Thesis

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Retrospective analysis of the clinical findings of horses with ocular squamous cell carcinoma

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Content

I. Introduction

In the last centuries horses more and more changed their position in society, from working and warfare animals to pet and sport partners (58). Because of that and economic possibilities of horse owners, health of horses became more important, to increase their life quality and extend their time for sports.
Nowadays in Germany horse riding is not an insignificant economic factor, the source of income of around 10.000 companies and establishments are in connection with equines. There are 3.89 million horse riders and 900000 horse owners. The estimated turnover of the industry was about 6.7 Milliard Euros in 2022 (59).

Veterinary medicine improved enormously in the last 50 years, because more animals are examined, and different diseases may be detected. The equine oncology and ophthalmology are growing constantly, but it is more difficult than in other parts of medicine to find comparable patients in a large number to find useful statistical results. Collection of patient data and results is possible in clinics popular for those specialities only. Statistical outcomes of studies with small samples are questioned. This is the reason that we carried out this study using the data from a special equine ophthalmology clinic in Germany.

The squamous cell carcinoma (SCC) is, after the equine sarcoid, the second most common neoplasia in equines and the most common ocular or periocular tumour. Genetics, age, breed, exposure to UV light is influencing the growth (11;15;20). Because of the increasing UV radiation, appearance of older equids, and more common histological biopsy taking this type of neoplasm can be diagnosed more often nowadays (4;23). Generally, it is a slow growing malignant tumour with low metastasis which may appear especially at the eyelids, the nictitans, and lateral limbus of the sclera (1;4;7;17;34;44). Different therapy methods and their combinations are used. It is not cleared which is the most ideal treatment. This study discusses especially the results of the usage of chemotherapeutic antibiotics Mitomycin C 0,04% eyedrops in the treatment of SCC.

II. Literature Review

Background

The term neoplasia (also called tumor/neoplasm) describes an abnormal mass, which can develop as a progressive and unrestrained tissue proliferation. The enlargement of the tumor continuous, even when the stimulus no longer appears. Cancer is the generic term for malignant masses. Malignant tumors grow into the surrounding tissue, which is named infiltration. Destruction is the term in case of tissue damage. Neoplasms are classified by their biological behaviour and tissue origin. Epithelial tumours develop from endocrine or exocrine glands, like the liver, or tissues covering the internal or external body surfaces such as mucosal epithelium or the cutaneous or mucosal squamous epithelium. Malignant epithelial tumours are called carcinoma (45). Squamous cell carcinoma (SCC) is a common type of neoplasm in bovines, equines, felines, canines, and humans (1; 2). It is a slowly developing malignant tumor of the epithelial cells (1; 7; 45). This neoplasm can be found especially in the skin, mucous membranes, and the eye of the equines, bovines and humans, middle and external ear, and oral cavity of felines and canines, and in the guttural pouch of older horses (1; 8; 23).

Prevalence of the SCC in equines varies in the different studies. In the study of Sundberg (n=236), from 1970 to 1974, 24.60% of all neoplasms (sarcoid, fibroma, lymphoma, melanoma, gonadal stromal tumour) were diagnosed as SCC. Analysis of Bastianello (n=378) between 1935 and 1974 was 23.5%. The result of Knowles (n=964) from 1982 to 2010 was 18.90% concerning SCC from all tumors (6). The retrospective study of Théon (n=573) concluded that 31.7% of cutaneous neoplasms of the periorbital area were SCC (9). All studies showed that SCC was the second most common tumor in equines after sarcoid (3; 6; 41). Nevertheless, SCC is the most frequent neoplasm of the eye and periorbital region in horses, representing 48 to 75% of tumors in this area (7; 41). Less frequently 8-38% of the patients suffer from ocular sarcoid. The other, approximately 7 to 9% of the neoplasms are melanoma, papilloma, and nerve sheath tumour around the eye (41).

The SCC grows over months or years until it becomes a malignant epithelial neoplasm. The complete regression of the neoplasm is not impossible since the body can develop an immune response against the tumor cells, but this is very rare. At the beginning of the development epidermal plaques can be detected, followed by papilloma formation (1; 30). The acute regression of these preneoplastic cells is 25-50%. Both lesions show acanthosis with dyskeratosis, epidermal downgrowth, and keratinization. Papillomas may grow up to 3 cm in diameter and may have marked parakeratosis, and hyperkeratosis in papillary formation (1). After that stage the carcinoma in situ may develop. The tumor cells grow through the lamina propria until the corneal or conjunctival epithelial cells (30).

Ocular and periocular SCC can occur on the eyelids, nictitating membrane, cornea, bulbar and palpebral conjunctiva, orbit and on the limbus (1; 2; 17; 41). The eyelids and the nictitans are the most often affected areas (17). A typical area is the edge of the lateral (dorsotemporal) limbus of the sclera in equines (4; 7; 17).

The exact pathogenesis of the ocular and adnexal squamous cell tumour is not clarified yet (10; 13; 41). The influence of genetic predispose, periocular skin colour, viral infection, hormonal and immunological status, gender, age of the equines, and environmental

circumstances, like ultraviolet light, on the growth of squamous cell carcinomas are discussed in different studies (7; 10; 13; 14; 36).

Genetic predisposition

The genetic predisposition is examined in some recent reviews. Several horsebreeds were found with ocular SCC: American Paint Horse, American Quarter Horse, Appaloosa, Arabian Horse, Belgian, Clydesdale, Hackney Pony, Haflinger, Missouri Fox Trotter, Morgan, New Forest Pony, Paso Fino, Percheron, Racking Horse, Rocky Mountain Horse, Shetland Pony, Standardbred, Suffolk, Tennessee Walking Horse, Thoroughbred, and Welsh Pony (15; 17; 18; 36; 54). An American study showed that the development of these neoplasms was more common in Appaloosas, and draft breeds, like Shire, Clydesdale, and Belgian Draft Horses than in American Quarter Horses (11; 15). Also, Haflinger, a common mountain breed of Austria which are chestnuts with a light mane and tail, are more often affected with ocular SCC than other breeds (14; 15; 19; 54).

A possible explanation for the frequent occurrence of SCC in Haflinger, Rocky Mountain Horses and Belgian Horses is a mutation in the damage-specific DNA (deoxyribonucleic acid) binding protein 2 (DDB2) allele on the ECA12 (equine chromosome 12) in the 483kb area (15; 16). 88% of the examined Haflingers with SCC on the nictitans were DDB2 homogenous carriers. The ratio of homozygous Haflingers diagnosed with limbal SCC was 77% (15).

The DDB2 allele codes for proteins, which are needed to repair the damaged DNA. Because of the occurrence of cyclobutan pyrimidine dimers and 4-6 photoproducts due to ultraviolet light, sun radiation is a potential risk for the development of SCC (4; 16; 17). If these damages appear in protein-coding chromosomes, which take part in the control of the cell-cycle and reparation, replication of the DNA, a carcinoma may grow. The protein coded by DDB2 adheres to the injured areas and mobilizes other proteins to repair them. In case of a mutation, the DDB2 is unable to recognize and fix broken DNA strains, therefore the SCC develops (16).

In a study from 2019 Singer-Berk looked for other gene variants around 483kb on the ECA12 allel. He examined the whole genome of six Haflingers suffered from ocular SCC and took a closer look at the genotype of 60 different variants. In conclusion none of them showed a connection with the phenotype of the cancer, except DDB2 (16). This study did not confirm that the variation of the DDB2 was a significant risk factor for the development of the ocular SCC in Arab horses, Appaloosas, and Percherons, but it is not excluded yet (16). There was only one carrier of DDB2 in the Rocky Mountain Horse breed diagnosed with limbal SCC documented, a larger survey is needed to find conclusion. Nevertheless, it would be beneficial to check the genome of equines used for breeding routinely to prevent the spreading of this neoplasm in Rocky Mountain Horses (15).

For genetic screening to rule out homozygous individuals for ocular or adnexal SCC, a minimum of 15-20 intact hairs inclusive follicles from the tail or mane and a whole blood sample containing EDTA are needed to examine the genome, therefore different manufactured kits are used, for example from Laboklin, Germany (www.laboklin.com) (15; 19).

Periocular pigmentation

Not only the breed, but also the pigmentation of the skin and hair play a role in the development of the ocular and adnexal SCC (17; 36). For example, breeds like the American Paint Horse or Appaloosa have a less pigmented ocular area, therefore they are more likely to suffer from SCC (10; 18; 36).

The hair colour chestnut is one of the most common skin colours documented in connection with this type of neoplasm (17). 32 to 46% of the effected horses are chestnuts (16; 17; 18). Black horses are unlikely to get affected (17; 18). The variant of melanocortin 1 receptor (MC1R) that causes the phenotype chestnut in horses stays in a connection with the development of ocular and periocular SCC (16; 19). On the surface of melanocytes G-protein coupled receptors can be found, they regulate the changing from eumelanin, dark pigment, to pheomelanin. The red colour, pheomelanin, appears in case of an eumelanin lack in chestnuts (19).

Age

Normally the ocular and periocular SCC occurs more often with a higher age in equines (11). The horses diagnosed with an ocular or adnexal SCC in different breeds were between 2.4-26.7 years of age, in average 12.1 years in a study (17). In the study of Lassaline (n=19) the average age of Haflingers' diagnosed with limbal SCC was 8.7 years. In comparison, this type of tumor occurs at lower age in this breed (14). Singer-Berk found, that homozygous DDB2 Haflingers and Belgians were 10 years old on average, homo- or heterozygous ones were 13 years old at the date of diagnosis (16).

Ultraviolet light of the solar radiation

Ultraviolet radiation is a potential risk factor for skin cancer in humans, cattle, horses, dogs, and cats, because of its ability to destroy genes (19; 20; 21). 93% of skin cancers in humans can be established to it (22). Ultraviolet light occurs in form of sunlight and solar radiation with 100 to 400 nanometers on the Earth (23). Between 2015 and 2019 was an increase of 15% of the intensity of the ultraviolet radiation, but it depends on the location, season, altitude, and latitude (4; 23). The reason for the hypersensitivity for radiation in human beings and animals in spring, is the weaning from sun light during the winter months. (56) A connection between SCC and constant sun light exposure can be established, particularly in outdoor workers (22). In above 90% of in-situ SCC human cases a "UV-signature" mutation could be found. Damaged p53 genes could be detected in 50% of the human cancer patients (24).

The radiation reaches the skin surface, the epidermal layer every day. It will be absorbed by the gene. The p53 tumor suppressor will be increased intranuclearly. The p53 encodes the phosphoprotein 53kd, which is important for the cell growth and protein expression. The p53 stops the G1-phase of the cell cycle to take enough time to repair the ultraviolet light damaged DNA. If there are cells with inactivated or disturbed p53, the cell cycle won't be stopped, therefore a replication of damaged cells or apoptosis (programmed cell death) will occur. The presence of the p53 antigen in paraffin or formalin fixed SCC tissue can be proven with immunohistology techniques (20; 24).

Viral infection

The Equus caballus papillomavirus 2 (EcPV2) infection is a risk factor for the genital SCC, but only in 25% of the patients with affected head and neck region (12; 16). In the study of Brandt there was no correlation between ocular SCC and EcPV2 detectable (12). Only one pony diagnosed with periocular SCC, and positive for EcPV2 and bovine papilloma virus test was documented yet (25).

Hormonal status

According to different studies, geldings are the most often affected horses followed by mares and stallions (11; 16; 19; 32; 41). Researchers try to answer the question if the hormonal status of castrated equines influences the growth of SCC. This hypothesis could not be tested so far because of the too small sample size of stallions (16).

Diagnosis

Nationale (signalment) of the horse is a principal point for the diagnosis of SCC on the eye. The coat colour, gender, pigmented skin areas, age, and the breed are documented. The history, clinical symptoms and used medication are discussed during the anamnesis. Horses are usually suffering for months, without any or little improvement after anti-inflammatory and analgesic treatment. They show inflammation on the eye, as well blepharospasm and epiphora/discharge (13; 26; 36; 41).

Physical examination and an ocular examination of both eyes with a slit lamp biomicroscope are indicated (10; 29; 19). In different studies, from 11.65% (n=5) to 19.8% of the horses both eyes were affected (11; 17; 34).

If there is a suspension of SCC, the nictitans, cornea, bulbar and palpebral conjunctiva, orbit, and the limbus should be examined thoroughly (7; 17; 41). The symmetry of the eyes is essential and compared. The bony orbit and eye muscles are palpated. The eyelids are examined, including the haired dermis, vibrissae, meibomian glands, blinking reflexes, and the palpebral conjunctiva. In a physiological condition the conjunctiva appears shiny and smooth. Abnormalities, such as swelling, hyperaemia and discolouration are recognized. In case of SCC, the nictitans shows surface irregularity. Eyes with less pigmentation of the third eyelid are more sensible to solar radiation (27; 13).

The normal cornea is clear and transparent. Tumor infiltration may invade the basement membrane of the epithelium and the stroma until the Descemet's membrane. The Descemet's membrane seems to be a strong barrier for cancer cells, a penetration of it, never has been reported. Carcinoma cells rather grow laterally to this barricade (27). Although limbal SCC may enlarge around the Descemet’s membrane and can penetrate the iridocorneal and uveoscleral tissue reaching the iris or eye muscles (10). The ophthalmic examination should focus on the temporal limbus of the bulb (4; 10; 54). In addition, the iris, ciliary body, vitreous and fundus should be exanimated.

Macroscopically the early detected SCC looks like a small bloody lesion or a plaque (7; 10). Later it appears as a cauliflower like shape in different sizes (10; 26). It is a non-movable mass in a pink to greyish-red colour (10; 13). Sometimes the neoplasm appears as a bleeding ulcer in a lobated form (10; 26). Due to eyelid disfunction or scraping on the cornea, a

corneal ulcer may develop (7). Secondary bacterial infection, ulcer or abscess formation and inflammatory signs could appear (7; 10).

A corneal SCC may have "cobblestone" shape and lead to white to yellowish mucoid discharge. The affected equines don't show heavy pain, just discomfort, that's why veterinarians should distinguish the tumour from corneal ulcer or keratitis (especially eosinophilic keratitis or immune-mediated keratitis) (29). Differential diagnosis are other tumor types: adenoma, adenocarcinoma, angiosarcoma, fibroma, fibrosarcoma, mastocytoma, melanocytoma, papilloma, plasmacytoma and schwannoma.

In a case report a Belgian Horse suffered from ocular SCC and hemangiosarcoma at the same time. The two tumor types developed in one mass and were considered because of the sunlight (28). As differentials, rare parasitic infections like Habronema microstoma, Habronema muscae, Onchocerca cervicalis and Thelezia lacrimalis should also be taken into consideration (10).

Cytology and histopathology

Cytology and histopathological examination are essential to determine the malignancy, prognosis, and the most accurate therapy. Malignancy is characterized by the number of mitotic figures, enlargement and abnormal shape of the tumor, increased synthesis of collagen fibres and angiogenesis (26; 45).

Excisional biopsy is taken and transported in formalin (29; 30). Carcinoma cells are usually stained with eosin-haematoxylin (10; 13). The fixing is made with paraffin. Microscopically SCC varies in morphology and appears as an infiltrative mass (10). Because of intracellular bridges there is a connection between epithelial cells and the surface. The tumor contains an eosinophilic centre and a basophilic periphery, where cells have wide, less eosinophilic cytoplasm and a heterochromatic less rounded cell nucleus (45). Keratinization of individual cells is possible, moreover an organized form could be detected in keratinized SCC (keratin pearl) (26).

In case of a good prognosis keratin pearls and a maximum of 2 mitotic figures in high power field magnification may be detectable. The connective tissue contains neutrophil granulocytes, plasma and lymphoid cells, the angiogenesis is minimal (26; 45). In case of poor prognosis, the tissue is less differentiated, contains a lot of abnormal mitotic figures and polymorphic cells (26). The nucleus is euchromatic and the nucleoli are easily visible (10). No keratinization can be found. If the SCC is ulcerated, neutrophil granulocytes and macrophages will be present. Immunohistochemically cytokeratin may be detected in this neoplasm (26).

Therapy

To treat ocular and periocular SCC there are different options, such as surgery (7; 10; 34), cryotherapy (17; 48; 54), high frequency hyperthermia (43), beta-radiation with Strontium 90 (29; 30; 31), gamma-radiation with Iridium 192 (32), laser ablation with carbon dioxide (18), and topical treatment with Mitomycin C (4; 48; 49; 50).

Combination of different treatment possibilities provides the most successful strategy (4; 29; 34). It is important to choose the adequate treatment method for each individual case. The

decision is defined by the size and location of the SCC, invasiveness, growth and treatment history, local enlargement, and metastasis (29; 39). The age, body condition, financial limitations, prognosis, and treatment availability must be taken into consideration (39; 46; 53).

Surgical excision

Surgical excision or debulking of the ocular or adnexal SCC is often used as a basic therapy. The size of the mass is reduced to improve treatment success with adjuvant options. Because of the invasiveness of this tumour type, it would not be considered as the only way of therapy (7; 10; 34).

Cryotherapy

Cryotherapy may be used as an additional treatment (17; 18; 35; 39). SCC of the conjunctiva or limbus cryotherapy with triple freeze/thaw is provided after surgery in human medicine (35). Moore described the double use of it in horses, depending on the size with or without surgical pre-treatment (39). The tip of the cryoprobe (frozen with -25°C cold liquid nitrogen) is applied to the lifted margin of the neoplasm (35; 39). Freezing destruction should extend 2mm wider in depth (39). After three seconds the probe will be removed and the tip refrozen. The affected people had a healed epithelial surface within one week. In the study of Khokhar the adjuvant therapy showed a recurrence rate of 9% in humans, which was considered low (35).

High frequency hyperthermia

This technique is regularly used in the bovine praxis, but may also be applied in precursor lesions, as an adjuvant therapy or for small neoplasms in the equine medicine (17; 36). Hyperthermia can be used easily in neoplasms smaller than 5cm in diameter and not infiltrating more than 20 to 50 mm deep into the palpebra or conjunctiva (43). There are probes available that heat up 1cm of the mass within 30 seconds to 50°C (36). In the study of Grier 80% of the bovine and horse patients were cured (43).

Beta-radiation with Strontium 90

Irradiation can be performed by a strontium 90 application (29). Radiation can be used in the tumor mass and inoperable regions (29; 30). 8000 to 10.000 radiation absorbed dose (50 rads/sec) or 80 to 100 Grays are applied to the carcinoma base after keratectomy. The dose used according to the size of the carcinoma and the radiosensitivity of the healthy cells (29; 31). Almost all beta particles can invade the ocular tissue as deep as 2-3 mm (30; 31). Beta-radiation seems to be safe, as it is used in the correct dose (17; 30). In the study of Mosunic, high dose of 250 Grays was used, 15.1% of the patients had recurrence (17). Other studies with lower doses showed higher recurrence rates (29; 34; 52).

After Beta-radiation there is a high risk for scarring and infection because of the large corneal wound created. Ollivier described for the first time the use of amniotic membrane graft (AMG) as a cover of corneal or limbal carcinoma excision site. AMG will be incorporated in the keratectomy wound with some degree of inflammation due to immunological reaction.

One week postoperatively the Fluorescein test was already negative. After some months the corneal lesion was mostly healed and transparent (30).

Gamma-radiation with Iridium 192

Iridium 192 is used for periocular SCCs that are larger than 3 mm. The possible penetration depth of iridium 192 implants is about 1 to 1,5 cm (17; 29; 31). The recovery time of large tumors may take as long as 1 year (32). In the report of Mosunic, where low doses of iridium 192 were used, the complication rate was minimal. Blepharitis, post radiation infection or discolouration of the periocular region was rarely detected (17). Theon applied 60 Grays as a minimum tumor dose. 10.3% of the cases had ulcers of the eyelids or cornea after radiation. Using higher dose the recurrence rate may decrease, but the post radiation complications increase (32; 52).

Laser ablation with carbon-dioxide

Laser ablation is a safe and effective adjuvant therapy option. Wavelength absorption in liquid containing tissue is the most relevant advantage of this technique (18; 51). Goldbaum documented that usage of CO_2 laser provides haemostasis, a decrease of inflammation and pain, exact cutting, and sterilization of tissue (51).

Rayner described cases that needed general anaesthesia for the treatment of corneal, scleral, and corneoscleral carcinomas, and 6-Watt CO_2 laser was used in combination with mitomycin C. 86% of cases was cured (49). Michau used CO_2 laser for corneolimbal SCC under general anaesthesia after keratectomy (18). After superficial keratectomy Tóth used a 2-3 Watt CO_2 laser to debride the entire keratectomy site in standing sedated horses. 80% of the cases were cured, 20% were enucleated due to tumor recurrence (4).

Bacillus Calmette-Guerin vaccine (BCG)

This immunotherapy uses bacterial Calmette-Guerin-Purified-Protein products. Mycobacterias' modified cell wall proteins are used as an injection, which as a foreign material will induce a non-specific cellular immune response (36). The activity of T-lymphocytes, macrophages and dendritic cells increased (7). In human medicine it is applied to treat in-situ bladder carcinoma or vaccination against tuberculosis. In horses sarcoid and melanoma can be treated with it (7; 36; 39; 41). Immunotherapy also can be applied in periocular SCC as intralesional injections (29; 37). In a case report McCalla successfully treated a pony for 17-weeks, though metastasis was in the lymphnodes (55). In bovine medicine the healing rate can be as great as 50-60% of treated cases. A retrospective study with large number of cases should be performed to evaluate this immunotherapy in horses (54).

Topical treatment with Mitomycin C

Mitomycin C is a chemotherapeutic drug, which is used to treat recurrent pterygium in human ophthalmology (4; 7; 48). The drug alkylates DNA and inhibits RNA, that will result in gene damage (7). As it was already described in human medicine, Rayner treated equine eyes with carbon dioxide ablation in combination with topical soaking of Mitomycin C for

5 minutes (48; 49). The healing rate was 86% (49). Complications were minimal (4; 7). Estell and Tóth applied 0.04% Mitomycin C TID or QID for 1 week postoperatively (4; 7). Keratectomy combined with topical Mitomycin accompanies with low recurrence rate in veterinary medicine (49; 50). Tóth's study found, that 95% of the clients were cured after this therapy (4). Destructive cryotherapy in combination with the antimitotic Mitomycin C also had good results in human medicine (48).

Treatment possibilities of eyelid SCC

Eyelid is the most affected ocular structure for SCC (17). Horses with non-pigmented eyelids are predisposed for eyelid SCC. A study from Colorado State University found that 27. 5% of effected horses had limbal, and 28.1% of them had nasal canthal, or third eyelid or combined SCC. Initially the tumor mass may look like a light pigmented, moist, erosive focal lesion or plaque. At this stage a biopsy should be performed, and metastasis should be excluded. As differentials, Habronema infection looks yellowish granulomas on the lid seasonally (36). With biopsy this can be excluded after microscopic examination, mast cells, eosinophilic and neutrophilic granulocytes, and plasma cells can be detected (39).

Rebhun described those neoplasms smaller than 1 cm, and 2 mm in depth had better prognosis and could be treated with cryotherapy, beta radiation, laser ablation, or radiofrequency hyperthermia. For larger lesions cryotherapy, cisplatin injections, laser therapy, interstitial radiotherapy, and radiofrequency hyperthermia could be used (36; 39; 18). Tumors larger than 2cm and deeper than 2mm needed combined and intensive treatment, furthermore the long-term prognosis was questionable. At the end-stage of the tumor, exenteration or enucleation must be performed (36). The entire tumor mass should be surgically removed. In unsuccessful excision further individual treatment possibility should be discussed with the owner (36; 39).

In a study Mosunic compared the recurrence rate of radiated and non-radiated palpebral masses. After radiation treatment no recurrence was documented, in contrast, when radiation was not applied, the recurrence rate was 54% (17).

Treatment possibilities of the third eyelid SCC

30% of the SCC patients had third eyelid tumor (42; 47). Mosunic found the third eyelid tumor as the second most involved area (17). The eyelid, cornea, or nasal canthus were involved in 50 to 60% of the patients (34; 42). Metastasis was seen more frequently compared to other sites (47). Early diagnosis, accurate therapy, and strict follow-up were essential for success (40).

A combination of surgery, and radiation or chemotherapy is advised (42). In a study Labelle found that a single surgical treatment of the third eyelid SCC had good prognosis and low recurrence rate, maybe due to careful patient selection (7; 33). The resection of the nictitans can be done under general anaesthesia or in standing with local anaesthesia (28; 33). No severe long-time complications, like keratoconjunctivitis sicca, ulceration or prolapse of the retrobulbar fat were found postoperatively (33). Tiny neoplasms could heal better, but larger ones that had resection difficulties, required additional therapy (33). King used additional radiation, cryotherapy, and hyperthermia (34). Ramirez described one horse with palpebral

SCC that was successfully treated with resection and with COX-2 inhibitor Piroxicam. To verify the effectiveness of Piroxicam, a larger number of patients must be examined (42).

Treatment possibilities of conjunctival SCC

Conjunctiva is one of the most involved parts in SCC (34; 36; 44). In the study of Mosunic 17.8% of the effected horses had limbal or bulbar conjunctival SCC (17). To treat conjunctival neoplasms surgery should be followed by any ancillary treatment (40). In case of an easily definable mass, a full resection is indicated, the recurrence rate is 25% (47). The surgery can be done in standing with topical anaesthesia and nerve blocks or under general anaesthesia. Conjunctivectomy should be performed with clear cut of 2 mm of the intact tissue with a blade or scissors (26). If the conjunctivectomy wound is larger than 1 cm, to prevent extensive granulation, grafting or suturing is performed (36).

Palpebral conjunctival large and deep masses postoperative Strontium 90 radiation, with the dose of 75 to 100 Grays per site, or hyperthermia can be used (36; 41). In humans cryotherapy triple freeze/thaw is provided after surgery (35). Carbon dioxide laser ablation in horses was described in the literature (41; 18). Mitomycin C (0,04%) was applied topically as an antineoplastic, antibiotic treatment, additionally to prevent postoperative fibrosis (4; 36).

There was only a single case with pigmented ocular SCC in a horse. The diagnosis was set up with histopathology, that excluded melanoma. Therapy included surgery without ancillary treatment (40). The prognosis of palpebral tumours is questionable, although a lot of possibilities for treatment are available (26).

Treatment possibilities of corneal SCC

If equine cornea becomes neoplastic, therapeutical options are limited (29). The application of medications as an only therapy is not enough effective (18). The infiltrative neoplasm may grow deep into the cornea, what could destroy the eye and may have a high recurrence rate. The metastasis is developing slowly (44). It is important to maintain the globe integrity and vison (30). The size and the invasiveness of the carcinoma determines the treatment possibilities (36; 47).

Because of the high chance for recurrence after keratectomy alone, the most accurate method is Beta-radiation therapy immediately after keratectomy, when the tumor’s thickness does not reach more than 3mm (17; 29). This technique is also used at limbal (30) and nictitans SCC (31). A single case report has been described with keratopathy after Strontium 90 radiation, which could had been due to the high dose and large surgical lesion (17).

Therapeutic management of small masses can be done with radiofrequency hyperthermia. Because of high temperature used, corneal cells may be damaged in case of large carcinoma. The BCG injection was described as too aggressive for these corneal cells (29). The destruction of small lesions is possible with cryotherapy, but in larger ones it is described as too risky (29). Other treatments can be laser ablation, topical Mitomycin C or enucleation (18).

The prognosis for superficial corneal SCC with a maximal width of 2mm and diameter of 1 cm is good. In case of large, multiple, metastatic tumor, or recurrence the prognosis is poor

(29; 36). After enucleation of the affected eye horses have a high chance for complete recovery (44).

Treatment possibilities of orbital SCC

Neoplasms of the orbit are rare, only 0.64% of the patients are affected (17; 41). The orbital involvement appears as enlargement of ocular or adnexal structures (36). Orbital tumours should be treated with an aggressive surgery. Diagnostic imaging of the orbit should be used. Metastasis must be excluded (37; 39). In a case report after an incomplete debulking Baptiste and Grahn used BCG and cisplatin injections locally, but it didn't help. A larger number of case reports is needed to evaluate the effectiveness of these therapies (37). Wyn- Jones used radioactive gold 198 grains (17; 34; 38). Other documented therapy forms are gamma-radiation, cryotherapy, and high frequency hyperthermia (26).

Orbital SCC is described to have the worst prognosis, because of the metastasis character (36; 37). This is also documented in dogs and human orbital tumours. The survival period is about nine months in horses, 12 months in dogs, and 10.2 months in humans (37).

Metastasis

Metastasis is the spread of tumor cells and further growth on other distant parts of the body (45). Metastasis of SCC usually develops slowly and at the late stage (5; 7). The carcinoma damages the limitrophe tissue and may have metastasis in the local lymph nodes, therefore they must be examined at clinical examination (5; 45; 7). The lung, thoracic muscles and spine can be involved (5; 18). Metastasis may be also detected in the central nervous system, cranium, heart, liver, and kidneys (7; 5). Estell documented 18.6% metastasis of ocular SCC (7). Other authors described 0.03 to 15.4% metastasis rate in ocular SCC (34; 44).

Prevention

Horses with non-pigmented periocular skin are more likely to suffer from ocular or adnexal SCC, so they should be protected from solar radiation. In a paddock a shade, facemask, when ridden, suntan lotion with high UV filter should be applied. Moore reported tattooing the non-pigmented skin in horses but was not useful because of the fast fading of it (39). Application of oral or topical piroxicam can be beneficial in the early stage of palpebral or conjunctival SCC, when surgery is not indicated, but it has not been tested in a large number of patients (42).

III. Aims

The aim of this TDK thesis is to describe the aetiopathogenesis, clinical signs, therapy options and results of ocular and periocular squamous cell carcinoma in a group of horses. The retrospective study includes patients of Tierärztliche Klinik für Pferde Domäne Karthaus in Dülmen, Germany from 2012 to 2022. In the study signalment of the patient, location of the tumour, treatment with or without Mitomycin C and duration of the therapy is reported.

IV. Materials and methods

Database

This retrospective study included 126 horses diagnosed with ocular SCC between 2012 and 2022. The data contains horses of different breeds between 3 and 38 years of age. The selection of the database was based on the clinical findings and suspected diagnosis of any stage of an ocular or periocular SCC. The examination and treatment were guided by Professor Dr. Dr. József Tóth at the clinic "Tierärztliches Kompetenzzentrum Karthaus GmbH, Abteilung Augenheilkunde". This TDK is based on the casereports including the anamneses, general and ocular examination, surgery, treatment, and follow up.

An Excel-table made available information about the breed, age, affected eye, localisation, duration, enucleation due to recurrence, histopathological findings, and therapy methods. The breeds Pura raza espanola, Irish horse, German sport horse, KWPN, Rheinländer, Oldenburger, Hannoverian, Westfalian, and Pinto are described as warmbloods, Shetland pony, Isländer, German sport pony, Welsh Pony, and Connemara as ponys, Paint horse and Appaloosa as Quarter Horses, and French horse, Tinker, Shire horse as coldbloods.

The age data is divided into groups: 1. group of horses: 0-5 years of age, 2. group: 6 -20 yrs of age, 3. group: above 20yrs of age. The management of the disease is expressed in weeks. The histopathological findings are summarized as SCC maligne, SCC in-situ and precancerous stage.

The SCC malign includes invasive and noninvasive SCC. Invasive SCCs are invading the basal layer to the subepithelial structures. Depending on the differentiation the typical keratin pearls, whorl formation, and intercellular bridges (well-differentiated) or accumulations of tumor cells and little keratinization were seen (poorly differentiated). Non-invasive SCCs are affecting the basilar layer of the epithelium. Typical are pleomorphic cells, mitotic figures, hyperchromatic nuclei, and decreased polarity. The SCC in situ (plaque) occurs mostly in the stratum spinosum, but every part of the epithelium can be included. The precancerous stage includes epithelial hyperplasia, dysplasia and inflammation (64).

Classification of the patients

Patients with the diagnosis of a limbal, corneal or conjunctival SCC were distributed into 2 groups according to the treatment used:

Group 1: Patients treated with only keratectomy or conjunctivectomy (surgery)

This group of horses included 63 horses of 126 cases (66 eyes), that were treated only surgically.

Group 2: Patients treated with keratectomy and Mitomycin C

This group of horses included 63 number of 126 cases (71 eyes) treated with keratectomy and Mitomycin C.

All patients which had finally enucleation due to recurrence are described later.

Histopathological examination

The owners were advised to get results from cytology or biopsy at the examination. Because of recurrence, previous histopathological results, enucleation, or financial reasons, not all cases had histopathological examination. During examination biopsy from the hyperplastic tissue was taken. In case of surgical excision or debulking, the excised tissue was sent to the lab in Formaldehyde 4%. According to the histological findings, the most feasible therapy was selected. Macroscopic and histological findings are seen in **Figures 1** and **2**.

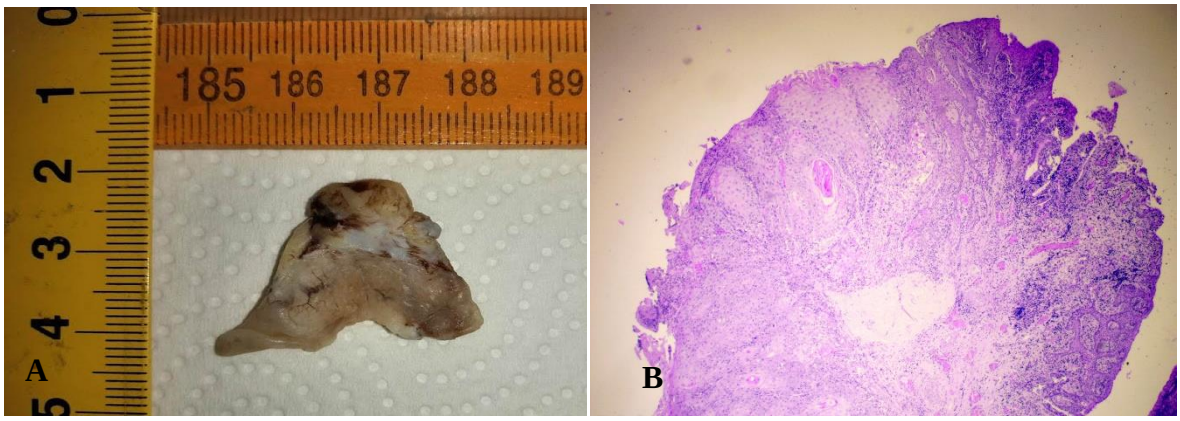

Figure 1*: (A) Macroscopic image of an excised nictitans with high-grade (Grade II.) SCC (carcinoma planocellulare keratoides infiltrans). (B) Epithelial subbasal cell proliferation with exulceration, without vascular or lymphovascular invasion (H&E stain*

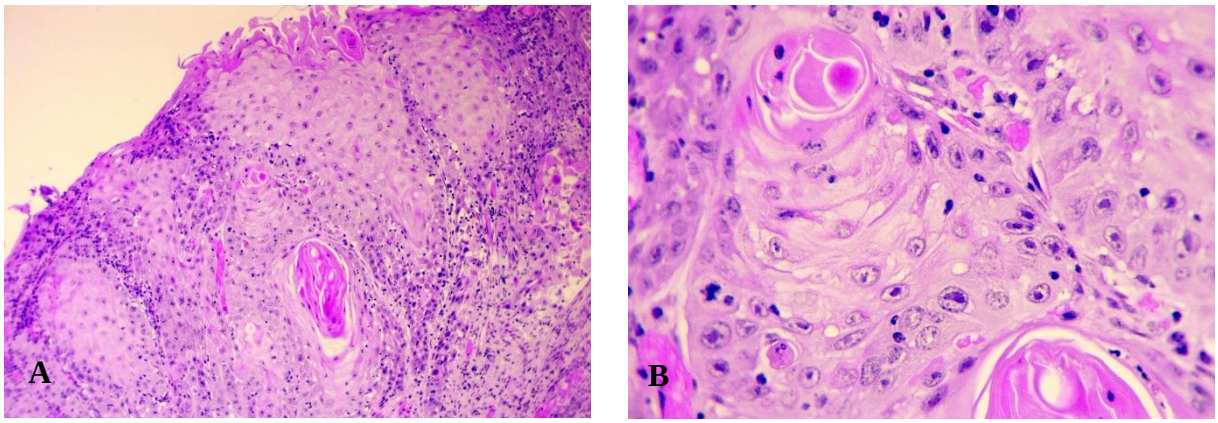

Figure 2*: (A) Anisocytosis, anisokaryosis, nucleo-, and nucleolomegalia (H&E stain, 100x) (B) Keratinisation, moderate mitotic index (8/10), (H&E stain, 400x) The therapy methods include surgery (keratectomy) and application of Mitomycin C eye drops or c*

History and examination

History about any previous ocular disorder, duration of the symptoms, previous treatment, and loss of vision were recorded. A general examination, especially on the cardiovascular system, was performed. The ophthalmic examinations were carried out by the same examiner (Prof. Tóth), using his protocol (Tóth: Augenheilkunde beim Pferd, Lehrbuch und Atlas) (57).

For ophthalmic examination intravenously injected detomidine (0.02mg/kg, Cepesedan®, CP Pharma Handelsgesellschaft GmbH, Burgdorf) was used for sedation in all horses. During the examination special inspection of the lids, nictitans, limbocorneal area of the temporal/dorsotemporal canthus, cornea, and sclera was performed.

Treatment

After examination the therapy plan was chosen by Prof. Tóth and discussed with the owner. Prof. Tóth summed the possible treatment options at that clinic, which served as reference: "Multimodal management of periorbital squamous cell carcinoma in horses with keratectomy, laser ablation and topical Mitomycin C" (4). According to this guideline, primer SCC and recurrent cases were treated with debulking and Mitomycin C, so adjunctive therapy was offered to the owner to prevent possible recurrence. After sedation horses were fixed with a headstand, and the nasolacrimal duct was flushed retrograde. The conjunctiva and nictitans were flushed with 0.5% povidone-iodine solution, then topical anaesthesia was performed with articainhydrochlorid/Epinephrin (Ubistesin 1/400000 40mg/ml+2,5mikrogramm/ml Injektionslösung; 3M Deutschland GmbH, Neuss). For retrobulbar and peribulbar nerve blocks the Optic, Lacrimal, and Zygomatic nerves were anaesthetised with proparacaine injection.

Keratectomy

Superficial lamellar keratectomy or keratoconjunctivectomy was performed in standing position. The first incision was done on the cornea at the limbal region as deep as the abnormal structure was visible. A special scalpel, Beaver blade (Ophthalmic Satin Crescent Knife, Alcon Novartis company, Alcon Laboratories, Texas USA) was used to provide sharp incision between the stromal cells to ensure proper healing. A clear-cut of a minimum of 2mm healthy conjunctiva and 1mm cornea around the lesion was made. Images of these stages are seen in **Figure 3**. Postoperatively topical 1 % atropine was applied to the affected eye once, when indicated SID further. Flunixin-meglumine 110mg/day (Flunidol ® RP 5%, CP-Pharma, Burgdorf) as NSAID was given orally SID.

Figure 3: *(A) Limbal carcinoma planocellulare (SCC). (B) Carcinoma planocellulare, intraoperative image (C) Carcinoma planocellulare, postoperative image right after surgery.*

Mitomycin C

63 horses (Group 2) after the keratectomy got Mitomycin C 0,04% eyedrops every 4 hours for 7 days, starting right after the surgery. Images of each stage can be seen in **Figure 4**. In case of recurrence, topical Mitomycin C was readministered after a 7-day break. Mitomycin C therapy was advised as an adjunctive therapy regardless of the horse or tumor. Due to previous studies showing a lower rate of neoplasm, Mitomycin C treatment was recommended to patient owners. The decision was made based on the owners' economic possibilities.

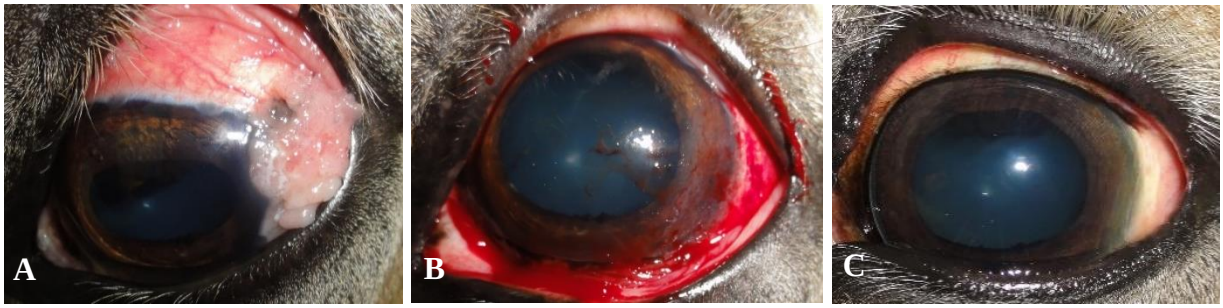

***Figure 4**: (A) Limbal carcinoma planocellulare (B) Carcinoma planocellulare, after surgery (C) Carcinoma planocellulare, after treatment*

V. Results

Description of the patients' data

Between 2012 and 2022 126 horses (137eyes) with suspected SCC were shown up in the clinic and were diagnosed and treated in the department of Ophthalmology.
In 2012, 2013, and 2014 four patients, respectively, in 2015 8, in 2016 15, in 2017 18, in 2018 25, in 2019 15, in 2020 17, in 2021 4, and in 2022 12 patients were examined (**Figure 5**). The number of patients were approximately distributed with a peak in 2018 with 25 patients.

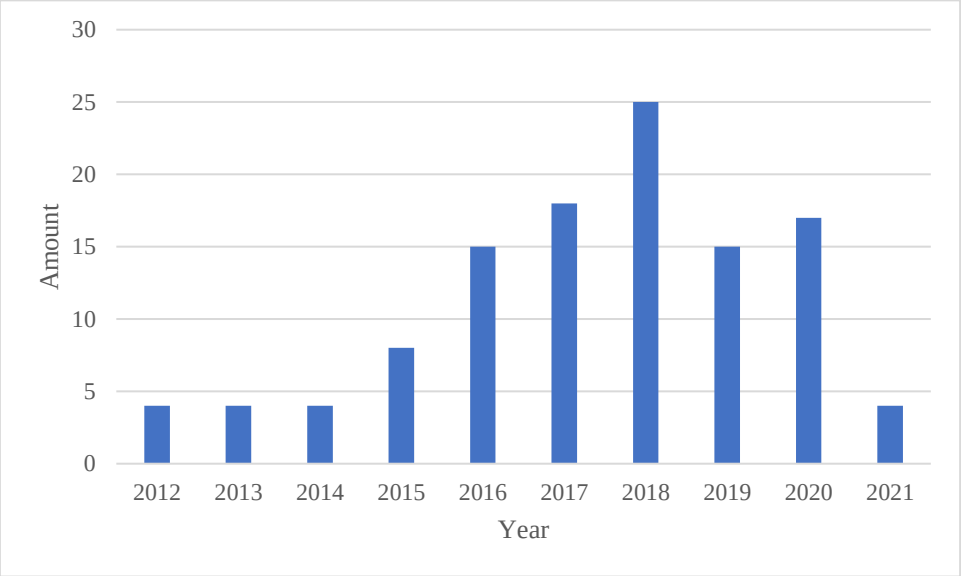

***Figure 5**: Number of patients with ocular or periocular SCC, year 2012-2022 (n=126)*

SCC lesions

Location of the lesions

91.27% of the patients had unilateral, and 8.7% (11 of 126) of patients bilateral lesions. The left eye was affected in 44.4% (56 of 126) of the patients, and the right eye in 46.8% (59 of 126) (**Figure 6**).

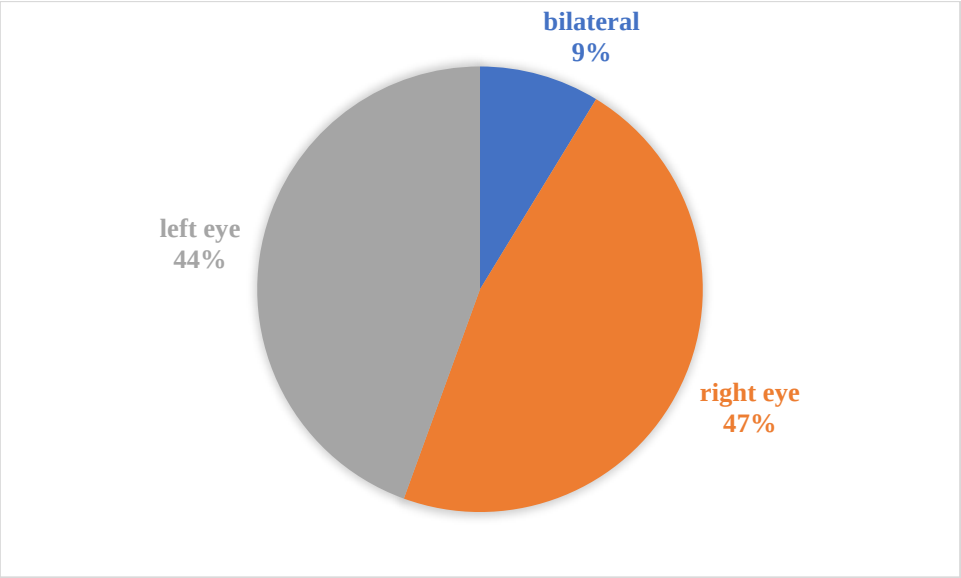

***Figure 6**: Patients distribution found with unilateral or bilateral lesions.*

Therapy

Group 1: Patients treated without Mitomycin C

50 % (63 of 126) horses were treated with surgery only.

Age

The middle age was 17.5 years. The oldest horse treated without Mitomycin C was 29 years old, the youngest 3 years old. The most represented age group was 6 to 20 years (**Figure 7**).

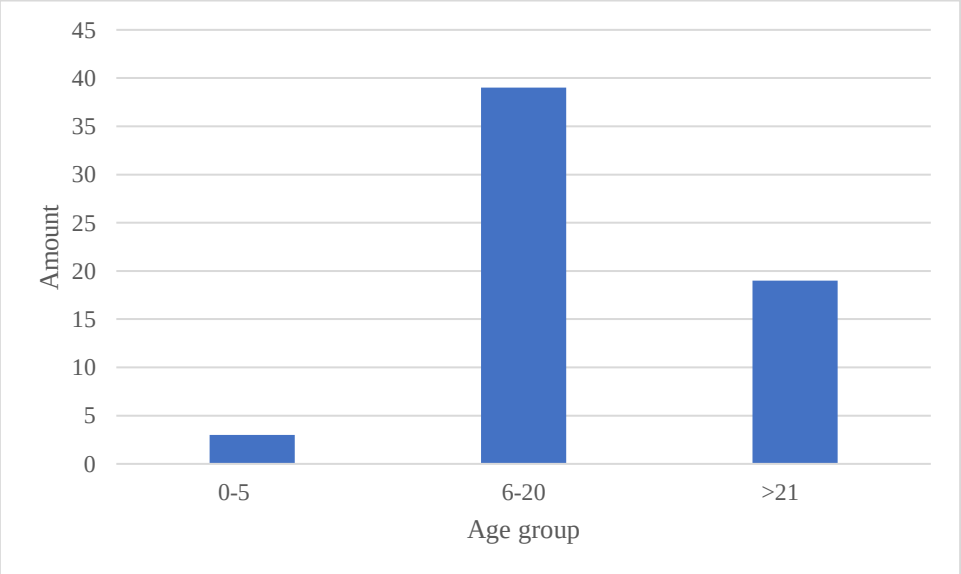

***Figure 7**: Distribution of age groups (Group 1)*

Breed

This group included 47.6% (30 of 63) Haflingers, 22.2% (14 of 63) warmbloods, 11.1% (7 of 63) ponies, 6.3% (4 of 63) coldbloods. 7.9% (5 of 63) quarter horses, 3.2% (2 of 63) Arab Horses, and 1.6% (one of 63) thoroughbred (**Figure 8**).

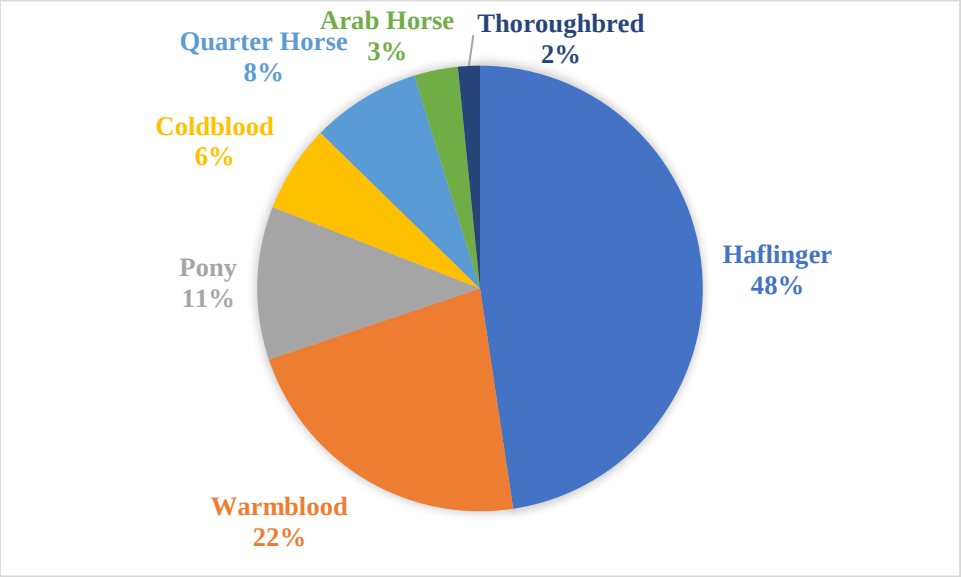

***Figure 8**: Breed distribution (Group 1)*

Location

Nineteen horses (No. 19/63), 30.71 % in Group 1. had SCC at the cornea and conjunctiva. 18 of 63 horses (28.57%) in this group had tumor at the nictitans. The third most common site was the conjunctiva (14 of 63, 22.23%). 7 horses of 63 (11.12%) had abnormalities at the lids, 3 at multiple locations (4.76%). Two horses had SCC only on the cornea (3.17%) (**Figure 9**).

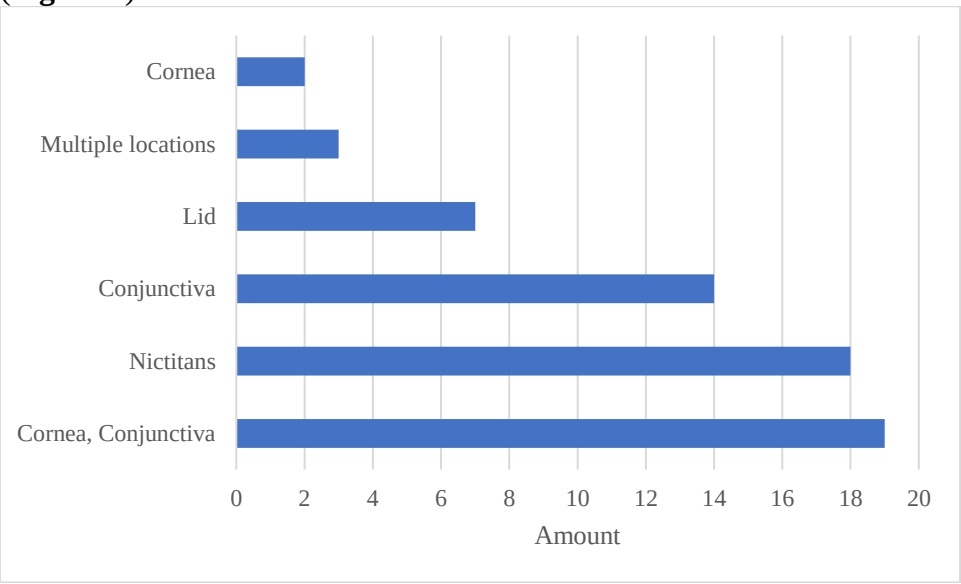

***Figure 9**: Location of the tumors (Group 1)*

Cytology

Macroscopic appearance of SCC was confirmed in 66.67% (42 of 63). 33 horses of 63 had a proven malign SCC (52.38%), 5 had an in situ (7.94%)., and 4 of 63 clients (6.35%) had tumor at precancerous stage.
33.34% of the cases had no histopathological examination either because of the owner's disagreement, or obvious macroscopic appearance (**Figure 10**).

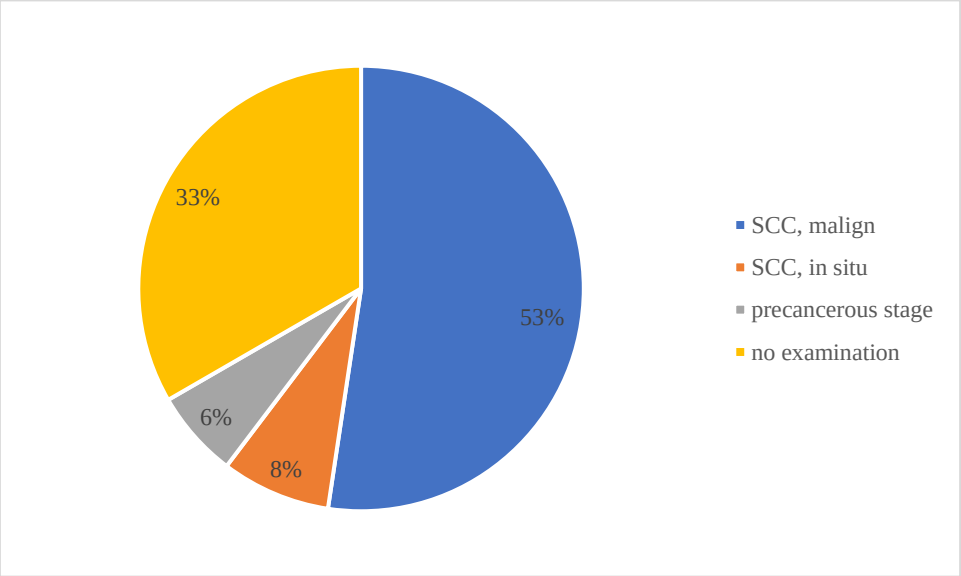

***Figure 10**: Results of histopathology (Group 1)*

Recurrence

One year after treatment horse owners informed about the recurrence of the SCC. Due to the lack of information from the owner, further details of the recurrence weren´t documented.
76.19% (48 of 63) horses recovered without recurrence, 23.81% (15 of 63) of patients showed regrowth at 1 year follow up (**Figure 11**).

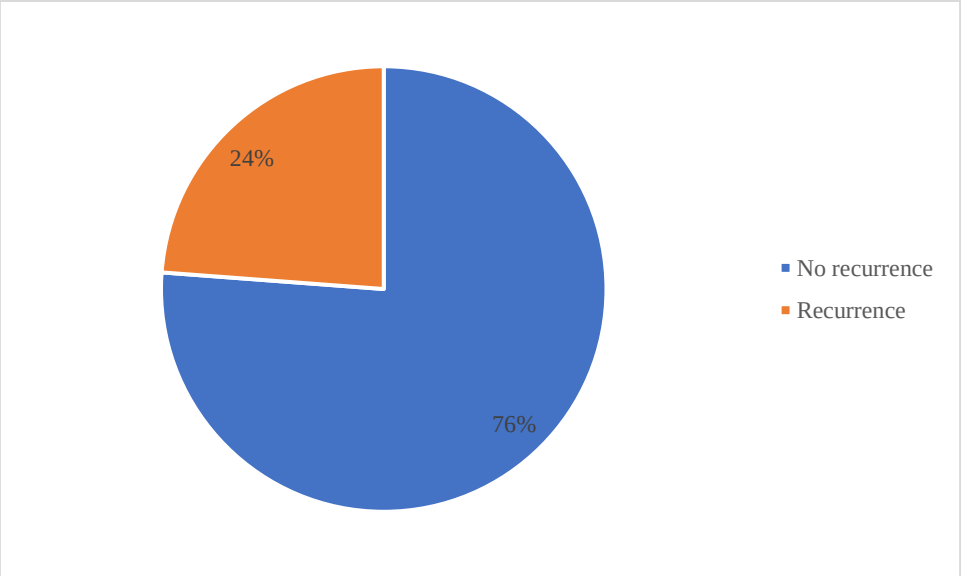

***Figure 11**: Recurrence rate (Group 1)*

Group 2: Patients treated with Mitomycin C

50% (63 of 126) the horses had treatment with Mitomycin C.

Age

Horses treated with Mitomycin C had a middle age of 15 years. The youngest was 4, and the oldest 38 years old. Most of the clients were 6 to 20 years old (47 of 63. 74,6%) (**Figure 12**).

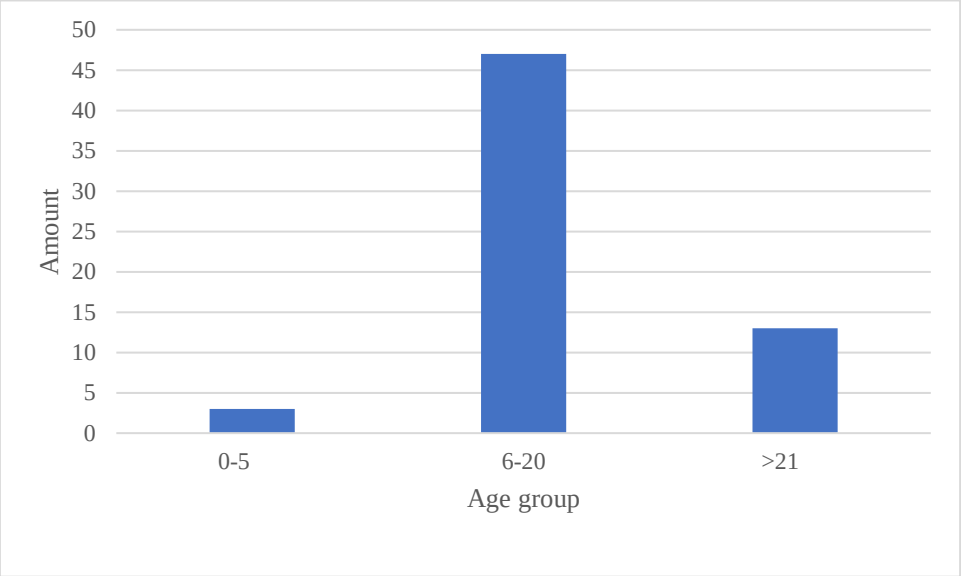

***Figure 12**: Distribution of age groups (Group 2)*

Breed

The most Mitomycin C treated breed group was the Haflinger (40 of 63 horses) (63.49%) followed by warmbloods (12 of 63 horses) (19.04%). 5 ponies (7.93%), 4 coldbloods (6.34%), and 2 Quarter Horses (3.17%) (**Figure 13**).

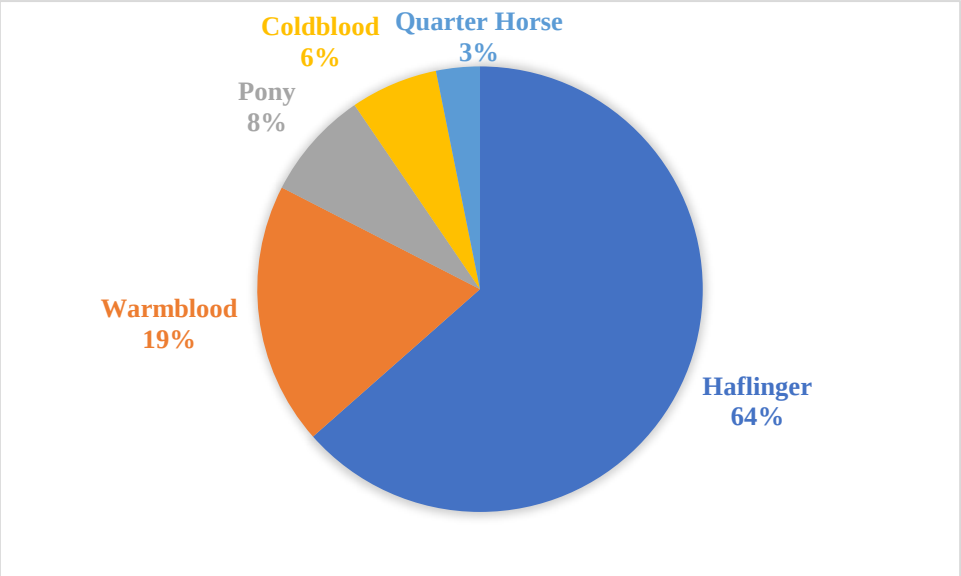

***Figure 13**: Breed distribution (Group 2)*

Location

Mitomycin C was used in 22 of 63 horses (34.93%) with corneal and conjunctival involvement. The second most common site was the nictitans (21 of 63, 33.3%). In 8 of 63 horses (12.69%) had SCC on the conjunctiva or at multiple locations. 1 horse with corneal, and another with skin involvement was treated (3.17%) (**Figure 14**).

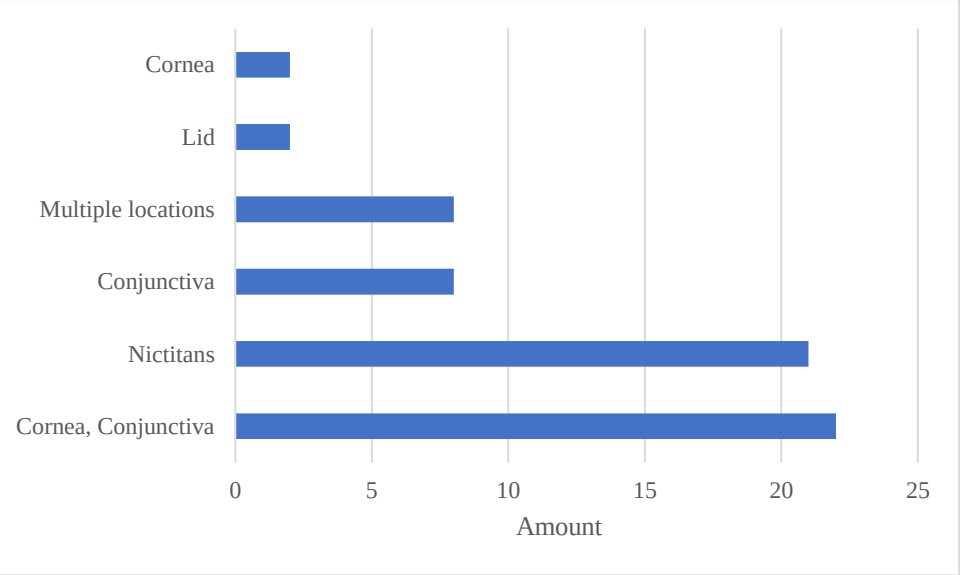

***Figure 14**: Location of SCC (Group 2)*

Cytology/histopathology

71.4% (45 of 63) of the patients treated with Mitomycin had a proven malign SCC. 9.52% (6 of 63) had carcinoma in situ. Precancerous stage was confirmed in 7.94% (5 of 63) of this group. One patient had either adenocarcinoma, or blastoma, or basosquamous SCC (5%). 6.5% (4 of 63) of the horses had no histopathological examination (**Figure 15**).

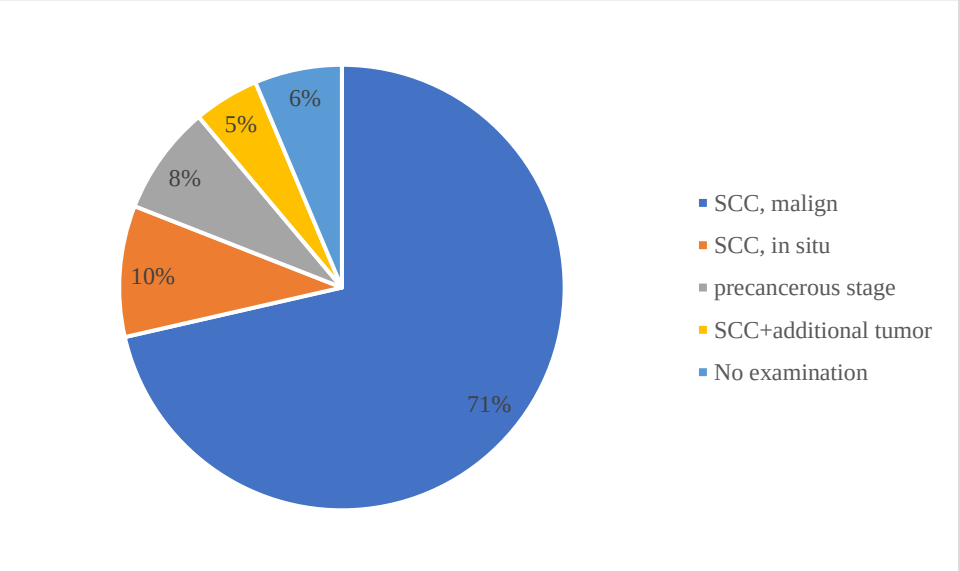

***Figure 15**: Results of histopathology (Group 2)*

Duration of the therapy

Most of the horses were treated for one week (43 of 63). Second most common duration was 4 weeks (11 of 63). Only 9 of 63 patients were treated longer than 4 weeks. The longest duration was 42 weeks (**Figure 16**).

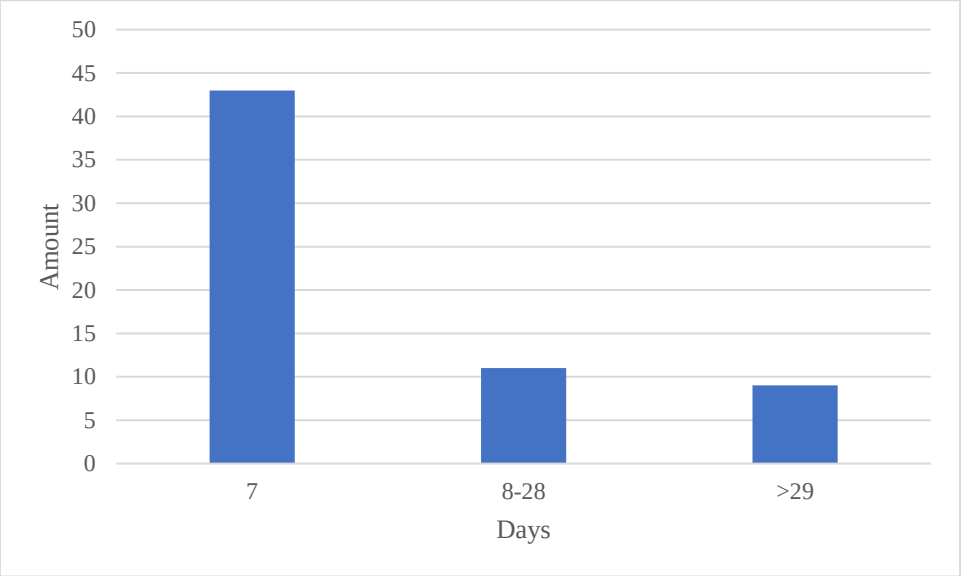

***Figure 16**: Duration of treatment (Group 2)*

Recurrence

27.0% (17 of 63) of the clients showed tumor recurrence in this group, while most of them remained healthy: 73% (46 of 63) (**Figure 17**).

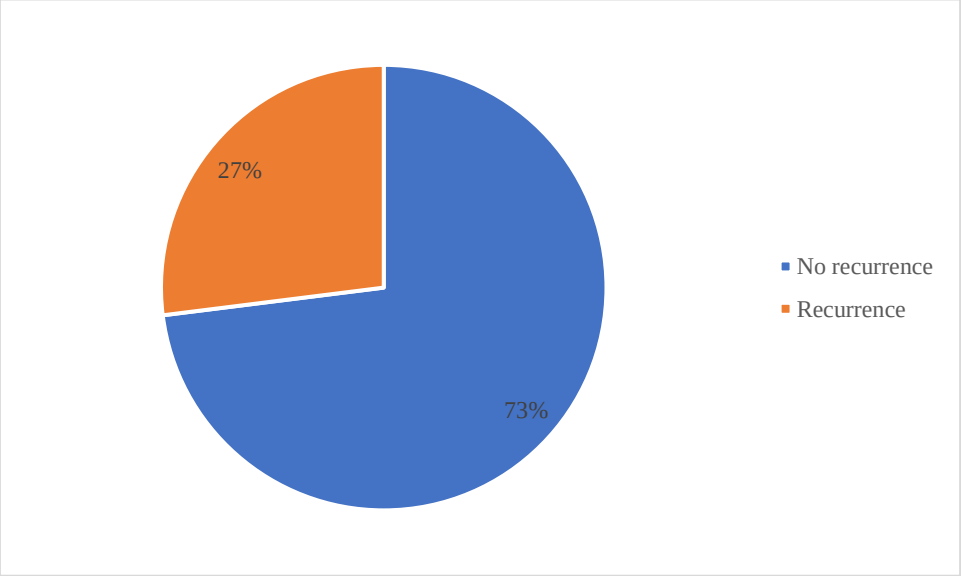

***Figure 17**: Recurrence rate (Group 2)*

Statistical analysis

The recurrence rates of patients in Group 1 and 2 were compared statistically to prove the effectiveness of Mitomycin usage. The ANOVA test was used, as the mean values were

normally distributed. The F-statistic measures the ratio of variation between group means to the variation within the groups. A lower F-statistic suggests that the means of the groups are similar relative to the variation within each group. The p-value is the probability of observing the given data if the null hypothesis (which states that all group means are equal) is true. F-statistic: 0.16, P-value: 0.68. In this case, the relatively high p-value (0.6852) shows that there is no significant difference. P:<0.05 was considered significant.

Enucleation

Enucleation was performed in 26 of 126 horses (20.63%), either because of poor prognosis, or advanced tumour growth, or uncontrollable pain and inflammation of the eye (**Figure 18**).

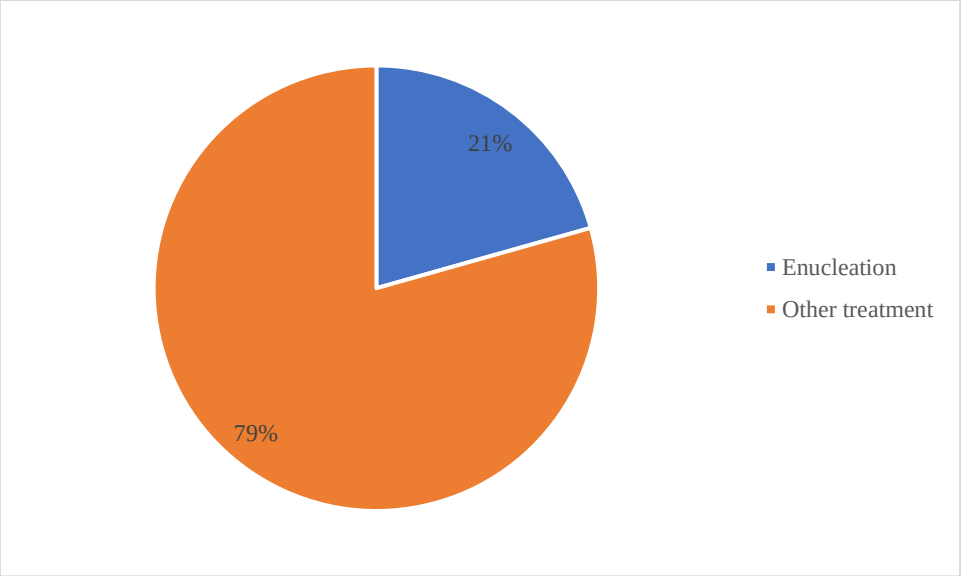

***Figure 18**: Enucleation*

Only 1 horse got Mitomycin C that had enucleation (3.84%), the other enucleated horses had only keratectomy (25/26 horses, 96.15%) (**Figure 19**).

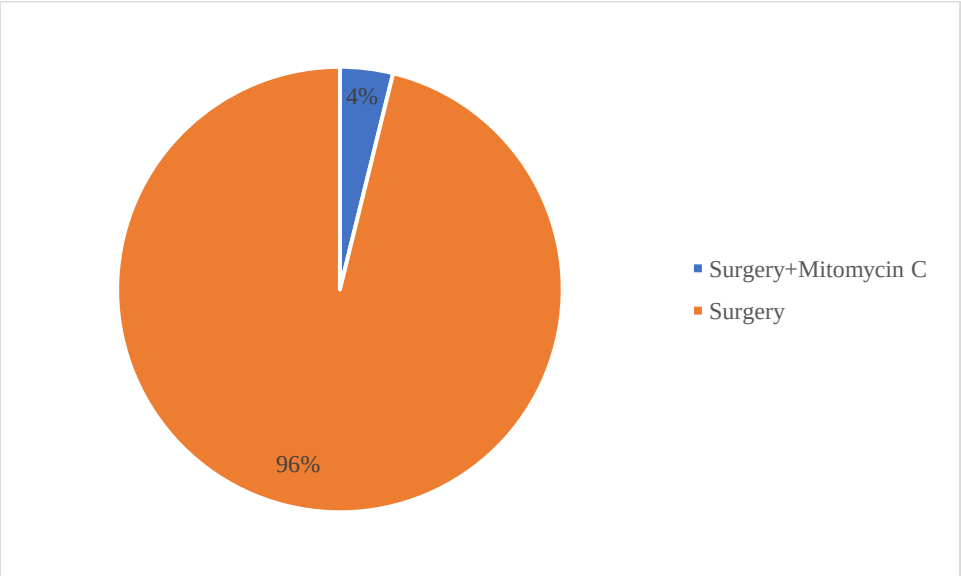

***Figure 19**: Previous treatment in the enucleation group*

To compere the treatment distribution among enucleated patients, again the ANOVA test has been performed. The F-statistic counts the ratio of variation between group means to the variation in the groups. A higher F-statistic explains that the means of the groups are more different relative to the variation in each section. The p-value is the probability of observing the dataset if the null hypothesis is true. The results are F-statistic: 35.29, P-value: 2.67×10^{-8}. The very small p-value (2.67×10^{-8}) shows strong evidence against the null hypothesis. Therefore, as a conclusion there is a significant difference.

VI. Discussion

Squamous cell carcinoma is a slow growing tumor which occurs in different species (1; 2). It is the most common tumor type in the equine eye and adnexa (7; 41). Metastasis is seen rarely (5; 7). The aetiogenesis of the disease is not cleared yet, but genetic predispose, age, breed, exposure to ultraviolet light and infections is put in connection with it (10;13;41). It is known that especially Haflinger Horses, coldbloods and Paint horses are more likely to get it (11;14;15). It can be described in the connection with a mutation in the DDB2-allel, mostly in Haflinger Horses (16). Aswell less periocular pigmentation and the fur colour chestnut are predisposing factors for the growth (17; 36). Affected patients are middle to higher aged (11; 16; 17). The risk of ultraviolet light of solar radiation is proven in humans. Apoptosis or unhealthy cells will appear if there is a disturbed p53 tumour suppressor. It is also expected in animals (4; 20; 22; 24).

The diagnosis is based on a thorough inspection and microscopical examination of biopsy samples. The first signs of growth can be plaque or a wound, followed by a cauliflower like appearance (10; 26). It may decrease the function of the eye, furthermore, cause inflammation (7;10).

There are different types of treatment options possible, but the combination of them shows better results, depending on the appearance of the lesion (4; 29; 39). Basic surgical removal is used to decrease the size of the neoplasm if it is possible (7; 10; 34). Other common therapies are cryotherapy (17), high frequency hyperthermia (43), beta-radiation with Strontium 90 (29), gamma-radiation with Iridium 192 (32), laser ablation with carbon dioxide (18), and topical treatment with mitomycin C (4). Mitomycin C is a chemotherapeutic antibiotic which is used in human medicine. Because of few side effects locally, 0.04% Mitomycin C is applied in equines as well to decrease the recurrence rate of SCC (4; 7; 48).

In our study the dataset contains 126 equine patients (137 eyes) courtesy of the ophthalmologist Professor Dr. Dr. József Tóth at the clinic “Tierärztliches Kompetenzzentrum Karthaus GmbH, Abteilung Augenheilkunde”. Our experiences are summarised as clinical aspects and results of the usage of Mitomycin C to treat SCC. The dataset includes data from 2012 to 2022, most of them had a cytological proven SCC. In another study of Panndorf, which was done in 1970, only 5 horses within 15 years showed ocular SCC (60). In 1976 Strafuss described 9 patients within 5 years (2). Keller informed about 195 patients in 15 years, from 1995 to 2010 (26). Due to the previously discussed increasing diagnostic possibilities and knowledge of horse owners, animals are subjected to more detailed examinations. Veterinary medicine improved diagnostic and treatment options in the last century and more ophthalmologists are known for their results. In middle Europe Professor Dr. Dr. József Tóth is popular for his excellent knowledge in equine ophthalmology. On the other part, the ultraviolet light of solar radiation increased in the last years. Especially workers, who have a high exposure to sunlight suffer from skin tumours (4; 23). Both may explain higher occurrence of ocular and adnexal SCC in the last 10 years in this study.

Data of both groups (treated with adjuvant-Group 2 or without Mitomycin C-Group 1) were compared and showed approximately the same results. The whole dataset contains patients with an age from 3 to 38 years. If they are spitted in age groups to simplify the opportunities

to testify, most of the patients were 6 to 20 years old in both treatment groups. The mean age was 16 years of all patients. If it is compared to other studies, younger mean age was found: In 1985 Monsunic informed about the mean age of 12.1 years (17). Michau described a mean age of 12.4 years in 2011 (18). Keller found a mean age of 13.4 years (26). The recent study contains more older equids; it stays in connection with the changed use of horses and higher willingness of owner to finance treatments, care about preventive care and better holding conditions. So, the horse population looks older in these years in Germany and in Europe. In general, horses between 6 and 20 years are used as sport partners and pets, that's why owners have everyday contact to their animals and detect diseases earlier. Very young and old horses usually live on pastures, that's why they don't get checked so frequently, and inconspicuous plaques or lesions are not detected.

More than half of the dataset included Haflinger horses. Second most common breed was the Warmblood. In the comparison with breeding statistics of the German Fédération Equestre Nationale, Haflinger horses are less common than warmbloods. In the year of 2022, 54.113 breeding warmbloods and 23. 901 breeding ponys and small horses, the breed Haflinger is included in it, were registered in the official system (59). Haflinger is an upcoming Austrian breed. In mountain areas these horses were excellent carrying and draft animals, that's why Germans started the breeding during the world wars. Nowadays they are used as allrounders and special lines for sports. They are light to dark chestnuts with white mane and tail (61). Their skin colour is the most affected in SCC (17). The breed is also known for a missense mutation at the DDB2-allel, which is a risk factor for SCC (16). Studies from different countries are describing other horse breeds as more common, it depends on geographic location of the clinic and on popularity of horse breeds in that country. Dugan detected a prevalence in Quarter horses, draft breeds, and Appaloosas in the USA (11).

Mosunic reported bilateral involvement of 19.8% (n=18) (63). In another study 5 of 43 cases showed both sided lesions (34). Our study also showed that most of the patients (91.2%) had unilateral growth. If more detailed localization is compared, in other studies the most common sites of SCC were the nictitans or lid, in contrast in our study, where SCC growth on the cornea and conjunctiva (30.17%, 34.93%) was detected most often, but followed by the nictitans in both groups (28.57%, 33.3%) (17; 34; 47; 63).

Group 1 (63 horses of 126) was treated with keratectomy or conjunctivetcomy (surgery). 47.6% of the subjects were Haflinger. 66.67% of Group 1 had a positive histopathological result. The dataset shows no recurrence in 24% of the cases. Overall, recurrence must be described in combination with therapy and duration as well as size and invasiveness of the SCC, as every tumor growth needs a specific individual treatment, that's why it is difficult to compare it. The other factor to consider is that there are too many variables that are different in each individual case to compare and interpret the results. If recurrence rates of other studies are compared, patient numbers, experience of the surgeon should be taken into consideration. In 1974 Gelatt and Myers reported 25% recurrence rate in surgery cases in affected conjunctiva (46). In another study, surgical excision alone had 44.10% recurrence rate (n=91) (17). We suppose that in our study the wide experience and great knowledge of the surgeon resulted low recurrence rate in the single therapy with surgical excision.

In this study additional Mitomycin C treatment was used in 50% (63 of 126) of the cases (Group 2). Mitomycin C can be used as topical eyedrops or applied with a soaked sponge as an additional treatment (49). In our study, when topical Mitomycin was used, 63.49% of the patients were Haflinger. 93.65% of this group showed a positive result in histopathology. The common treatment duration was one week. The results describe the recurrence rate as 27% of the cases. In comparison to the statistical outcome of these 2 groups, Mitomycin C doesn't influence the recurrence significantly (P: 0.68).

We found similar recurrence rates to other reports in case of effectiveness. Rayner explained 30% recurrence rate with Mitomycin C single treatment (n=10) (49). Malalana reported a recurrence rate of 25% with single Mitomycin C treatment, 23% in combination with surgical excision (n=14) (50). Another study found, that 95% of the clients were cured after surgery with adjuvant Mitomycin C therapy for 1 week (4). Aswell it is used successfully in human medicine. Because of less side effects it is useful and effective. Gupta described no clinical recurrence in 90 human patients, treated with surgery and Mitomycin C, in 56.8 months follow up study (65). In another human report 100% healing in 22 months was described (66). In a report with a long term follow up of two years no recurrence was documented (35).

Twenty-six of 126 horses of the database needed enucleation, only 1 horse was treated with keratectomy and Mitomycin C topically before. Rayner described as well only one horse post Mitomycin C treatment which needed eye extraction (n=10) (49). Another report showed 13 enucleations of 91 horses, which didn't receive an adjuvant therapy before (17). Because of comparable results of other studies, we can come to the conclusion that the adjuvant therapy with Mitomycin C decreases the chance of enucleation (P: 2.67×10^{-8}).

If all studies also are taken into account, Mitomycin C shows a stabile and low recurrence rate and very low enucleation rates. It can be assumed that Mitomycin C as a therapy in the majority of patients, depending on the tumor, supports healing and does not lead to tumour recurrence or enucleation indication. As discussed, recurrence decreases with combination of therapy options. As single therapy it may be used in smaller sized abnormalities and early detected plaques. Among human and veterinary ophthalmologists, topical Mitomycin C is a popular drug due to its low side effects and good results. In his long career as an ophthalmologist, Prof. Tóth has used a number of different treatment methods. In his experience, depending on the tumor size, multimodal treatment with surgery and postoperative topical Mitomycin C is the most successful combination. Malalana also used primarily surgery and postoperatively Mitomycin C treatment topically and received good results.

As this is a retrospective study certain limitations are present because of data collection and owners information. Further studies are needed to describe the aetiopathogenesis, especially the possible influence of ultraviolet light of solar radiation in each case. Aswell new popular horsebreeds and their genetic lines should be checked for genetic predisposition, to improve breeding results. Especially in chestnuts or more common effected breeds, during a yearly check or vaccination program veterinarians should check the eyes regularly to recognize SCC as early as possible.

VII. Abstracts

English Abstract

The ocular and periocular squamous cell carcinoma (SCC) is a slowly growing tumour. It is the second most common neoplasm in equines, and the most common in the eye and adnexa. Metastasis is rarely recognized and is only in severe cases detectable. The growth starts in plaques and epithel hyperplasia. The typical shape is cauliflower like. The pathogenesis is not cleared yet, but genetic predisposition, periocular depigmentation, age, ultraviolet light of solar radiation, viral infection and hormonal status influence the appearance of SCC. Different therapies are preformed depending on the location, size, invasiveness, and shape of the neoplasm. Usually, a combination of different treatment possibilities are used to decrease the recurrence rate. Surgical removal, cryotherapy, high frequency hyperthermia, beta-radiation with Strontium 90, gamma- radiation with Iridium 192, laser ablation with carbon-dioxide, Bacillus Calmette-Guerin vaccine and topical treatment with Mitomycin C is described for the therapy of SCC.

This study includes 126 equine patients of a German Equine Hospital ("Tierärztliches Kompetenzzentrum Karthaus GmbH, Abteilung Augenheilkunde"). The retrospective study reports different ocular SCC cases and their treatment possibilities to compare surgical removal and adjunctive therapies. The dataset was split in two groups: group of horses treated with and without Mitomycin C. Data were analysed and compared regarding the age, breed, location, cytology, and recurrence using descriptive statistics. Minimum, maximum, and mean age of the patients were 3, 38 and 16,26 years of age. Most common breed was the Haflinger (55,6%). Unilateral tumour growth was mostly detected in 91,2% of the cases. Cornea and conjunctiva were affected in 32,53% of the cases. Majority of the cases (78%) was confirmed microscopically, each one horse additionally with blastoma, adenocarcinoma, or basosquamous SCC was detected. Recurrence rate was 25,4%.

In the group of Mitomycin treated patients had a minimum, maximum, and mean age was: 4, 38, and 15 years of age, respectively. 63,49% of them were Haflinger and most common site was cornea and conjunctiva. (34,92%). Recurrence rate was 27%.

Horses treated without Mitomycin C had a minimum, maximum, and mean age was 3, 29, and 17,5 years. 47,6% were Haflinger. Overrepresented location was the cornea and conjunctiva. (30,17%) The recurrence rate was 23,8%.

As a conclusion we used surgical excision in combination with the topical application of mitomycin C. This treatment did not show a significant improvement in results compared to exclusive surgical treatment. However, this multimodal therapy significantly reduced the need for subsequent enucleation.

Hungarian Abstract
Az okuláris és szemkörnyéki laphámsejtes karcinóma (SCC) egy lassan növekvő daganat. Az SCC a lovak második leggyakoribb daganata, és a leggyakoribb daganat a szem és a környező szövetek területén. Metasztázisok ritkán mutathatók ki, és csak súlyos esetekben diagnosztizálhatók. Kezdetben plakkok és epithelhiperplázia formájában jelenik meg, majd kifejlődik egy tipikus karfiolszerű forma. A patogenezis még nem teljesen tisztázott, de genetikai hajlam, szemkörnyéki depigmentáció, életkor, napsugárzás, ultraviola fény, vírusfertőzés, és hormonális állapot is befolyásolhatja az SCC kialakulását. A neoplazma helyétől, méretétől, invazivitásától, és alakjától függően különböző terápiás módszereket alkalmaznak. Általában a kiújulás kockázatának csökkentése érdekében különböző kezelési lehetőségek kombinációját alkalmazzák, mint például sebészeti eltávolítás, krioterápia, nagyfrekvenciás hipertermia, béta-sugárzás Strontium 90-nel, gamma-sugárzás Iridium 192-vel, lézeres abláció szén-dioxiddal, Bacillus Calmette-Guerin vakcina, és helyi kezelés Mitomycin C-vel.

Ez a tanulmány 126 lóbeteg adatain alapul, akik egy német lókórházban („Tierärztliches Kompetenzzentrum Karthaus GmbH, Abteilung Augenheilkunde") kezeltek. A retrospektív vizsgálat különböző okuláris SCC eseteket és azok kezelési lehetőségeit mutatja be, összehasonlítva a műtéti eltávolítást és a kiegészítő terápiákat. Az adatkészletet két csoportra osztották: Mitomicin C-vel kezelt és nem kezelt lovak csoportjára. Az adatokat életkor, fajta, hely, citológia és recidíva szempontjából elemezték és összehasonlították, leíró statisztikákat alkalmazva. A betegek életkorának tartománya 3 és 38 év között mozgott, átlagosan pedig 16,26 éves. A leggyakoribb fajta a Haflinger volt (55,6%). Az esetek 91,2%-ában többnyire egyoldalú daganatnövekedést figyeltek meg. A szaruhártya és a kötőhártya az esetek 32,53%-ában volt érintett.

A betegek túlnyomó többsége (78%) mikroszkóposan igazolták. Ezen felül egy lónál blastómaképződést, egy másiknál adenokarcinómát, és egy harmadiknál basosquamous SCC-t észleltek. A kiújulási arány 25,4% volt. A mitomicinnel kezelt betegek csoportjában a minimális, maximum és átlagéletkor 4, 38, és 15 év volt. Közülük 63,49%-uk Haflinger fajtájú volt, és leggyakrabban a szaruhártya és a kötőhártya területe volt érintett (34,92%). A kiújulási arány 27% volt. A mitomicin C nélkül kezelt lovak minimális, maximum és átlagéletkora 3, 29, és 17,5 év volt, közülük 47,6%-a Haflinger fajtájú volt. A leggyakoribb érintett területek továbbra is a szaruhártya és a kötőhártya voltak (30,17%). Az ismétlődési arány 23,8% volt.

Következtetésként a sebészi kivágást a mitomycin C helyi alkalmazásával kombinálva alkalmaztuk. Ez a kezelés nem mutatott szignifikáns eredményjavulást a kizárólagos sebészeti kezeléshez képest. Ez a multimodális terápia azonban jelentősen csökkentette a későbbi enukleáció szükségességét.

VIII. References

1 Brian P. Wilcock, Bradley L. Njaa (1963): Spezial Senses. In: Jubb, Kennedy, and Palmer, pathology of domestic animals volume 1, Missouri, Elsevier.

2 Blodi, F. C., & Ramsey, F. K. (1967). Ocular Tumors in Domestic Animals. American Journal of Ophthalmology, 64(3), 627/109–633/115. doi:10.1016/0002-9394(67)905683Strafuss, a. c. (1976): squamous cell carcinoma in horses. j am vet med assoc 168, p. 61-62.

4 Prof J., Hollerrieder J., Buijs L. (2021) Multimodale Behandlung des Carcinoma planocellulare am Pferdeauge mit Keratektomie, Diodenlaser-Ablation und Mitomycin. Pferdeheilkunde 37, 278-283; DOI 10.211836/PEM20210309

5 S.Zanichelli, G. Pezzoli, M. Del Bue, P. Botti, P. Scrollavezza (1994) Observations on Squamous Cell Carcinoma in the Horse Pferdeheilkunde 10, 219-225

6 Knowles, E. J., Tremaine, W. H., Pearson, G. R., & Mair, T. S. (2015). A database survey of equine tumours in the United Kingdom. Equine Veterinary Journal, 48(3), 280–284. doi:10.1111/evj.12421

7 Estell, Krista (2017). Periocular Neoplasia in the Horse. Veterinary Clinics of North America: Equine Practice, 33(3), 551–562. doi:10.1016/j.cveq.2017.08.004

8 Arthurs, Callum; Suarez-Bonnet, Alejandro; Willis, Claire; Xie, Boyu; Machulla, Natalie; Mair, Tim S.; Cao, Kevin; Millar, Michael; Thrasivoulou, Christopher; Priestnall, Simon L.; Ahmed, Aamir (2020). Equine penile squamous cell carcinoma: expression of biomarker proteins and EcPV2. Scientific Reports, 10(1), 7863–. doi:10.1038/s41598-020-64014-3

9 Long-term outcome associated with intratumoral chemotherapy with cisplatin for cutaneous tumors in equidae: 573 cases (1995–2004) Alain P. Théon, dr med vet, ms, dacvr; W. David Wilson, bvms, ms, dacvim; K. Gary Magdesian, dvm, dacvim, dacvecc, dacvcp; Nicola Pusterla, dvm, phd, dacvim; Jack R. Snyder, dvm, phd, dacvs; Larry D. Galuppo, dvm, dacvs

10 Simone Kaps; Marianne Richter; Martin Philipp; Madeleine Bart; Corinna Eule; Bernhard M. Spiess (2005). Primary invasive ocular squamous cell carcinoma in a horse. , 8(3), 193–197. doi:10.1111/j.1463-5224.2005.00358.x

11 Dugan SJ, Curtis CR, Roberts SM et al. Epidemiologic study of ocular/adnexal squamous cell carcinoma in horses. Journal of the American Veterinary Medical Association 1991; 198: 251–256. 12 Brandt, S (2021). Papillomvirusinfektion als Ursache von Hauttumoren beim Pferd. Pferdespiegel 2021; 24:171-181

12 Brandt, S (2021). Papillomvirusinfektion als Ursache von Hauttumoren beim Pferd. Pferdespiegel 2021; 24:171-181

13 Drazek M., Lew M., Lew S., Szarek J., Balicki I., Della Salda L. (2015): Equine ocular squamous cell carcinoma: a case report. Veterinarni Medicina, 60: 379-386.

14 Lassaline, Mary; Cranford, Taryn L.; Latimer, Claire A.; Bellone, Rebecca R. (2015). Limbal squamous cell carcinoma in Haflinger horses. Veterinary Ophthalmology, 18(5), 404–408. doi:10.1111/vop.12229

15 Knickelbein, Kelly E.; Lassaline, Mary E.; Bellone, Rebecca R. (2018). Limbal squamous

cell carcinoma in a Rocky Mountain Horse: Case report and investigation of genetic contribution. Veterinary Ophthalmology, (), –. doi:10.1111/vop.12612
16 Singer-Berk, Moriel H.; Knickelbein, Kelly E.; Lounsberry, Zachary T.; Crausaz, Margo; Vig, Savanna; Joshi, Nikhil; Britton, Monica; Settles, Matthew L.; Reilly, Christopher M.; Bentley, Ellison; Nunnery, Catherine; Dwyer, Ann; Lassaline, Mary E.; Bellone, Rebecca R. (2019). Additional Evidence for DDB2 T338M as a Genetic Risk Factor for Ocular Squamous Cell Carcinoma in Horses. International Journal of Genomics, 2019(), 1–10. doi:10.1155/2019/3610965
17 C. B. Mosunic, P. A. Moore, K. P. Carmicheal et al., "Effects of treatment with and without adjuvant radiation therapy on recurrence of ocular and adnexal squamous cell carcinoma in horses: 157 cases (1985–2002),"Journal of the American Veterinary Medical Association, vol. 225, no. 11, pp. 1733–1738, 2004.
18 T. M. Michau, M. G. Davidson, and B. C. Gilger, "Carbon dioxide laser photoablation adjunctive therapy following superficial lamellar keratectomy and bulbar conjunctivectomy for the treatment of corneolimbal squamous cell carcinoma in horses: a review of 24 cases," Veterinary Ophthalmology, vol. 15, no. 4, pp. 245–253, 2012.
19 Bellone, Rebecca R; Liu, Jiayin; Petersen, Jessica L; Mack, Maura; Singer-Berk, Moriel; Drögemüller, Cord; Malvick, Julia; Wallner, Barbara; Brem, Gottfried; Penedo, M Cecilia; Lassaline, Mary (2017). A missense mutation in damage specific DNA binding protein 2 is a genetic risk factor for limbal squamous cell carcinoma in horses. International Journal of Cancer, (), –. doi:10.1002/ijc.30744
20 Teifke, J.P.; Löhr, C.V. (1996). Immunohistochemical detection of P53 overexpression in paraffin wax-embedded squamous cell carcinomas of cattle, horses, cats and dogs. , 114(2), 205–210. doi:10.1016/S0021-9975(96)80010-7
21 Brash, D. E.; Rudolph, J. A.; Simon, J. A.; Lin, A.; McKenna, G. J.; Baden, H. P.; Halperin, A. J.; Ponten, J. (1991). A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma.. Proceedings of the National Academy of Sciences, 88(22), 10124–10128. doi:10.1073/pnas.88.22.10124
22 Gallagher RP, Lee TK, Bajdik CD et al (2010) Ultraviolet radiation. Chronic Dis Can 29:S51-S68
23Watson, M., Holman, D. M., & Maguire-Eisen, M. (2016). Ultraviolet Radiation Exposure and Its Impact on Skin Cancer Risk. Seminars in Oncology Nursing, 32(3), 241–254. doi:10.1016/j.soncn.2016.05.005
24 Ortonne, J.-P. (2002). From actinic keratosis to squamous cell carcinoma. British Journal of Dermatology, 146(s61), 20–23. doi:10.1046/j.1365-2133.146.s61.6.x
25 KAINZBAUER, C., RUSHTON, J., TOBER, R., SCASE, T., NELL, B., SYKORA, S., & BRANDT, S. (2011). Bovine papillomavirus type 1 and Equus caballus papillomavirus 2 in equine squamous cell carcinoma of the head and neck in a Connemara mare. Equine Veterinary Journal, 44(1), 112–115. doi:10.1111/j.2042-3306.2010.00358.x
26 Keller, M. (2011). Retrospektive Studie zum Vorkommen des equinen okulären Plattenepithelkarzinoms an der LMU München in den Jahren 1995-2010. Tierärztliche Fakultät der Ludwig-Maximilians-Universität München.
27 Carastro, S. M. (2004). Equine ocular anatomy and ophthalmic examination. Veterinary Clinics of North America: Equine Practice, 20(2), 285–299. doi:10.1016/j.cveq.2004.04.013

28 Patricia M. Gearhart; Barbara A. Steficek; Simon M. Peteresen-Jones (2007). Hemangiosarcoma and squamous cell carcinoma in the third eyelid of a horse. , 10(2), 121–126. doi:10.1111/j.1463-5224.2007.00510.x
29 REBHUN, W. C. (1990). Treatment of Advanced Squamous Cell Carcinomas Involving the Equine Cornea. Veterinary Surgery, 19(4), 297–302. doi:10.1111/j.1532-950x.1990.tb01191.x
30 Ollivier, F. J., Kallberg, M. E., Plummer, C. E., Barrie, K. P., O'Reilly, S., Taylor, D. P., … Brooks, D. E. (2006). Amniotic membrane transplantation for corneal surface reconstruction after excision of corneolimbal squamous cell carcinomas in nine horses. Veterinary Ophthalmology, 9(6), 404–413. doi:10.1111/j.1463-5224.2006.00480.x
31 Théon, Alain P. (1998). Radiation Therapy in the Horse. Veterinary Clinics of North America: Equine Practice, 14(3), 673–688. doi:10.1016/S0749-0739(17)30192-X
32 THÉON, A. P., & PASCOE, J. R. (1995). Iridium-192 interstitial brachytherapy for equine periocular tumours: treatment results and prognostic factors in 115 horses. Equine Veterinary Journal, 27(2), 117–121. doi:10.1111/j.2042-3306.1995.tb03046.x
33 A. L. LABELLE; A. G. METZLER; D. A. WILKIE (2011). Nictitating membrane resection in the horse: A comparison of long-term outcomes using local vs. general anaesthesia. , 43(Supplement s40), 42–45. doi:10.1111/j.2042-3306.2011.00486.x
34 KING, T. C., PRIEHS, D. R., GUM, G. G., & MILLER, T. R. (1991). Therapeutic management of ocular squamous cell carcinoma in the horse: 43 cases (1979-1989). Equine Veterinary Journal, 23(6), 449–452. doi:10.1111/j.2042-3306.1991.tb03759.x
35 J. Murli Manoher, Praveena, Anju Kochar, Anil Chauhan. Topical 0.02% Mitomycin C for management of primary cornealconjunctival intraepithelial neoplasia as primary therapy: A long term follow up. IAIM, 2016; 3(8): 16-22.
36 Rebhun, William C. (1998). Tumors of the Eye and Ocular Adnexal Tissues. Veterinary Clinics of North America: Equine Practice, 14(3), 579–606. doi:10.1016/S0749-0739(17)30188-8
37 BAPTISTE, K. E. und GRAHN, B. H. (2000): Equine orbital neoplasia: a review of 10 cases (1983-1998). Can Vet J 41, S. 291-295.
38 WYN-JONES, G. (1979): Treatment of periocular tumours of horses using radioactive gold198 grains. Equine Vet J 11, S. 3-10.
39 Moore, C. P. (1992). Eyelid and Nasolacrimal Disease. Veterinary Clinics of North America: Equine Practice, 8(3), 499–519. doi:10.1016/s0749-0739(17)30438-8
40 McCowan, C., & Stanley, R. G. (2004). Pigmented squamous cell carcinoma of the conjunctiva of a horse. Veterinary Ophthalmology, 7(6), 421–423. doi:10.1111/j.1463-5224.2004.04040.x
41 Dugan SJ. Ocular Neoplasia. Vet Clin N Am Equine Pract. 1992;8:609-626.
42 Iwabe S, Ramírez-López L, Juárez-Sánchez M. The use of piroxicam as an adjunctive treatment for squamous cell carcinoma in the third eyelid of a horse.. Vet Mex. 2009;40(4):389-395.
43 GRIER, R. L., BREWER, W. G., Jr., PAUL, S. R. und THEILEN, G. H. (1980): Treatment of bovine and equine ocular squamous cell carcinoma by radiofrequency hyperthermia. J Am Vet Med Assoc 177, S. 55-61.44 SEVERIN, G. A. (1996): Severin´s Ophthalmology Notes (3). Fort Collins.

45 HEWICKER- TRAUTWEIN, M., GRUBER, A.D., WOHLSEIN, D. (2007): Tumoren. In: Baumgärtner, W. (Ed.), Pathohistologie für die Tiermedizin, S. Stuttgart, Enke Verlag.
46 GELATT, K. N., MYERS, V. S., Jr., PERMAN, V. und JESSEN, C. (1974): Conjunctival squamous cell carcinoma in the horse. J Am Vet Med Assoc 165, S. 617-20.
47 GELATT, K. N. (1975): Corneolimbal squamous cell carcinoma in a horse. Vet Med Small Anim Clin 70, S. 53.
48 Khokhar, S., Soni, A., SinghSethi, H., Sudan, R., Sony, P., & Pangtey, M. S. (2002). Combined Surgery, Cryotherapy, and Mitomycin-C for Recurrent Ocular Surface Squamous Neoplasia. Cornea, 21(2), 189–191. doi:10.1097/00003226-200203000-00012
49 SG RAYNER; N. VAN ZYL (2006). The use of mitomycin C as an adjunctive treatment for equine ocular squamous cell carcinoma. , 84(1-2), 43–46. doi:10.1111/j.1751-0813.2006.tb13124.x
50 Malalana, F., Knottenbelt, D., & McKane, S. (2010). Mitomycin C, with or without surgery, for the treatment of ocular squamous cell carcinoma in horses. Veterinary Record, 167(10), 373–376. doi:10.1136/vr.c3815
51 Goldbaum, A. M., & Woog, J. J. (1997). The CO2 laser in oculoplastic surgery. Survey of Ophthalmology, 42(3), 255–267. doi:10.1016/s0039-6257(97)00097-0
52 FRAUNFELDER, H. C., BLEVINS, W. E. und PAGE, E. H. (1982): SR-90 for treatment of periocular squamous cell carcinoma in the horse. J Am Vet Med Assoc 180, S. 307-309.
53 WILKIE, D. A. (1991): Ophthalmic procedures and surgery in the standing horse. Vet Clin North Am Equine Pract 7, S. 535-47.
54 Gerco Bosch; Wim R. Klein (2005). Superficial keratectomy and cryosurgery as therapy for limbal neoplasms in 13 horses. , 8(4), 241–246. doi:10.1111/j.1463-5224.2005.00395.x
55 McCalla TL, Moore CP, Collier LL. Immunotherapy of periocular squamous cell carcinoma with metastasis in a pony. Journal American Veterinary Medical Association 1992; 200: 1678–1681
56 Köpke P. (2007) Solare UV-Strahlung und ihre Wirkung auf den Menschen. Promet 33, 95-108
57 Tóth, Hollerrieder, Sótonyi (2010): Augenheilkunde beim Pferd, Lehrbuch und Atlas. Schattauer GmbH, Stuttgart Germany, S.33-63.
58 https://en.wikipedia.org/wiki/Horse(15.08.2023 21:14).
59 Bundesverband für Pferdesport und Pferdezucht – Fédération Equestre Nationale (FN) – (2022): Deutsche Reiterliche Vereinigung E.V., Deutschland.
60 PANNDORF, H. (1970): Statistischer Beitrag zum Geschwulstvorkommen im Patientengut einer Großtierklinik. Mh Vet Med 25, S. 99-104.
61 https://de.wikipedia.org/wiki/Haflinger(02.08.2023 22:09).
63 Mosunic B. (2003): Equine ocular squamous cell carcinoma: retrospective and diagnostic studies, Georgia.
64 Gilger, Brian C. Equine ophthalmology. 2nd ed. St. Louis, Mo: Elsevier Saunders, 2011.
65 Gupta A, Muecke J. Treatment of ocular surface squamous neo-plasia with Mitomycin C. Br J Ophthalmol. 2010; 94:555–8.
66 Shields CL, Naseripour M, Shields JA. Topical mitomycin C for extensive, recurrent conjunctival-corneal squamous cell carcinoma. Am J Ophthalmol., 2002; 133: 601–6.

IX. Figures List

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I, the undersigned Zita Makra DVM, as the supervisor, declare that I have read and approved the thesis " Retrospective analysis of the clinical findings of horses with ocular squamous cell carcinoma" of the student Johanna Rothensteiner (6^{h} year), and support her participation in the Scientlfic Student Conference of the University of Veterinary Medicine in 2023. Furthermore, I declare that the uploaded TDK thesis has been successfully checked for plagiarism and that any matches found comply with the University guidelines/rules.

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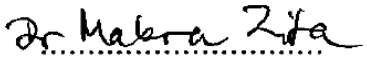

Announcement

Hereby Johanna Rothensteiner declare that my thesis titled: "Retrospective analysis of the clinical findings of horses with ocular squamous cell carcinoma" is completely identical in terms of content and form to my TDK work presented at the TDK Conference in 2023.

Thesis progress report for veterinary students

Name of student: Johanna Rothensteiner.....

Neptun code of the student: D1DJR7.....

Name and title of the supervisor: Dr. Makra Zita.....

Department: Department and Clinic of Equine Medicine

Thesis title: Retrospective analysis of the clinical findings of horses with ocular squamous cell carcinoma .....

Consultation – 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022.	09.	29.	Discussion of the topic	Dr Makra Zita
2.	2022.	10.	20.	Major parts of the thesis	Dr Makra Zita
3.	2022.	11.	17.	Collection of literature	Dr Makra Zita
4.					
5.					

Grade achieved at the end of the first semester: 4 (good).....

Consultation – 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
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1.	2023.	09.	14.	Discussion of the data	Dr Makra Zita
2.	2023.	10.	06.	Evaluation of the results, text correction	Dr Makra Zita
3.	2023.	10.	12.	Final correction of the text	Dr Makra Zita
4.					
5.					

Grade achieved at the end of the second semester: 3 (grade three).....

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

Dr Kalcsa Zita
.....
signature of the supervisor

Signature of the student: Johanna Rothensteiner

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Date of handing the thesis in 2023 11. 15.