Equine Sarcoids in Lipizzaner horses

Diploma Thesis

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1. Introduction

1.1. Definition of Equine sarcoids

Equine Sarcoids are the most common skin lesions in equids (Chambers et al., 2003a). They occur in horses, donkeys, mules and zebras (Knottenbelt, 2003). A higher incidence in donkeys and mules is reported (Yu, 2007). There is a prevalence of 1-8% worldwide (Knottenbelt, 2005). 20% of all equine tumors and 90% of equine skin neoplasia are sarcoids. Although the mortality is low, sarcoids influence the material value and often compromise the use of the affected horses (Goodrich et al., 1998).

The name "sarcoid" means "flesh-like" and should suggest the proliferative sarcomatous appearance of lesions (Knottenbelt, 1995). Sarcoids are true semi-malignant neoplasia with a characteristic gross appearance (Goodrich et al., 1998). They are not painful and also non-pruritic (Hamann et al., 2005). Typical predilection sites include the neck, head (periorbita and ear), axilla, distal limb, ventral abdomen and groin region, however, sarcoids can appear at any cutaneous site. They are not metastatic but often show aggressive and infiltrative growth, often recur following ineffective treatment and commonly multiply on an individual (Goodrich et al., 1998; Knottenbelt, 2005). Spontaneous regression is only rarely observed (Broström, 1995). Most lesions tend to develop to a more aggressive type within time, especially when exposed to trauma (Hamann et al., 2005).

The average age of disease development is reported to range between 3 to 6 years (Goodrich et al., 1998) and 1 to 7 years (Yu, 2006), the onset of the disease at a higher age is rarely reported. In horses, no predisposition of colour and gender is reported (Broström, 1995). In donkeys however, age and gender seem to be associated with the development of sarcoid lesions: young males are more often affected by sarcoids than older males or females (Goodrich et al., 1998). In some regions of the world sarcoids are considered as a contagious disease (Knottenbelt et al., 1995). Some breeds, e.g. standardbreds, appear to be more resistant to sarcoids than other breeds such as thoroughbreds (Chambers et al., 2003a)

In the UK, horses bearing 10 to 1000 lesions of occult or verrucous type are often observed. In continental Europe and the USA, the majority of affected horses bear one single or only several lesions on average (Knottenbelt, 1995). In a study conducted by Broström (1995) 66% of Swedish halfbred horses showed only solitary lesions. In addition, a relationship between localization and size of tumors has been shown: sarcoids located at the distal limbs were significantly larger than the mean size, lesions on the head appeared consistently smaller. The average tumor size of 2-5 cm was encountered in 60% of affected animals (Broström, 1995).

1.2. Clinical classification

Equine sarcoids show a very typical gross appearance (Goodrich et al., 1998). It is the only dermatologic disease which represents itself in so many different ways (Hamann et al., 2005). Although former literature only describes 4 different types (McConaghy et al. 1994), most authors refer to 6 different sarcoid types today (Knottenbelt, 1995; Pascoe & Knottenbelt, 1999).

1.2.1. Occult Sarcoids

Occult sarcoids represent a superficial, mild tumor form which often remains quiescent over many years (Knottenbelt, 2005). They have a variable appearance, but commonly show alopecia and mild hyperkeratosis (Hamann et al., 2005). Occult sarcoids may contain rough areas and also sometimes small nodules (Knottenbelt, 2005), but in most cases they appear as circular hairless areas with an alopecic, grey and scaly surface (Knottenbelt et al., 1995). Sometimes they are also hyperpigmented (Yu, 2006). In some cases clinical signs only consist of a slightly thickened skin or changes in hair (Knottenbelt, 2005).

Upon trauma and/or influence of yet unknown factors, occult sarcoids may develop to more aggressive types (Knottenbelt, 2005), possibly because injury stimulates the proliferation of fibroblasts (Hamann et al., 2005). In many cases, occult sarcoids develop to verrucous forms (Knottenbelt, 2005). Occult sarcoids may be confounded with dermatophytosis, especially Trichophytos (Hamann et al., 2005), simple skin rubs or ectoparasitic infestation e. g. by lice (Knottenbelt et al., 1995).

1.2.2. Verrucous Sarcoids

Verrucous sarcoids have a very rough, hyperkeratotic surface and are sometimes of very prominent size (Knottenbelt, 2005). The dermis appears grey and the surrounding tissue shows more or less alopecia (Hamann et al., 2005). Typical locations of verrucous sarcoids are the face, neck, groin and axillae (Yu, 2006). They rarely occur on the distal extremities but may sometimes affect the coronary band (Knottenbelt, 2005).

Sometimes, especially when affecting the eyelid, verrucous lesions tend to invade the muscle and to compromise its function (Knottenbelt, 2005). Upon trauma, verrucous sarcoids may develop to more aggressive forms (Hamann et al., 2005). This sarcoid type can be confounded with equine papillomatosis, irritation-induced hyperkeratosis or even squamous cell carcinomas (Knottenbelt et al., 1995).

1.2.3. Nodular sarcoids

This type of sarcoid consists of solid nodules of various sizes (Hamann et al., 2005). They appear well-defined, spherical and firm (Knottenbelt, 2005), and are covered by grossly normal skin which sometimes may be thinner or shiny in appearance (Knottenbelt et al., 1995). Type A nodular sarcoids are covered by a freely movable skin, and they are also movable against underlying tissues. Type B nodular sarcoids show dermal involvement and sometimes also an invasion of deeper tissue layers. These two types may also be present in a mixed form and can merge to a cord of nodules (Knottenbelt, 2005).

Nodular sarcoids are most common located at the eyelid and groin region (Hamann et al., 2005). As soon as they start to ulcerate they usually develop into fibroblastic lesions (Knottenbelt et al., 1995). Traumata may induce transformation to fibroblastic sarcoids (Yu, 2006). Nodular sarcoids can be confounded with benign dermal swellings, fibromas, melanomas, neurofibromas (Knottenbelt et al., 1995) or the cutaneous type of lymphomas (Hamann et al., 2005).

1.2.4. Fibroblastic sarcoids

The fibroblastic sarcoid is the most aggressive form of this tumor (Knottenbelt et al., 1995). It often occurs at sites of previous trauma or less aggressive sarcoid types and has a fleshy appearance (Knottenbelt, 2005). Due to their ulcerative character they are frequently subject to secondary infections (Hamann et al., 2005). Knottenbelt distinguishes two types: The pedunculated Type 1 is further divided in subtype 1a, which has only a narrow skin pedicle, and subtype 1b which has a deep, invasive root. Type 2 fibroblastic sarcoids are sessile tumors with a broad, locally invasive base. Due to their extension, which may be underestimated at first sight, fibroblastic sarcoids are difficult to treat and often recur (Knottenbelt, 2005). Fibroblastic sarcoids are rapidly growing invasive tumors (Hamann et al., 2005) that can be confounded with exuberant granulation tissue or a staphylococcus pyogranuloma infection (Knottenbelt, 2005). Other differential diagnoses are fibrosarcomas or neurofibrosarcomas, squamous cell carcinomas and cutaneous botryomycotic lesions (Knottenbelt et al., 1995).

1.2.5. Mixed sarcoids

Mixed sarcoids may represent a progressive tumor stage and always comprise different sarcoid types in various combinations (Knottenbelt, 2005). They may consist of confluent, relatively benign verrucous and occult lesions or contain a more aggressive mixture of verrucous, nodular and fibroblastic tumours.

1.2.6. Malevolent Sarcoids

This type was first described by Knottenbelt in 1995. It describes an invasive growth which also infiltrates lymphatic vessels and may appear as confluent masses along these vessels. Invasion of local lymphnodes may equally occur (Knottenbelt et al., 1995). This malignant type of sarcoid also extends widely into surrounding dermis and epidermis (Yu, 2006). Sometimes, tumor strands are likely to originate from a primary lesion (Knottenbelt, 2005). Malevolent sarcoids are rare and may be mistaken for the cutaneous form of lymphoma (Hamann et al., 2005).

One should always weight the benefits of tumor tissue collection, because taking biopsies may trigger tumor growth and the development of more aggressive types of sarcoids (Knottenbelt, 2005). In contrast to this widely accepted suggestion, a round

table discussion of the North American Veterinary Dermatology Forum recently recommended to take biopsies to confirm diagnosis (Yu, 2006). Typical histopathological signs of sarcoids include long rete pegs of tumor tissue which extend to intact dermis (Knottenbelt et al., 1995). They are present in about 50% of all sarcoids, likewise other signs considered as "typical" e.g. epidermal hyperplasia or "picket fence" formations are also present in only 46-54% of tumors (Martens et al., 2000). The only characteristics which is consistently observed in all sarcoids is an increased density of dermal fibroblasts containing bovine papillomavirus type 1 or 2 (BPV1, BPV2) DNA (Martens et al., 2000).

1.3. Methods of treatment

The treatment of sarcoids is often difficult and a high rate of recurrence is described (Goodrich et al., 1998). The main problem of finding an adequate therapy may be the incomplete understanding of sarcoid pathogenesis (McConaghy et al., 1994). In body areas where sarcoids cause discomfort and where removal is difficult, disease represents a therapeutic challenge (McConaghy et al., 1994). Early tumor detection and therapy are crucial for successful eradication of disease (Knottenbelt, 2005; Haman et al. 2005). The method of therapy should be chosen carefully, depending on type, severity and localization of sarcoids (Knottenbelt, 2005). Recurrent tumors have a worse prognosis than primary lesions (Haman et al., 2005). A combination of different treatment methods often represents the most effective therapeutic option (Haman et al., 2005).

1.3.1. Surgical Methods

Surgical excision is a common method to treat equine sarcoids (Knottenbelt et al., 1995). Excision may be performed by using a scalpel, electrocautery or a CO2-laser. In any case, a non-touch-technique should be applied (Martens et al., 2001). Depending on tumor location wound closure or even complete excision may not be possible and lead to a poor cosmetic outcome (Knottenbelt et al., 1995). High rates of recurrence are reported when surgical excision is ineffectively performed (Knottenbelt et al., 1995). In New Zealand, only 28% of horses were treated successfully by surgical excision (McConaghy et al., 1994). Another study reports a three years recurrence rate of 50% following surgery (Goodrich et al., 1998). Hence prognosis

should be guarded if only surgery is performed. In addition, wound healing complications may occur (Haman et al., 2005). In 90 % of cases, recurring lesions are more aggressive than initial tumors (Knottenbelt et al., 1995). The outcome of surgery generally depends on the completeness of resection and the ability of the surgeon to discern the tumor margins (Knottenbelt et al., 1995).

Another reported method of surgical intervention consists in ligating the tumor. This method is easy to perform in the field but may not remove the entirety of the lesion. Ligation is hence recommended for treatment of pedunculated noninvasive lesions only (Haman et al., 2005).

Cryotherapy using liquid nitrogen is often reported as an effective method for sarcoid treatment (Martens et al., 2001). High success rates are reported when used after ineffective surgical excision or in combination with surgical debulking (McConaghy et al., 1994). Cryotherapy should not be used for extensive areas and large masses (Knottenbelt et al., 1995). It may damage underlying tissues and cause septic arthritis when applied in vicinity of joints (McConaghy et al., 1994). Hence surrounding skin should be protected and tissue temperature should be controled by using thermocouple needles (Martens et al., 2001). Cryosurgery wounds always heal *per secundam intentionem* (Hamann et al., 2005). Immediate side effects such as wound swelling and discharge often occur (McConaghy et al., 1994). Long term effects such as alopecia, leukotrichia and scarring may lead to a poor cosmetic outcome (Knottenbelt et al., 1995).

Hamann et al. (2005) also reported the use of thermotherapy which targets superficial cell layers and is hence recommended for treatment of superficial lesions only (Hamann et al., 2005).

1.3.2. Immunotherapy

Bacillus Calmette-Guérin (BCG) has been commonly used for treatment of periocular sarcoids (Komaromy, 2004; Knottenbelt et al., 2000) as a live-attenuated vaccine or modified BCG cell wall preparations (McConaghy et al., 1994). The mechanism of BCG treatment remains unclear (Knottenbelt et al., 1995) but is thought to likely stimulate the host´s immune system and to enhance the cellular immune response

(Hamann et al., 2005). Lesions should be surgically debulked before treatment (Knottenbelt et al., 1995; Martens et al., 2001). Intralesionally given injections lead to better results than perilesional BCG administration (Knottenbelt et al., 2000). BCG dosage is crucial, 1 ml/cm³ tissue is usually applied (Hamann et al., 2005, Komaromy, 2004). Treatment is performed in one-, two- or three-week intervals (McConaghy et al., 1994; Knottenbelt et al., 2000; Komaromy, 2004). Anaphylactic reactions may occur in some cases (Hamann et al., 2005; Knottenbelt et al., 1995). A prophylactic administration of flunixin-meglumine and dexamethasone after the third BCG administration may help avoiding them (Knottenbelt et al., 2000). Horses sometimes show slight depression due to immunoreactions (Komaromy, 2004). Local swellings and ulceration may occur, but no long term complications have been reported so far (McConaghy et al., 1994). Use of BCG in a pregnant mare showed no negative influence on pregnancy, foaling or the health of the foal (Komaromy, 2004).

Autogenous vaccines like those used in equine papillomatosis seem to represent another attractive approach in theory. In practice however, they enhance the number of lesions and the severity of disease (Knottenbelt et al., 1995). Treatment with Interleukin 2 seems more promising but only in combination with chemotherapy (Spoormakers et al., 2003). Imiquimod as an immune response modulator appears to be a convenient and safe treatment option. In a pilot study, 19 sarcoids were treated with Imiquimod 3 times per week for 16 weeks. 60 % of treated tumors resolved completely and 80% showed a 75% size reduction. The cosmetic outcome was satisfactory, side effects included alopecia, erythema and exudation, all resolved within 30 days (Nogueira et al., 2006). Unfortunately, this agent is extremely expensive and hence not affordable by the majority of horse owners.

1.3.3. Radiotherapy

This method commonly implies the use of Iridium isotope 192 which is a medium energy gamma radiator that also emits beta rays (Byam-Cook et al., 2006). Other gamma radiators used are gold-198 and radium-226 (McConaghy et al., 1994; Knottenbelt et al., 1995). Radiotherapy is restricted in use due to the high cost and required safety management (Knottenbelt et al., 1995). Only referral centers with appropriate facilities can apply this method (Byam-Cook et al., 2006) which requires special isolation boxes and minimum contact during treatment (McConaghy et al.,

1994). The maximum radiation intensity emitted by Iridium-192 is below 0,67 MeV so medium shielding for personnel is required (Byam-Cook et al., 2006). The radiation implant consists of a platinum sheathed wire which can be either straight or hairpin shaped (Knottenbelt et al., 2000; Byam-Cook et al., 2006). It is inserted directly into the lesion which can be done under general anesthesia or on the standing horse under deep sedation (Knottenbelt et al., 2000). The implants are left in situ until the recommended dosage of radiation ranging between 50 (low dose radiation protocol) and 90 Gy is delivered (Knottenbelt et al., 2000; Byam-Cook et al., 2006). Lesions undergo slow and progressive atrophy and necrosis with mild adverse reactions which include alopecia, leucotrichia and scarring (Byam-Cook et al., 2006). In periorbital sarcoids, the integrity of eyelids can be preserved and good to excellent curative and cosmetic results can be reached (Byam-Cook et al., 2006).

The use of beta radiation is restricted to very small and superficial single lesions. Mainly Strontium-90 is used and administered directly into the tumor for 5 days with an overall dosage of 100Gy (Knottenbelt et al., 2000).

1.3.4. Chemotherapy

Topical chemotherapy has been a common method for over 100 years to treat sarcoid-like lesions (Knottenbelt et al., 1995). The big advantage of topical therapy is that high concentrations of agent can be applied with lower systemic side effects (Theon et al., 1993). Cytotoxic agents such as copper sulfate and silver nitrate can lead to remarkable success but also to dramatic failures (Knottenbelt et al., 1995). Nowadays, cisplatin, a broadspectrum antineoplastic agent, is commonly used in sarcoid therapy by direct injection into the lesion (Theon et al., 1993). In addition cisplatin-coated implants are available today (Knottenbelt et al., 1995). Cisplatin in combination with medical sesame oil is used to avoid diffusion to adjacent tissues – cisplatin binds to sesame oil 17 times better than to phosphate buffer saline (PBS) (Theon et al., 1993). Water-in-oil emulsions are neutral and hence well-tolerated by tissues, show no cytotoxic side effects and provide a slow release of cisplatin (Theon et al., 1993). The recommended dose of delivered cisplatin is 1mg per cm^3 tumor tissue which should be achieved by placing multiple needles in rows on the whole tumor (Theon et al., 2007). Tumor eradication rates of up to 100% are reported (Rees, 2004). In a study from Theon et al. (2007) a 4 years effectivity of 96.3 % is described for cisplatin treatment

with a higher incidence of disease recurrence for large and previously treated tumors (Theon et al., 2007). To increase intratumoral cisplatin diffusion, electrochemotherapy can be performed (Tamzali et al., 2001). In a preliminary study, specially designed electrodes were used for a pulsed stimulation of tumors 5 minutes after cisplatin injection. This treatment was repeated several times in an interval of two weeks, leading to sarcoid regression after four sessions with no recurrence within 2 years (Tamzali et al., 2001). Recently, biodegradable cisplatin-loaded Dextran beads have been developed. They are implanted in the tumor or the tumor bed post-excision and assure constant cisplatin delivery for two weeks, while beads slowly degrade. High effectivity of cisplatin beads could be demonstrated for treatment of sarcoids, melanoma and squamous cell carcinoma (Hewes and Sullins, 2006).

Another well known chemotherapeutical agent is 5-Fluorouracil. In sarcoid therapy it is used in a dosage of 50 mg/cm³ tissue when injected intratumorally. As for treatment of other tumors, success depends on previous treatments and tumor size, with good prognosis for primary tumors (86.7%) (Steward et al., 2006).

XXTerra™ (Larson Laboratories, INC., Fort Collins, Colorado) is made of Sanguinaria canadensis (papaveraceae family) (Felbert et al., 2005). Sanguinaria products have been used by Native Americans to treat various conditions, including warts (Rees, 2004). The ingredients sanguinarin and chelerythrin are reported to lead to cell apoptosis (Adhami et al., 2003). In one reported periocular sarcoid the treatment with XXTerra™ lead to ulceration and necrosis, complete regression and no regrowth within 16 month. Althought there are no scientific success rates reported, XXTerra™ may have curative effects in some cases (Felbert et al., 2005).

1.4. BPV as major causal agent in sarcoids

The idea of an infectious agent causing equine sarcoids is nearly as old as the description of the tumor itself. Jackson suggested a viral origin in 1936, which was further supported by epidemic outbreaks described 1966 by Ragland (Ragland et al., 1966). Today, bovine papillomaviruses of type 1 (BPV1), and to a lesser extend type 2 (BPV2), are widely accepted as causative factors of sarcoid development and maintenance in equids (Chambers et al., 2003a).

1.4.1 BPV

Papillomaviruses are small, double-stranded, non-enveloped DNA viruses. BPV1 and 2 have a genome of 7945 bp containing 8 open reading frames (ORFs) coding for early functional proteins E1, E2, E4, E5, E6 and E7, and late capsid proteins L1 and L2. The L1 and E6 ORFs are separated by a long control region containing the necessary cis-regulatory elements for the replication and transcription of the viral DNA (Campo, 2006). In cattle, BPV mainly causes benign warts which usually resolve without treatment (Campo, 1997). In some cases however, bovine BPV-induced lesions undergo malignant transformation to persistent treatment-refractory tumors, leading to the affected animals being sculled (Campo, 2006). Malignant transformation upon papillomavirus infection is equally encountered in other mammalian species and humans. Additional tumorigenic factors such as immunosuppression seem to co-induce malignancy (Campo, 2002). BPV has thus become a common model for studying the influence of environmental factors as well as the viral mechanisms which lead to neoplastic transformation (Campo, 1997).

Cross species-infection by papilloma viruses represents a rare phenomenon as papillomaviruses are usually strictly species-specific. Infection of equids by BPV hence represents the only example of a natural papillomavirus cross-infection known to date (Chambers et al., 2003a). In contrast to infection in cattle, intact BPV virion could never be detected in equids, suggesting cross infection as a dead end pathway for the virus (Nasir and Reid, 2006). BPV DNA has been demonstrated in up to 100 % of sarcoid lesions and expression of early and occasionally late genes has been shown (Carr et al., 2001a; Nasir and Reid, 1999; Nixon et al., 2005). Given the postulated absence of virion in the foreign equine host, infection is considered as the result of an abortive infection with multiple copies of episomal BPV genome residing within nuclei of infected cells (Chambers et al., 2003).

1.4.2. Transmission of BPV

Transmission of BPV still remains unclear. Inoculation with cow wart extract or purified virus induced sarcoid-like fibrosarcomas (Olson and Cook, 1951; Ragland and Spencer, 1969; Voss, 1969), however lesions regressed spontaneously and horses developed antibodies against BPV, which is rarely observed in naturally induced

disease (Nasir and Reid, 2006). BPV DNA has been detected in face flies, suggesting that insects feeding on wounds may contribute to virus spread (Kemp-Symonds, 2000; Chambers et al., 2003a). The surrounding environment does not seem to play a role in transmission, as swabs collected from feeding tubes and stable walls have tested negative for BPV DNA as long as no cattle were present in the same barn (Bogaert et al., 2005). Sarcoids as a transmissible disease like the tasmanian devil face tumor could be excluded since a study showed no difference between DNA of host cells and sarcoid cells which would be expected when foreign infected cells are transplanted as allografts (Gobeil et al., 2007). Detection of equine papilloma virus (EqPV-1) DNA in sarcoids failed in 9 out of 10 samples, indicating that EqPV-1 is not involved in sarcoid pathogenesis (Postey et al., 2007). Moreover, exposure to EqPV-1 particles provided no protection against sarcoid development (Knottenbelt, 2007). BPV DNA was also detected in some cases of equine dermatitis, but the significance of this finding remains unclear (Nasir and Reid, 2006).

1.4.3. BPV major transforming gene E5

In various studies the intralesional expression of viral genes in sarcoids could be shown (Nasir and Reid, 1999, Carr et al., 2001a; Nixon et al., 2005). Malignant transformation might be linked to deregulation of early gene expression (Chambers et al., 2003a). In a study conducted on 20 sarcoid specimens, 19 of these contained at least two different early gene products, and E2 protein has been found in 18 specimens (Nasir and Reid, 1999). In another study 22 out of 23 sarcoid samples contained E5 protein, thus providing further evidence for viral involvement in sarcoid development (Carr et al., 2001b).

E5 is a hydrophobic protein consisting of 44 amino acids (aa) which is mainly located in the endoplasmatic reticulum (ER) and in the Golgi apparatus (GA) (DiMaio and Mattoon, 2001). It is already expressed during early infection and constitutes the major BPV oncoprotein. E5 leads to anchorage independence, down-regulation of gap junctions, a lower contact inhibition and an increase of cell proliferation in low serum medium *in vitro* (Ashrafi et al., 2002). E5 forms a stable complex with PDGF-beta receptor in infected cells (DiMaio and Mattoon, 2001). This interaction activates a cascade which results in an up-regulation of internal growth signals (Chambers et al., 2003b). E5 further binds to H+-ATPase which controls the pH in the GA (DiMaio and

Mattoon, 2001). This alkalization interrupts the intracellular transport system, leads to GA fragmentation and retention of major histocompatibility class I (MHC I) molecules (Ashrafi et al., 2002). The ability to retain MHC I abrogates cell surface presentation of viral proteins and hence contributes to evasion of the virus from the host's immunosurveillance (Ashrafi et al, 2002). Finally, E5 binds to 16K Ductin/subunit c, thus compromising gap junction-mediated cell communication and isolating cells from their cellular neighborhood (Chambers et al., 2003). These findings support the major role of E5 in transformation to neoplastic lesions (DiMaio and Mattoon, 2001).

In sarcoid-affected horses, the E5 open reading frame contains several point mutations in comparison to E5 sequences derived from affected bovines. These mutations are mostly silent, but may promote higher codon usage and thus more efficient translation (Chambers et al., 2003b; Nasir and Reid, 2006). Sarcoid-specific sequence variants are also reported for the LCR (Nasir et al., 2007), thus suggesting an adaptation to the equine host (Chambers et al., 2003; Nasir et al., 2007). In contrast to these findings, earlier reports describe an absolute identity between bovine virus types and those isolated from equine sarcoids (Carr et al., 2001b). However, Brandt et al. (accepted manuscript) independently detected published E5 variants in sarcoid-affected Austrian breeds with consistency, thus confirming presence of sarcoid-specific genetic variants of the E5 gene.

1.4.4. Immunosurveillance and genetic predisposition

BPV viruses are poorly immunogenic in horses, which may be due to the postulated lack of particle formation and E5-mediated inhibition of MHC I expression (Ashrafi et al., 2002). Regression of naturally occurring sarcoids upon immune stimulation further supports the concept of reduced immunogenicity of BPV in the infected horse (Nasir and Reid, 2006). Genetic predisposition may be another factor promoting sarcoid development. In rabbits, a genetic correlation of distinct MHC II haplotypes with tumor development has been demonstrated (Campo, 2002). In horses, a higher susceptibility of horses harboring the Equine Leucocyte Antigen (ELA) W3 haplotype has been shown in relation to sarcoid disease (Broström et al., 1988; Lazary et al., 1985; Broström, 1995). In contrast to these findings a study on hereditary disease in Swiss Mountain draft horses did not demonstrate any clinical correlation between sires and

their offspring in regard to prevalence of sarcoids (Mele et al., 2007). This breed also showed none of the previously described ELA haplotypes.

1.4.5. BPV detection

BPV DNA has been first demonstrated in sarcoids by Southern blot (Lancaster et al., 1979; Amtmann et al., 1980; Trenfield et al., 1985). With the use of a more sensitive polymerase chain reaction (PCR), BPV DNA as been detected in up to 100% of investigated lesions (Otten et al., 1993; Bloch et al., 1994; Carr et al., 2001a, b; Martens et al., 2001a, b; Bogaert et al., 2005). It has also been traced in apparently intact skin of sarcoid-bearing horses (Carr et al., 2001a; Bogaert et al., 2005) leading to the speculation that BPV might latently reside in fibroblasts until factors such as trauma would initiate its transforming activity. There is ample evidence that BPV DNA is absent in sarcoid-free individuals or non-sarcoid equine tumors (Otten et al., 1993; Carr et al., 2001a, b). However, occasional presence of BPV in unaffected horses living in close contact with sarcoid-affected stable-mates has been recently described (Bogaert et al., 2005). In addition, BPV DNA has been found in some cases of dermatitis (Angelos et al., 1991; Chambers et al., 2003; Yuan et al., 2007). Blood might represent another reservoir of BPV, since viral DNA could also be demonstrated in peripheral blood mononuclear cells (PBMCs) of equine sarcoid patients (Brandt et al., accepted manuscript).

1.5. Field study on Lipizzaner horses

Based on this recent finding, the purpose of the study presented here was to examine a cohort of 20 Lipizzaner horses for the presence of sarcoid-like lesions and to compare obtained clinical data to results obtained by BPV PCR from PBMC DNA isolates obtained from these 20 individuals in 2002.

2. Material and Methods

2.1. Clinical evaluation and sample collection

An entity of 62 horses was evaluated for skin lesions at Austrian National Stud in Piber, Styria, during a four day period in May 2007. Because there was no possibility to obtain bloodsamples of all of these horses at this time the inclusion criteria for this study was the presence of a blood sample taken in 2002. A cohort of 20 horses matched these criteria. All of the horses were of Lipizzaner breed, their age ranging from 13 to 28 years. 12 of them were broodmares and 8 of them stallions.

All horses underwent a full clinical evaluation which was completed by the author and four other 5th year students under supervision of Prof. Dr. Theresia Licka. Evaluated parameters included body temperature, heart rate, respiratory frequency, mucous membrane color, capillary refill time and hydration, spontaneous or induced cough, cardiac murmurs, broncho-alveolar sounds and gastro-intestinal borborygmis. If possible, consistency of feces was evaluated. Skin elasticity was measured on the upper eyelid and on four defined spots on the neck, the time until the skin fold elapsed being recorded. Some of this data was used for other studies. Evaluation parameters and results are presented in Appendix 1, Evaluation Sheets.

All skin lesions, including scars, crusts, putative melanomas, alopecic and scaling areas were recorded, their location and shape was described and their size was given in mm. Pain, mobility and consistency as well as possible ulceration or exsudation were registered. In several cases a descriptive mode was chosen to complete evaluation sheets. Only sarcoid-like lesions were measured with a ruler and photographed. The number of the photo was indicated on the evaluation chart as to assure their correct assignment to corresponding horses. Suspicion of encountered lesions was determined by the author and confirmed by Prof. Dr. Licka on basis of their clinical appearance and location.

2.2. Blood sample processing

Whole blood (2 x 8 ml/individual) was collected in 8 ml BD Vacutainer® CPT™ Cell Preparation Tubes with Sodium Citrate (Becton Dickinson) from each individual. Following centrifugation at 2400 rpm at 4 °C for 15 min, 6 ml of thus obtained

supernatant were discarded and remaining 2 ml containing PBMCs were transferred to 2 ml reaction tubes and centrifuged at 2400 rpm at 4 °C for 10 min. Then, the entire supernatant was discarded and PBMCs were resuspended in 2 ml of PBS (phosphate buffered saline, pH 7.4). Following centrifugation at 2400 rpm at 4 °C for 10 min, the supernatant was removed and PBMCs were resuspended in 200 µl of PBS. Subsequently, DNA purification was carried out by using a DNeasy extraction kit according to instructions of manufacturer (Qiagen). Briefly, 20 µl proteinase K and 200 µl Buffer AL from this kit were added to each tube. After mixing thoroughly by vortexing, tubes were incubated at 56 °C for 10 min. Following addition of 200 µl of 96 % ethanol/tube and vortexing, mixtures were pipetted into DNeasy Mini spin columns placed in a 2 ml collection tube, and centrifuged at 8000 rpm for 1 min. Then flow-through was discarded and columns were filled with 500 µl of washing buffer AW1 and centrifuged at 8000 rpm for 1 min. Flow-through was discarded again, columns were filled with 500 µl of washing buffer AW2 and centrifuged at 14000 rpm for 3 min. Then columns were placed in clean 1.5 ml tubes and 200 µl of elution buffer AE/column were added. Following incubation at room temperature for 1 min, purified PBMC DNA was eluted by centrifugation for 1 min at 8000 rpm and stored at -20 °C prior to PCR.

2.3. PCR analysis

To determine the presence of BPV-1/-2 DNA in PBMC DNA derived from the 20 Lipizzaner horses, 2 µl-DNA aliquots were subjected to E5 PCR by using BPV-1/-2 consensus primers selected from published BPV-1 (GenBank accession no. X02346) and BPV-2 sequences (GenBank accession no. M20219). E5-specific primers (5´B1/2-E5: 5´cactacctcctggaatgaacatttcc 3´; 3´B1/2-E5: 5´ctaccttwggtatcacatctggtgg 3´) were designed for amplification of a 499 bp (BPV-1) or 497 bp (BPV-2) fragment spanning the E5 open reading frame (ORF). A first PCR reaction was carried out in 0.5 ml MµlTI Ultra PCR tubes (Sörenson™, Bioscience Inc.), each containing 9.5 % dimethyl sulfoxide, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 1.5 mM of each dNTP, 100 pmol of sense and antisense primer and 2 µl of DNA template in a volume of 49 µl, using two drops of mineral oil as top layer. Reaction tubes were placed unlocked in an Eppendorf Mastercycler (Eppendorf). Following a manual hot start at 95 °C for 5 min and addition of 1 U of Taq Polymerase (Roche Life Science), tubes were closed and a very stringent amplification program consisting of seven touch-down cycles [92

°C for 30 sec/67-58 °C for 45 sec (-1.5 °C/cycle)/72 °C for 45 sec] followed by 40 standard cycles (92 °C for 30 sec/58°C for 45 sec/72°C for 45 sec) was performed. PCR products were visualized on 2% tris-acetate agarose gels by ethidium bromide staining. Sarcoid DNA of an affected individual was used as positive control. Sterile water and skin DNA isolates from 4 healthy horses served as no template and negative controls, respectively. Beta-actin PCR performed under conditions described above (primers 5´EBA 476: 5´tcacccacactgtgcccatctacg 3´; 3´EBA 1090: 5´cgtcrtactcctgcttgctgatcc 3´; GenBank accession no. AF035774) confirmed successful DNA purification for all isolates (not shown). Subsequently, a second PCR was carried out as described. However, a less stringent temperature program (56°C instead of 58°C for primer annealing) has been used in this case. This additional experiment was performed to assure that the virus would not escape detection due to possible genetic deviations from the bovine E5 reference sequences selected for primer design. Sarcoid DNA, skin DNA from a healthy donor and sterile water were used as appropriate controls.

From 8 PBMC DNA isolates (horses 13 to 20) a blind E5 PCR was already conducted in 2005 by Dr. Sabine Brandt from the Equine Biotechnology Unit of the Veterinary University Vienna, Austria. Obtained results have been kindly provided and included in the results section, table 1.

3. Results

3.1. Clinically determined skin lesions

We evaluated 20 horses for skin lesions. Eight of them (all stallions) were retired show horses, the remaining 12 were broodmares with or without foal. The age ranged from 13 to 28 years, with a mean of 19.15 years and a standard deviation of 4.78. The clinical parameters of all of them were within normal limits. Seven of them (35 %) displayed skin lesions which may represent sarcoids (Tab. 1, individuals with skin lesions are highlighted in yellow).

Horse #1 (isolate P10AM) had three small suspect lesions. One of the lesions was on the left side of the neck in the caudal third, about 5 mm in diameter and was non-movable. In the right axilla there was a 1 cm^2 sized alopecic lesion and on the left side of the thorax there was another nodular, hyperkeratotic movable lesion 5 mm in diameter (Fig. 1-3)

Figure 1: Horse 1, lesion on caudal neck

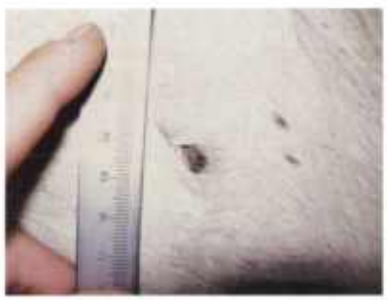


Figure 2: Horse 1, lesion on chest

Figure 3: Horse 1, axillar lesion

Horse #2 (P11ME) had suspect lesions in both axillae. The size of these lesions ranged from 5 to 20 mm in diameter and more than likely represented occult sarcoids with a small verrucous part. Another lesion was seen about one hand rostral from the ear base and appeared to be an occult sarcoid 5 mm in size (Fig. 4 and 5)

Figure 4: Horse 2, lesion rostral to the ear

Figure 5: Horse 2, axillar lesion right

Horse #4 (P29MA) had a small circular verrucous lesion (4 mm in diameter) on the lateral edge of the right ear. Two very similar lesions were seen on the crest and the left thoracic wall, respectively (Fig. 6-8)

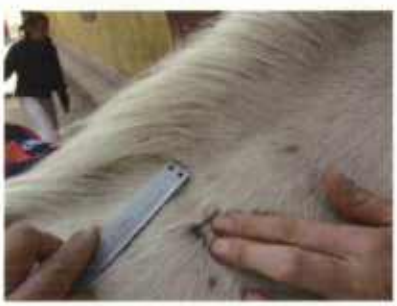


Figure 6: Horse 4, lesion on the neck

Figure 7: Horse 4, lesion on the right ear

Figure 8: Horse 4, lesion on the thoracic wall

Horse #6 (P48BR) had a hyperkeratotic lesion caudal to the right olecranon. Lateral to her left eye there was a nodular lesion 2 mm and on the base of the left ear was an alopecic 10 x 4 mm lesion. (Fig. 9 and 10)

Figure 9: Horse 6, lesion caudal right Olecranon

Figure 10: Horse 6, lesions left temporal region

Horse #11 (P79AN) had one suspect lesion on the ventral abdomen. It was a verrucous looking wart on the left side of the ventral midline with a diameter of 7 mm. In addition, this mare had another smaller lesion on the thoracic wall at the level of the 17th ICS (intercostal space) at level of the hip. (Fig. 11 and 12)

Figure 11: Horse 11, lesion on ventral abdomen

Figure 12: Horse 11, lesion in ICS 17

Horse #13 (W001SM) had an ulcerated lesion on the ventral abdomen. The lesion was mildly hemorrhagic and had a diameter of approximately 1.2 cm. (Fig. 13)

Figure 13: Horse 13, ulcerated lesion on the ventral abdomen

Horse #14 (W010NK) had multiple suspect lesions. Both axillae showed slightly hyperkeratotic hairless areas 2 x 3 cm in size. On the scrotum he had a verrucous looking non-ulcerated lesion 1 x 2 cm in size (Fig. 14 and 15)

Figure 14: Horse 14, scrotal lesion

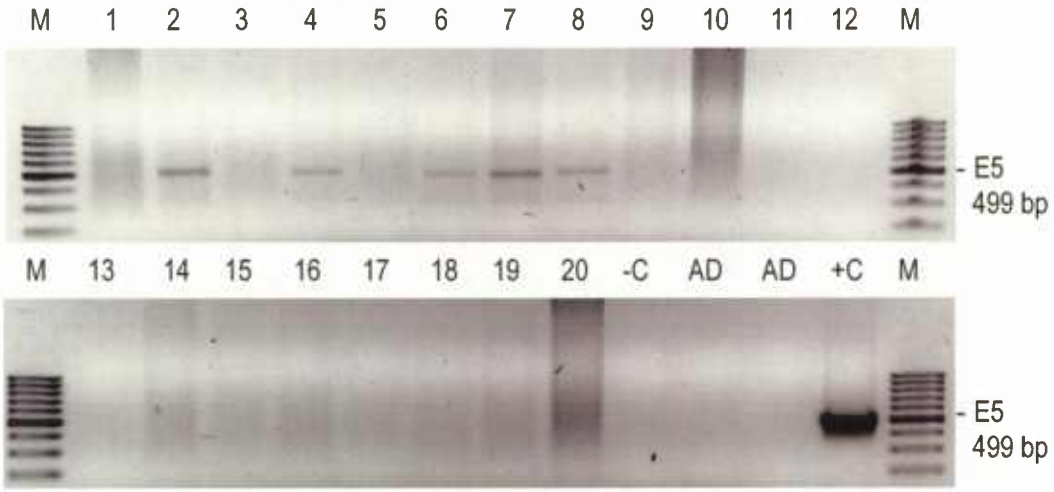
Figure 15: Horse 14, alopecic area in left axilla

For clinical evaluation sheets see appendix 1.

3. 2. PCR results

A preliminary PCR reaction performed in 2005 detected no E5 in PBMCs derived from individuals #13 - 20 (Brandt et al., accepted manuscript). The first PCR experiment conducted from these 8 individuals and an additional 12 PBMC DNA isolates derived from the here defined study group was conducted under the same stringent temperature conditions and scored negative for all investigated samples, whereas an anticipated product obtained for the positive control (sarcoid DNA) testified for success of the amplification reaction (Table 1). When repeating the reaction with a decreased primer annealing temperature of 56 °C, 5 PBMC DNA isolates obtained from broodmares #2, 4, 6, 7 and 8, respectively, scored positive for the viral E5 gene, as did the positive control (Fig. 16; Table 1)

Figure 16: PCR scores positive for 5 PBMC DNA isolates when using a primer annealing temperature of 56°C for detection of the BPV-1/2 E5 gene



M: GeneRuler 100 bp DNA ladder (Fermentas); 1-20: PBMC DNA isolates obtained from a cohort of 20 Lipizzan horses that have been clinically examined for presence of skin lesions. -C: negative control (skin DNA from a healthy horse); AD: sterile water (no template control); +C: positive control (sarcoid DNA)

PCR results obtained throughout this study and by a preliminary experiment carried out in 2005, as well as specifications regarding the 20 Lipizzaner horses constituting the study group are summarized in table 1.

Table 1: E5 PCR from PBMC DNA isolates of 20 clinically examined horses

Horse	DNA isolate	Gender	year of birth	skin lesions	E5 PCR 2005 (AT: 58°C)*	1. E5 PCR (AT: 58°C)	2. E5 PCR (AT: 56°C)
1	P10AM	f	1991	yes	nd	0	0
2	P11ME	f	1992	yes	nd	0	1
3	P25BE	f	1991	no	nd	0	0
4	P29MA	f	1989	yes	nd	0	1
5	P34TR	f	1993	no	nd	0	0
6	P48BR	f	1989	yes	nd	0	1
7	P61AL	f	1989	no	nd	0	1
8	P66BI	f	1992	no	nd	0	1
9	P70BO	f	1992	no	nd	0	0
10	P72TH	f	1990	no	nd	0	0
11	P79AN	f	1989	yes	nd	0	0
12	P83CO	f	1994	no	nd	0	0
13	W001SM	m	1979	yes	0	0	0
14	W010NK	m	1979	yes	0	0	0
15	W017CA	m	1986	no	0	0	0
16	W025NN	m	1979	no	0	0	0
17	W029ST	m	1984	no	0	0	0
18	W044MB	m	1982	no	0	0	0
19	W045MB	m	1989	no	0	0	0
20	W052CT	m	1988	no	0	0	0

f: female (brood mare); m: male (stallion); nd: not done; 1: BPV-1/2 E5 positive; 0: BPV-1/2 E5 negative
AT: primer annealing temperature; *PCR data obtained in 2005 and provided by Dr. S. Brandt

4. Discussion

Sarcoids represent the most frequently encountered tumor type in horses, donkeys and mules. Hence, sarcoid disease is scientifically investigated over many years in order to elucidate the biology of disease pathogenesis and maintenance. In addition, different diagnostic and therapeutic approaches have been developed to detect and fight this problematic disease. Yet, many aspects still remain unclear including horizontal and vertical transmission of the virus, particle formation and virus latency in the foreign equid host. Despite the fact that histological examination of neoplastic tissue is considered as the most reliable diagnostic method, diagnosis is preferentially based on the clinical appearance of lesions (Knottenbelt et al., 1995) because any mechanical irritation of sarcoids including collection of biopsies may trigger tumor progression to more aggressive types. Accordingly, the entire lesion including intact margins should be excised when conducting histological analysis of affected tissue.

BPV DNA is not only detectable in tumor tissue but also in apparently intact skin distant from the lesion (Carr et al., 2001b; Bogaert et al., 2005; Brandt et al., 2007) and in PBMCs of sarcoid-bearing horses (Brandt et al., accepted manuscript). PCR-mediated detection of BPV DNA from superficial tumor swabs, intact skin biopsies or PBMCs may hence represent an interesting diagnostic alternative. Moreover, these findings suggest that viral DNA may latently reside in blood cells and fibroblasts until factors such as trauma initiate its transcriptional and thus transforming activity. Based on this hypothesis, it can be speculated that systemic infection may even be crucial for initiation of disease. To assess this possibility, a cohort of 20 horses was clinically examined for the presence of putative sarcoids. Then, PBMC DNA isolates obtained from these individuals at an earlier point in time (2002) were investigated for the presence of the major BPV-1/2 gene E5 by using a highly sensitive PCR protocol. E5 has been selected as detection target since it is consistently found in sarcoids, intact skin and also PBMCs of affected equines.

In 2005, a blind E5 PCR was performed from enciphered PBMC DNA isolates derived from 66 Lipizzaner stallions, including individuals #13 to 20. The latter scored negative, indicating an absence of BPV-1/2-infection at the time of blood collection in 2002. Analysis of provided clinical records confirmed freedom from BPV-induced

malignancies for these 8 individuals (Brandt et al., accepted manuscript). Both PCR reactions performed in the frame of this actual study from the same PBMC DNA preparations were consistent with PCR results obtained in 2005. No E5 was detected in these specimens independently from selected temperature conditions, whereas the sarcoid DNA control clearly scored positive for E5 in both reactions. Given that quantitative E5 PCR recently carried out by us has revealed <100 E5 molecules per 1.2×10^5 PBMCs obtained from sarcoid-affected horses, it is thinkable that viral DNA may have escaped from detection in the case of two stallions displaying small skin lesions at the present time (horses #13 and 14). If these lesions indeed correspond to sarcoids, a more likely explanation is that they have acquired infection some time after blood collection. Alternatively, observed skin lesions may represent other skin disorders or transient abrasions as suspected in case of individual #13.

PBMCs of broodmares #1 and #11 prepared in 2002 equally scored negative by E5 PCR. Both mares now display small skin lesions. It cannot be ruled out that they may correspond to sarcoids that developed upon infection acquired after blood collection. However, given the high occurrence of melanoma in grey horses of Lipizzaner breed (Seltenhammer et al., 2003), the advanced age (17 and 19 years, respectively) of the two mares and the gross-appearance of the detected nodules, the latter are more likely to represent melanomas.

An inverse scenario was encountered when comparing clinical examination results to those obtained by PCR for individuals #7 and 8. Whereas the first reaction conducted under stringent temperature conditions revealed absence of E5 in these two investigated PBMC isolates, a second reaction performed with a decreased primer annealing temperature of 56 °C scored positive, albeit on a reduced level. Several authors have described E5 sequence variations for BPV DNA isolates obtained from equids (Chambers et al., 2003b; Nasir and Reid, 2006; Brandt et al., accepted manuscript). If such variations occur within primer recognition sites, E5 detection can be hampered when using bovine PV-1/2 specific primers under stringent hybridization conditions as we did in our first PCR experiment. The second PCR (AT: 56°C) yielded E5-specific amplimers of expected size (497/499 bp) and a small amount of a ~380 bp product, the latter probably originating from partly unspecific primer annealing at 56°C. Controls derived from virus-free individuals tested consistently negative by both

reactions, thus demonstrating absence of cross-contamination. This result appears to reflect an authentic infection with no apparent disease induced as yet. It confirms the recent observation that BPV-1/2 can be occasionally detected in apparently healthy equines (Bogaert et al., 2005).

Finally, interesting data was obtained for PBMC DNA isolates derived from broodmares #2, 4 and 6 displaying skin lesions. Whereas the first amplification reaction conducted under stringent temperature conditions scored negative, the second PCR (AT: 56°C) experiment detected E5 in PBMC isolates derived from these three horses (Fig. 16). From their general appearance, lesions observed in these horses were clinically the most likely to represent sarcoids. Although presence of BPV-1/2 E5 detected in PBMC DNA isolates from 2002 does not allow a reliable sarcoid-diagnosis, it constitutes a strong argument for presence of disease. Providing that lesions observed in mares #2, 4 and 6 are true sarcoids, this finding further suggests latent BPV-1/2 infection may contribute to disease pathogenesis.

No blood was collected during clinical examination of the 20 probands, since equipment allowing immediate sample processing has not been available at the stud. This is unfortunate as PCR analysis of fresh PBMC DNA isolates and comparison of data to results obtained for isolates prepared in 2002 would be of great scientific interest. Such an experiment should indeed be undertaken, including analysis of intact skin biopsies and superficial swabs collected from lesions of these animals as to determine the exact nature of observed skin lesions and validate our conclusions.

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6. Abstract

6.1. English

Equine Sarcoids are the most common skin neoplasia in horses. They have a very typical clinical appearance and are often found at sites of previous trauma or scarring. Many authors refer to a classification system of six different types, which include occult, verrucous, nodular, fibroblastic, mixed and malevolent sarcoids. The treatment is challenging and recurrence of the disease is often observed. Although many aspects of disease have been studied intensively, the biology of virus infection and tumor pathogenesis are still poorly understood. BPV types 1 and 2 (BPV1, BPV2) are recognized causative factors for sarcoid development and maintenance. BPV DNA is detected in up to 100% of lesions as well as in apparently intact skin and PBMCs of affected equines, thus possibly contributing to the theory of virus latency. BPV early and late genes are also expressed within tumors, thus further supporting direct involvement of BPV1 and 2 in sarcoid pathogenesis. Intact particles have not been detected in lesions to this point, thus disease is regarded as the result of an abortive virus infection with BPV DNA residing episomally within infected cells.

This study compared the clinical data of 20 horses of Lipizzaner breed to data obtained by BPV1/2 E5 PCR of DNA isolates purified from peripheral blood mononuclear cells (PBMCs) of these horses in 2002. Whereas a first PCR carried out under highly stringent temperature conditions scored negative for the major viral oncogene E5 in all investigated PBMCs, a second reaction carried out under less stringent conditions scored E5 positive for 5 PBMC DNA isolates. Two of the positive samples had been obtained from individuals with no apparent lesions, whereas the three remaining samples were derived from broodmares with sarcoid-like skin disorders. Providing that these horses bear true sarcoids, our findings further confirm that disease is associated with the presence of latent viral DNA in blood cells of affected individuals and that apparently healthy horses can be virus carriers.

6.2. German

Das Equine Sarkoid ist der häufigste Hauttumor bei Equiden. Es hat ein typisches Aussehen und tritt bevorzugt in Folge von Traumata auf. Viele Autoren beziehen sich

heute auf ein Klassifikationssystem, das sechs unterschiedliche Tumortypen umfasst, nämlich okkulte, verruköse, noduläre, fibroblastische, gemischte und malevolente Sarkoide. Die Behandlung von Sarkoiden gestaltet sich oft schwierig und Tumoren neigen häufig zur Rezidivierung. Obwohl wissenschaftlich seit Jahren zahlreiche Anstrengungen unternommen werden, die Erkrankung besser zu verstehen, sind nach wie vor zahlreiche Fragen offen. Bovine Papillomviren der Typen 1 und 2 (BPV1, BPV2) sind heute als Hauptverursacher von Sarkoiden anerkannt. BPV DNS ist in bis zu 100 % der Läsionen nachweisbar, ausserdem findet man virale DNS in scheinbar intakter Haut und mononukleären Blutzellen (PBMCs) erkrankter Pferde. Auch Virusprotein ist in Tumoren detektierbar, was die pathogene Rolle des Virus hinsichtlich der Entstehung und Persistenz von Sarkoiden zusätzlich unterstreicht. Intakte Viruspartikel wurden in Pferden bislang nicht nachgewiesen, so dass man annimmt, dass Sarkoide aus einer abortiven Infektion resultieren, in welcher Virus-DNS episomal in infizierten Zellen vorliegt.

In der hier beschriebenen Studie haben wir klinisch 20 Lipizzaner hinsichtlich Sarkoid-artiger Veränderungen untersucht und erhaltene Ergebnisse mit Daten verglichen, die durch BPV1/2 E5 PCR von Blutzell-DNS gewonnen wurden, die wir im Jahre 2002 aus Vollblut dieser 20 Tieren isoliert hatten. Während eine erste PCR unter höchst stringenten Temperaturbedingungen ausschließlich negative Resultate erbrachte, wies eine zweite, unter weniger stringenten Verhältnissen realisierte Reaktion, in fünf Fällen das virale BPV1/2 Hauptonkogen E5 eindeutig nach. Zwei der E5-positiven PBMC-DNS-Proben stammen von Tieren ohne feststellbarer Hautveränderungen, die anderen drei E5-positiven Proben wurden aus Stuten gewonnen, die heute Sarkoid-artige Läsionen aufweisen. Sofern es sich bei diesen tatsächlich um echte Sarkoide handelt, untermauert diese Arbeit, dass die Erkrankung mit latenter Präsenz von BPV1/2 DNS in Blutzellen infizierter Tiere assoziiert ist und dass es auch scheinbar gesunde Tiere gibt, die Virusträger sind.

7. Appendix I – Clinical Evaluation Sheets

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Arabella Fotoapparat Nr.: ANNA							**Datum, Uhrzeit:** 4.5.		
Patient Nr.: HEDEA 92 Alter: Verwendungszweck: 287–300									
Anamnese:									
INTERNISTISCHE UNTERSUCHUNG									
Allgemeinverhalten	o.B.								
Ernährungszustand	Body score index								
Haarkleid	o.B.								
Hautoberfläche	o.B.	**Hautveränderungen** * **Foto Nr.:**							
		Lage							
		Höhe mm							
		Konstistenz							
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.			
		Verschieblich	ja		nein				
		Oberfläche							
		Exsudat							
Hautelastizität *	Oberes Augenlid			Hals li			Hals re		
	links	rechts		A	B	C	A	B	C
Verstrichen in sec	1	1		>3	>3	>3	>3	>3	>3
Hautdicke mm	0,4								
Hauttemperatur	o.B.								
IKT °C	37,5°C								
Auge	o.B.								
Lidbindehaut	Farbe: mgr. ♀.						Feuchtigkeit: fng		
Maulschleimhaut	Farbe: bv						Feuchtigkeit: leg		
KFZ sec.	1								
Nase + Nasennebenhöhlen	o.B.								
Maulhöhle + Zähne	o.B.								
Obere Halsgegend, Husten	o.B.								
Blutangebot	o.B.								
Lymphknoten	o.B.								
Puls /min.	36								
Atmung /min	18								
Herz Auskultation	o.B.								
Perkussion Herz									
Lunge Auskultation	o.B.								
Mittlere caud. Lungengrenze ICR	12 ICR								
Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig			
Harnabsatz	o.B.								

* 3cm ventro-caud vom li Auge Ø 5mm derb, aufsitzend, nabelf. Einziehung unter beiden Achseln je 3 Veränderungen versch Größe von 5–20 mm Ø, Haarlos, knotig i.d. Mitte - Siehe Foto derb

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: AG Fotoapparat Nr.:	**Datum, Uhrzeit:** 3.5.2007 13[1]T
Patient Nr.: ANCONA 1999 Alter: Verwendungszweck: ♀	
Anamnese: FOTOS 111-114, 120-124 HVE	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.							
Ernährungszustand	Body score index 3	re Lendenseite re Flanke re. 17 ICR, Höhe Hüfthöcker, kugelig, derb, schuppig; 2-3 mm Ø;						
Haarkleid	o.B.							
Hautoberfläche	o.B.	**Hautveränderungen**	**Foto Nr.:**					
		Lage	re relen Wade, 10 cent groß					
		Höhe mm	2 mm					
		Konstistenz	derb					
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.		
		Verschieblich	gegen Ha. nicht	nein				
		Oberfläche	feinst schuppig					
		Exsudat						

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	1	1	>3	>3	>3	>3	>3	>3
Hautdicke mm	0,6			1			1	

Hauttemperatur	o.B.			
IKT °C	37,6 °C			
Auge	o.B.			
Lidbindehaut	Farbe: mgr. ger.	ggr. per	Feuchtigkeit:	f+g
Maulschleimhaut	Farbe: br		Feuchtigkeit:	f+g
KFZ sec.	2 2			
Nase + Nasennebenhöhlen	o.B.			
Maulhöhle + Zähne	o.B.			
Obere Halsgegend, Husten	o.B.			
Blutangebot	o.B. prompt			
Lymphknoten	o.B.			
Puls /min.	48			
Atmung /min	16			
Herz Auskultation	o B			
Perkussion Herz				
Lunge Auskultation	o B			
Mittlere caud. Lungengrenze ICR	12			

Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Lisa, Cla, Tes						**Datum, Uhrzeit:** 35.09			
Fotoapparat Nr.: Ana									
Patient Nr.: Neapolitano kitty II 79.									
Alter:									
Verwendungszweck: Pensionspferd									
Anamnese: Fotos 1B 56-68									
INTERNISTISCHE UNTERSUCHUNG									
Allgemeinverhalten	o.B.								
Ernährungszustand	Body score index 3-4 ggr. Unterbauchödem → Dissezierende volle Kruste; Areal haarlose Stellen ca 2x3cm								
Haarkleid	o.B.	Sehr dicht behaart, Präputium ugr. ödematisiert, nicht gut +pla							
Hautoberfläche	o.B.	**Hautveränderungen** Foto Nr.:							
		Lage	Hodensack ca 8x2x1cm axial						
		Höhe mm	3						
		Konstistenz	brüchig						
		Schmerzhaft	nein	Ggr.		Mgr.		Hgr.	
		Verschieblich	ja			nein			
		Oberfläche	verrucös						
		Exsudat	nein						
Hautelastizität *	Oberes Augenlid			Hals li			Hals re		
	links	rechts		A	B	C	A	B	C
Verstrichen in sec	2	1		73	73	73	73	73	73
Hautdicke mm	0,7								
Hauttemperatur	o.B.								
IKT °C	37,3								
Auge	o.B.								
Lidbindehaut	Farbe: ggr ger					Feuchtigkeit: oB			
Maulschleimhaut	Farbe: ggr ger ger					Feuchtigkeit: oB			
KFZ sec.	2								
Nase + Nasennebenhöhlen	o.B.								
Maulhöhle + Zähne	o.B.								
Obere Halsgegend, Husten	o.B.	u. Zirkulation, aus. u.B. u.a.							
Blutangebot	o.B.								
Lymphknoten	o.B.	abzutasten							
Puls /min.	28								
Atmung /min	12								
Herz Auskultation	oB								
Perkussion Herz									
Lunge Auskultation	ggr. v. V.								
Mittlere caud. Lungengrenze ICR	11								
Kotkonsistenz	gebaltt	hart	weich	breiig	flüssig	wässrig			
Harnabsatz	o.B.								

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Arabella/Uva Fotoapparat Nr.: P: Julia	**Datum, Uhrzeit:** 3.5.
Patient Nr.: Maskula Alter: 89 Verwendungszweck: tragend. Foto ab 96 – 101	
Anamnese:	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.	r+a.
Ernährungszustand	Body score index 4	→ HE li Achilles, 2cm Ø, 20cm prox Sprunggel., nicht ganz eine Schulterblattbreite vor Schulterblatt, knochenhart, Haut verschiebl. Spindelf. 1½ x 0,5 cm. / 1x1cm. 2 Finger breit prox. Hügel. Schweifrübe → re Handbreit am Widerist, 7 cm Ø, Oberfl. ggr krustig, derb, gr. Haut nicht versch.! → Foto 107
Haarkleid	o.B.	
Hautoberfläche *	o.B.	**Hautveränderungen** **Foto Nr.:**

Hautveränderungen				
Lage				
Höhe mm				
Konsistenz				
Schmerzhaft	nein	Ggr.	Mgr.	Hgr.
Verschieblich	ja		nein	
Oberfläche				
Exsudat				

Hautelastizität *	Oberes Augenlid links	Oberes Augenlid rechts	Hals li A	Hals li B	Hals li C	Hals re A	Hals re B	Hals re C
Verstrichen in sec	1"	1"	>3	3	>3	>3	>3	>3
Hautdicke mm	0,6							

Hauttemperatur	o.B.	regelm.
IKT °C	37,8	
Auge	o.B. ✓	
Lidbindehaut	Farbe: blr	Feuchtigkeit: feucht
Maulschleimhaut	Farbe: blr	Feuchtigkeit: feucht
KFZ sec.	<2	
Nase + Nasennebenhöhlen	o.B. ✓	
Maulhöhle + Zähne	o.B.	401 labial ggr. ausgebrochen. 403 rostrolabial dto 101-108 ggr. dto
Obere Halsgegend, Husten	o.B.	Parotis ggr. v.v. durchtastbar → n. auslösb.
Blutangebot	o.B.	
Lymphknoten	o.B.	ggr. s. gelappt verschieblich
Puls /min.	60	
Atmung /min	24	
Herz Auskultation	o.B	
Perkussion Herz		
Lunge Auskultation	li. ggr. v.v. rechts o.B.	
Mittlere caud. Lungengrenze ICR		

Kotkonsistenz	geballt	hart	weich (circled)	breiig	flüssig	wässrig
Harnabsatz	o.B.	ggr. Koth2O				

* re Uvic ca 1,5cm Ø verhallt, frei beweglich
re Ohr caud. Rand 5mm Ø 3mm abstehen, krustig Foto 108
li 3Fi linker Scapula Höhe Widerrist [illegible] re Ohr !

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: AG Fotoapparat Nr.:	**Datum, Uhrzeit:** 8.5.07 14 30
Patient Nr.: BRIOSA Alter: Verwendungszweck: 89	li Rückbein: 4 fl unter Tsp. markige Kante 7 x 3 mm li 20 cm vor dem Knie, dahinter, 2 x 1, Fahrspeck.
Anamnese: 270 - Ende Juni Anabella Kanne -1-4	li Auge: lat 2 x 2 mm Kante li VE: med. 15 cm prox. d. Karpale, 2 x 2 mm gr. Kante

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.	
Ernährungszustand	Body score index 3	kaud. re Oberschenkel, Rel. vom Stamm, hypertrophiert, Muskulatur verdickt, + verdächtig li Umfang 10 x 2 mm harte Stelle Pigmentverlust um kleine Augen + Maul
Haarkleid	o.B.	
Hautoberfläche	o.B.	**Hautveränderungen** **Foto Nr.:**

Lage				
Höhe mm				
Konstistenz				
Schmerzhaft	nein	Ggr.	Mgr.	Hgr.
Verschieblich	ja		nein	
Oberfläche				
Exsudat				

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	1	1	> 3	2	> 3	> 3	> 3	> 3
Hautdicke mm	2,5 cm							

Hauttemperatur	o.B.	
IKT °C	37,7 °C	
Auge	o.B.	
Lidbindehaut	Farbe: re ggr. g. li mgr. g.	Feuchtigkeit: f + g
Maulschleimhaut	Farbe: bv	Feuchtigkeit: f. g
KFZ sec.	4	
Nase + Nasennebenhöhlen	o.B.	
Maulhöhle + Zähne	o.B.	
Obere Halsgegend, Husten	o.B.	Parotis ggr. umfangvermehrt, mittl. derb, selten Husten mit Auswürfen
Blutangebot	o.B.	< 5 li + re
Lymphknoten	o.B.	solegent + gut verschieblich
Puls /min.	38	
Atmung /min	18	
Herz Auskultation	o.B.	
Perkussion Herz		
Lunge Auskultation	re ggr. v. v. li o.B.	
Mittlere caud. Lungengrenze ICR	14	

Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: CH/AK/AN Fotoapparat Nr.:	**Datum, Uhrzeit:**
Patient Nr.: SLAVY MANTUA '79 Alter: Verwendungszweck: ♂ FOTOS 320 – 331	
Anamnese:	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.							
Ernährungszustand	Body score index Präputium ödematisiert → multiple intracutane Knötchen im Unterhals/Brustbereich							
Haarkleid	o.B.							
Hautoberfläche	o.B.	**Hautveränderungen**		**Foto Nr.:** 326				
		Lage	li Brustwand					
		Höhe mm	5					
		Konstistenz	derb					
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.		
		Verschieblich	ja		nein			
		Oberfläche	borstig ~~glatt~~ ~~blutig~~ ggr ulcerierend					
		Exsudat	ggr blutig					

Hautelastizität °	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	1	1	>3	>3	>3	>3	>3	>3
Hautdicke mm	0,6							

Hauttemperatur	o.B.
IKT °C	38,3
Auge	o.B.
Lidbindehaut	Farbe: br — Feuchtigkeit: gf
Maulschleimhaut	Farbe: br — Feuchtigkeit: gf
KFZ sec.	2
Nase + Nasennebenhöhlen	o.B.
Maulhöhle + Zähne	o.B.
Obere Halsgegend, Husten	o.B.
Blutangebot	o.B.
Lymphknoten	o.B.
Puls /min.	44
Atmung /min	20
Herz Auskultation	o.B.
Perkussion Herz	
Lunge Auskultation	ggr verschärftes Vesikuläratmen
Mittlere caud. Lungengrenze ICR	10.

Kotkonsistenz	geballt	hart	welch	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Anna, Lina, Julia Fotoapparat Nr.:				**Datum, Uhrzeit:** 3.5. 7 30						
Patient Nr.: SIGLAVY Alter: 1984 → TRAMPETA Verwendungszweck: Pensionshengst										
Anamnese: ab 49 - 55										
INTERNISTISCHE UNTERSUCHUNG										
Allgemeinverhalten	o.B.	ruhig								
Ernährungszustand	Body score index 3 beidseits Sprunggelenk ~~Carp~~ UV + Abschürfung im Bereich Calcaneus li Auge med 5mm ø Hautabschürfung re Halsseite 1/2 Drosselrinne knotige UV ø 1cm									
Haarkleid	o.B.									
Hautoberfläche ggf. Umfang messen	o.B.	**Hautveränderungen**	**Foto Nr.:**							
		Lage	ingr. Zellulitis am Kruppbis Sitzbein							
		Höhe mm								
		Konsistenz								
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.				
		Verschieblich	ja		nein					
		Oberfläche								
		Exsudat								
Hautelastizität *	Oberes Augenlid			Hals li			Hals re			
	links	rechts		A	B	C	A	B	C	
Verstrichen in sec	1	1		>3	>3	>3	>3	>3	>3	
Hautdicke mm	0,6									
Hauttemperatur	o.B.									
IKT °C	37,3									
Auge	o.B.									
Lidbindehaut	Farbe: ggr. gerötet					Feuchtigkeit:				
Maulschleimhaut	Farbe: blr.					Feuchtigkeit:				
KFZ sec.	2									
Nase + Nasennebenhöhlen	o.B.									
Maulhöhle + Zähne	o.B.	1 abgebrochene								
Obere Halsgegend, Husten	o.B.	Narbig ggr UV								
Blutangebot	o.B.									
Lymphknoten	o.B.									
Puls /min.	28									
Atmung /min	~~28~~ 18									
Herz Auskultation	o.B.									
Perkussion Herz										
Lunge Auskultation	o.B.									
Mittlere caud. Lungengrenze ICR	13.									
Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig				
Harnabsatz	o.B.									

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Arq, Chr., Ter Fotoapparat Nr.: Isabella							**Datum, Uhrzeit:** 5.5.07		
Patient Nr.: Conversano Amata 1986 Alter: Verwendungszweck: Pensionspferd Foto: 43–48									
Anamnese:									
INTERNISTISCHE UNTERSUCHUNG									
Allgemeinverhalten	o.B.								
Ernährungszustand	Body score index 3-4								
Haarkleid	o.B.								
Hautoberfläche	o.B.	**Hautveränderungen**		**Foto Nr.:**					
		Lage							
		Höhe mm							
		Konstistenz							
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.			
		Verschieblich	ja		nein				
		Oberfläche							
		Exsudat							
Hautelastizität °	Oberes Augenlid			Hals li			Hals re		
	links 1	rechts 1		A	B	C	A	B	C
Verstrichen in sec	0			>3	>3	>3	>3	>3	>3
Hautdicke mm	0,6								
Hauttemperatur	o.B.								
IKT °C	37,9°								
Auge	o.B.								
Lidbindehaut	Farbe: OB			Feuchtigkeit: OB feucht					
Maulschleimhaut	Farbe: blassrosa			Feuchtigkeit:					
KFZ sec.	<2								
Nase + Nasennebenhöhlen	o.B.	N. blassrosa OB							
Maulhöhle + Zähne	o.B.	OB							
Obere Halsgegend, Husten	o.B.	viel Durchfeuchtet							
Blutangebot	o.B.	Ltr							
Lymphknoten	o.B.								
Puls /min.	44								
Atmung /min	12								
Herz Auskultation	OB								
Perkussion Herz									
Lunge Auskultation	OB								
Mittlere caud. Lungengrenze ICR	13								
Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig			
Harnabsatz	o.B.								

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: AG Fotoapparat Nr.:	Datum, Uhrzeit: 8³⁰ 3.5.2007
Patient Nr.: CONVERSANO TOSCANA II - 48 (24.04.1988) Alter: Verwendungszweck: ♂	
Anamnese:	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	(o.B.)						
Ernährungszustand	Body score index 3 Verletzung, blutig 1×1 cm, lat. Sprunggelenk Unterseite Schweifrübe 6 erbsengroße Haarlose						
Haarkleid	(o.B.)						
Hautoberfläche re Schulter: Knötchen von 1cm×1mm knotenhart (Ø) gegen Unterlage nicht verschieblich, darauf auch ein kleines; + viel eine	(o.B.)	Hautveränderungen	Foto Nr.:				
		Lage	li Halsseite caud. 1/3, dors. Mähnenkamm				
		Höhe mm	erhaben				
		Konsistenz	hart				
		Schmerzhaft	(nein)	Ggr.	Mgr.	Hgr.	
		Verschieblich	(ja) gegen Haut + Unterhaut				
		Oberfläche					
		Exsudat					

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	0,6		>3	>3	3	>3	2	>3
Hautdicke mm				1			1	

Hauttemperatur	(o.B)	
IKT °C	37,2	
Auge	(o.B.) ggr. ger.	
Lidbindehaut	Farbe:	Feuchtigkeit: f g
Maulschleimhaut	Farbe: bv	Feuchtigkeit: f g
KFZ sec.	1	
Nase + Nasennebenhöhlen	(o.B)	
Maulhöhle + Zähne	(o.B)	
Obere Halsgegend, Husten	(o.B)	ggr. Umfangsvermehrung Parotis
Blutangebot	(o.B.)	
Lymphknoten	(o.B)	
Puls /min.	28	
Atmung /min	12	
Herz Auskultation	o.B.	
Perkussion Herz		
Lunge Auskultation	oB	
Mittlere caud. Lungengrenze ICR	13	

Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Anna, Isabella, Theresia Fotoapparat Nr.:	**Datum, Uhrzeit:** 3-5-
Patient Nr.: Biaudella 92 ♀ Alter: Verwendungszweck:	
Anamnese: FOTO: diabolia M – 15	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.								
Ernährungszustand	Body score index 3 Unterl. kann kurzzeitig aufsitzend warzenartig li: 5Fi ↑ Ellb. Skelettmuskelhypotr., subcutan, Vers.								
Haarkleid	o.B.								
Hautoberfläche	o.B.	**Hautveränderungen**		**Foto Nr.:**					
		Lage							
		Höhe mm							
		Konstistenz							
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.			
		Verschieblich	ja		nein				
		Oberfläche							
		Exsudat							
Hautelastizität *	Oberes Augenlid		Hals li			Hals re			
	links	rechts	A	B	C	A	B	C	
Verstrichen in sec	1	1	>3	>3	>3	>3	>3	>3	
Hautdicke mm	0,4								
Hauttemperatur	o.B.								
IKT °C	37,7								
Auge	o.B.								
Lidbindehaut	Farbe: o.B		Feuchtigkeit: f+g						
Maulschleimhaut	Farbe: o.B		Feuchtigkeit: f+g						
KFZ sec.	1								
Nase + Nasennebenhöhlen	o.B.								
Maulhöhle + Zähne	o.B.								
Obere Halsgegend, Husten	o.B.								
Blutangebot	o.B.								
Lymphknoten	o.B.								
Puls /min.	36								
Atmung /min	18								
Herz Auskultation	o.B								
Perkussion Herz									
Lunge Auskultation	o.B								
Mittlere caud. Lungengrenze ICR	14								
Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig			
Harnabsatz	o.B.								

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Ure, Christ, Julia Fotoapparat Nr.: 264 – (270)	**Datum, Uhrzeit:** 2.5 13 55
Patient Nr.: THEODOROSTA Alter: ♀ 1990 Verwendungszweck: ~~Kat~~ 101-264 – 101269	
Anamnese:	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.	r + R
Ernährungszustand	Body score index 3	→ Narbe Hinter Schulterblatt mitte Thorax ca 25cm lang u. 15 cm breit
Haarkleid	o.B.	
Hautoberfläche	o.B.	**Hautveränderungen** Foto Nr.:
		Lage
		Höhe mm
		Konstistenz
		Schmerzhaft: nein / Ggr. / Mgr. / Hgr.
		Verschieblich: ja / nein
		Oberfläche
		Exsudat

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	1	1	2	2	2	2	2	2
Hautdicke mm	0,5							

Hauttemperatur	o.B.	
IKT °C	37,6	
Auge	o.B.	
Lidbindehaut	Farbe: ggr. gr. beidseits	Feuchtigkeit: ✓
Maulschleimhaut	Farbe: bl.	Feuchtigkeit: ✓
KFZ sec.	2	
Nase + Nasennebenhöhlen	o.B.	
Maulhöhle + Zähne	o.B.	
Obere Halsgegend, Husten	o.B.	nicht druckschmerzhaft, nicht sh., kein Husten
Blutangebot	o.B.	re + li
Lymphknoten	o.B.	
Puls /min.	40	
Atmung /min	12	
Herz Auskultation	o.B.	
Perkussion Herz		
Lunge Auskultation	ggr. versch. ves.	
Mittlere caud. Lungengrenze ICR	11. KR	
Kotkonsistenz	geballt / hart / weich / breiig / flüssig / wässrig	
Harnabsatz	o.B.	

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Fotoapparat Nr.:	**Datum, Uhrzeit**:
Patient Nr.: Bellamira Alter: 12 ♀ Verwendungszweck: ~~Foto 101-251~~ → 10.1257	
Anamnese:	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.							
Ernährungszustand	Body score index 3							
Haarkleid	o.B.							
Hautoberfläche	o.B.	**Hautveränderungen**	**Foto Nr.:**					
		Lage Schwellung 2 Finger Anus						
		Höhe mm	~~10~~ 8 mm					
		Konsistenz						
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.		
		Verschieblich	ja		nein			
		Oberfläche						
		Exsudat						

Hautelastizität *	Oberes Augenlid		Hals li sec.			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec. !	2	2	>3	>3	2	>3	>3	2
Hautdicke mm	4mm							

Hauttemperatur	o.B.	Kopf m
IKT °C		
Auge	~~o.B.~~	o
Lidbindehaut	Farbe: ggr. gr	Feuchtigkeit: o
Maulschleimhaut	Farbe: o.	Feuchtigkeit: o
KFZ sec.	1"	
Nase + Nasennebenhöhlen	~~o.B.~~	
Maulhöhle + Zähne	o.B.	ggr. prognatia inferior
Obere Halsgegend, Husten	o.B.	Struma ggr. verg. nicht durchtastbar kein
Blutangebot	~~o.B.~~	prompt
Lymphknoten	~~o.B.~~	o.
Puls /min.	60	
Atmung /min	32	
Herz Auskultation	o.B.	
Perkussion Herz		
Lunge Auskultation	R ggr. V.V. / li. o.B.	
Mittlere caud. Lungengrenze ICR	11	

Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: Protokoll: Julia Vinay Christ Fotoapparat Nr.:	**Datum, Uhrzeit:** 2.5.
Patient Nr.: Trompeta Alter: Verwendungszweck: Z.S. 1793 ♀ 756	
Anamnese:	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.	r.a.
Ernährungszustand	Body score index → 3 • re Thx mittig C-förmig (Narbe) • Narbe li Flanke prox → • Narbe prox tuber calcanei 5 cm lang 1 cm breit von prox. nach dist. bds / Haarlos Sitzbeinhöcker re	
Haarkleid	o.B.	
Hautoberfläche	o.B.	

Hautveränderungen	**Foto Nr.:**			
Lage				
Höhe mm				
Konsistenz				
Schmerzhaft	nein	Ggr.	Mgr.	Hgr.
Verschieblich	ja		nein	
Oberfläche				
Exsudat				

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	~~3~~ 1	1	>3	2	>3	>3	2	>3
Hautdicke mm	0,6							

Hauttemperatur	o.B.	
IKT °C	37,7	
Auge	o.B.	
Lidbindehaut	Farbe: g. ger	Feuchtigkeit: ✓
Maulschleimhaut	Farbe: ob	Feuchtigkeit: ✓
KFZ sec.	~~2~~ < 1	
Nase + Nasennebenhöhlen	o.B. ✓	
Maulhöhle + Zähne	o.B.	
Obere Halsgegend, Husten	o.B. ✓	
Blutangebot	o.B. ✓	
Lymphknoten	o.B.	ggr. schmerzhaft, gelappt & verschiebl.
Puls /min.	40	
Atmung /min	12	
Herz Auskultation	o.B.	
Perkussion Herz		
Lunge Auskultation	mgr. v.V. – hgr.	
Mittlere caud. Lungengrenze ICR	12.	

Kotkonsistenz	~~geballt~~	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: AG Fotoapparat Nr.: UH	**Datum, Uhrzeit:** 2.5.2007 10°°
Patient Nr.: NEAPOLITANO NIHA I-80 Alter: Verwendungszweck: 1979 ♂	11.4.1979

Anamnese:
21 – 86

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.							
Ernährungszustand	Body score index 3							
Haarkleid	o.B.							
Hautoberfläche	o.B.	**Hautveränderungen**	**Foto Nr.:**					
		Lage						
		Höhe mm						
		Konstistenz						
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.		
		Verschieblich	ja		nein			
		Oberfläche						
		Exsudat						

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec			>3	>3	>3	>3	>3	>3
Hautdicke mm	0,6			2			2	

Hauttemperatur	o.B.	
IKT °C	37,4°C	
Auge	o.B.	
Lidbindehaut	Farbe: mgr. ger RE / ggr. LI	Feuchtigkeit: f + g
Maulschleimhaut	Farbe: lr	Feuchtigkeit: f + g
KFZ sec.	3sec	
Nase + Nasennebenhöhlen	o.B.	
Maulhöhle + Zähne	o.B.	
Obere Halsgegend, Husten	o.B.	
Blutangebot	o.B. 5sec	
Lymphknoten	o.B.	
Puls /min.	44	
Atmung /min	14	
Herz Auskultation	o.B.	
Perkussion Herz		
Lunge Auskultation	li: / re:	
Mittlere caud. Lungengrenze ICR	12 ICR	

Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: UH Fotoapparat Nr.:						**Datum, Uhrzeit:** 2.5.			
Patient Nr.: MAESTOSO BELLAMIRA 37 Alter: Verwendungszweck: 1982 ♂									
Anamnese: Fotos: 75–80									
INTERNISTISCHE UNTERSUCHUNG									
Allgemeinverhalten	o.B.	r+a							
Ernährungszustand	Body score index 3 Unterseite Schwanzrübe: ø 1×2 cm, ø 2×2 mm, 5×0,5 cm ø knotige UV ø 4 cm haarlose Stelle im Scheitelbereich re hinter prox.-med. d. Sprunggelenks 4 cm lange V-förmige Narbe								
Haarkleid	o.B.								
✶ **Hautoberfläche**	o.B.	**Hautveränderungen**	**Foto Nr.:**						
		Lage							
		Höhe mm							
		Konstistenz							
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.			
		Verschieblich	ja	nein					
		Oberfläche							
		Exsudat							
Hautelastizität *	Oberes Augenlid			Hals li			Hals re		
	links	rechts		A	B	C	A	B	C
Verstrichen in sec	1	1		>3	>3	>3	>3	>3	>3
Hautdicke mm	0,6								
Hauttemperatur	o.B.								
IKT °C	37,6								
Auge	o.B.	ggr. Ikter. Sklera							
Lidbindehaut	Farbe: ggr. ikterisch			Feuchtigkeit: ✓					
Maulschleimhaut	Farbe: ggr. ikterisch			Feuchtigkeit:					
KFZ sec.	2								
Nase + Nasennebenhöhlen	o.B.								
Maulhöhle + Zähne	o.B.	Prognatie mf. ggr., 303 abgebrochen, Zahnstein, 101 fingivitis							
Obere Halsgegend, Husten	o.B.	Parotis OD nicht abtastbar, OB							
Blutangebot	o.B.	> 5 sec							
Lymphknoten	o.B.								
Puls /min.	28								
Atmung /min	18								
Herz Auskultation	systolisches HG								
Perkussion Herz									
Lunge Auskultation	ggr. -ingr. vorschärf. vesikulär bds. re								
Mittlere caud. Lungengrenze ICR	~~14~~ 13								
Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig			
Harnabsatz	o.B.								

✶ re, ventr. Halsbereich bis Drosselrinne multiple knotige UV, teils Holzgrinne zu entfernen
2×2 cm große UV am Unterkieferrändern

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers: U Fotoapparat Nr.: 87 – 90 Th	**Datum, Uhrzeit:** 3.5. 11⁰⁰
Patient Nr.: CONFIDERA Alter: Verwendungszweck: mobil tropend 1994	
Anamnese:	

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.	↑↑Q
Ernährungszustand	Body score Index 4 → ☆. Narbe über Achillessehne re + li ca 7 cm lang	
Haarkleid	(o.B.)	
Hautoberfläche	o.B.	**Hautveränderungen** **Foto Nr.:**
		Lage
		Höhe mm
		Konstistenz
		Schmerzhaft: nein / Ggr. / Mgr. / Hgr.
		Verschieblich: ja / nein
		Oberfläche
		Exsudat

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	<1	<1	>3	>3	>3	>3	>3	>3
Hautdicke mm	5 mm							

Hauttemperatur	(o.B.)	
IKT °C	37,9	
Auge	(o.B.)	
Lidbindehaut	Farbe: blr	Feuchtigkeit: ✓
Maulschleimhaut	Farbe: ber.	Feuchtigkeit: —
KFZ sec.	<2	
Nase + Nasennebenhöhlen	o.B.	beidseits ggr. seröser Nasenausfluss vorn OD
Maulhöhle + Zähne	(o.B.)	
Obere Halsgegend, Husten	(o.B.)	
Blutangebot	(o.B.)	
Lymphknoten	(o.B.)	
Puls /min.	60	
Atmung /min	24	
Herz Auskultation	gepolkter 2. HT	
Perkussion Herz		
Lunge Auskultation	ggr – mgr verstk. ves.	
Mittlere caud. Lungengrenze ICR	12.	

Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

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Protokoll für Bestandsuntersuchung in Pieber 2007

Name des Untersuchers:	**Datum, Uhrzeit:**
Fotoapparat Nr.: Haut	14[10] 4.5.
Patient Nr.:	
Alter:	
Verwendungszweck: BONA 1992 / Rinde [illegible]	AMABILA 1991
Anamnese:	Foto ab 313 – 317

INTERNISTISCHE UNTERSUCHUNG

Allgemeinverhalten	o.B.							
Ernährungszustand	Body score index 2 ① dist 1/3 Hals links: knotig, hart ∅ 0,5 cm unter Haut, nicht verschieb Haut darüber — darüber dann 2mm Hartverdickung, verdickt, narbig							
Haarkleid	o.B.							
Hautoberfläche	o.B.	**Hautveränderungen**	**Foto Nr.:** ab 313					
		Lage	li Brust lat. d. Clavicula					
		Höhe mm	3mm / ∅ ca 5 mm					
		Konstistenz	elub - knotig					
		Schmerzhaft	nein	Ggr.	Mgr.	Hgr.		
		Verschieblich	ja		nein			
		Oberfläche	hyperkeratot ggr					
		Exsudat	nein					

Hautelastizität *	Oberes Augenlid		Hals li			Hals re		
	links	rechts	A	B	C	A	B	C
Verstrichen in sec	1	2	>3	>3	>3	>3	>3	>3
Hautdicke mm	0,44cm							

Hauttemperatur	o.B.					
IKT °C	37,7					
Auge	o.B.					
Lidbindehaut	Farbe: ggr gor + ikt			Feuchtigkeit: ~		
Maulschleimhaut	Farbe: kr + ggr ikt.			Feuchtigkeit: ~		
KFZ sec.	2					
Nase + Nasennebenhöhlen	o.B.					
Maulhöhle + Zähne	o.B.					
Obere Halsgegend, Husten	o.B.	Rasselg ggr ↑ nicht eitrig.				
Blutangebot	o.B.					
Lymphknoten	o.B.					
Puls /min.	64					
Atmung /min	18					
Herz Auskultation	o.B.					
Perkussion Herz						
Lunge Auskultation	mgr v.v.					
Mittlere caud. Lungengrenze ICR	10					
Kotkonsistenz	geballt	hart	weich	breiig	flüssig	wässrig
Harnabsatz	o.B.					

② li Achselhöhle med 1 cm ∅ hoarlos