chambers *et al.*, 2003a). This is suported by the fact that experimental inoculation of sarcoid extracts in cattle does not induce warts (Ragland & Spencer, 1969).

Previous sarcoid studies, using in situ hybridization had only shown BPV DNA in the nuclei of fibroblasts, especially at the dermo-epidermal junction and not in the epidermis, which contrasts with BPV infection in cattle (Teifke *et al.*, 1994), where only in the first stages of infection BPV DNA is found in the fibroblasts (Campo, 1997). In cattle skin, a productive infection only occurs in well-differentiated keratinocytes. Here considerable replication and formation of complete virus particles takes place (Campo, 1997; Carr *et al.*, 2001b). Nevertheless, the presence of BPV in keratinocytes from latently infected normal skin samples and occult sarcoids has recently been demonstrated, but not in keratinocytes from advanced sarcoids, which could indicate that BPV infection starts in keratinocytes, as in most papillomavirus infections (Bogaert, Martens, Kast Wijbe, Van Mark & De Cook, 2010). However, when equine sarcoids progress to a more advanced state, a shift of BPV seems to occur towards the dermal layers and infection disappears from the epidermis. This specific equine epithelial environment does not seem to be able to support the complete virus life cycle production, resulting in an abortive infection and can even favor cancer progression (Doorbar, 2006). This unique feature has not, until date, been shown in other species, and

could contribute to the unusual position that BPV takes in the family of papillomaviruses as being the only known papillomavirus responsible for cross-species infection in large mammals (Bogaert *et al.*, 2010; Borzacchiello, 2007; Campo, 2002).

It has also been shown that intra-type sequence variation occurs within papillomavirus types, which can affect the cellular location and function of oncoproteins, such as E5, and therefore affect the transforming ability of the virus, as found for HPV (Giannoudis & Herrington, 2001). This may, as well, explain the different pathogenesis of the equine sarcoid compared to papillomas induced by BPV in cattle. However, this remains to be established (Chambers *et al.*, 2003b; Reid, Gettinby, Fowler & Ikin, 1994a).

The presence of BPV DNA and mRNA has been demonstrated in some cases of equine inflammatory skin conditions (Yuan, Philbey, Gault, Campo & Nasir, 2007b), as well as in normal skin of sarcoid free horses (Bogaert *et al.*, 2005; Bogaert *et al.*, 2008d) showing that latent BPV infection in horses is wide spread. This may be an explanation for the high recurrence rates after sarcoid tumors surgical excision (Carr *et al.*, 2001a).

Therefore, normal ephitelia is considered as a site of latent infection. However, this may not be the only site for latent papillomvirus infection. In cattle, BPV DNA has already been detected in circulating lymphocytes and latent BPV infection of lymphocytes experimentally established (Campo, Jarrett, O'Neil & Barron, 1994; Stocco dos Santos *et al.*, 1998) and, in a recent study, BPV DNA was also demonstrated in peripheral blood mononuclear cells (PBMCs) of equine sarcoid patients (Brandt, Haralambus, Schoster, Kirnbauer & Stanek, 2008a). This finding could explain how this disease can spread from one part of the body to another, resulting in the development of multiple tumors (Martens *et al.*, 2009a). However, although BPV-DNA plays a major role in equine sarcoids development, the presence of the viral DNA alone is not sufficient to cause cell transformation and, exactly what triggers equine sarcoid development after BPV latent infection remains unclear (Martens *et al.*, 2009a).

For instance, in cattle, papilloma lesions are benign lesions that often regress, however occasionally they can persist and progress to cancer, but in order to do so the presence of co-factors, either environmental or genetic, are needed (Knottenbelt, 2007; Nasir & Campo, 2008). This has been experimentally demonstrated for cancer of the urinary bladder and cancer of the upper alimentary canal in cattle feeding on bracken fern (*Pteridium aquilinum*) (Nasir & Campo, 2008).

In equids, skin trauma may certainly play an important role since it not only allows direct access of BPV to the basal keratinocytes and epidermal fibroblasts, but also, because it is possible that viral genes expression is induced through the production of inflammatory cytokines, and formation of different growth factors necessary for skin regeneration, leading to papilloma formation, which has already been demonstrated in cattle (Campo *et al.*, 1994). Furthermore, equine sarcoid lesions have been frequently reported to develop at previous

wound sites (Broström, 1995a; Knottenbelt *et al.*, 1995; Torrontegui & Reid, 1994). Moreover, many sarcoid lesions develop with no evidence of skin trauma, eventhough one cannot exclude microtrauma caused, for example, by insect bites (Bogaert *et al.*, 2010).

When an equine fibroblast gets infected by BPV-1 or -2, a number of cellular changes can happen resulting in cell transformation and uncontrolled growth. First of all, the MHC involved in immune regulation plays a major 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; Marchetti *et al.*, 2009; Yuan *et al.*, 2008a).

Other cellular proteins are also involved, such as p53, c-myc and, in Arabian horses, DNA-PKcs, and probably many more.

Equine sarcoid appears to be a tumour due not to cell hyperproliferation but to lack of apoptosis, since the markers of cell proliferation, such as cyclins and their respective kinases, are not different from normal skin (Nixon *et al.*, 2005; Yuan, Gallagher, Gault, Campo & Nasir, 2007a). The tumour suppressor gene p53 codes for a nuclear phosphoprotein which in case of genetic damage will activate the transcription of genes inhibiting the cell cycle progression, resulting in DNA repair. Consequently, this gene enhances the genetic stability of the cell playing a critical role in tumor suppression (Nasir & Reid, 1999). In human cancer, somatic mutation of p53 is the most commonly observed genetic alteration (Levine *et al.*, 1994). However, Bucher *et al.* (1996), and Nasir and Reid (1999) failed to detect p53 gene mutation in animals with equine sarcoid lesions. Nonetheless, more recently, researchers demonstrated aberrant perinuclear localization of p53 in 44% of equine sarcoid lesions, suggesting that mutational independent inactivation of p53 occurs commonly in sarcoids. The significance of these findings is yet to be elucidated (Martens, De Moor, Demeulemeester & Ducatelle, 2000a). Other possible explanations for p53 inactivation are complex formation with viral or cellular proteins or, cytoplasmic sequestration of p53, leading to inability to exert it function in the nucleus (Nasir & Reid, 1999).

An increased activity of the oncogene c-myc has been found in sarcoid tumors, as well as an association between the level of such increased activity and the aggressiveness of the tumor lesions (Théon & Carr, 2001[7]).

[7] In Nel, 2007

In Arabian Horses the high prevalence of equine sarcoids is attributed to the presence of the heterozygotic defective DNA protein kinase catalytic subunit (DNA-PKcs) allele, responsible for severe combined immunodeficiency (Ding *et al*., 2002).

Epidemiology

Prevalence and Incidence

Prevalence and incidence are both epidemiologic measures that help characterizing a disease. Prevalence expresses the amount of disease or condition in a known population at a designated period of time. Incidence, on the other hand, measures the number of new cases of a disease or condition in a known population over a certain period of time. Thus, prevalence depends on incidence and duration of a disease (Roe & Doll, nk).

Equine sarcoids represent 12% to 67% of all equine tumors and 36% to 70 % of all skin tumors in horses (Jackson, 1936; Miller & Campbell, 1982; Ragland *et al.*, 1970; Teifke *et al.*, 1994; Weiss, 1974).

A prevalence of 0,5% to 2,2% has been reported in clinical populations (Goodrich *et al.*,1998; Marti *et al.*, 1993; Mohammed *et al.*, 1992; Ragland *et al.*, 1970). However, it is difficult to estimate the real prevalence and incidence of equine sarcoids because some reports are made by clinicians working in referral hospitals which can increase the figures due to reference bias (being specialized in sarcoid treatment these authors have a higher case load). On the other hand, not all horses with sarcoids are referred for treatment and small tumors may be underdiagnosed. Others may be treated at home by local practitioners or left untreated. Still others can be misdiagnosed as fibromas, papillomas, fibropappilomas, fibrossarcomas, neurofibromas, keloid or exuberant granulation tissue (Broström, 1995a).

Professor Knottenbelt (2005), from the University of Liverpool states that 1% to 8% of horses will be affected by the disease.

Natural transmission

Nowadays, it is known that BPV plays a major role in equine sarcoid pathogenesis (Bogaert *et al.*, 2005; Bogaert, Martens, Depoorter & Gasthuys, 2008a; Chambers *et al.*, 2003a).

Although the natural mechanism of infection in horses is not yet clarified, many hypotheses have been proposed.

Possible natural transmission routs include contamination of the environment with subsequent trauma and infection of the skin (Carr, 2009), direct or indirect transmission from affected animals or even within the same animal to different locations of the body by biting, rubbing, fomites or via stable management practices. (Bogaert *et al*., 2005; Chambers *et al.*, 2003a; Jackson, 1936; Scott & Miler, 2003), indirect transmission from cattle to horses, for

example housing horses in cattle stables or transmission by fomites or animal caretakers (Bogaert *et al.*, 2005), and insect vectors (Finlay *et al.*, 2009; Kempt-Synonds & Kirk, 2007[8]).

Reid *et al.* (1994a) carried out an epidemiologic study on a large herd of donkeys noticing that animals housed with sarcoid affected individuals reveled an increased occurrence of sarcoids, which also suggests the possibility of direct transmission.

In an attempt to investigate animal to animal transmission researchers performed a sequence analysis of BPV-1 genomes isolated from sarcoids from four donkeys. The animals were housed in pairs and in each pair one donkey was sarcoid affected and the other one was sarcoid free. In both pairs, sarcoid free donkeys developed sarcoids. Tumors from all four donkeys were analyzed for the presence of BPV-1 variation and it was noticed that the viral sequences within each pair were identical but distinct between the pairs, providing strong evidence for animal to animal transmission. (Nasir & Campo, 2008).

An increased incidence of sarcoids was also noticed between horses of the Swedish cavalry, during their first two years in service, when associated with older horses in an ingrain horse environment (Broström, 1995a).

Insects acting as a virus vector for BPV infection of the horse would explain not only some epidemiological features (such as development of the tumors in places of the body that are common feeding sites for insects such as around the eye, ventral abdomen and paragenital region, distal limbs or wound sites), but also the multiplicity of tumors on one horse, spontaneous development of sarcoids on intact skin and absence of contact with cattle or affected *Equidae* (Knottenbelt *et al.*, 1995; Pascoe & Knottenbelt, 1999; Reid *et al.*, 1994a).

Kemp-Symonds and Kirk (2007)[8] isolated BPV-1 and BPV-2 DNA from 98% of face flies, *Musca autumnalis*. Furthermore, the same viral DNA sequences were detected in the horses from which the flies were removed.

Finlay *et al.* (2009) were also able to isolate BPV-1 DNA both from, biting flies, *Stomoxys calcitrans*, and non-biting flies, *Fannia carnicularis* and *Musca domestica*.

Knottenbelt *et al.* (1995) even proposed that the different flies from different geographical areas could possibly be implicated in regional variations in numbers and types of sarcoids.

Furthermore, some researchers (Bogaert *et al.*, 2005) tried to evaluate environmental contamination as a source of viral particles using a molecular technique to detect BPV-DNA in the habitual surroundings of sarcoid affected horses and their stable mates. In this study 44% of sarcoid-free horses living in close contact with affected horses were found positive for BPV-DNA, 4 out of 6 skin swabs taken from the normal skin of a horse living in close contact with a cow that developed a papillomatosis infection were also positive for BPV-DNA (whereas the samples taken from that same horse before the cow got infected were all

[8] In Bogaert *et al.*, 2008a

negative) and, a sample obtained from the stable wall that housed that horse was also positive for BPV-DNA after the papillomatosis infection occurred.
All this findings appear to confirm the hypotheses that the surroundings of sarcoid-affected animals or insect vectors play a role in BPV transmission in horses.
Nevertheless, Broström *et al.* (1988), Carr *et al*. (2001a) and Gerber *et al.* (1988), agreed on the fact that contact with the virus alone is probably not sufficient for tumor development, believing that skin trauma, the immunological status and the genetic constitution of the individual horse also play an important role.

Incubation Period

Olson (1948) reported an incubation period of 6 months, in a case of auto transmission of sarcoid tissue in equids by topically inoculating scarified skin. However, in transmission studies conduced by Ragland and Spencer (1969), incubation period varied from 10 to 39 days, being normally less than 14 days. Moreover, Voss (1969) indicated that this period varied between homologous and autologous transmission, being respectfully 115 and 57 days.

Seasonality

It is difficult to determine if transmission is seasonal, especially if one consideres the highly variable latent period between infection and clinical manifestation of the disease. However, sarcoids are commonly reported to grow during the winter time whereas in summer they tend to multiply on individuals (Knottenbelt, 2001).

Age

Although sarcoids can occur in horses at any age, unlike most reports on tumors in other species, they often occur in young adult horses. Most affected horses are under 6 years of age, but there is an incidence peak between 3 and 6 years of age (Knottenbelt, 2005; Marti *et al.*, 1993; Torrontegui & Reid, 1994). A gradual increase in incidence is observed up to the age of 15 years, followed by a decrease in prevalence (Mohammed *et al.*, 1992). A retrospective study carried out in the Pacific Northwest 2006 showed that the mean age at diagnosis was 9 years (Valentine, 2006) which may suggest some difference between geographic regions. A statistical assessment on donkeys indicated that the highest incidence rate occurs in male donkeys between 0,5 and 3 years of age (Reid & Gettinby, 1996). One possible explanation for this age range is that older horses and donkeys, acquire a certain level of immunity resulting in spontaneous regression of existing tumors and prevention of

new ones to develop. Another reason could be that genetically susceptible animals develop the tumors earlier in live (Torrontegui & Reid, 1994).

Gender

According to most authors, there is no gender predisposition to sarcoids (McConaghy, *et al.*, 1994b; Scott & Miller, 2003). However, Mohammed and coworkers (1992) demonstrated that geldings were at higher risk when compared to mares or stallions. In another study carried out by Reid and Gettinby (1996) in a population of donkeys the highest prevalence was detected among males. Reid and Mohamed (1997) have also reported an increased risk among male donkeys, with sarcoids occurring most commonly on the paragenital region (Reid *et al.*, 1994a). Possible explanations are that the wounds left by castration were contaminated by flies caring a fly born pathogen or that the surgical procedure itself may have been the cause of contamination. The similarity of risk between stallions and mares, and the reported fact that sarcoid tumors commonly occur in traumatized areas or near previous wounds gives support to this view (Jackson, 1936; Mohammed *et al.*, 1992; Olson, 1948; Ragland *et al.*, 1970).

Other explanation could be that sex hormones are involved in the pathogenesis, which has already been evidenced in the pathogenesis of papillomaviral-associated disease in humans (Pater, Mittal & Pater, 1994[9]).

Breed

There is a clear breed predisposition for the development of equine sarcoids.

Angelos *et al.* (1988) detected that in North America sarcoids were twice as likely to develop in Quarter Horses comparing to Thoroughbreds and, half as likely to develop in Standardbreads compared to Thoroughbreds. Mohammed *et al.* (1992) also reported that Appaloosas, Arabians and Quarter Horses are at increased risk and that standardbreds are at decreased risk. However, European authors state that Arabians and Thoroughbreds are more likely to be affected and that Quarter horses are less susceptible (Knottenbelt, 2008c).

Broström (1995a), Meredith *et al.* (1986) and Mohammed *et al.* (1992) agree on the fact that Standardbreds are rather resistant.

There is some evidence that 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 (Knottenbelt, 2005). However, no breed is free from developing sarcoids (Dutka, 2007).

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[9] In Reid & Mohammed, 1997

One possible explanation resides in the fact that breed populations and use of the horses are different in different geographical areas. Thus, the different genetic background of these breeds gives a more plausible explanation (Lazary, Gerber, Glatt & Straub, 1985; Meredith *et al.*, 1986).
Another explanation, regarding Quarter Horses and Appaloosas, might be that they are often used on large cattle farms which results in a higher risk to get in contact with BPV (Angelos *et al.*, 1988; Mohammed *et al.*, 1992).
Still another explanation resides on the fact that some of these breeds often work on rough surfaces resulting in frequent injury to the limbs and the possibility for sarcoid development in these wounds (Broström, 1995a).

Genetic background

In a North American study, Quarter Horses had a significantly higher risk of developing equine sarcoids relative to Thoroughbreds, while Standardbreds relative to Thoroughbreds had a significantly lower risk. (Angelos *et al.*, 1988). Even though some individual variation can occur, genetic background differs mainly between breeds, and it is a plausible explanation for the breed predisposition found in this disease (see chapter “Genetic factors”) (Lazary *et al.*, 1985; Meredith *et al.*, 1986).
A strong genetic predisposition to sarcoids has been identified in which over 80% of sarcoid-affected horses have specific MHC alleles (MHCI and MHCII) linked to ELA. Broström (1995b) identified an association between clinical manifestation of sarcoids and the presence of certain ELA-specificities. Both A3W13 and W13 alone was associated with higher prevalence of sarcoids and W13 also with higher recurrence rates after surgery and A5 with earlier onset (Broström 1995b).
Recently researchers have revealed significant sarcoid related genes (Lesté-Lasserre, 2010; Yuan *et al*, 2008b).

Geographic region

Even though sarcoids are worldwide in distribution it seems to be some differences in some parameters of distribution between geographic regions. Nevertheless this is difficult to evaluate, because results are highly influenced by several factors such as type of horse and breed populations, use of the horse, distance to veterinary clinics and referal hospitals among others.
When Knottenbel *et al.* (1995) tried to compare Sarcoid lesions seen in the UK to the ones reported in other parts of the globe he noticed that, for example, in Australia it was less common to find horses with large numbers of sarcoid and that most studies reported from continental Europe and North America showed an average of between two and eight sarcoid

lesions *per* horse. In the UK, it was much more likely to find multiple lesions in horses (ranging from 10 to several thousand) compared to the rest of the world.

He also noticed that also the character of the lesions appeared to have a geographical variation. Occult and verrucous lesions seemed particularly common in the UK but unusual in Africa, Australia and North America.

Common sites for sarcoid occurrence appear to vary with geographic region and climate. According to Carr (2009), most tumors are observed on the distal limbs and face in the United States and Australia, whereas in England and Switzerland they mainly develop on the trunk.

Even though BPV-1 and BPV-2 DNA has been isolated in sarcoids all over the world, either one or both types in the same horse, it seems that BPV-1 DNA is more frequently isolated in Europe, 74-93% of all examinated samples, compared to only 7 - 26 % BPV-2 (Angelos *et al.*, 1991; Bogaert *et al.*, 2007; Martens *et al.*, 2000a; Martens, De Moor, Demeulemeester & Peelman, 2001a; Nasir & Reid, 1999; Otten *et al.*, 1993; Teifke *et al.*, 1994). However, in America both types were either seen in equal proportions (Teifke *et al.*, 1994) or BPV-2 DNA would be more frequently isolated (63%-80%) (Carr *et al.*, 2001a; Wobeser *et al.*, 2010).

In horses with multiple tumors both BPV types can be demonstrated in the same animal (Carr *et al.*, 2001a; Martens *et al.*, 2001a), but until recently only one BPV type could be found in a single tumor. Conversely, researchers have recently detected both BPV types in the same sarcoid sample (Bogaert *et al.*, 2010; Wobeser *et al.*, 2010).

BPV types different from types 1 and 2 have been identified in equine sarcoid lesions in Australia and Brazil (Silva *et al.*, 2010; Trenfield *et al.*, 1985).

Tumor location

Sarcoids may occur anywhere on the body, but predilection sites include the face (muzzle, ears, and periocular region), neck, ventral abdomen (including the paragenital region), distal limbs, and areas of previous injury and/or sites predisposed to trauma (Carr, 2009; Finlay *et al.*, 2009; Jackson, 1936; Torrontegui & Reid, 1994).

There are references stating that in northern cooler climates sarcoids seem to occur primarily on the head and abdomen, whereas in warmer climates they commonly occur on the limbs (Foy, Rashmir-raven & Brashier, 2002; Marti *et al.*, 1993; Scott & Miller, 2003). Different authors, nevertheless, state that it is not known whether climatic differences play a role in this variation or not (Carr, 2009).

Broström (1995a) referred that tumor localization has a significant relationship to their size, with tumors developing on the head being, mostly, smaller whereas tumors developing on limps are larger when compared to all other locations.

Tumor location has also been reported to affect prognosis, having worse prognosis sarcoids located on the periorbital region and distal limbs (Carr, 2009).

Clinical manifestations and differential diagnosis

There are several different clinical manifestations of equine sarcoids, which have variable macroscopic appearances, growth patterns and behavior. They can range, for example, from locally aggressive fast growing and ulcerative fibroblastic lesions to slow growing occult lesions or even, less frequently, spontaneous regression lesions, even in the same horse (Broström 1995a; McConaghy, Davis & Hodgson, 1994a; Nasir & Reid, 1999; Ragland *et al.*, 1970).

Being a disease where clinical characteristics can vary widely, some confusion exists in the literature, and many different classifications have been proposed over the years. Only the most recent ones will be mentioned and the most accepted one will be used in this work.

Kobluk and coworkers (1995), in a scientific book entitled “The Horse: Diseases & Clinical Management” state that only two types had been proposed until that date, verrucous and fibroblastic, and, that both forms could occur in the same animal and even in the same tumor site. Pascoe and Knottenbelt (1999), in “Manual of Equine Dermatology” described a classification including six different types of equine sarcoids, occult, verrucouse, nodular, fibroblastic, mixed (verrucous, nodular and fibroblastic) and malevolent. Nevertheless, Knottenbelt and his team had already described six distinct forms of the disease in an article published in 1995. Even though, in “Equine Dermatology” book, Scott and Miler (2003) only took four morphologic types of sarcoids in consideration, occult (flat), verrucous (wart-like), fibroblastic (proud flesh-like) and mixed verrucous and fibroblastic. Nowadays the classification baring six types of sarcoids proposed by Knottenbelt *et al.* (1995) is recognized by most clinicians and it has been recently reviewed by Knottenbelt (2005).

It is important to recognize that, even though these six different forms are generally identifiable, this tumor has an unpredictable pattern. Sarcoids can stay stable for years or exhibit a rapid and aggressive growth with infiltration of surrounding skin or even progress rapidly from less severe forms to more aggressive types, especially if traumatized.

It has been suggested that both cell and host factors are responsible in combination for the variety of forms (Knottenbelt, 2008c).

Occult sarcoids

Lesions are limited to the epidermis showing as flat circular areas characterized by alopecia and roughened mild hyperkeratosis or, grey scaly appearance (Knottenbelt *et al.*, 1995). One or more small nodules can be observed, with 2-5mm in diameter (Figure 1). In some cases, it

can be very subtle, with no more than slight changes in hair coat pigmentation, reduced hair density and slightly thickened skin with thin hair coat (Knottenbelt, 2005; Pascoe & Knottenbelt, 1999). This relatively benign form of sarcoid is normally characterized by a slow growing pattern, but it can evolve rapidly into a more aggressive type, such as verrucose or fibroblastic types, either spontaneously or following an insult such as biopsy or erroneous treatment (Charles, 2005; Pascoe & Knottenbelt, 1999).

Predilection sites for occult sarcoids include the skin of the face, especially around the mouth and eyes, the neck and other relatively hair-less areas of the body, including axilla, armpit, inside of the forearm, medial thigh and groin areas.

Differential diagnosis include dermatophytosis (ringworm), blisters, burns, rub marks and chronic skin rubbing (from tack or stables) and also ectoparasites (lices) (Knottenbelt *et al.*, 1995; Pascoe & Knottenbelt, 1999).

Figure 1: Occult sarcoid

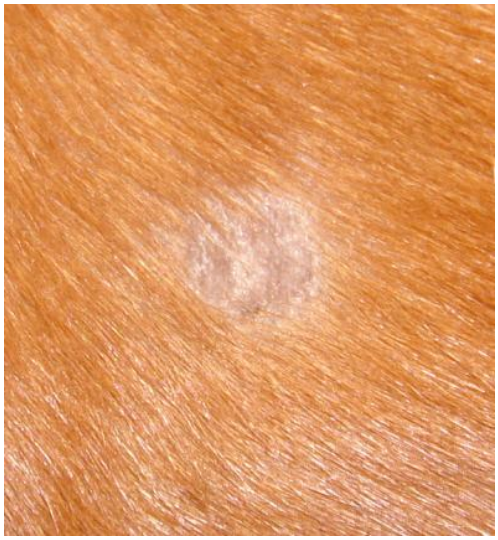


Photo by the author

Verrucouse (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 (Figure 2). 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. Probably this type of tumor is the origin of the name “wart”, wrongly applied to the equine sarcoids.

Sites commonly affected comprise face and ear base, axillae and groin/sheath areas.

Common differential diagnosis include equine papilomatosis (grass warts), hyperkeratosis (for example, as consequence of chronic sweet itch), chronic blistering (horsepox) and squamous cell carcinoma (Charles, 2005; Knottenbelt *et al.*, 1995; Scott & Miller, 2003).

Figure 2: Verrucouse sarcoid

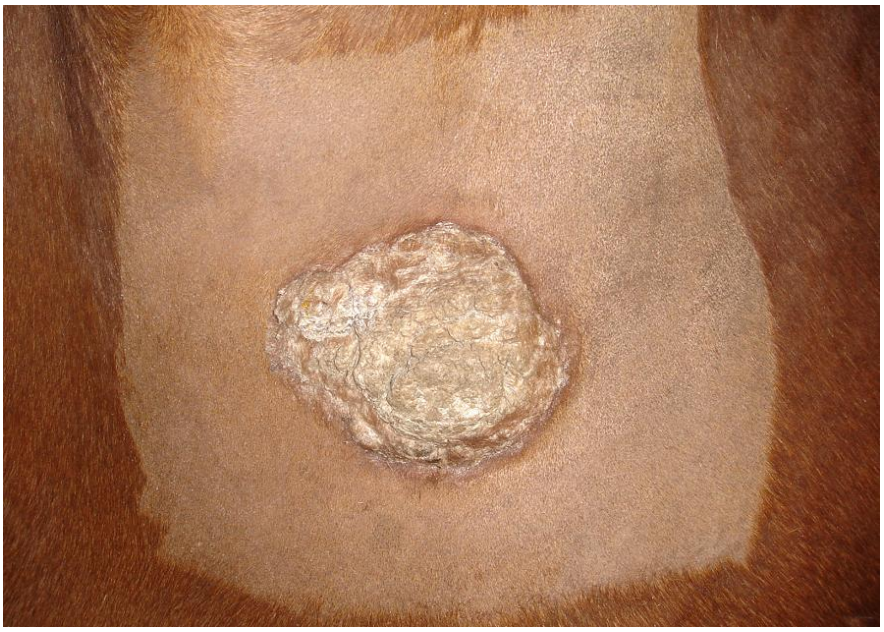


Photo by the author

Nodular sarcoid

This type of sarcoids is generally described as firm, but normally easily moveable, and well defined spherical nodules, with 5-20mm in diameter or bigger, covered by intact and apparently normal skin. The number of nodules varies broadly, from single lesions to several hundreds. Skin may become thin over large nodules. They 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, meaning that they have independent movement. Type B nodules (Figure 3) bare obvious dermal involvement: the overlying skin cannot be moved independently.

Where ulceration occurs, lesions can quickly develop into a more aggressive fibroblastic type of sarcoid. An iatrogenic or accidental insult can, as well, lead to a fibroblastic response (Charles, 2005; Knottenbelt, 2005; Pascoe & Knottenbelt, 1999).

The lesions are most often located in the eyelid, groin and sheath areas.

Differential diagnosis include fibroma, neurofibroma (particularly in the upper eyelid), equine eosinophilic granuloma, melanoma, allergic collagen necrosis, dermoid cysts and H*ypoderma spp.* (warble fly) cysts (Knottenbelt *et al.*, 1995).

Figure 3: Nodular type B sarcoid

Photo by the author

Fibroblastic sarcoid

Fibroblastic sarcoid tumors characteristically present as aggressive and ulcerated fleshy like masses commonly infiltrating local tissues, although they cannot metastasize. This type of tumors is liable to trauma, haemorrhage and local infection with bacteria or maggots and has the appearance of a true neoplasm (Knottenbelt *et al.*, 1995). Fibroblastic sarcoid can evolve from any other type after accidental or iatrogenic manipulation such as biopsy or erroneous treatment (Knottenbelt, 2008c).

Two different types, type 1 and type 2, of fibroblastic lesions can be described. Type 1 or pedunculated, can occur at any site of the body and is subdivided into two distinct clinical groups, type 1a and type 1b. Type 1a tumours have a narrow pedicle made of skin and subcutaneous tissue and don't bare any root beyond the skin attachment which, in most cases, can make treatment simpler (Knottenbelt, 2005). Type 1b is characterized by a narrow neck with an infiltrative root. Treatment is always problematic. Type 2 or sessile can occur anywhere in the skin and is common in wounds with hypergranulating tissue that do not seem to be healing properly, especially on distal limbs and face (Figure 4). Nevertheless, sarcoid transformation at wound sites can be difficult to identify, even by biopsy, as they can be layered by granulation tissue resulting in a diagnostic and therapeutic challenge. This type has a local invasive base and frequently results from insult to other less aggressive types of sarcoids and tends to worsen with time and repeated insult.

Predilection sites include the eyelid, lower limbs and coronet, groin, sites of skin wounds at any location and sites of any other type of sarcoids subjected to accidental or iatrogenic injury or insult.

Differential diagnoses are exuberant granulation tissue, botryomycosis, habronemiases, fibrosarcoma, neurofibroma/neurofibrosarcoma and squamous cell carcinoma (palpebral form) (Knottenbelt *et al.*, 1995; Scott & Miller, 2003).

Figure 4: Fibroblastic type 2 sarcoid

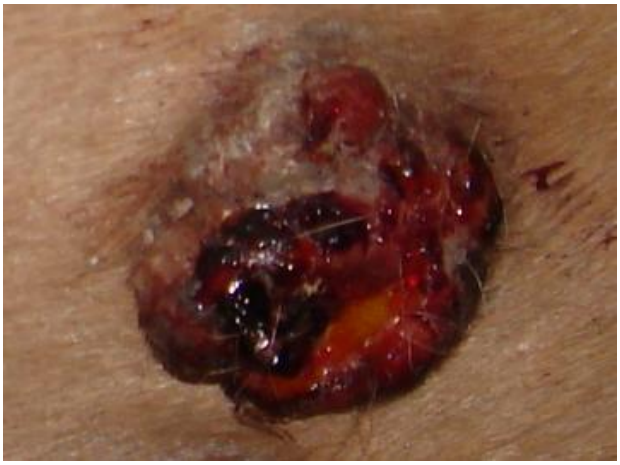


Photo by the author

Mixed (occult, verrucouse, Nodular, and Fibroblastic) sarcoid

Mixed sarcoid are an irregular combination of two or more of the above types of equine sarcoid tumors, probably representing a progressive or transient state between the verrucose/occult types and the fibroblastic/nodular types (Pascoe & Knottenbelt, 1999).

This type becomes progressively more aggressive as a common consequence of repeated trauma, such as accidental injuries, inappropriate treatments or even biopsy, contributing to it an increase in fibroblastic transformation (Knottenbelt, 2005).

Most common sites include the face, eyelid, axillae, groin and medial thigh (Knottenbelt *et al.*, 1995; Pascoe & Knottenbelt, 1999).

Figure 5: Mixed fibroblastic and verrucous sarcoid

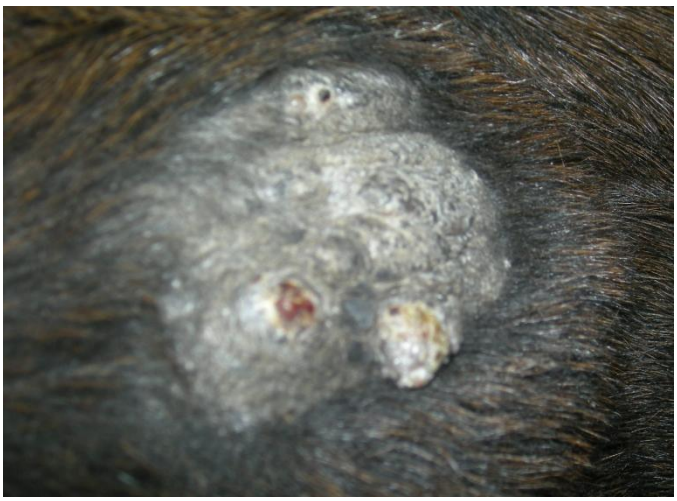


Photo by courtesy of Dr. Carina Pettersson

Malignant or Malevolent sarcoid

Malevolent sarcoid is a very rare and aggressive type of tumor and has only been described by one group of clinicians (Knottenbelt *et al.*, 1995). This is a particularly invasive type of tumor which can infiltrate into lymphatic vessels resulting in multiple tumor masses along these vessels (where cords of tumor can be palpable). Local lymph nodes might also be affected (Charles, 2005). It is particularly dangerous, not least because there is no current treatment available for it. Malevolent type can result from spontaneous transformation, but most commonly evolves from any of the other types following a history of repeated trauma (Knottenbelt, 2005).

Malignant type is most commonly encountered in the skin of the jaw and face, elbow and medial thigh areas. In the area around the eye can occur a particularly dangerous form.

Differential diagnosis include lymphangitis and lymphosarcoma (Knottenbelt *et al.,* 1995; Knottenbelt, 2005).

Diagnosis and histopathologic features

Diagnosis of equine sarcoid can be accessed in three different ways: clinical examination, biopsy followed by histopathology and, detection of BPV DNA (Bogaert *et al.*, 2008a; Carr, 2009).

An individual lesion on a horse might be difficult to diagnose although a thorough clinical examination and anamnesis (including duration of the problem, localization of the lesion, evolution, age and breed of the horse) combined with the clinician's experience should be sufficient in most cases. Furthermore, the large majority of horses have multiple tumors with characteristic features of the various types of sarcoid on an individual horse which makes the diagnosis simple because there are no other diseases with the same range of clinical features and types (Knottenbelt, 2008c; Pascoe & Knottenbelt, 1999). Accidental or surgical wounds that fail to heal especially if the horse has sarcoid lesions elsewhere on the body may be associated with sarcoid transformation (Knottenbelt, 2008c).

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 (Carr, 2009).

In those cases where some diagnostic confusion is present, for example because of atypical tumor characteristics or lack of clinical experience, lab-assisted diagnosis might be required (Bogaert *et al.*, 2008a).

Even though histopathological examination is often diagnostic, taking a sample biopsy might trigger a rapid and uncontrollable growth and ulceration at the tumor site (Knottenbelt & Matthews, 2001). Another option is to perform a total excisional biopsy, with full wide margins, using a "non-touch", surgical excision technique as if the lesion was truly a sarcoid tumor followed by histological confirmation rather than a sample biopsy. However, surgical excision is not the therapy of choice for all equine sarcoids and in some cases it may even be wise not to interfere (Pascoe & Knottenbelt, 1999). On the other hand, it might also be difficult to diagnose if sarcoid is diffusely mixed with granulation tissue. In these cases an experienced pathologist is often required in order to access a correct diagnosis (Knottenbelt, 2008b).

Typical histopathological signs of sarcoids include dermal proliferation of fusiform or spindle-shaped fibroblasts (forming whorls or interlacing bundles), epidermal hyperplasia and hyperkeratosis as well as rete peg formation[10] and, at the dermal-epidermal junction, a 'picket fence' formation (Charles, 2005; Marti *et al.*, 1993; Ragland *et al.*, 1970). However, in a study trying to determine the histopathological characteristics of five clinical types of

[10] broad invaginations (up to more than 20 cells) of epidermal cells into the dermis (Goodrich *et al.*, 1998)

sarcoids, researchers found that the classic epithelial changes of hyperkeratosis, hyperplasia, elongated rete pegs and 'picket fence' were only found in 79%, 54%, 46% and 48% of sarcoids, respectively (Martens *et al.*, 2000a). The only coherency found in this study regarding histopathological characteristics of all sarcoid samples examined was an increased density of dermal fibroblasts.

A more recent diagnostic approach is the detection of BPV DNA by PCR (Bogaert *et al.*, 2006). Depending on the study, BPV DNA can be detected between 98% and 100% of sarcoid tissue samples (Carr *et al.*, 2001a; Martens *et al.*, 2001a).

However, in another study it was detected in only 88% and 91% of swabs and scrapings from sarcoids respectively and it was found unsuitable for the occult type (Martens *et al.*, 2001b).

PCR detection of BPV DNA has many advantages such as being a non-invasive technique, having easy sampling and minimal trauma to the tumor (Carr, 2009; Knottenbelt & Matthews, 2001). Nevertheless, it can be performed on histopathological samples and it has already been useful to demonstrate the presence of BPV DNA in erroneous histopathologically diagnosed cases of fibropapilloma, fibrossarcoma and pyogranulomatous dermatitis (Angelos *et al.*, 1991). Disadvantages include the 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 (Bogaert *et al.*, 2008c; Knottenbelt & Matthews, 2001; Martens *et al.*, 2001b).

Prognosis

Prognosis for equine sarcoids is always very guarded and veterinarians should warn the owners for possible serious complications that can arise both from the disease itself and from the treatment (Knottenbelt, 2008c; Pascoe & Knottenbelt, 1999).

The sooner the diagnosis is reached the sooner a proper treatment can be instituted which binds with a better prognosis. Nonetheless, one should be aware that if the diagnosis is not entirely correct it can have disastrous consequences, meaning that each unsuccessful treatment attempt only makes the prognosis worsen (Pascoe & Knottenbelt, 1999).

Regarding the number of tumors, single tumors are known to have a better prognosis when compared to multiple ones (Goodrich *et al.*, 1998; Lane, 1977).

Anatomical location of the tumor has also been reported to affect prognosis, with sarcoid tumors located on the periorbital region and distal limbs having a worse prognosis when compared to other locations (Carr, 2009; Knottenbelt, 2005, 2008b). Recurrence rates were significantly associated with certain ELA-specificities, early onset, long duration, large size of the tumors and localization to lower extremities (Broström, 1995b).

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 (Marti *et al.*, 1993), as so, some veterinarians prefer to benign neglect tumors that are small and do not cause the horse discomfort or impede its use (Goodrich *et al.*, 1998; Knottenbelt *et al.*, 1995).

Several treatment methods have been described throughout the years, with variable success rates, but there is no current treatment known to be 100% effective. Therefore, the confidence of owners in conventional treatments may have decreased leading them to believe in, or at least to want to try some anedotical propaganda found on the internet such as homeopathy or even toothpaste. In fact, it is difficult to assess the efficacy of different treatments because most early studies have not been controlled and are based on referral populations of *equidae* treated at major veterinary clinics or hospitals. Referral populations, as stated previously, may not represent the overall population of horses with equine sarcoids seen in the field but a subset of tumors that may represent fast-growing, recurrent, or multiple tumors that veterinary practitioners in the field have been unable to treat successfully (Bogaert, Martens, Depoorter & Gasthuys, 2008b; Goodrich *et al.*, 1998).

The choice of treatment and the outcome results may vary with the cost of treatment, underlying characteristics of the sarcoid tumor, such as its volume or multiplicity, it's anatomical localization and clinical type, with factors inerrant to the animal, such as the horse's value (sentimental or economic) and use, with factors related to the owner, like his/hers economic capacity and will to treat the horse, as well as with factors related to the veterinary, such as its competences and technical resources available at his/her reach. Prognosis depends on site and method of treatment and it is generally accepted that it gets significantly worse (about 40%) if one or more unsuccessful attempts have been made previously (Knottenbelt & Walker, 1994). Therefore, considering all these variables, and knowing that there is no universal treatment for every type of sarcoids, the treatment modality chosen for each horse and subset of tumors must be accessed individually with the owner's informed consent and, the best available treatment option, with the highest chance of success, should always be used at the first treatment attempt (Pascoe & Knottenbelt, 1999). Nevertheless, one should know that not all horses with sarcoids can be cured, in horses that have a large number of tumors, or with a very large tumor surface, it might not be possible to treat (Bogaert *et al.*, 2008b).

Treatment must remove every single abnormal cell. If one single cell is left behind, recurrence, sooner or later, is inevitable, with regrowths being often more aggressive.

Currently there is no effective treatment for the malevolent form of equine sarcoids (Knottenbelt, 2005).

It is very important to carefully monitor the site post treatment, since recurring or aggravation can often occur and also because it is always possible for the horse to develop new sarcoids on other locations of the body (Bogaert *et al.*, 2008b; Broström, 1995a).

Current Treatment Methods

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 by subsequent necrosis.
This method is only really applicable to nodular type A sarcoids in which the capsule is loose, meaning that the tumor can be moved independently of the skin. Nodular lesions in the groin area have particular satisfying results (Knottenbelt, 2009; Pascoe & Knottenbelt, 1999). Pascoe and Knottenbelt (1999) have had 40-60% of success rates when using this method.
This method is not amenable for flat or sessile lesions (occult, verrucose, fibroblastic and mixed), or extensive lesions and those where the margins of the tumor cannot be accurately defined (Knottenbelt, 2008c, 2009).

Surgical excision

Surgical excision of equine sarcoids has been applied for decades with variable success (Table 5). High recurrent rates ranging from 50% to 76,5% have been reported with regrowths being often more aggressive, with deeper invasion and increased vascularity (Broström, 1995a; Martens, De Moor, Vlaminck, Pille & Steenhaut, 2001c; McConaghy *et al.*, 1994b).
Recession of wide normal skin margins is of major importance because sarcoids are locally invasive and failure to remove all tumor cells may explain the high recurrence rates (Ragland *et al.*, 1970). Therefore, if excision is performed under general anesthesia, the surgeon is provided with a clear view of the tumor, and a wider excision margin and measures to avoid auto-inoculation (such as changing gloves and surgical instruments after tumor removal, before closure of the wound) can be taken which significantly reduces recurrence rates to 18% to 20% (Broström, 1995a; Martens *et al.*, 2001c), when compared to the 72% to 76,5% of recurrence obtained in tranquilized standing animals operated under local anesthesia (Broström, 1995a; McConaghy *et al.*, 1994b).
Martens and other researchers (2001a) concluded that the prognosis for successful resolution of equine sarcoids was significantly greater if the outer surgical margin did not contain BPV DNA. In this study surgical margins were evaluated at 4, 8, 12, and 16 mm, and contained BPV DNA in, respectively, 95%, 73%, 39%, and 33% of tumors. Despite the fact that the width of surgical excision was not correlated with recurrence in this study

researchers believe that sarcoids are better excised widely (16 mm if possible). Nevertheless, recurrence only developed in tumors containing BPV DNA in the widest excisional margin, suggesting that the presence of BPV DNA in the margin is more important than margin width in predicting the outcome. Unfortunately, PCR testing of the surgical margins requires time, and results are not available until after surgery is complete, however, results can still be evaluated so sarcoids with a positive surgical margin can benefit from additional therapy to reduce the probability of local recurrence (Martens *et al.*, 2001a).

The ideal sarcoid tumors amenable for surgical excision are those that are freely movable from underlying tissues and where size and location permits wide surgical excision and complete wound closure (Martens, Bogaert, Depoorter & Vanderstraeten, 2009b). Superficial, occult, verrucose and nodular lesions can be efficiently treated by excision (Knottenbelt, 2008b). Nonetheless, nodular sarcoids located on the eyelid are potentially very dangerous because they commonly have extensive ramifications through adjacent tissues, which cannot be accessed just by looking at the tumor, therefore, surgery is often inadequate in these cases due to cosmetic reasons or subsequent interference with normal function (Knottenbelt, 2008b). Conventional excision of tumors located on the axillar region are more tricky when compared to other locations because, despite the excess of skin that seems to be present at this location, the large mobility and the absence of a firm subcutaneous layer on the axillar region often result in wound dehiscence. For tumors located on the distal limbs this option is also not usually viable due to cosmetic reasons and lack of tissue to proper wound closure. Indeed, considering the infectious etiology of this disease and the possibility of viral contamination from the environment, the pursuit for a complete wound closure seems logical.

Fibroblastic and mixed sarcoids are generally not suitable for surgical excision alone. Prognosis following surgery can be improved if combined with other treatment modality such as cryosurgery, topical cytotoxic compounds, intra-tumoral chemotherapy, or radiotherapy (Knottenbelt, 2009; McConaghy, 1994b).

Table 5: Summary of results from surgical therapy of equine sarcoids in several studies

Investigator	Number of patients treated (nr of lesions)	Tumor location	Tumor types	Tumor dimensions (cm^2)	Follow up In months	Available for follow up	Success rate	Recurrence rate	Remarks
McConaghy *et al.* (1994b)	18 (NK)	Limbs, head, body	NK	NK	6 - 120	100% after initial treatment	28% (5/18)	72%	0,5 – 1cm margins of normal tissue
Broström (1995a)	17 (NK)	Head, neck, chest, abdom, limbs, groin	Mostly fibroblastic	NK <2 - >5 cm	6 – 12	100%	23,5%	13 (76,5%)	Excision in tranquilized standing horses
	30 (NK)						80%	6 (20%)	Excision under General Anesthesia (non-touching technique to avoid auto-inoculation, and ensure wide margins recession)
Martens *et al.* (2001c)	25 (67) 12 of which were previously treated	Head, trunk, limbs	Occult, verrucouse, fibroblastic, nodular and mixed	0,2 - 114	6 - 60	88% (22 horses with a total of 57 tumors)	82% (18 horses with a total of 47 tumors)	18% (10 lesions in 4 horses)	

NK- not known

Cryosurgery/Criotherapy

This method has been commonly employed both in veterinary (Table 6) and human medicine for the treatment of malignancies (Bogaert *et al.*, 2008b). Success rates of 42% to 100% have been reported (Fretz & Barber, 1980; Klein *et al.*, 1986b; Lane, 1977; Martens *et al.*, 2001c; McConaghy *et al.*, 1994b). It has been, occasionally, observed spontaneous regression of non-treated sarcoids on the same horse, which indicates that destruction of one tumor can result in an immune response against other distant sarcoid tumors (Fretz & Barber, 1980; Lane, 1977). Nevertheless, the role of cryoantigenes has not been subsequently substantiated (Knottenbelt *et al.*, 1995; Pascoe & Knottenbelt, 1999).

In cryosurgery, cell death is obtained by repeated freeze-thaw cycles, with optimal cryonecrosis achieved by 2-3 cycles of rapid freezing (-30ºC to -25ºC) and slow thawing of the tissue (Martens *et al.*, 2009b; White & Yu, 2006). Prior to freezing, tumors should be debulked to the level of surrounding skin, or even lower followed by freezing of the tumor base and surrounding normal skin margin with liquid nitrogen, the most commonly used cryogen. Temperature control is essential and can be obtained by thermocouple needles, also used to monitor depth (Bogaert *et al.*, 2008b).

Cryosurgery is indicated to tumors that do not allow wide surgical excision or those that are not freely movable for underlying structures (Martens *et al.*, 2009b).

Major disadvantages to this method include the prolonged anesthesia time required and duration of the intervention, especially in large surface tumors, the need to careful placement of the thermocouples, the cosmetic outcome, such as local scarring, leukodermia and leukotrichia, and the possible edema, hyperemia or even damage of underlying structures (Fretz & Holmberg, 1980). The latter might result in loss of function, such as facial nerve paralysis, septic arthritis, loss of the upper eyelid, and globe evisceration (Fretz & Barber, 1980; Lane, 1977). Therefore, cryosurgery is less suitable for tumors located on the periorbital area, or for large sarcoid areas and infiltrative lesions, such as tumors located near large vessels, nerves, ligaments, tendons or joints (Fretz & Barber, 1980; Knottenbelt *et al.*, 1995; Martens *et al.*, 2009b; McConaghy *et al.*, 1994b).

Table 6: Summary of results from cryosurgery therapy for equine sarcoids in several studies

Investigator	Number of patients treated (nr of lesions)	Debulking before treatment	Tumor location	Tumor types	Tumor dimensions (cm^2)	Follow up in months	Available for follow up	Success rate	Recurrence	Remarks
Lane (1977)	50 (480)	NK	Head, neak, axilla and girth, limbs, ventral abdomen and groin	NK	NK	6 - 48	94% (47 horses)	70,2%	12,7% (Failure in 6 of the horses)	No horse with more than 20 lesions was completely cured Suggest that spontaneous regression of untreated tumors in 3 horses due to cryo-immune reaction
Fretz and Barber (1980)	50 (204)	In all lesions prior to freezing	Head, neck, thorax+gip and limbs	Sessile , pedunculated and sessile verrucouse-	NK	12	100%	68%	32%	Overcaution resulted in inadequate necrosis which led to local recurrence (eyelid) The use of isolators was found useless and unsafe
Klein *et al.* (1986b)	10 (26) Notes: each tx – 2 freeze-thaw cycles. In total - 1-5 cycles, 2-3 weeks apart until complete tumor regression	Large tumors were 1st frozen followed by cytoreductive surgery and double freeze-thaw cycle (n=5)	Head, breast, abdomen, groin and limbs	NK	2 - 84	6 - 35	100%	100% obtained between1,5 and 7,5 months	0%	
McConaghy *et al.* (1994b)	37 (NK)	Yes, in all 37 cases	Head and limbs	NK	NK	6 – 120	100% after initial treatment	42%	58%	
Martens *et al.* (2001c)	15 (19)	Only for large sarcoid masses	head, trunk and limbs	verrucouse, fibroblastic, and mixed	1 - 296	6 - 60	14 horses with a total of 18 sarcoids	79% (11 horses with 14 sarcoids in total)	21,4% (4 lesions in 3 horses)	

NK- not known

Laser Surgery

Laser stands for Light Amplification by Stimulated Emission of Radiation, and functions by the different ways the instrument can concentrate and generate light wavelengths (Table 7).
This technique has been used to remove or debulk tumors masses and it is usually performed on sedated animals in standing position, under local anesthesia (Carstanjen, Jordan & Lepage, 1997). The advantages are ease, speed, precision, and decreased contamination of healthy areas with viral or tumoral cells, causing less damage to surrounding tissue, as well as less pain, bleeding and inflammation, when compared to conventional surgical excision (Carr, 2009; Palmer, 2002).
The disadvantages are the high cost of the instrumentation, the requirement of specialized training, and expert technical assistance, lack of specificity for neoplastic cell destruction, and possible bad cosmetic outcome, due to leukotrichia, cutaneous scar tissue, or both (Carstanjen *et al.*, 1997).
Two types of lasers are commonly used in equine therapy: the carbon dioxide laser (CO_2 laser) and the diode laser. The laser light interacts with tissue very differently. The carbon dioxide laser, probably the most commonly used laser, interacts minimally with tissues, exerting its effects when it comes in contact with water and does not penetrate more than 1-2 mm. The diode laser, on the other hand, interacts with colored proteins. It can pass through clear water with no interaction or absortion until it hits something with color. Thus is much more effective in areas of significant bleeding or when large tumors are present. It also has the advantage of passing easily through an endoscope and therefore be used as a surgical instrument in places that traditionally have very poor access, as for example in upper respiratory surgeries (Washington State University [WSU], 2006).
A retrospective study to evaluate the CO_2 laser in the treatment of single and multiple sarcoids in 60 animals reported an overall recurrence rate of 38% (Carstanjen *et al.*, 1997).
In the 2008 BEVA congress, other researchers (Kemp-Symonds & Nixon, 2008) referred having a 94% success rate one year after using this method to treat equine sarcoids. According to Carstanjen *et al.* (1997) and, Scott and Miller (2003), animals with multiple sarcoids are more likely to suffer from recurrences.

Table 7: Summary of results from Laser therapy of equine sarcoids in several studies

Investigator	Number of patients treated (nr of lesions)	Tumor location	Tumor types	Tumor dimensions (cm^2)	Follow up In months	Success rate	Recurrence	Remarks
Carstanjen *et al.* (1997)	60 (160) 20 of which were previously treated	Head, neck, chest, girth, abdomen, groin, limbs	Occult, verrucous, fibroblastic, and mixed verrucouse and fibroblastic	NK	6 - 20	62%	38% (in 23 animals) 2 weeks – 11months (mean 3,4months) 58% (if considering that 35 animals developed new lesions, recurrence or both)	Animals with multiple sarcoids were more predisposed to recurrence. Donkeys showed a significant lower recurrence rate than horses
Martens *et al.* (2001c)	28 (81) 16 of which were previously treated	Head, trunk, limbs	occult, verrucouse, fibroblastic, nodular and mixed	0,1 – 81,6	6 – 60 (available on 100% of the animals)	71% (20 horses with 72 tumors in total)	28,5% (in 8 horses with 9 lesions)	
McCauley, Hawlins, Adams and Fessler (2002)	15 (at least 27) 6 horses have been previously treated 7 horses had more than 1 tumor	Head, trunk, neck, limbs	NK	NK	0,5 - 4	67% (in 10/15 horses)	33% (in 5 /15 horses, 2 wks-4mo after surgery)	Recurrence related to the power output used for laser excision Whenever possible a 2 to 3cm margin of normal appearing tissue should be excised
Kempt-Symond and Nixon (2008)	24 (NK)	NK	fibroblastic, nodular, occult and mixed	NK	12 (available for 16 animals – 66,7%)	94% (15 out of 16 animals)	6,3%	Recurrence in only 1 horse with advanced state of disease, with multiple large, ulcerated widely disseminated tumors

NK- not known

Radiofrequency hyperthermia

Radiofrequency current-induced hyperthermia is a technique that raises temperature to 50ºC for 30 seconds, affecting tumor growth, since neoplastic cells are more sensitive to heat than normal cells. 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 (Goodrich *et al.*, 1998; Hewes & Sullins, 2009). This makes the method best suitable for periocular sarcoids. Successful treatments have been reported for superficial verrucouse or occult sarcoids (Knottenbelt, 2009).

In one study, this technique was reported to induce tumor regression in 3 sarcoid tumors, with no recurrence 7 to 12 months after the last treatment (Hoffman, Kainer & Shideler, 1983).

Knottenbelt and Kelly (2000) tried to use a method similar to the one described by Hoffman *et al.* (1983) to treat two periorbital sarcoids, but neither case had a successful outcome. These researchers stated that this might have been rather a reflection of the inadequate experience, than due to the inneficacy of method itself. However, it is difficult to assess the efficacy of this method to treat sarcoids, since only few reports could be found on the literarure.

Immunotherapies/Immunological methods

These methods have been widely applied (Table 8) 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 (Bogaert *et al.*, 2008b; Hewes & Sullins, 2009).

As stated by Knottenbelt (2009) in the 37th annual meeting of the French Equine Veterinary Association, "any method that can establish a normal rejection mechanism will result in a dramatic and satisfactory resolution of the sarcoids from the whole body surface".

Several immunotherapeutic methods have been applied throughout the years.

Bovine wart vaccine was found useless in the treatment of equine sarcoids (Pascoe, 2005; Ragland *et al.*, 1970). Pox vaccines, applied into the sarcoid lesion were also a failure (Pascoe, 2005).

Autogenous vaccines involve the transfer of sarcoid tumors, tumors homogenates, or cell-free extrats. Hallamaa (2007) and, Tallberg, Kinnunen, Palkama, Saario and Borgström (1994) had success rates of 74% and 100%, respectively, after repeated immunizations, using autologous polymerized equine sarcoid tumor tissue, combined with oral supplementation of folic acid and stannic chloride ($SnCl_4$). In both of these studies,

recurrence was more often seen when bio-immunotherapy was not used as the first therapeutic approach. However, other researchers have reported worsening of the condition or development of new sarcoid tumors after autovaccination, thus considering this technique to be inappropriate for equine sarcoid treatment (Knottenbelt *et al.*, 1995; Knottenbelt, 2009; Pascoe & Knottenbelt, 1999; Roberts, 1970; White & Yu, 2006).

Chimeric virus-like particles (CVLPs) can also be used to produce vaccines. In a recent trial, where sarcoid-bearing donkeys were vaccinated with CVLPs of BPV type 1, results showed a tendency towards enhanced tumor regression and reduced progression in the vaccinated group, when compared to the placebo control group (Ashrafi *et al.*, 2008). Sarcoid regression was observed in 4 out of 8 (50%) vaccinated animals against only in 2 out of 9 control animals.

In another study where sarcoid-bearing horses were vaccinated with an identical CVLPs vaccine, but without any placebo-control, sarcoid regression was also observed, in 8 out of 12 horses (66,6%), and many sarcoids remained stationary (Mattil-Fritz *et al.*, 2008). Nevertheless, several new tumors developed.

Bacillus of Calmette-Guérin (BCG), an attenuated strain of *Mycobacterium bovis*, is the agent most commonly employed for immunotherapy of equine sarcoids.

Live organisms, killed bacilli, mycobacterium cell wall extracts, and propionibacterial cell wall extracts have been combined with variable adjuvants for intralesional injection in sarcoid tumors and in some tumors in human beings (Marti *et al.*, 1993). These products are thought to stimulate the host lymphocyte and natural killer cells, thus leading to recognition of tumor cell-specific antigens as foreign and tumor cell destruction (Marti *et al*., 1993; White & Yu, 2006). The antineoplastic response of BCG involves a delayed type hypersensitivity response against the neoplasm into which it is injected. The tumor is destroyed by macrophages, which generate a proteolytic activity and synthesize cytotoxic oxygen-derived free radicals, and by cytotoxic lymphokines produced by sensitized T lymphocytes and natural killer cells.

The tumor should be thoroughly saturated with vaccine, and to maximize results repeated treatment is often indicated (Komáromy, Andrew, Brooks, Detrisac & Gelatt, 2004; Lavach, Severin & Lueker, 1984).

Success rates range from 59% to 100% in several studies (Klein *et al.*, 1986b; Lavach *et al.*, 1985; Martens *et al.*, 2001c; Vanselow, Abetz & Jackson, 1988), and it varies with tumor size and location. Small tumors and the ones located on the periorbital region usually have a better outcome (Knottenbelt & Kelly, 2000), with most studies reporting success rates of up to 100% for periorbital lesions (Komáromy *et al.*, 2004; Lavach *et al.*, 1984; Zeidner & Bracken, 1985). On the other hand, large or multiple lesions, particularly those on the limbs and in the axillary region, are usually less responsive to immunotherapy and even considered contraindicated because of possible tumor exacerbation (Goodrich *et al.*, 1998;

Klein, 1986a; Knottenbelt, 2009; Komáromy *et al.*, 2004; McConaghy *et al.*, 1994a; Murphy, Severin, Lavach, Helper & Luker, 1979). BCG therapy is far less effective for occult and verrucouse tumors (Knottenbelt & Kelly, 2000).

Although excellent success rates have been seen with treatment of periocular sarcoids, local response to intralesional BCG injection may be dramatic, especially after two or more injections. Severe local inflammatory reactions can occur, including lymphangitis, septic arthritis, and necrosis and ulceration of the tumor. Extensive swelling and edema, fevers up to 40ºC, increased white blood cell count (WBCC), and general malaise can also occur in some cases. Other complications include death from anaphylactic shock after two or more injections. Therefore, premedication with flunixin meglunine and corticosteroids is recommended (Goodrich *et al.*, 1998). However, this is more likely to occur with repeated use of the live form of BCG therapy and, for that reason their use is no longer recommended (McCaughy, Davis & Hodgson, 1994a). This modality of treatment has the advantage of being practical, because expensive equipment and hospital environment are not necessary, and the cost is not prohibitive (Goodrich *et al.*, 1998).

Similarly, *Propionibacterium acnes*, formerly known as *Corynebacterium parvum*, and normaly used for treatment of chronic respiratory disease is a non-specific immunostimulant that may induce macrophage activation and lymphokine production, increase natural killer cell activity, and enhance cell mediated immunity against immunogenic components, therefore it has been applied in equine sarcoid therapy, albeit with anecdotal evidence. Protocols vary from intralesional to IV injections once weekly for 6–8 weeks. Susceptible lesions generally show improvement after two to three treatments, and eventually, suffer necrosis (Yu, 2006). Nevertheless, some authors express some doubts about its true efficacy (Rush, 2006).

Inactivated *Corynebacterium pseudotuberculosis* solution (ICP) has also been used to treat equine sarcoids. Its mechanism of action is based on unspecific immune-stimulation mediated by the mucolic acid, a substance produced by the bacteria, which is responsible for the activation of a cell-mediated immune response, and, therefore induce tumor regression. In a study presented at the 11th International Congress of World Equine Veterinary Association, in Brazil, researchers reported that the use of an inactivated CP solution by the subcutaneous route was effective in reducing the size and healing equine sarcoids in a horse, ten months after the beginning of the treatment (Cavicchioli, Peres, Okano, Silva & Zanella, 2009).

An extract of mistletoe (*Viscum album austriacus*) has also been reported to have some efficacy in the treatment of equine sarcoid. The treatment consists on repeated injections of increasing doses of the extract. The mechanism of action of this extract is thought to be based on mistletoes lectins and viscotoxins acting at high concentration by direct cytotoxic inhibition of the tumor growth and, at low dosages as an immunostimulator (Clottu, 2008). In

a double-blind placebo controlled study carried out by Clottu (2008), an immunotherapy dosage of mistletoe extract (Iscador ® P) was used in order to evaluate its effectiveness in the treatment of equine sarcoid. This researcher reported 28,1% of complete remission (9 out of 32 treated horses) and 40,6% of improvement (13 out of 32 horses) in the treated group contrasting with 14,3% (3 out of 21 horses) of complete remission in the placebo control group. Nevertheless, at the end of trial 78,1% of the horses were considered stable, improved or clinically treated. Clottu (2008) concluded that Iscador ® P is a safe and effective treatment for equine sarcoid, particularly in cases of multiple tumors where complete surgery cannot be conducted.

Another form of immune stimulation is the autografting method. This is an autologous implantation technique in which frozen cubes of tumor tissue derived from a sarcoid lesion on the same horse are implanted subcutaneously under the main (Epsy, 2008). This technique was first used in the 1940's but had no convincing results (Knottenbelt, 2009). On the 54th Annual Convention of the American Association of Equine Practitioners, Espy (2008) reported having an 80% regression on sarcoid-affected horses treated by this method, with the horse followed the longest in follow-up care being sarcoid-free for 5 years. In this study tumor regression took 90 to 180 days to occur. Although this technique seems very promising, further testing is required to corroborate these results, especially because the sample size of 15 horses does not allow diffinite conclusions (Epsy, 2008; Knottenbelt, 2009).

Table 8: Summary of results from several studies of immunotherapic methods applied to equine sarcoid treatment

Investigator		Number of patients (nr of lesions)	Tumor location	Tumor types	Tumor dimension (cm^2)	Follow up In months (mean)	Nr of vaccinations (mean)	Success rate	Recurrence / lack of response	Remarks
BCG	Murphy *et al.* (1979)	7	Periocular	NK	5 - 24	9 - 24	3 – 6 (4)	100%	0% until 9 – 24 mo	The longest period of remission was 24 mo, and the shortest 9 mo
	Lavach *et al.* (1984)	32	Periocular	NK	NK	Maximum 84	NK – 9 (4)	100%	0%	100% successful treatment response until end of trial
	Klein *et al.* (1986b)	Live BCG 10 (29) Note: Tumor cure range 2 -14 mo (mean 8,6)	Head, shoulder, breast, abdomen, groin, and limbs	NK	4 – 91	6 – 29 (18)	1 – 6 (3,6)	60% Tumor basis - 83% of succ.	30%	Regression appeared to correlate with size and localization Horses with a positive delayed type hypersensitivity reaction to PPD before the start of treatment showed a tendency to more favourable prognosis than PPD negative horses.
		BCG–CW 10 (16) Note: Tumor cure range 1 - 7 mo (mean 3,3)	Head, shoulder, breast, abdomen, grump, groin, limbs		2 - 48	4 – 40 (19,5)	2 – 6 (3,4)	70% Tumor basis - 69% of succ.	10%	
	Vanselow *et al.* (1988)	61 (NK) Note:Large tumors were surgically trimmed before treatment	In all parts of the body.	All types: verrucous, fibroblastic and mixed	0,2 – 20 cm in diameter	Maximum 24	1 - 4	59% of complete regression	23% lack of response or regrowth	No recurrence up to 2 years after treatment Lack of treatment response in multiple or large bulky tumors
	Martens *et al.* (2001c)	27 (30)	Head, trunk, extremities	Occult, verrucouse, fibroblastic, nodular, and mixed	0,8 - 85	6 – 60	3-9	67% (18 horses with 21 sarcoids in total)	33,3% (9 tumors from 9 horses)	Ulcerated fibroblastic sarcoids were debulked before treatment
	Komáromy *et al.* (2004)	1 (multiple)	Periocular	Nodular	NK	7	5	100%	0%	tumor free 2 years after the last treatment
Iscador® P	Clottu (2008)	Total 53 (163) Treated with: Iscador – 32 (95) Placebo controls - 21 (68)	Abdomen, axilla, thorax, Head, neck, groin, limbs	Occult, verrucouse, fibroblastic, nodular, and mixed	NK	12	45 Note:with rising concentrations of Iscador®P extract, from 0,1mg/ml to 20mg/ml	78,1% stable, improved or clinically treated horses at the end of trial 28,1% (tumor free)	NK	Safe and effective treatment for sarcoids, particularly in cases of multiple sarcoids

Topical Immune Modulation

Imiquimod 5% cream (Aldara™, 3M Pharmaceuticals[11]), an imidazoquinoline amine, is the first representative of a new class of immune response modifiers with potent antiviral and antitumor activity in animals (Table 9) and humans (Manzione, Formiga & Nadal, 2010; Nogueira *et al.*, 2006). It acts by binding to the toll like receptor 7 (TLR-7) present on dendritic cells, macrophages, and monocytes. Subsequent activation of these cells leads to release of pro-inflamatory cytokines and cell-mediated activity. An antiviral state is created by upregulation of NK-cell activity. Additionally, imiquimod appears to enhance the functional maturation and migration of Langerhans cells to regional lymph nodes, promoting antigen presentation to naïve T cells (Manzione *et al*., 2010; Navi & Huntley, 2004).

In humans, imiquimod was first used to topically treat genital warts, also caused by a papillomavirus (HPV), with successful results (Beutner *et al.*, 1998).

In a pilot study trying to evaluate the efficacy of imiquimod 5% cream in the treatment of equine sarcoid, 60% of tumors were completely resolved between 8 to 32 weeks after beginning treatment, and 80% of the tumors showed more than 75% reduction in size (Nogueira *et al.*, 2006).

In a case report presented at the 11th International Congress of World Equine Veterinary Association, a protocol of cryosurgery followed by a long-term treatment with immune modifier Imiquimod 12,5% cream was considered efficient and safe in the treatment of verrucous ulcerated sarcoids over a total period of 159 days (Massoco, Foz & Fernandes, 2009).

In a pilot study from the Swedish University of Agricultural Sciences (SLU), Pettersson (2008) concluded that 73% of the tumors treated with Aldara™ regressed completely at a maximum period of 35 weeks of follow up, increasing to 90% if mere size reduction was included. In some of the cases without complete regression treatment period was extended beyond end of the study.

It may be the therapy of choice for tumors in certain location, such as the limbs, perineal and periorbital areas, where other treatments may be inappropriate (Nogueira *et al.*, 2006).

Advantages of this therapy include convenient application by owners without special equipment and facilities, thus cost is not prohibitive, being a noninvasive treatment protocol, and having a satisfactory cosmetic outcome.

Disadvantages include long treatment duration and possible adverse side effects such as pain, ulceration and exsudation, erythema, erosion, depigmentation and/or leukotrichia, hyperpigmentation, scaring, and alopecia, often confined to the treatment area (Bogaert *et al.*, 2008b; Nogueira *et al.*, 2006).

[11] Imiquimod is also found under the names of Zyclara (3,75% imiquimod) and Beselna (5% imiquimod).

Topical citotoxic approach

Xxterra™ (Larson Laboratories, INC., Fort Collins, Colorado) is a topic caustic ointment of an herbal extract of bloodroot (*Sanguineria canadensis*), zinc chloride, and water. The preparation was first made in the 70's at Colorado State University, United States, by a veterinarian named Ken Larson to treat equine sarcoids. According to manufacturer this cream is effective in over 95% of cases, but 20% of horses need a second treatment 60 days after the first (Larson Laboratories, 1982)[12].

The mechanism of action is not entirely known, but it is thought to destroy the tumor by altering its antigens, and apparently stimulating the host's immune system to recognize them as foreign (White & Yu, 2006). The manufacturer also refers to a study where a horse with four sarcoid tumors was treated with Xxterra™ on three of the lesions, but all four were resolved, suggesting that this drug also has a positive systemic effect. Total failures have been observed in rare instances and have been attributed to primary immune deficiencies. Xxterra™ appears to be safe on normal skin (White & Yu, 2006).

In a study conducted at SLU, Uppsala, Sweden, 93% of the tumors treated with Xxterra™ responded with size reduction or complete regression (Table 9). For the non-responders (7%) treatment was changed to Aldara. Advantages and disadvantages are similar to the ones obtained with imiquimod treatment (Pettersson, 2008). Nevertheless, Xxterra™ is not recommended for sarcoids located in the face due to risk of intense scarring (Dr. K. Bergvall, personal comuncation, 17th of February, 2010).

Similar products are also available, such as Animex™, an escharotic salve that also contains bloodroot extract, as well as puccoon (*Lithospermum canescens*), gromwell (*Lithospermum officinale*), distilled water, and trace minerals. This product has been used to treat various types of skin lesions, including sarcoids, and is considered by some to be the treatment of choice for small sarcoids that can be easily bandaged (Foy *et al.*, 2002).

[12] It is sold on the internet (http://www.vetlineequine.com/xxterra.html), but only to veterinarians or by prescription.

Table 9: Summary of results from topical immune modulation therapy and citotoxic approach studies for equine sarcoids

Investigator		Number of patients treated (nr of lesions)		Tumor location	Tumor types	Available for follow up	Follow up (Weeks)	Success rate	Remarks
Nogueira *et al.* (2006)	Imiquimod 5% cream - Aldara™	15 (19) 3x/wk until complete resolution or for 32 wks, whichever occurred first		Head, neck, thorax, groin, limbs	Occult, nodular, fibroblastic, mixed, verrucous	79%	4 – 60	60% completely resolved within 16 wks (9/15 tumors) In total, 80% (12/15 tumors) reduced more than 75% in size in 8 to 32 wks, and 20% (3/15 tumors) did not respond to tx	Of the tumors that completely resolved, none recurred during follow-up Limitation - Too short post treatment evaluation
Petterson (2008)	Imiquimod Aldara	19 (130) 41 lesions were treated, the other 89 lesions were used as control A total of 8 horses with multiple sarcoids were trated by both modalities 4 lesions were treated by both modalities	17 (30)	Head, limbs and shoulders, neck, trunk and genitalia	Occult, nodular, fibroblastic, mixed, and verrucous	100%	1 - 35	73% resolved, Note:90% regressed in size 10% did not respond to treatment, and were instead treated with xxterra and resolved after 8 wks	The tumors that did not respond to tx were all fibroblastic and located on the distal hind limbs of one single horse
	Xxterra		10 (15)					93% responded with size reduction or complete resolution 7% did not respond to treatment, and treatement was changed to Aldara	
Massoco *et al.* (2009)	Imiquimod 12,5% cream	1 Sx excision, followed by 3 freeze-thaw cycles. 9 days later started the imiquimod 12,5% tx, every day for 30 days. Subsequently 3x/wk for 60 days. Followed by a 60 days period of weekly applications, until the wound was completely healed		Laryngeal and parotid regions, and lateral neck	Ulcerated verrucous	100%	NK	100%	The protocol of cryosurgery with immune modifier Imiquimod 12,5% cream for sarcoid treatment was efficient and safe

NK- not known

Chemotherapy

Antimitotic and cytotoxic chemicals have been used in the management of equine sarcoids. These drugs can be administrated locally or systemically, and local chemotherapy can be applied topically or intralesionally. The rationale for local chemotherapy is based on achieving a high drug concentration in the tumor over time while sparing normal tissue, and thereby minimizing toxicity because a low systemic exposure is obtained (Théon, 1998a).

Despite increasing use of this method to treat cancer in human beings and animals, the use of antineoplastic agents has found limited applications in equine oncology, mostly because of cost and adverse side effects of the drugs (Théon, Pascoe, Carlson & Krag, 1993). Disadvantages also include need for safety precautions. A serious human health concern is the potential for carcinogenicity and teratogenicity of all those handling treated horses, including extruded drug from the patient's sweat, urine, and/or feces. At this time, post-chemotherapeutic treatment quarantine times have not been addressed (White & Yu, 2006) even though it is recommended that patient waste should be disposed of in properly labeled biohazard bags or plastic sharps containers for 24 to 48 hours following chemotherapy administration (Burns & Couto, 2009).

5-Fluorouracil (5-FU), AW3-Ludes, or AW4-Ludes can be used as a topical form of chemoterapy to treat equine sarcoids. 5-Fu, available as a topical cream, is suitable for single, small occult or verrucous sarcoids but also for larger areas of occult or verrucous tumors that are not amenable for any other form of treatment (Bogaert *et al.*, 2008b; Hewes & Sullins, 2009). According to Théon (1998a) 5-FU should be limited to flat lesions due to poor diffusion and inadequate distribution from the surface to the deep margins. Topical 5-FU is applied daily for 30-90 days, or less frequently under a badage (Bogaert *et al.*, 2008b; Roberts, 1970). Knottenbelt and Kelly (2000) obtained a success rate of 66% for periocular lesions (Table 10). It can also be mixed with podophylin, an irritant cathartic, to aid the penetration through the epidermis. Marked inflammatory reaction can occur in the treatment area, but scaring is usually minimal (Pascoe & Knottenbelt, 1999).

AW3-Ludes or AW4-Ludes are topical ointments that contain a variety of heavy metals, 5-FU and thiouracil, and its application is limited to veterinary use, and more specifically it can only be obtained at the Liverpool University Hospital, in the United Kingdom. These drugs can be applied on successive or alternate days for 3 to 5 treatments, depending on size, number and nature of the sarcoids (Knottenbelt & Walker, 1994). Adverse reactions include severe local irritation and scaring. With the initial version of the cream, a resolution rate of over 80% was reported in cases treated for the first time by this compound, but expected efficacy would drop by 30% to 40% for each previous unsuccessful treatment (Knottenbel & Walker, 1994). The invasive and destructive nature of this material makes it unsuitable for tumors located around the eyes (Knottenbelt & Kelly, 2000).

Intralesional injection of cytotoxic drugs allows higher intratumoral concentrations and accurate placement of the drug within the tumor, enhancing its beneficial effects (Théon, 1998a). The drug most commonly applied by this manner to the horse is cisplatin, either in injectable suspension or in absorbable beds (Hewes & Sullins, 2009).

A standard treatment protocol for cisplatin intratumoral administration includes 4 injections at 2 week intervals. Théon and colleagues (1993) evaluated intratumoral treatments of cisplatin in 19 horses with sarcoids and found that, one year after, 87% of the horses were free from recurrence (Table 11). In another study by the same group, 27 horses (32 tumor lesions, including 22 sarcoids) were treated with surgical debulking or excision combined with perioperative intratumoral injection of cisplatin and sesame oil emulsion. Relapse-free rate was 94±6% at 1 year, with a mean relapse free interval of 45±2 months for animals with sarcoids. No adverse effect, regarding wound healing, was observed (Théon, Pascoe & Meagher, 1994b).

Other researchers tried to compare the therapeutic effects of local intratumoral administration of 5 to 10 low doses of Interleucina-2 (IL-2) with those of a combination of cisplatin and a single high dose of IL-2 in the treatment of 22 equine sarcoids (Spoormakers *et al.*, 2003). In this study, complete regression was observed in 53% of tumors treated with cisplatin/IL-2, and in 14% of tumors treated with IL-2, thus it has not improved efficacy over cisplatin injections alone (Spoormakers *et al.*, 2003; White & Yu, 2006).

This therapeutic modality can be effective as the sole treatment for small sarcoids, but for larger tumors the combined use with surgery is recommended (Théon *et al.*, 1994b). Nevertheless, in areas where surgery may be impossible this modality spares structures proximal to the tumor, such as eyes, joint capsules, and tendon sheats (Goodrich & Semevolos, 2000). This technique is easy, feasible, and does not require sophisticated treatment or training. Side effects are local and self-limiting, and include inflammation, tissue sloughing and swelling, focal ulceration, and potential secondary peri-infectional infections (Théon, 1998a; White & Yu, 2006).

Implantation of biodegradable cisplatin beads in and around the wound at the time of surgery can also be used. They are easy and precise to implant, and a success rate close to 90% can be obtained with a mean of 2 consecutive implantations with one month interval, which is an advantage compared to the 4 injections required for the oily cisplatin emulsion (Table 11). The beds release cisplatin over 30 days. Disadvantages include the costs of the beads and the fact that for extensive lesions, more than 2 implantation sessions are often needed (Hewes & Sullins, 2006).

Alternative cisplatin formulations with almond oil or epinephrine as well as local intratumoral chemotherapy with bleomycin and 5-FU are described but appear to be less effective (Bouré, Krawiecki & Thoulon, 1991; Doyle, 1998; Knottenbelt & Kelly, 2000; Stewart, Brush & Davis, 2006; Théon *et al.*, 2007).

Table 10: Summary results from topical chemotherapy study for equine sarcoid

Investigator		Number of patients treated	Tumor location	Tumor types	Available for follow up	Follow up In months	Success rate
Knottenbelt and Kelly (2000)	Intralesion emulsion of Cisplatin and almond oil	18	Periorbital	fibroblastic, extensive nodular	100%	NK	33% resolved (all nodular lesions of limited size) 55,5% improved but recurred 12 to 26 weeks after finishing treatment
	AW4-Ludes (10% 5-FU + Rosemary oil + heavy metals)	146 (159 lesions)		Very small superficial verrucouse or occult sarcoids	100%	NK	35%
	Topical 5% 5-Fluorouracil cream	9		Occult or verrucouse	100%	NK	67% 33% improved temporarily with recurrences in a fibroblastic form over 6-36 mo

NK- not known

Table 11: Summary results from chemotherapy studies for equine sarcoids

Investigator		Number of sarcoid tumors	Tumor location	Tumor dimension (cm^3)	Tumor type	Available for follow up	Follow up In months	Success rate	Remarks
Théon *et al.* (1993)	Intralesion cisplatin in sesame oil	19	Periorbital, peribuccal, trunk or limbs	3 – 104	verrucous, fibroblastic and mixed	NK	12 (5 - 24)	87% relapse free rate at one year (mean of relapse free time 21,6 mo)	simple, safe , and effective chemotherapy should be continued beyond the time of apparent healing.
Hewes and Sullins (2006)	Cisplatin beads	22 1 -8 tx	Head, neck, abdomen, girth, groin, axilla, limbs	NK	NK	NK	24	84,6% relapse free 24 mo after treatment	Effective tx for equidae with various cutaneous neoplasms. Simpler alternative to cisplatin injections
Théon *et al.* (2007)	Intratumoral cisplatin in sesame oil	409 (386 horses)	periorbital, face (pinnae), trunk, neck, limbs and groin.		verrucous, fibroblastic andmixed	100%	48 (25 - 74)	96,3% of cure rate after 1 course of treatment 10% developed new tumors outside the treated area	Tx efficacy was lower for large tumors, those with gross postoperative residual disease, and those that had been previously treated with other modalities.
Stewart *et al.* (2006)	Intratumoral 5-FU	23 (14 horses) Tumors >5cm3 were prior debulked	Head, neck, body and limbs	0,25 – 33	NK	100% at 6 mo 93% 13 animals at 36 mo	6 and 36	71,4% resolved at 6 months At 36 mo: 61,5% resolved and 38,5% recurred or persisted	Smaller sarcoids (<13,5 cm3) were more likely to resolve than larger ones. Sarcoids that were not responsive to previous treatments were more likely to recur after 36 months

NK- not known; mo - months

Electrochemotherapy

Electrochemotherapy (Table 12) uses electric field pulses to induce a transient permeabilization of the cell plasma membrane and therefore induce a significant increase of antitumoral drug concentration and toxicity in tumor cells, which enhances the effectiveness of chemotherapeutic agents (Tamzali, Teissie & Rols, 2001). This technique has been used with cisplatin and bleomycin (Tamzali *et al.*, 2001).

Tamzali and colleagues (2001) treated 3 sarcoid-affected horses under short duration general anesthesia where intratumoral injections of cisplatin were followed by short and intense electric pulses directly applied on the skin, at the tumor sites. Two to four successive treatments were applied at two-week intervals. All treated lesions responded with complete regression, and after two years of follow up, success rate was still of 100%. In another study conducted by the same researchers 25 horses with 46 individual tumors where treated by the same method, but tumors were treated slightly differently, according to size, with lesions greater than 5cm in diameter being subjected to prior surgical excision (Tamzali, Teissie & Rols, 2003). Success rates were also of 100% after one to three and a half years of follow up. No negative effects were observed in both studies. In the latest study, small lesions with less than 1 cm in diameter responded faster than larger ones (with more than 1cm), reducing after one single treatment. However, this approach has the disadvantages of requiring special equipment and general anesthesia (Tamzali *et al.*, 2001, 2003; Théon *et al.*, 2007).

Table 12: Summary of results from electrochemotherapy of equine sarcoids

Investigator	Number of horses (Nr of tumors)	Tumor location	Tumor dimension (diameter)	Tumor types	Available for follow up	Follow up In months	Success rate	Remarks
Tamzali *et al.* (2001) cisplatin	3 (10)	Head and limbs	1,5 – 5 cm	Fibroblastic and verrucous	100%	24	100%	No relapse was observed 2 years after last treatment. Small lesions with diameter<10mm responded faster to tx than larger ones with diameter>10mm

Photodynamic therapy

Photodynamic therapy (PDT) 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 (Table 13). Following activation, free radicals and the highly reactive singlet oxygen are formed, resulting in tumor cells destruction (Martens, De Moor, Waelkens, Merlevede & Witte, 2000b). PDT is a very selective cancer treatment, especially when compared with other modalities such as radiation or systemic chemotherapy. In order to get a therapeutic tissue effect, the photosensitizer, light of the appropriate wavelength, and oxygen must be in the tissue at the same time, if these conditions are not established no oxidizing radicals will be formed, and therefore no effect will be obtained. Since the photosensitizer concentrates in the tumor cells, the normal surrounding tissue is spared, and multiple treatments may result in minimal toxicity (Gustafson, 2004).

Martens and colleagues (2000b) trying to evaluate the use of hypericin[13] for photodynamic therapy of equine sarcoids obtained a 90% reduction in tumor volume, 2 months after therapy. Nevertheless, 7 months after treatment a gradual regrowth was observed resulting in a final volume reduction of 75 – 78%. However, these results suggest that photodynamic therapy using hypericin is a usefull non-invasive treatment for equine sarcoids.

Four years later a preliminary investigation into photodynamic therapy was performed at Oregon State University to evaluate the use of topical or locally injected delta-aminolevulinic acid (ALA) for the treatment of equine sarcoids (Gustafson, 2004). In this study 13 out of 18 treated tumors had a positive treatment effect. The entire tumor was treated in 11 of the 13 lesions, resulting in 4 complete remissions, 4 partial remissions and 3 that did not achieve remission. Nevertheless, it was concluded that topical and locally injected ALA produced some localized PDT treatment effects but did not result in sufficient depth of kill to treat large tumors.

Adverse side effect include local edema, ulceration, the development of a darkened scab of necrotic tissue on the surface of the treated tumor, possible damage to blood vessels, and systemic toxicity especially if the photosensitizer is administrated systemically. Some photosensitizers can also cause long term photosensitization which may require restrictions of exposure to direct sunlight. This technique seems less suitable for occult sarcoids, if the photosensitizer is applied topically. In this type of tumor the epithelium is intact which prevents adequate absorption of the drug (Gustafson, 2004).

[13] Hypericin is a photoactive pigment naturally present in the plant *Hypericum perforatum* (St John's wort), and is responsible for phototoxicity in grazing animals

Table 13: Summary results from photodynamic therapy of equine sarcoids

Investigator	Number of animals (Nr of tumors)	Tumor location	Tumor dimensions (cm^3)	Tumor types	Available for follow up	Follow up	Success rate	Remarks
Martens *et al.* (2000b)	1 (3)	Head (2 on the lower lip, 1 on the upper eyelid)	0,7 – 4,1	Fibroblastic, mixed	100%	At the end of tx	81% reduction in tumor volume	This results indicate that PDT using hypericin may have a potencial for non invasive tx of equine sacoids
						2 mo after tx	90% reduction in tumor volume	
						7 mo after tx	a gradual regrowth was observed resulting in a final volume reduction of 75 – 78%	

mo – months; tx - treatment

Radiotherapy

Radiotherapy (Table 14) is the use of ionizing radiation to selectively destroy tissue. It is considered the gold standard treatment for equine sarcoid (Knottenbelt, 2009). Cell death occurs by injuring DNA to prevent replication and by injuring critical proteins to prevent cell function. The sensitivity of cells to radiation is in direct proportion to their reproductive activity, and inversely proportional to their degree of differentiation, therefore, fast growing tumors, are more responsive to radiation than slow growing masses (Blackwood & Dobson, 1994; Hewes & Sullins, 2009; Wyn-Jones, 1983).

This technique is very suitable for tumors located in areas where surgical excision would have an unacceptable functional outcome, such as eyelids and lips, and good results have been reported for recurrent tumors (Blackwood & Dobson, 1994; Bogaert *et al.*, 2008b).

There are two forms of radiation available: brachytherapy and teletherapy.

Brachytherapy, short distance radiation therapy or pleisotherapy involves the implantation of sealed radioactive sources (β or ɣ radiation) within body tissues or cavities to treat tumors at short source-object distances. The primary advantage of this technique is that high radiation doses can be given directly to the tumor with minimal damage to the surrounding tissues (Henson & Dobson 2004).

Examples include strontium-90, and interstitial brachytherapy (using gold^{198}, radium^{226}, iridium^{192} or iodine^{125} sources), with interstitial brachytherapy with iridium^{192} being the most frequently used (Bogaert *et al.*, 2008b; Knottenbelt *et al.*, 1995). Since iridium^{192} has a long half-life, the sealed radioactive source needs to be removed once the required dose is administered, usually 5 to 6 days after being implanted.

With stroncium^{90}, the radiation source is β radiation and its use is restricted to small superficial lesions, since this kind of radiation only penetrates within 2mm of the probe. Success rates of 100% have been reported (Knottenbelt & Kelly, 2000). Minimal complications occur with this radiation source because it has minimal penetration into the tissues (Hewes & Sullin, 2009).

Interstitial brachytherapy with iridium^{192} is mostly used in horse neoplasms located on the head, distal extremities, and genitalia. In this case the radiation source is ɣ radiation. Success rates reported vary from 86,6% to 100% in periocular sarcoids, and 86,7% to 94% in non-ocular tumors (Byam-Cook, Henson & Slater, 2006; Knottenbelt & Kelly, 2000; Turrel, Stover & Gyorgyfalvy, 1985; Wyn-Jones, 1983).

Complications include necrosis, infection, and some cosmetic defaults, such as leukotrichia, alopecia, minimal scaring, cataract, keratitis, and corneal ulceration (Byam-Cook *et al.*, 2006; Théon & Pascoe, 1994a; Turrel *et al.*, 1985).

Teletherapy, or external beam therapy is a method in which the radiation source is at long distance from the tumor - 80 to 100cm (Théon, 1998b). This method is far less frequently

applied, mainly for logistic and capital cost reasons, but facilities are being developed in United Kingdom and Germany (Knottenbelt, 2009).

These techniques have the disadvantages of requiring anesthesia, special facilities and trained personnel, patient isolation during the treating period, and of being extremely expensive, and limited in availability, and most of all they represent a possible health problem to the operators, but on the other hand response rates are great and good cosmetic results are usually achieved (Bogaert *et al.*, 2008b; Knottenbelt, 2009).

Table 14: Summary of results of radiotherapy of equine sarcoids in several studies

Investigator		Number of patients treated (nr of lesions)	Tumor location	Tumor types	Available for follow up		Follow up In months	Success rate	Remarks
Turrel *et al.* (1985)	ɣ radiation with Ir^{192}	22 (23) 14 horses were previous unsuccessfully tx 22/23 tumors had complete response to therapy 52 – 93 Gy 4 – 14 d	Head (periocular, pinnae and lip's comissure), and limbs	Verrucous, and fibroblastic Volume:5-400 cm^3	73% 16/22 animals		6 – 41 (mean 20)	94% after 12 mo	Strongly indicated for sarcoids that recur, have aggressive behavior , or are located in areas of potential cometic disfigurement or functional loss. 14 tumors were only treated with Ir^{192}, 6 with sx + Ir^{192}, 3 hyperthermia + Ir^{192}
Théon and Pascoe (1994a)	ɣ radiation with Ir^{192}	56 (63) 27 horses were previously treated with at least one cytoreductive procedure 17horses had cytoreductive surgery immediately before treatment to improve implantation 60 Gy 4,8 – 8,9 d	periorbital	NK	100%		Mean 24	86,6% after 12 mo 74% after 60 mo	The tumor size was found to affect prognosis in terms of tumor recurrence Should be part of a combined treatment modality for lesions greater than 5 cm in diameter.
Knottenbelt and Kelly (2000)	ɣ radiation with Ir^{192}	66 70 Gy – 90 Gy / 10-14 d	periorbital	Nodular, fibroblastic, mixed	100%	80% 53 horses	36 – 168	98% (52 horses resolved)	A common feature of the healing process was a slow progressive resolution over 6 – 12 months
						20% 13 horses	12	92% (12 horses resolved)	
	β radiation	3 100 Gy/5d		Occult and verrucouse	100%		12 - 48	100% (all treated lesions resolved but new lesions appeared at close range or different sites)	
Byam-Cook *et al.* (2006)	ɣ radiation with Ir^{192}	18 (23) 8 periorbital tumors, and 15 non-ocular 35,2 – 64 Gy	Periorbital, limbs, axilla, groin, abdomen, flank and head (pinnea, lips)	Occult, nodular, fibroblastic, verrucouse, and mixed,	100%		100% of remission for periocular sarcoids with median recurrence free interval of 14,5 mo, (range from 4 to 34 mo) 86,7% of remission for non-ocular sarcoids with median recurrence free interval of 55 mo, (range from 0 to 91 mo)		Good to excellent cosmetic results. 'Gold standard' for the treatment of periocular sarcoids and strongly recommended for non-ocular sarcoids that have proved non-responsive to other treatments

NK- not known; mo - months Gy-Gray

Factors affecting choice of treatment

No current treatment is universally accepted as being effective for all types of sarcoids (Table 15).

Therefore, each case must be evaluated individually. The wide range of treatment options for equine sarcoids may be confusing when a veterinarian whishes to consider all the potential treatment options and choose the optimal therapeutic course for his patient.

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 horse, the cost of treatment, the type, behavior, location and number or extent of the tumors, time between tumor development and presentation, previous treatments and history, the owner and patient compliance, cosmetic considerations, facilities and practicality or expertise and potential complications and the risk of therapy both for the patient and owner (Goodrich *et al.*, 1998; Knottenbelt, 2009; Pascoe, 2005). Nevertheless, veterinarians should work with owners in order to choose the most appropriate therapy for each case, based on a shared treatment decision and the owner's informed consent.

The value of the horse, actual or sentimental, might condition some owners to choose between treatment options presented by the veterinarians or even between to treat or not to treat the affected animal (Knottenbelt, 2008b; Pascoe, 2005).

The cost of treatment is often the major concern to owners and the best treatment is sometimes the most expensive one, however, the benefits have to be weighed against the financial sacrifice. Cost of treatment varies greatly and is determined by many factors, including size, location and number of tumors present, choice of therapy, and whether general anesthesia or sedation combined with local anesthesia is required. Another factor that must be taken in consideration, and probably the most important one, is whether or not new treatments are required due to tumor recurrence or development of new tumors in other locations (Goodrich *et al.*, 1998).

Tumor type must be taken in consideration because no therapeutic method is universally effective, as so, some types are amenable to be treated by, for example, surgery whilst others are far less so. The clinical behavior also matters enormously because there is always the risk that interference with the tumor might transform it in a more malignant form but there is no way of identifying which behavior an individual lesion will take further on (Knottenbelt, 2009). In fact, as stated by Knottenbelt (2001), the only predictable thing about sarcoids is that it is unpredictable.

Tumor location often determines how the lesion should be managed due to anatomical constraints. Some areas of the body such as the eyelids and the limbs have very limited spare skin which conditions the therapeutic methods that can be applied. Thus, a lesion on

the eyelid that looks superficial and benign may actually be deep and extremely dangerous (Knottenbelt & Kelly, 2000). It is contraindicated to treat periocular tumors by surgical removal, debulking or freezing due to the risk of damaging an important ocular structure. Nonetheless, other therapeutic methods have had good success, such as BCG immunotherapy and, interstitial brachytherapy (Table15) (Byam-Cook *et al.*, 2006; Goodrich *et al.*, 1998; Lavach *et al*., 1984). Tumors located on the limbs may also be a challenge because it is difficult, or sometimes even impossible, to remove all the neoplastic tissue without damaging important vascular, bone, or synovial structures and have enough skin to cover it, especially if the lesion is not small.

Larger tumors or a high number of lesions in a single horse also limits what can be done. It is often impossible to remove a large area of skin and be able to close it without serious complications.

Time between tumor development and presentation must be the shortest in cancer therapy because early treatment is much more effective than late treatment. The failure of owners and, unfortunately, sometimes veterinarians, to address or refer it in proper time may result in presentation of the cases only when it is too late and virtually impossible to treat. However, sometimes even a scared owner can miss the signs of equine sarcoids or at least attribute less significance to them because it can be either so benign or so subtle. Inappropriate treatments, as stated previously, can also lead to complications and lower prognosis. The first treatment attempt should be performed with the best available option that has the highest chance of success (Knottenbelt & Walker, 1994).

In order for a treatment to be successful, owner and patient compliance is essential. The treatment needs to be used in the correct manner and its application can either be compromised by the owner's lack of memory or time or by the horse's intolerance to a painful and/or prolonged treatment.

Some owners do not easily accept the cosmetic changes left in their animals by some treatment methods and as so they must be properly informed of that possibility before the treatment starts.

Cryotherapy often leaves an area of tissue scarring due to destruction of hair follicles and affected tissue, and hair regrowth is often white. If the tumor is located on or near an important structure such as an eyelid, it can result on the structure damaged or lack of functionality (Fretz & Barber, 1980; Fretz & Holmberg, 1980; Knottenbelt *et al.*, 1995; Martens *et al.*, 2009b; McConaghy *et al.*, 1994b).

Surgical excision with CO_2 laser leaves a, often dry and hard, brownish yellow surface, and/or leukotrichia when used (Carstanjen *et al.*, 1997; McCauley *et al.*, 2002).

Immunotherapy makes tissue to slough and exudates during the period of tumoral injection and may also leave scarring but the cosmetic outcome depends on the area of tissue

injected and the number of treatments required (Klein *et al.*, 1986b; Komáromy *et al.*, 2004; Vanselow *et al.*, 1988).

Intralesional brachytherapy also depends on the amount of tissue that needs to be treated and may result in permanent epilation or regrowth of white hair (Théon & Pascoe, 1994a; Turrel *et al.*, 1985).

Treating sarcoids has always been problematic but it is clear that horses treated by experienced veterinarians have a better prognosis and that sophisticated facilities are more likely to be able to offer an adequate treatment to each case. Nevertheless, the best option may sometimes be economically or practically impossible. For example radiation treatment carries a good prognosis but is very limited because of its high cost, difficulty of getting licenses and health safety issues, being only available at limited major referral hospitals as, for example, in The Liverpool University and The Animal Health Trust. Even though, it is only applicable to relatively small tumors in suitable sites, such as the eyelids and limbs, otherwise cost would be prohibitive. The prognosis with surgical excision might improve if combined with other treatment options such as chemotherapy or if the scalp is replaced by a CO_2 or diode laser.

Tumor regrowth is the most common complication when treating equine sarcoids. Nevertheless, combined methods are often used in order to minimize it (Goodrich *et al.*, 1998). Other potential complications include the coexistence of other factors such as granulation tissue, infection, presence of other tumors at the same location, and transmission of the disease to other animals or to other locations on the same animal possibly by flies. These may be misleading when histopathological characteristics or clinical appearance varies. Furthermore flies can and must be controlled in order to decrease the chances of transmission.

Incisional dehiscence and perilesional swelling might also occur after surgical debridement or cisplatin injections, respectively (Goodrich *et al.*, 1998).

The risk of therapy, both for patient and owner must be weighed against the treatment's efficacy. For example, brachytherapy has a risk of exposure to the client but also to the surgeon implanting the material, therefore, most cases are isolated in special facilities until the implants are removed and special proceedings must be followed (Goodrich *et al.*, 1998).

Table 15: Comparison of treatment methods applied to equine sarcoids

Treatment modality		Advantadges	Disadvantadges	Indications	Success rates
Ligature		Ease, inexpensive.	Limited application.	Only for nodular type A sarcoids.	40-60%
Standing Surgery		Ease, inexpensive.	Can favor iatrogenic or auto-inoculation.	Superficial, occult, verrucose and nodular lesions Ideally for freely movable tumors, and where size and location permits wide surgical excision and complete wound closure.	23,5-28%
Conventional Surgery		Ease. Under GA a non-touching technique can be applied, avoiding auto-inoculation, and a wide margin recession is better performed than in standing sx.	Expensive. Not suitable for extensive and widely ramificated tumors or those located in places that do not permit proper wound closure (eyelid, axillar region, legs) – where cosmetic or functional damage may be at stake.		80-82%
Cryosurgery		Inexpensive, ease of use, lack of hemorrhage, and less pain and scaring when compared to conventional surgery. Possible prevention of dissemination of tumor cells and development of cryoantigens.	Prolonged anesthesia and intervention time required. Cosmetic and/or functional damage to underlying structures, therefore not suitable for large and infiltrative tumors located on the limbs and eyelids.	For tumors that do not allow wide surgical excision or those that are not freely movable from underlying structures.	42-100%
Laser Surgery		Ease, speed, precision, and minimal direct tissue contact (decreased contamination), as well as less pain, bleeding and inflammation, when compared to conventional surgery.	Possible bad cosmetic outcome. Initial high cost of the laser, need of specialized personnel, and safety considerations.	For Lesions where proper wound closure is not possible.	62-94%
Radiofrequency hyperthermia		Minimal side effects.	Limited area treated per application.	Suitable for periocular tumors.	Few data
Immunotherapy	**BCG**	Practical. Cost not prohibitive. Inhibitory effect on tumor growth.	Possible secondary effects includes severe local and systemic inflammatory reaction and death (live vaccine not recommended). Repeated treatment is often indicated.	Considered as a therapy of choice for periorbital lesions.	59-100%
	Mistletoe extract (Iscador® P)	Safe and effective.	Repeated inoculations needed.	For cases of multiple tumors where complete surgery cannot be conducted.	78,1%

Table 15 (continuation) – Comparison of treatment methods applied to equine sarcoids

Imiquimod (Aldara™)		Inexpensive. Ease- at owners care. Non-invasive. satisfactory cosmetic outcome.	Long treatment duration and possible adverse side effects and lack of compliance.	Possible therapy of choice for tumors located on the limbs, perineal and periorbital areas.	80-90%
Bloodrot extract (Xxterra ™)			Not recommended to sensitive areas (such as eyes and ears) due to risk of scarring	Apparently good for every clinical types	93-95%
Chemotherapy	**Topical**	Convenience, easy to apply, low cost.	Limited availability and applicability. Possile adverse reactions.		66-80%
	Intralesional	Inexpensive. Higher intratumoral concentrations and accurate placement. Relatively well tolerated.	Human health hazards/concern. Multi-treatments often required. Larger tumors care for prior surgical excision .	However, suitable for places were surgery is not an option.	77-90%
electrochemotherapy		Well tolerated, with excellent success rates. No side effects.	Need of GA. Successive treatments required.	Suitable for fibroblastic and verrucous tumors. (Smaller lesion respond faster to treatment).	100%
Photodynamic therapy		Relatively inexpensive. Potential non-invasive and selective cancer treatment. Minimal damage to surrounding tissue and minimal toxicity. Hypericin – reduction in tumor size. ALA – does not cause long term photosensitization.	Limitations – depth of treatment, not suitable for occult sarcoids if applied locally. Possible adverse side effects, including photosensitization which may require restrictions of exposure to direct sunlight.	May be useful to treat tumors that have been debulked or to help reduce recurrence on tumors with narrow margins of excision. Needs further testing.	36% of complete remission Up to 90% on size reduction
Radiotherapy		High radiation doses can be given directly to the tumor, sparring normal surrounding tissues. With implants the tumor is continuously irradiated, which may improve the cell killing efficiency. Accomplished in a relatively short period of time.	Expense, need for especial facilities and training, radioactive hazards, invasive. Secondary effects.	'Gold standard' for periocular sarcoids. Strongly recommended for the treatment of tumors, that have proved non-responsive to other treatments.	74-100%

GA – general anesthesia; Sx- surgery; Tx – treatment

Materials and methods

Equine sarcoid cases

Fifteen horses of mixed age, sex and breed, diagnosed with single or multiple equine sarcoid tumors and examined during the practice period of the author, either for a follow up evaluation or for a treatment proceeding were included on this study. Horses were withdrawn from the study when the histopathological diagnosis did not confirm the clinical diagnosis of sarcoids.

Eleven horses (numbers 1 to 11) were observed in Sweden, either at a referral teaching Hospital in Uppsala or in the field, and four (numbers 12 to 15) in England, at a referral hospital in Newmarket (Table 16).

Diagnosis was based on clinical appearance according to Knottenbelt *et al.* (1995). In eleven horses, at least one treated tumor was also examined histopathogically, confirming the diagnosis.

On the 15 horses there were a total of 64 tumors before the beginning of treatment, from which 29 were treated, and the others were either left as controls or were treated by benign neglect regarding their small size. Only 1 of the horses was previously treated by a different modality, being the remaining 14 horses treated for equine sarcoids for the first time.

Five different therapeutic approaches (single or combined) were used in this study: ointment application of imiquimod (Aldara™) or of an herbal extract from bloodroot (Xxterra™), surgical excision after interrupting treatment with imiquimod, ligation and, laser surgery.

For horses treated with imiquimod or the bloodroot extract, the criteria of selection of either drug was made randomly, with the exception of tumors located on the head, where imiquimod was automatically chosen, since the bloodroot extract has been reported to cause intense scarring at this location.

Surgical excision after incomplete treatment with imiquimod was performed on a competing horse, at owners request, on a tumor located on the girth area that blead everytime the horse had the sadle on, in order to shorten the treatment time required and allow the horse to compete.

Ligation was chosen over ointment application to treat a nodular, pedunculated, type A sarcoid (in its attachement to the skin) located on the groin area since it was considered to be the best suitable method, in this case.

Laser surgery was the therapy of choice for sarcoids at the referral hospital in Newmarket.

Follow up information was obtained by veterinarian evaluation and registered. Data concerning the age and breed of the horses, the total number of tumors present and treated on each horse, as well as, their type, size and location are mentioned in Table 16, and the methods of treatment employed in the management of these cases are described.

Treatment method with Imiquimod (Aldara™):

The tumor area was washed, with mild soap and water, and then dried before each application of the ointment in a thin layer, over the affected area. Gloves were used in order to avoid ointment contact with the skin of the operator. The horses were treated 3 times a week, on non-consecutive days, for up to 32 weeks[14], or until complete resolution, whatever came first according to Nogueira *et al.* (2006). Meloxicam (in sachets) was administered *PO*, by the owners, when needed.

Treatment method for the bloodroot extract (Xxterra™):

The affected area was cleaned with mild soap and water, and then dried, before each application of the ointment. Gloves were used in order to avoid contact between the ointment and operator's skin.

In cases where bandaging was possible a thick layer of Xxterra™, 3 – 5 mm, was applied on the tumor's surface. Then it was covered with gauze without applying pressure, and the bandage was left for 4 to 6 days. After this period the bandage was removed and the area inspected, and treatment repeated if needed.

In cases where bandaging was not possible a thick layer (3 – 5 mm) was applied daily on the tumor surface for 4 to 6 days, and then treatment was repeated every 4 days until it was completely resolved (Pettersson, 2008). Meloxicam (in sachets) was administered *PO*, by the owners, when needed.

Treatment method for ligation:

A rubber elastic band was applied as a ligature.

Treatment method for laser surgery:

Prior to laser surgery horses were sedated with detomidine (0,01-0,02 mg/Kg, IV) and buthorphanol (0,02- 0,04 mg/Kg, IV), and locally anesthetized with mepivacaine.

The tumor area was surgically cleaned and the tumors were excised with a diode laser beam, (980 nm wavelength, 0-25 watts power, Ceramoptec manufacturer). Besides the sarcoid tumor, a margin of normal skin of about 1 cm width was removed. All personnel in the surgery room wore laser safety protection glasses.

After surgery, horses were given phenylbutazone (4,4 mg/Kg, IV or *PO*, q 12 h), oxitetracycline (6,6mg/kg IV (/IM) q 12h for 5 days) or penicillin (10mg/kg IM for 5-8 days) and a fly repellent was applied - Swat original. The wound was left to heal by second intention.

[14] Except for one of the tumors on horse number 7 that was treated for 36 weeks at veterinarian consideration

Statistical analysis

Descriptive statistics was used to perform comparisons in this work.

Equine sarcoid inquire

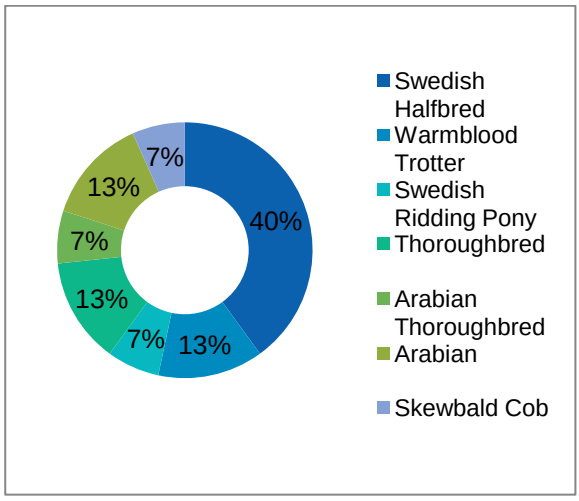
An on-line inquire (Appendix) in relation to equine sarcoid was performed to Portuguese veterinarians, concerning aspects such as frequence of the disease, diagnostic and treatment methods, as well as immediate success and, recurrence rates. Results are shown on Graghics 5 to 10.

Results

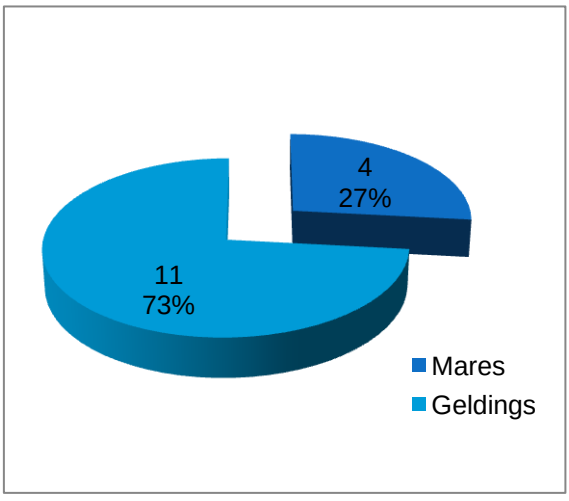
Equine sarcoid cases

From the 15 horses included in this study 6 were Swedish Halfbreds, 2 Thoroughbreds, 1 Arabian Thoroughbred, 2 Arabians, 2 Warmblood Trotters, 1 Swedish Ridding Pony, and 1 Skewbald Cob (Graphic 1). Nine of the horses had single (60%) and 6 (40%) had multiple equine sarcoid tumors. According to gender, there were 11 geldings and 4 mares (Graphic 2).

Graphic 1: Breed



Graphic 2: Gender



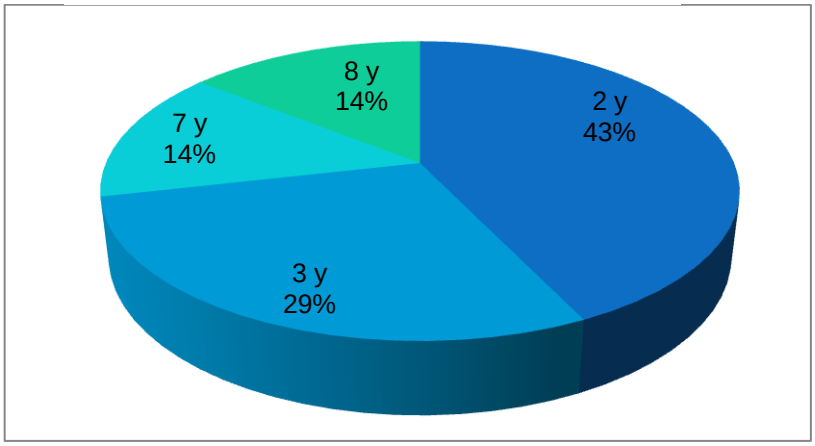
From the 29 treated tumors 14 were treated with imiquimod (Aldara™), from which 1 was surgically excised after a brief period of application of the drug, 5 were treated with an herbal extract of bloodroot (Xxterra™), 1 by ligation, and 9 by laser surgery, as mentioned in Table 16.

Three of these horses were treated simultaneously with imiquimod and the bloodroot extract on different locations.

Treated tumor types included 3 occult, 7 nodular, 11 fibroblastic, 4 verrucous and 4 mixed.

The age of the animals included in this work ranged from 5 to 20 years (mean 11,3; median 10; mode 12). Information concerning the age of the animals when tumor was first detected was known in 7 horses (47%). From these, 86% were 7 years old or younger (Graphic 3).

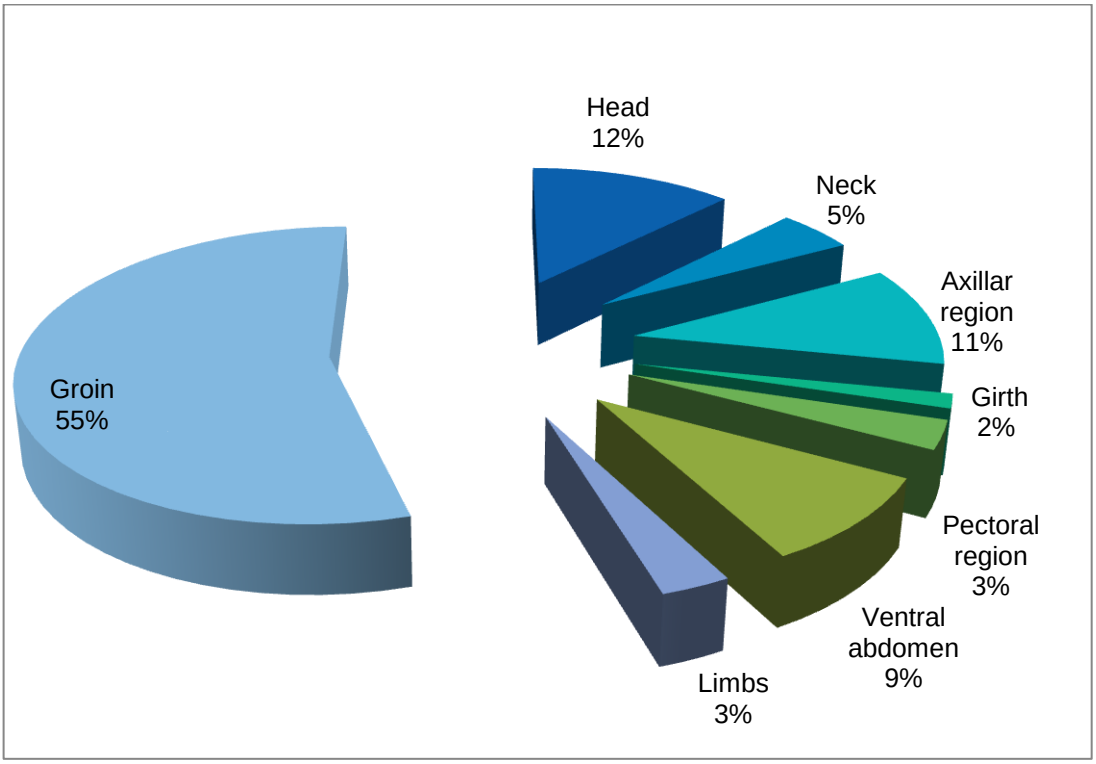
Graphic 3: Animal's age at tumor onset



N=7

Affected areas included the head (pinna area), neck, axillar, girth, and pectoral regions, ventral abdomen, limbs, and groin (Graphic 4).

Graphic 4: Tumor location



Follow up evaluation was performed on 10 horses. For horses 1-8 and, 9 follow up evaluation was performed, respectfully, at 24 and at 30 months after treatment ended, and for horse number 11 it was performed 1,5 months after treatment started.

Table 16: Characteristics of horses, sarcoid tumors, and treatments included in this work

Horse	Age	Gender	Breed	Age at tumors onset	Total nr of tumors present and location	Nr of tumors treated	Tumor type	Approximate size	Previous treatment	Treatment	Follow up (Months)
1	7	gelding	Swedish Halfbred	2ys	1 on the groin area	1	Fibroblastic	3 cm^3	No	Xxterra	24
2	19	mare	Swedish Halfbred	NK (came with the tumors when the owners bought her at the age of 12)	7 all on the right ear	3	Fibroblastic	9 – 43,4 cm^3	No	Aldara	24
3	12	gelding	Swedish Halfbred	8ys	1 on the left axilla	1	Mixed fibroblastic and verrucous	20 cm^3	No	Aldara	24
4	8	gelding	Arabian thoroughbred	3 ys	14 on the: groin area, girth, ventral abdomen and axilla	2 (girth region and ventral abdomen)	Fibroblastic, mixed nodular and verrucous	5 – 48 cm^3	No	Girth- incomplete tx with Aldara followed by surgical excision Abdomen-Xxterra	24
5	8	gelding	Swedish Halfbred	3 ys	6 tumors in total located on the head, neck, groin, axilla, and pectoral regions	5 (neck, groin (2), pectoral, and axilla regions)	Occult, verrucous (2), fibroblastic, and mixed nodular and verrucous	0,5 – 60 cm^3	No	Neck+ Axilla- Aldara Groin+pectoral– Xxterra Groin(penis) - ligation	24
6	6	gelding	Warmblood trotter	2 ys	1 on the axillar area	1	Fibroblastic	45 cm^3	No	Aldara	24
7	20	gelding	Swedish ridding pony	NK (came with the tumors when the owners bought him at the age of 14)	15 on the groin and axillar areas	2 (groin)	Nodular	12 – 140 cm^3	Surgery 5 ys ago but it recurred	Aldara	24
8	20	mare	Swedish Halfbred	NK (came with the tumors when the owners bought her at the age of 12)	7 tumors, located on the groin, neck, and axilla	2 (neck and groin)	Mixed occult, verrucous and fibroblastic, and fibroblastic	126 – 210 cm^3	No	Neck- Aldara Groin-Xxterra	24
9	5	gelding	Swedish Halfbred	2 ys	1 on the cranial aspect of the left fore	1	Fibroblastic	NK	No	Aldara	30
10	18	gelding	Arabian	NK (came with the tumors when the owners bought him at the age of 4)	1 on the ventral abdomen, close to the *linea alba*	1	Nodular	12 cm^3	No	Aldara	(-)
11	10	gelding	Warmblood trotter	7 ys	1 on the groin area	1	Verrucous	15 cm^3	No	Aldara	1,5
12	6	gelding	Thoroughbred	NK	2 small tumors on the groin area, and 4 bigger tumors located on the ventral abdom	6	Occult (2), fibroblastic (1) and nodular (3)	0,05 – 35 cm^3	No	Laser surgery	(-)
13	12	mare	Thoroughbred	NK	1 on the groin area	1	Nodular	360 cm^3	No	Laser surgery	(-)
14	12	gelding	Skewbald cob	NK	1 on the groin area	1	Fibroblasic	NK	No	Laser surgery	(-)
15	6	mare	Arabian	NK	1 on the base of the neck	1	Verrucous	68,4 cm^3	No	Laser surgery	(-)

NK- not known; ys- years; tx-treatment; (-) not applicable

Results concerning treatment and follow up evaluation are summarized on Table 17, and treatment efficacy is given on a tumor basis.

All of the 5 tumors treated with the bloodroot extract (Xxterra^{TM}) regressed completely, with no signs of recurrence present 24 months after finishing treatment (Figure 6). Thereby, the success rate for this method was 100%, for a 24 month period of follow up. The only sequelae left by this modality of treatment was a thickened scar tissue formation and mild alopecia in all the treated animals, both only present at the treatment site.

Figure 6: Horse number 1, before starting treatment with Xxterra^{TM} (A), and at the 24 months follow up examination (B)

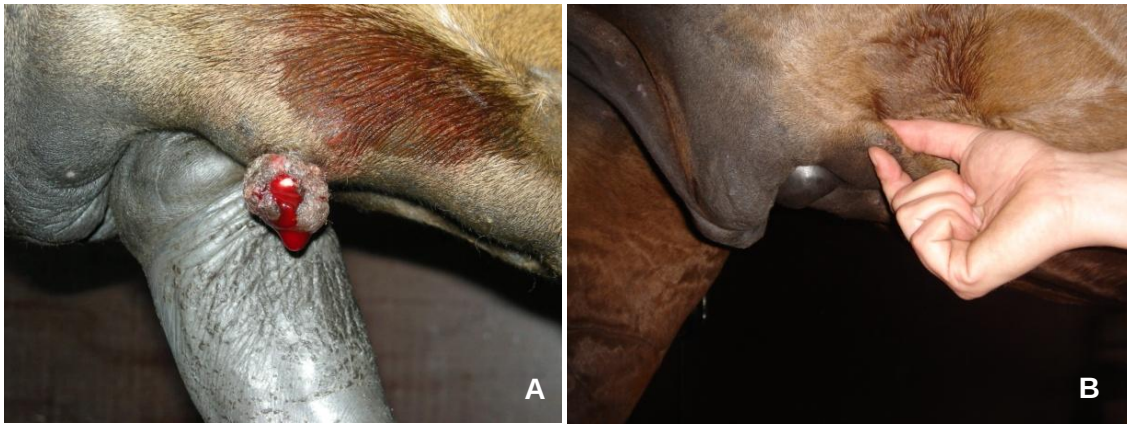


Photo by courtesy of Dr. Carina Pettersson Photo by the author

From the 14 tumors treated with imiquimod (Aldara^{TM}), 2 shrank in size, 1 had no follow up examination, 1 was surgical excised before finishing treatment with imiquimod, and 10 regressed completely, with no signs of recurrence present up to 30 months after treatment ended (Figures 7 and 8). All tumors with follow up information either had a partial regression in size, or regressed completely. Nevertheless, 4 of the horses, with a total of 4 tumors, were excluded from treatment efficacy assessment: horse number 3, due to owners lack of compliance, horse number 4, since treatment was not finished with imiquimod but with surgical excision, at owners request, horse number 10, because no follow up examination was made during the practice period of the author, and horse number 11, since the only follow up evaluation was made 1,5 months after the beginning of treatment, and so treatment was not completed, and thereby its efficacy could not be evaluated. Thereby, the success rate obtained by this method for the 10 tumors that completed treatment was 100%, with no signs of recurrence present up to 30 months after treatment ended. Only a thickened scar tissue formation, and mild alopecia were observed at the treatment site.

Treatment duration was accessed for 100% of the horses (93,3% of the tumors), that completed treatment with these 2 formulas (Table 17). Treatment duration using imiquimod ranged from 3 to 36 weeks and took an average of 21,2 weeks compared to the 4 to 10 weeks of treatment with an average of 7,2 weeks when using the bloodroot extract.

Imiquimod took in average about 3 times longer to treat when compared to the bloodroot extract.

Figure 7: Horse number 2, before (A), and 24 months after (B) treatment with Aldara™

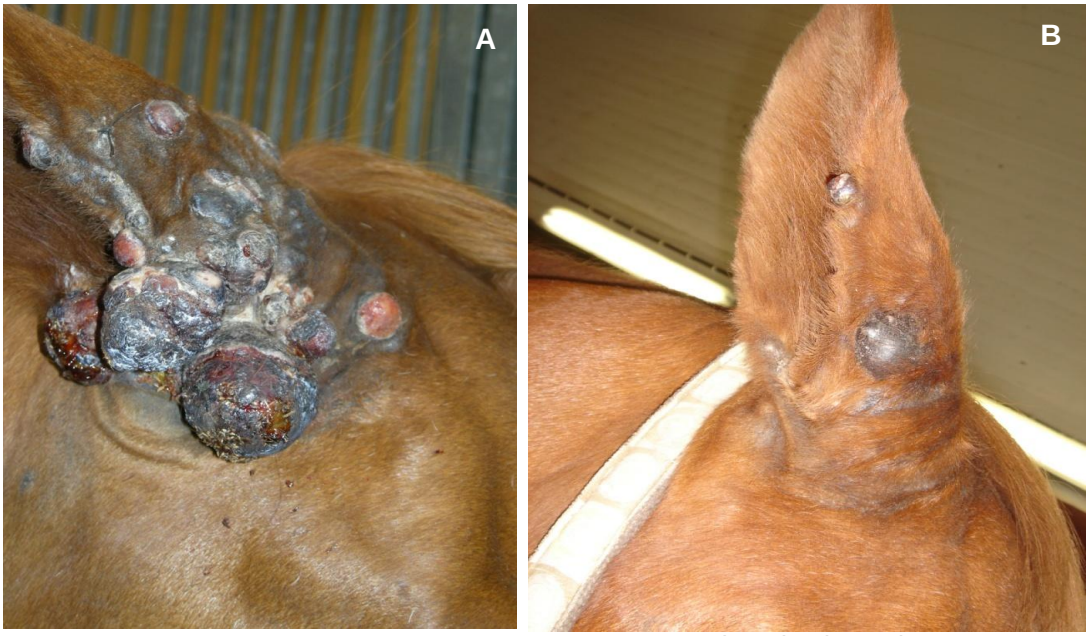


Photo by courtesy of Dr. Carina Pettersson

Photo by the author

Figure 8: Horse number 6, before (A), and 24 months after (B) treatment with Aldara™

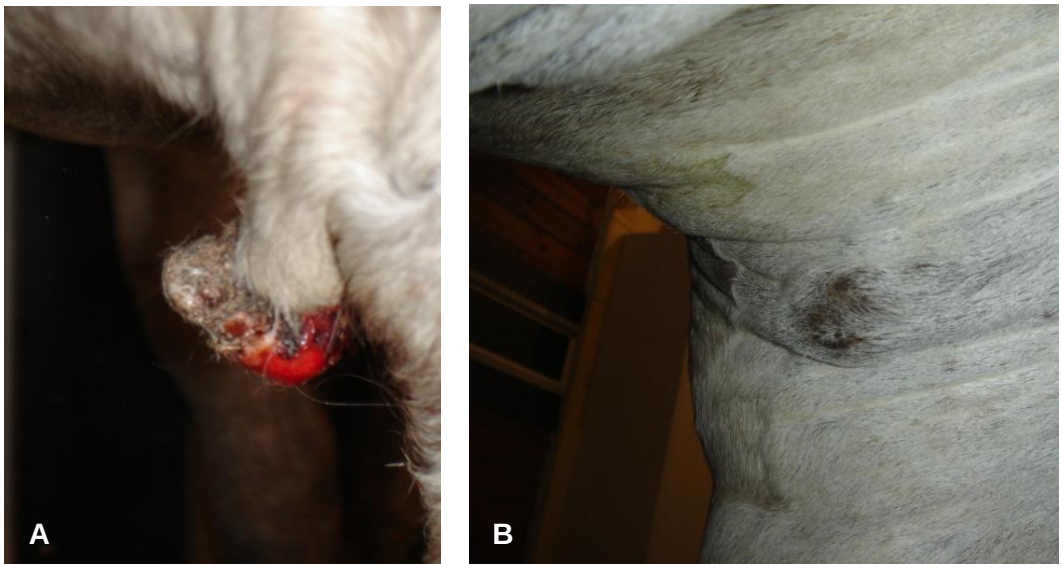


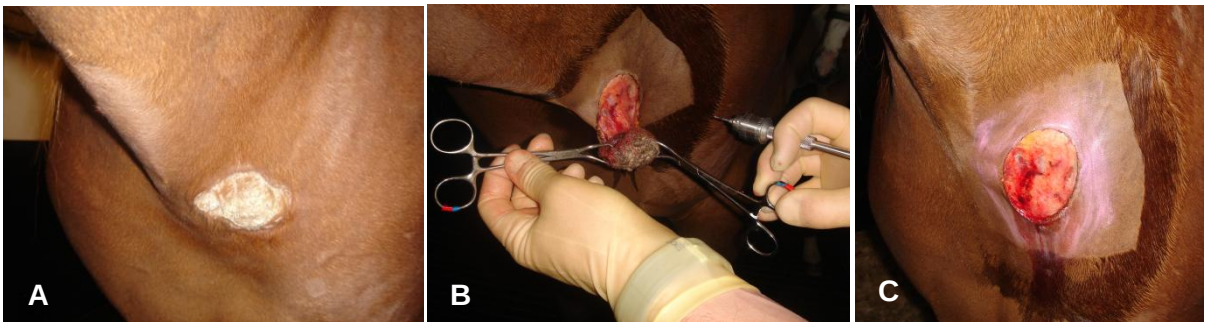
Photo by courtesy of Dr. Carina Pettersson

Photo by the author

One horse (number 5) was treated by ligation, on one of the tumors, and recurrence was not seen 24 months after treatment ended.

Horses treated by laser surgery showed good immediate results, regarding tumor removal, nevertheless, the surgical wound was left to heal by second intention, showing a huge scar, as shown in Figure 9c.

Figure 9: Horse number 16, before (A), during (B) and immediately following (C) laser surgery



Photos by the author

Table 17: Summary of tumor treatments and treatment outcome

Horse	Tumor type	Treatment	Treatment duration (Weeks)	Follow up (Months)	outcome
1	Fibroblastic	Xxterra	4	24	Complete regression. Scar tissue and mild alopecia present at the treated area. A new tumor mass has developed at the coronary band.
2	Fibroblastic	Aldara	20 (x2) 16 (x1)	24	All treated tumors regressed. Scar tissue and alopecia present at the treated area. Two new tumors developed in the back of the ear.
3	Mixed fibroblastic and verrucous	Aldara	(-)	24	The treated tumor shrank, but was still present. Nevertheless, treatment was not completed by lack of owner's compliance. 2 new tumors appeared (pectoral region and ventral abdomen).
4	Fibroblastic, mixed nodular and verrucous	Girth- incomplete treatment with Aldara followed by surgical excision Abdomen -Xxterra	NK 8	24	Both of the treated tumors were only present as scars and mild alopecia. 2 new tumors appeared on the abdomen.
5	Occult, fibroblastic, verrucous, and mixed nodular and verrucous	Neck+ Axilla- Aldara Groin+pectoral– Xxterra Groin(penis) - ligation	16 and 32 8 and 10 NK	24	All tumors regressed, only a thick skin scar and alopecia were observed at the treated areas. The only non-treated tumor regressed spontaneously.
6	Fibroblastic	Aldara	28	24	Complete regression. Only a thick skin scar and alopecia were noticed.
7	Nodular	Aldara	NK and 36	24	Both tumors regressed completely. Only a thick skin scar and alopecia were present.
8	Mixed occult verrucous and fibroblastic, and fibroblastic	Neck- Aldara Groin-Xxterra	20 6	24	Both treated tumors regressed completely. Only a thick skin scar and mild alopecia were noticed. Several new tumors developed on the groin area.
9	Fibroblastic	Aldara	3	30	Complete regression. Only a thick skin scar was noticed At the treated area.
10	Nodular	Aldara	NK/(-)	(-)	(-)
11	Verrucous	Aldara	NK/(-)	1,5	The tumor regressed until 1/3 of its initial size.
12	Occult, fibroblastic and nodular	Laser surgery		(-)	(-)
13	Nodular	Laser surgery		(-)	(-)
14	Fibroblasic	Laser surgery		(-)	(-)
15	Verrucous	Laser surgery		(-)	(-)

NK – not known; (-) not applicable

Equine sarcoid inquire

The on-line inquire performed to Portuguese veterinarians had the adhesion of 15 practitioners.

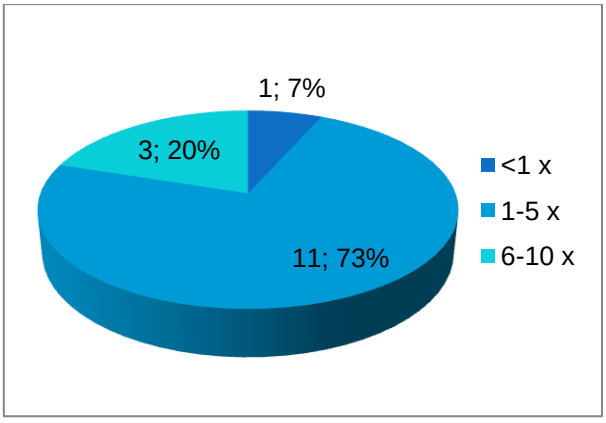
From the 15 veterinarians inquired, 11 (73%) reported to have 1 to 5 new equine sarcoid cases per year, 3 (20%) had 6 to 10 cases and, 1 (7%) had less than 1 case per year (Graphic 5).

Most of the veterinarians (47%) claim to diagnose this disease by biopsy and histopathology, and/or clinical assessment, 40% only by clinical assessment, and 13% simply by biopsy followed by histopathology (Graphic 6).

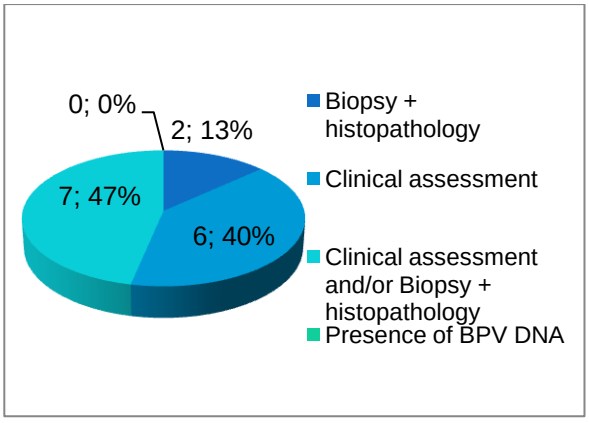
The majority of inquired practitioners (53,3%) used only surgical techniques in order to control equine sarcoid tumors on their patients, 6,7% use only non-surgical approaches, and 40% use both (Graphic 7). Surgical methods varied from ligature to conventional field surgery, or cryosurgery, having the concern to remove wide margins of normal tissue, whenever possible. Non-surgical methods used by these veterians include local chemotherapy with 5-FU, cisplatin and AW4-Ludes, topical approaches with Xxterra, Lotagen or green mud, cryotherapy, and immunotherapy with intralesion BCG injections. Five professionals (33,3%) always used the same therapeutical surgical technique. However, 10 practitioners (66,7%) use different therapeutical options to treate these tumors (Graphic 8) based on: results obtained from a previous unsuccessful treatment attempt (60%), tumor type (70%), localization (100%), or extention (90%), as well as, cost of treatment (50%) or histopathologist advice (10%). Immediate therapeutical success was qualified as good by 56% of the professionals inquired and satisfactory by the remaining 38% (Graphic 9).

Recurrence rates (Graphic 10) lower than 10% are reported by 38% of the enquirees, 11 to 30% by 38%, 31 to 50% by 13% and, more than 50% by 6% of the practitioners.

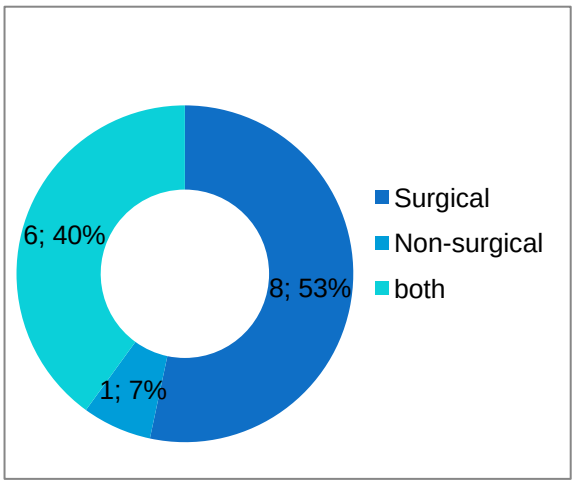
Graphic 5: Cases of equine sarcoid diagnosed per year by the 15 veterinarians



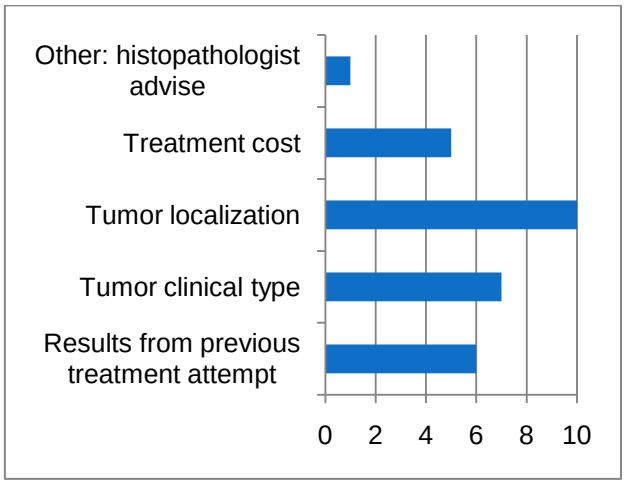
Graphic 6: Diagnostic methods of sarcoids used by the 15 veterinarians



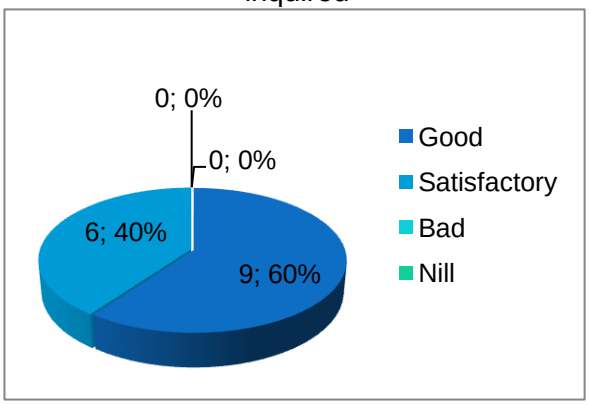
Graphic 7: Treatment approaches



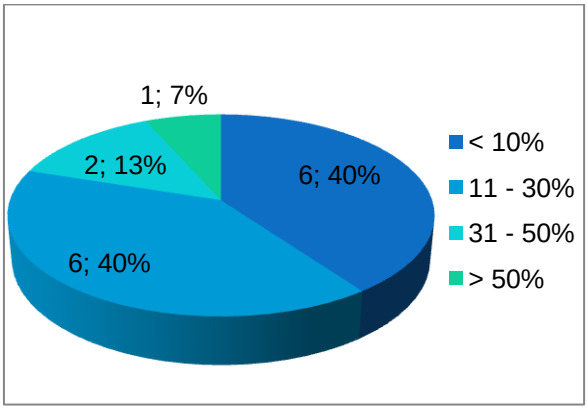
Graphic 8: Given reasons for using different treatment approaches



Graphic 9: Immediate therapeutical success obtained by the 15 veterinarians inquired



Graphic 10: Recurrence rates observed by the inquired practitioners



Discussion

Equine sarcoid cases

Equine sarcoid is the most common skin tumor in horses (Marti *et al.*, 1993; Mohammed *et al.*, 1992). This tumor represents a therapeutical challenge to the veterinary practitioner and it is commonly located in areas that can cause discomfort to the horse and where removal is often difficult or even impossible to perform (McConaghy *et al.*, 1994b). Thus, researchers are trying for decades to pursuit a universal treatment with high success rates and low recurrence rates, however, no treatment was found to be 100% effective for all clinical manifestations and locations of the disease, which can be easily understood considering the high number of treatments now available.

In the present work 5 different therapeutic approaches (single or combined) were used in order to treat equine sarcoids: ointment application of imiquimod (Aldara^{TM}) or an herbal extract from bloodroot (Xxterra^{TM}), surgical excision after incomplete treatment with imiquimod, ligation and, laser surgery.

Single tumors were found in the majority of the studied population (60%), what is in agreement with other studies (Broström, 1995a; Fretz & Barber, 1980; Nogueira *et al.*, 2006). Even though equine sarcoid tumors can affect every breed, gender, age or localization, some predisposition has been identified. At higher risk are Quarter horses, Appaloosas, and Arabians, followed by Thoroughbreds, while Standardbreds are rather resistant (Angelos *et al.*, 1988; Meredith *et al.*, 1986; Mohammed *et al.*, 1992). Horses from breeds carrying haplotypes ELA A3 (MHC I), ELA W13 (MHC II) or, the heterozigotic defective DNA-PKcs, such as the Swedish Halfbred, the Swiss Warmblood, the Irish Warmblood, the Selle Français, the Thoroughbred and the Arabian are more susceptible to equine sarcoid development (Broström *et al.*, 1988; Broström, 1995b; Ding *et al.*, 2002; Lazary *et al.*, 1994; Meredith *et al.*, 1986). The most representative breeds included in this work reflect some of the breeds mentioned above. Even though, an individual variation can occur. Considering this, it would be interesting to test the horses for the presence of these haplotypes.

A higher tendency for sarcoid development have been reported in geldings (McCauley *et al.*, 2002; Mohammed *et al.*, 1992), which is also reflected in this work, with 73% of the horses being geldings, against 27% of mares. The exact reason why geldings are more affected than mares or stallions is not known. Possible explanations can be that wounds left by castration are contaminated by flies carrying the bovine papilloma virus, since BPV DNA has already been isolated from 4 different fly species by Finlay *et al.* (2009) and, Kemp-Symonds (2007)[15] or that the surgical procedure itself may be the cause of contamination. One way to

[15] In Bogaert *et al.*, 2008a

prevent this from happening is by castrating horses out of the warmer season, when the number of flies are lower in the environment, and also by applying a fly repellent next to the surgical wound. An iatrogenic contamination during castration or other surgical procedure can be easily avoided by proper instrument sterilization. It has also been proposed that sexual hormones could be involved in the pathogenesis, as it has already been evidenced in human papillomaviral-associated disease (Pater, Mittal & Pater, 1994[16]).

This tumor can affect horses at any age (Marti *et al.*, 1993; Ragland *et al.*, 1970). In this work, the majority of cases (86%) at tumor onset were in 7 year old and younger animals, which is in agreement with other researchers that have reported a higher prevalence in younger individuals (Carstajen *et al.*, 1997; Clottu, 2008; Cotchin, 1977; Knottenbelt, 2005; Marti *et al.*, 1993; Reid *et al.*, 1994a; Torrontegui & Reid, 1994), with the exception of Ragland *et al.* (1970) that reported a higher prevalence in older animals. Only 14% of the studied population was over 7 years of age at tumor onset.

The location pattern of tumors in the present study is in agreement with distributions described by other European investigators, were the majority of tumors were located on the head and trunk, rather than on the extremities (Broström, 1995a; Byam-Cook *et al.*, 2006; Clottu, 2008; Martens *et al.*, 2001c).

Knottenbelt (2008b) believes that notwithstanding the genetic susceptibility, no horse can be considered to be totally exempt from the condition and that it is reasonable to try to exert breeding pressure against the disease by avoiding the breeding of two affected horses.

If one considers that sometimes affected horses, as in the present work, have sarcoid tumors located on the groin area (Reid *et al.*, 1994a) and, that this is a transmissible disease, it is probably wise to keep such horses away from natural cover and therefore avoid a possible transmission by contact. Moreover, if the mare is affected it is easy to presume that a nursing foal can easily get infected.

Nevertheless, when sarcoid affected horses are kept in a group, transmission can be prevented, up to a certain level, by using different tacks or brushes, on affected and non-affected animals as well as keeping the horses in different boxes, and by caretakers personal hygiene. Contact with cattle, especially if suffering from acute or recent BPV infection should also be avoided if possible (Martens *et al.*, 2009a).

In agreement to findings in other studies (Byam-Cook *et al.*, 2006; Clottu, 2008; Martens *et al.*, 2001c) all clinical types of equine sarcoids, except for the malevolent form, were observed in the present work, with the fibroblastic type being the most often treated, which is reasonable since it has been suggested that sarcoids develop initially as one of the less aggressive types and then transform into the more aggressive fibroblastic form, being more likely to receive diagnostic and therapeutic attention (Broström, 1995b; Byam-Cook *et al.*,

[16] In Reid & Mohammed, 1997

2006; Knottenbelt *et al.*, 1995; Marti *et al.*, 1993; Scott & Miler, 2003; Théon *et al.*,1993). In the present work this form represents 37,9% of the treated tumors.

Most of the horses included in this study were treated for the first time (93,8%), which is also in agreement with other studies (Carstanjen *et al.*, 1997; McCauley *et al.*, 2002; McConaghy *et al.*, 1994b), and may also reflect the efficacy of the treatments employed.

Since not all tumors from the same animal were treated, results were given in a tumor basis, rather than in an animal one.

Cases treated with Xxterra™ had an excellent outcome, with 100% of success rate. No signs of recurrence were observed on the last follow up visit, 2 years after treatment ended. These results were similar to the ones mentioned by Ken Larson, the manufacturer of Xxterra™ (Larson Laboratories, 1982).

Tumors treated with Aldara™ also had an excellent outcome, as well as a success rate of 100% with no signs of recurrence observed on the last follow up visit, up to 30 months after treatment ended. Results obtained in this study were better than the ones reported by Nogueira *et al.* (2006), and Pettersson (2008).

Good cosmetic results were obtained in all cases treated by these two modalities, with side effects resuming to a thickened skin scar and mild alopecia.

No correlation was found between the number of tumors present and treatment outcome, neither between tumor's volume, type or, location and treatment outcome, contrasting with what was mentioned by other researchers (Stewart *et al.*, 2006; Tamzali *et al.*, 2001; Théon *et al.*, 1994a), since all lesions resolved and showed no signs of recurrence up to 30 months after treatment ended. Nevertheless, even though volume did not influence treatment outcome, smaller lesions tended to resolve faster than bigger ones.

When comparing these 2 ointments, treatment duration with imiquimod took significantly longer time (approximately 3 times longer) than with Xxterra™, which can significantly interfere with owners and/or animals compliance. The same trend in treatment duration was obtained by other authors (Nogueira *et al.*, 2006; Pettersson, 2008).

Pettersson (2008) mentioned that if one of these drugs is not effective, in treating one tumor or horse, changing treatment to the other drug seems to have good results. Even though this was not appraised in the current work, since excellent results were obtained with either drug in all tumor types and locations that completed treatment, it is an aspect that worths to be mentioned.

There are both advantages and disadvantages when treating the same horse with the two ointments. The benefit is that one can assess which drug is more suitable for that particular horse, tumor location or, type. The shortcoming is that the possible systemic effects of each agent cannot be evaluated (Pettersson, 2008). However, no obvious systemic effects on untreated sarcoids could be observed in treated horses.

Treatment duration, as well as other differences between the 2 drugs, might also be due to tumor duration, size, type and location, as well as to individual differences in immune response to tumors (Petersson, 2008). Nevertheless, in order to make any conclusions further trials need to be performed.

When used as a single treatment approach, both Aldara™ and Xxterra™ require a relatively long period of treatment which, as mentioned previously, can interfere with owners and/or animals compliance.

One of the horses included in this work (horse number 4) started treatment with Aldara™, but since it was a competing horse and owners wanted to shorten treatment duration in order to compete with the horse sooner, they decided to stop imiquimod treatment and to remove the tumor surgically instead. In the present work, surgical excision after incomplete treatment with Aldara™ showed good results with lack of recurrence 24 months after treatment ended, yet one cannot draw any conclusions from this since it was assessed only in 1 horse and it can be attributed to the horses immune system or be influenced by the brief Aldara™ treatment, and also because this particular animal was also treated with Xxterra™, and there is a possibility that this drug might have a positive systemic effect as suggested by its manufacturer, Ken Larson (Larson Laboratories, 1982).

Therefore, this makes one think that if results are needed sooner, the combined treatment of surgical excision and application of either of these drugs might be an alternative.

There is also the possibility to surgically remove the tumor first and then apply either drug to shorten the treatment time required, or to apply either drug, surgically remove the tumor and then continue applying the ointment, at least, until complete wound healing.

Even though, either of these sequential treatments using surgical excision and the application of one of the 2 ointments can be an alternative. The reported fact that injury may trigger sarcoid lesions to develop into a more aggressive form of the disease (Ragland *et al.*, 1970) could be a limitation to this approach. Moreover, today too little is known about these 2 ointments, when applied to horses and, without an appropriated clinical trial, one cannot be sure about the influence of this sequential treatment on outcome and duration (shortened or extended) of equine sarcoid and its treatment.

Several new sarcoid tumors developed in horses treated with Aldara™ and Xxterra™, but at different locations from the treated tumors, which is understandable since, this disease is thought to be transmissible, to have a phase of viral latency in the skin and in PBMCs, and also because not all the tumors, present in the same horse, were treated. Even though equine sarcoid tumors do not methastize, they invade locally surrounding skin tissue, and sometimes even muscle (Bogaert *et al.*, 2008; Knottenbelt & Kelly, 2000; Olson, 1948). Thereby there is always the possibility of new tumor development, if the virus is still present in the skin or PBMCs. As mentioned by Knottenbelt (2005), this disease is best regarded as a form of skin cancer, so treatment is best considered as a control measure than as a cure.

After treatment has finished and, ideally also before treatment starts, if it can be promptely instituted, it would be of most interest to make a skin biopsy in order to determine: a) the presence of histopathologycal characteristics of the tumor and, b) the presence or, amount of virus present at the treated area and, normal surrounding skin, trying to correlate it with treatment efficacy and possible relapse, similarly to what was performed by Martens *et al.* (2001a). It would also be interesting to test the blood for the presence of BPV DNA in PBMCs and see if it relates to horses having multiple tumors as reported by others (Brandt *et al.*, 2008a; Martens *et al.*, 2009a).

Nevertheless, the development of new tumors seems to contradict a possible systemic effect of either drug. In this work, the development of new tumors occurred in 7 year old and older animals, in contrast to other reports (Carstanjen *et al.*, 1997). This could seem to contradict the reported fact that younger horses are more susceptible towards the condition, and the suggestion that older horses may develop some kind of immune resistance towards it (Broström, 1995a; Reid & Gettinby, 1996). On the other hand, it may also mean that treatment was not performed soon enough, allowing the virus to disseminate in the skin or blood and making it harder to control the disease. Another possible explanation resides on the fact that this is a contagious disease, with a viral component (BPV types 1 and/or 2), that by itself has an impact on the animal immunity and that older individuals may also have their immune system somehow compromised, contributing additionally to immunossupression and allowing viral replication and invasion.

Still, another possible and more plausible explanation reside on the fact that new tumors only developed in horses from 2 breeds, Swedish Halfbred and Arabian thoroughbred, known to be more predisposed towards the development of sarcoid tumors on a genetic basis linked to the immune system. As mentioned previously, the predisposition for sarcoids in Swedish Halfbreds and Thoroughbreds is related to the presence of the haplotype ELA W13 (MHC II), and in Arabians to the presence of the heterozygotic defective DNA-PKcs allele, responsible for severe combined immunodeficiency (Broström, 1995b; Ding *et al.*, 2002).

Researchers have reported spontaneous regression ranging from 2,2% to 32% (Broström, 1995a; Clottu, 2008; Martens *et al.*, 2001c; Pettersson, 2008). In the present work, only one tumor, from horse number 5, showed spontaneous regression, which makes a 2,86% of spontaneous regression from the initial 35 non-treated tumors, and is in agreement with other reports. This might support that Xxterra™ has a positive systemic effect, as suggested by its manufacturer, but since it happened only in one case, and the horse was also treated with Aldara™, this could not be verified and can actually be attributed to either drug, or the host's immune system.

Ligation was used to treat one tumor from horse number 5, and no signs of recurrence were observed 24 months after treatment. Nevertheless, efficacy cannot be estimated for this modality of treatment since only one tumor was treated by this method, and because this

horse was also treated with Aldara™ and Xxterra™, and therefore its efficacy could be attributed to a positive systemic effect from either drug. However, anecdotical observations report that ligation could be an effective treatment for pedunculated sarcoids.

Laser surgery was used to treat a total of 9 equine sarcoid tumors on 4 horses, and even though horses were not evaluated during the practice period of the author, veterinarians from the Hospital where this treatment was performed reported a success rate of 90% on the first year after treatment, and of 80% on the second year based on data from 110 horses followed for 5 years (Dr. R. Payne, personal communication, 9th of June, 2010), which is in agreement with results reported by other researchers for sarcoid laser treatment (Kempt-Symond *et al.*, 2008).

Cost of treatment is an important factor when considering which therapy to choose, as mentioned earlier. Imiquimod (Aldara™), 250mg, is sold in boxes with 12 sachets each (Autoridade Nacional do Medicamento e Produtos de Saúde [Infarmed], 2010), and one bag is enough to treat an area of 20cm^2 (Pettersson, 2008). This means that final cost will depend up on tumor size and duration of treatment. For example, for a 20 cm^2 occult sarcoid treated for 8 weeks treatment will have a final cost comparable to cryosurgery and standing surgery prices. Even though treatment using only this late method is not recommended, due to the high recurrence rates reported, it can be used as an adjuvant method. Nevertheless, if the tumor is twice that size – 40 cm^2, and treatment duration takes for example 16 weeks, total cost will be equivalent to prices of surgery under general anesthesia.

Xxterra herbal paste is sold[17] in vials of 28,35g (1 OZ) and 56,7g (2 OZ) and total cost of treatment will also depend on tumor size and treatment time required, which can only be assessed individually.

Again, the sequential treatment of surgical excision followed by the application of one of the 2 ointments could be an alternative. However its possible limitations should also be considered, as mentioned previously.

Benefits when using these 2 ointments, besides the good cosmetic and general results, low (Nogueira *et al.*, 2006; Pettersson, 2008) or, lack of recurrence rates as obtained in the present study, and relatively low cost are that this methods are simple, non-invasive and, can be performed at home by owner's care. Disadvantages include long duration of treatment, discomfort to the horse when touching the affected area and, sometimes there is a problem concerning lack of animals and/or owners compliance, as happened with horse number 3.

Ligation is quite inexpensive when compared to other treatment methods but has the limitation of being suitable only for nodular type A sarcoids (Knottenbelt, 2009).

Treatment with diode laser surgery including horse's appointment, tumor assessing, advising owners and hospital care will cost between cryosurgery and surgery under general

[17] http://www.entirelypets.com/xxterra.html

anesthesia prices. The advantages when using this method, as mentioned before, are ease, speed, precision, less pain and damage to surrounding tissues (bleed and inflammation), as well as, reduced contamination when compared to other surgical techniques (Palmer, 2002). On the other hand, disadvantages include the high cost of the equipment, the requirement of specialized trained personnel, lack of specificity towards tumor cell destruction, and a cosmetic outcome, not tolerable by some owners, such as a vast cutaneous scar tissue (Figure 9c), and often leukotrichia, (Carstanjen *et al.*, 1997).

Even though studies from different treatment modalities cannot be directly compared with each other, other treatment methods described in the literature have reported success rates of 100% (Klein *et al.*, 1986b; Knottenbelt & Kelly, 2000; Komáromy *et al.*, 2004; Lavach *et al.*, 1984; Massoco *et al.*, 2009; Murphy *et al.*, 1979; Tallberg *et al.*, 1994; Tamzali *et al.*, 2001). Current therapies of choice include BCG immunotherapy, radiotherapy and chemotherapy, among other promising ones.

Nevertheless, most often, the number of cases studied or the follow-up periods are rather short, information concerning recurrence is not always clear or, in other cases, it seems that most of the treated tumors recurred after a certain period of time or have limited applicability to certain tumor types, dimensions or body locations (Klein *et al.*, 1986b; Komáromy *et al.*, 2004; Murphy *et al.*, 1979; Tallberg *et al.*, 1994).

In many published studies, treatment efficacy seems to correlate with tumor size, number, type, location and, previous unsuccessful treatment attempts (Stewart *et al.*, 2006; Théon *et al.*, 2007). Contrastingly, in the present work, completed treatments with either Aldara™ or Xxterra™ showed 100% of success rate up to 30 months after treatment ended, for all types, sizes and, locations. Nevertheless, a bigger trial, comprising a larger sample, should be performed in order to corroborate these results.

The only publications found in literature with similar results, in number of cases, follow-up period as well as treatment outcomes, were with electrochemotherapy (Tamzali *et al.*, 2001), and periocular radiotherapy (Byam-Cook *et al.*, 2006). However, in the study performed by Tamzali *et al.* (2001) not all tumor types or locations were considered. Nonetheless, there is a health safety issue at stake when using these methods (electrochemotherapy and radiotherapy), which is not a concern when using Aldara™ or Xxterra™. Moreover, until now, post-treatment quarantine times have not been addressed for animals (White & Yu, 2006).

Even though, numerous studies have evaluated potential prognostic and predictive markers for veterinary neoplastic diseases, until recently there were no established guidelines or standards for the conduct and reporting of prognostic studies in veterinary medicine, and this lack of standardization is one of the reasons why evaluation and comparison of studies is often difficult. However it has been recently developed through an initiative of the American College of Veterinary Pathologists' Oncology Committee recommended guidelines for the conduct and evaluation of prognostic studies in veterinary oncology (Webster *et al.*, 2011).

Unfortunately this recent established guidelines have not been appraised in the current work, since the practice toil from which the present thesis was based on had already finished by then. Since this is of great interest to all the veterinary community this aspect could not be forgotten, and, hopefully, newer studies will be better guided in a next future.

Nowadays it is mostly accepted that BPV plays a major role in equine sarcoid pathogenesis, nevertheless, its exact mechanism, and precisely which external factors might trigger the disease are not entirely known.

It has been demonstrated some genetic predisposition towards the condition, in certain families (James, 1968; Gerber *et al.*, 1988), and in horses carrying certain ELA-specificities (A5, A3W13 and W13) (Broström *et al.*, 1988; Lazary *et al.*, 1994; Meredith *et al.*, 1986), which doesn't mean that animals lacking this alleles will not develop sarcoids, only that the ones that have it are at higher risk of developing the disease. This might lead one to think that it is possible that the 6 distinct clinical manifestations of this tumor, and the disease itself, might be due to individual differences in the immune response, as well as to some external trigger factors, besides the presence of the virus.

In fact, there is a group of human disorders, the spondyloarthropaties, that have a similar behavior. These disorders share certain clinical features and an association with the human leucocyte antigen (HLA) B27 allele, and include ankylosing spondylitis, reactive arthritis, psoriatic arthritis and spondylitis, enteropathic arthritis and spondilitis, juvenile-onset spondyloarthritis, and undifferentiated spondyloarthritis (Taurog, 2005).

Studies about these human syndromes were performed *in vivo*, using transgenic rats for both HLA-B27, and human β2-microglobulin. These animals developed an inflammatory disease that mimics the human spondyloarthropathy, characterized by diarrhea, arthritis, nail changes, psoriasis-like skin lesions, and male genital tract lesions that begins at proximately 10 weeks of age (Inman & Scofield, 1994). Taurog *et al.* (1993) reported that when these kind of transgenic rats were raised in a germ-free environment they had an altered expression of the disease. These animals only developed a limited set of symptoms, such as nail and skin changes, as well as genital inflammation, which indicates that other independent factors, such as gut flora, are involved in the development of arthritis and gut inflammation, other major clinical manifestations of the condition.

Similarly, in equine sarcoids both genetic and external factors contribute to the expression of the disease. A similar trial could be designed in order to assess the contribution of both ambiental and genetic factors and to better understand this disease.

Equine sarcoid inquire

The on-line inquire performed was answered by 15 Portuguese equine veterinarians and showed that there are no significant differences in the way this disease is managed in Portugal as compared to other countries. Most of the practitioners questioned (80%) have less than 5 sarcoid cases per year and all of them diagnose this disease either by clinical assessement and/or biopsy followed by histopathology. The majority of veterinarians inquired choose and perform therapeutical methods and refere immediate therapeutic success and recurrency rates similar to those described in literature. Methods most employed involve surgical techniques, ranging from ligature, to conventional surgery, or cryosurgery. Non-surgical methods performed involve local chemotherapy with 5-FU, cisplatin and Aw4-Ludes, topical approaches with Xxterra^{TM}, Lotagen or green mud, cryotherapy, and immunotherapy with intralesion BCG injections. Lotagen and green mud, where the only 2 methods that could not be supported by literature for equine sarcoid therapy.

However, this is an opinion study, and as so it only gives us an idea of what is performed in Portugal and it cannot be extrapolated to the entire country. In order to assess the accurate prevalence and management of this disease in Portugal further analysis must be performed.

Conclusions

Equine sarcoid is the most common skin tumor in horses and, there is still a lot to learn about this disease.

In the present work, treatment with imiquimod (Aldara^{TM}), as well as with the bloodroot extract (Xxterra^{TM}), was found rather effective and safe for all locations, dimensions, and types of equine sarcoid tumors, since no signs of recurrence were noticed up to 30 months after finishing treatment. Besides, these two methods are simple, non-invasive and quite inexpensive for equine sarcoid therapy, and can be performed at home by owners care. Disadvantaged include long duration of treatment, discomfort to the horse when touching the inflammated affected area and, sometimes, owners or animals lack of compliance might be a problem.

Even though other treatment methods described in literature have reported high success rates, most often, the number of cases studied or the follow-up periods are rather short or information concerning recurrence is not always clear. In other cases, it seems that most of the treated tumors recurred after a certain period of time or have limited applicability to certain tumor types, dimensions or body locations. As well, some are quite expensive, or can even cause health hazards, which is not a concern when using imiquimod or the bloodroot extract.

When comparing these 2 ointments, imiquimod was noticed to take approximately 3 times longer to treat, than with the bloodroot extract. Good cosmetic results were obtained in all cases treated by these two modalities, with sequelae resuming to a thickened skin scar and mild alopecia. Therefore, these 2 ointments appear to be a promising treatment for equine sarcoids. Nevertheless, a trial appraising a bigger sample of horses and larger number of different types of equine sarcoid tumors should be performed in order to corroborate these results.

These 2 modalities of treatment do not necessitate the extirpation of the tumor tissue. Yet, considering that treatment duration was found relatively long for both ointments, and if results are expected sooner the sequential treatment of surgical excision and application of one of these 2 ointments could be an alternative. As so, it would be of most interest to perform a parallel study in order to evaluate if this sequential treatment has a positive effect in reducing treatment duration.

No conclusion concerning treatment efficacy could be drawn from tumors treated by ligation or laser surgery in this study.

The opinion study about sarcoid gave an idea of what is performed in Portugal and showed that there are no major differences between the management of this disease in Portugal when compared to other countries, however, it cannot be extrapolated to the entire country

and therefore no major conclusions can be obtained. In order to assess the accurate prevalence and management of this disease in Portugal further investigation must be done.

The etiopathogenesis of this disease is known to be multifactorial. As so, and in order to achieve a better understanding and a better control of this disease some considerations and suggestions should be made.

First of all, since a genetic component has been identified, it would be interesting to test the affected horses used in this work for the presence of certain haplotypes, concretely ELA A3, ELA W13 and heterozygotic defective DNA-PKcs allele, and see if they are found in these animals.

Second, in order to better monitor treatment evolution, for imiquimod and the bloodroot extract, at least after treatment has finished and, ideally also before treatment starts, if it can be promptly instituted, it would be of most interest to make a skin biopsy in order to determine: a) the presence of histopathologycal characteristics of the tumor and, b) the presence or, amount of virus present at the treated area and, normal surrounding skin.

It would also be interesting to test the blood for the presence of BPV DNA in PBMCs and see if it relates to horses having multiple tumors.

Third, since this is a transmissible disease where a viral component, BPV, is accepted by most as being envolved in the etiopathogenesis of equine sarcoids, basic hygienic measures should be employed, such as using different tacks or brushes between affected and non-affected horses, keeping affected horses in different boxes, and by caretakers personal hygiene. Contact with cattle, should also be avoided, if possible.

For the same reasons, and since sarcoids are often encountered more in geldings than in mares or stallions, castrations should be preferably performed in colder months, when there are less or no flies present in the environment, and a fly repellent should be applied close to the wound. Still, embreeding pressure should be avoided, because notwithstanding the genetic susceptibility, sometimes sarcoids are located in the perigenital area, as was found in the present work, so it is easy to understand that it can be transmited by contact to the other horse if breeding is made through natural cover, as well as to the offspring if the female is the affetect horse and nurses its foal.

Nevertheless, it must be kept in mind that no horse is free from developing the condition and that BPV transmission can never be avoided completely because this is a very resistant virus in the environment and, there is always the possibility of a fomite or a vector transmission, as it has already been identified by BPV DNA detection in the stables environment as well as in flies.

In conclusion, although it is true that BPV is needed for sarcoid development, many infected horses do not develop the disease. As so, further research is needed in order to understand which other environmental or gentic factors increase the possibility of tumor development.

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Appendix

On-line inquire performed to Portuguese veterinarian practitioners

How often do you diagnose equine sarcoids?

- ○ <1x/year
- ○ 1-5x/Year
- ○ 6-10x/year
- ○ >10x/year

How do you diagnose equine sarcoids?

- ☐ Biopsy + histopathology
- ☐ Clinically
- ☐ BPV DNA analysis

How do you treat equine sarcoids?

- ☐ Surgical method
- ☐ Non-surgical method

Which protocols do you use to treat equine sarcoids?

Do you always use the same therapeutic approach?

- ○ Yes
- ○ No

If your answer is no, in what do you base to apply different treatments?

- ☐ Results from a previous therapeutic approach
- ☐ Clinical form of sarcoids
- ☐ Tumor localization
- ☐ Tumor extention
- ☐ Treatment cost

Other:

How do you qualify the immediate therapeutic success obtained by you?

- ○ Good
- ○ Satisfactory
- ○ Bad
- ○ Nill

In your experience, how often does it recur?

- ○ <10%
- ○ 11-30%
- ○ 31-50%
- ○ >50%