Ophthalmic complications of radiation therapy of tumors of the head in dogs and cats

Research project Veterinary Medicine, Utrecht University

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Research period: 10-3-2014 – 30-06-2014

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Abstract
Irradiation is an important part of the complete therapy for nasal, paranasal, and brain tumors, and is becoming increasingly available in companion animal medicine. The goal of radiation therapy is to induce an increased degree of cell death inside the tumor, while minimizing side effects. The structures of the eyes are sensitive to acute as well as to late side effects. In this article the literature on ophthalmic complications of radiation therapy is summarized. The aim of this study was to investigate the incidence and nature of early and late onset ophthalmic complications in 15 patients of the Utrecht University Clinic for Companion Animals in The Netherlands and to compare these to the complications described in veterinary literature.
The research consisted of a retrospective part to obtain a general view of the late complications, and a prospective part for evaluation of the acute complications. During the research period, running from March 10th 2014 to June 30th 2014, a questionnaire was completed by all 15 patient owners, of which seven patients were invited for ophthalmologic examination. Of four of the remaining eight patients, information on ophthalmic examination was derived from the patient file. Ophthalmic examination took place prior to the start of radiation therapy (n=3), and at three time points after irradiation (at the last day of radiation treatment (n=6) and at 2-4 weeks after treatment (n=8) for early complications, and at 15-49 weeks after treatment (n=3) for late complications). At the first post-therapy examination, only mild acute responses were seen in four out of six patients. The acute reactions mainly consisted of conjunctival hyperemia and epiphora. In two patients signs had progressed by the second post-therapy examination. Corneal edema (n=5/8), epiphora (n=5/8) and conjunctival hyperemia (n=5/8) were seen. One patient showed bilateral moderately swollen eyelids at the first post-treatment examination, which unilaterally progressed to one severely swollen eyelids at the second post-treatment examination. One patient with no vitreal liquefaction at the first post-treatment examination had developed moderate vitreal liquefaction at the second examination. Several late complications were seen in the three patients visiting the clinic for a third post-treatment ophthalmic examination. Two of these three patients were diagnosed with keratoconjunctivitis sicca. An additional third patient was diagnosed with keratoconjunctivitis sicca (n=1/8) at the second post-treatment examination, but this patient wasn't seen for the third checkup. One patient was diagnosed with keratoconjunctivitis sicca prior to radiation therapy (at four years of age).
Cataract was seen in two patients, but did not seem progressive (n=2/3 pre-treatment, n=0/5 at first post-treatment examination, n=1/8 at second post-treatment examination and n=1/3 at third post-treatment examination). By the third post-therapy examination, one out of three patients had completely lost vision, and showed retinal abnormalities in both eyes, with retinal hemorrhages in the left eye and complete retinal detachment in the right eye. One of the other patients also showed retinal hemorrhages, but vision was not impaired. One patient showed only mild changes with some conjunctival follicles in both eyes and no retinal changes in either eye. Only two owners (n=2/15) mentioned loss of vision in the questionnaire. During the research period some fluctuations in median tear production and median IOP was seen. The fluctuations in individual patients and variation between patients might be interpreted as normal fluctuations and variation, because no particular trend was visible in the collected data on tear production and IOP.

Taken into account the length of the period between exposure to radiation and the clinical occurrence of retinopathy, this research was only an preliminary and descriptive study. Further research with more patients and longer follow-up times is necessary to determine precisely which acute and late ophthalmic complications of cranial radiotherapy occur in canine and feline patients of the Utrecht University Clinic for Companion Animals, and which additional preventive measures could help in reducing the occurrence and severity of acute and late complications.

Introduction

Irradiation is an important part of the complete therapy for nasal, paranasal, and brain tumors, and is becoming increasingly available in companion animal medicine. (1-3)

The absorbed dose of radiation is relevant for both the therapeutic efficiency and the toxicity of the radiation. The absorbed dose is measured in Gray (Gy). In veterinary medicine tumors usually receive a cumulative dose of 40 to 60 Gy (4000 to 6000 cGy). In order to reduce the negative effects of irradiation, the cumulative dose of radiation therapy is divided into multiple smaller doses. For example, a cumulative dose of 40 Gy can be divided into individual doses of 2 Gy, administered 5 days a week, during 4 weeks. Two days a week without irradiation are scheduled for rest and repair of damaged tissue. (2)

The goal of radiation therapy is to induce an increased degree of cell death inside the tumor, while minimizing side effects. The tissues surrounding the tumor have a limited tolerance for irradiation. The severity of the reaction to irradiation in normal tissues doesn't depend on the cell type, but on the amount of proliferation in the tissues at the moment of irradiation. This is due to the fact that cells in mitosis and in phase G1 of the cell cycle have an increased sensitivity to radiation. When there is a high rate of cell proliferation, the tissue is called radiosensitive. Tissues with a low rate of proliferation are called radioresistant. (1-3)

The intention of radiation therapy in small animal patients can be curative or palliative. Curative means providing cure or long-term tumor control. In curative protocols the goal is to maximize chances of local tumor control, while minimizing risk of significant late radiation side effects. Palliative therapy aims at providing relief of symptoms while resulting in little to no acute radiation side effects. (3)

Side effects of radiation therapy can be seen at different time points. Acute side effects can be seen within hours to days after exposure to radiation and are at their worst at two weeks after finishing radiation treatment. These side effects usually resolve quickly in approximately four weeks and are therefore considered acceptable. The number and severity of side effects increase when there is not enough time for rest and repair between the individual doses. A dose that is too high will also induce more severe side effects. (1,2,4) Frequently occurring acute side effects include mucositis, dermatitis and keratitis. (3,5-7)

Besides acute side effects, late side effects occur. The late side effects occur typically in tissues with a low rate of proliferation and can be the result of damage to vascular endothelial cells and connective tissue. These cells can be found in almost all tissues and therefore these side effects can occur almost anywhere in the body. Another factor that may be involved in the onset of cell death and the occurrence of late side effects is the increased release of tumor necrosis factor-α (TNF-α), which may produce distant cytotoxic effects. (2)

The late side effects can emerge months or even years after radiation therapy and are

irreversible. Examples of late side effects are fibrosis, necrosis, non-healing ulcerations, damage to the central nervous system and blindness due to retinopathy and retinal detachment. (1,3,5)
In cats occurrence of acute side effects is less frequent than in dogs. Late side effects are difficult to monitor in cats, because there are not many tumors in cats with a favorable long-term prognosis after radiation therapy.

Nearly all tissues in the eye are susceptible to radiation-induced damage. The eyes are located closely to the nasal cavity, and due to this location ocular complications of irradiation of nasal tumors have been reported in humans, monkeys, mice, rats, rabbits, frogs, dogs, and cats. (8-17) The acute and late ophthalmic complications include conjunctivitis, blepharitis, corneal ulcerations and keratitis, keratoconjunctivitis sicca (KCS), lagophthalmos due to scar tissue and secondary ectropion, neovascular glaucoma, scleral necrosis, cataracts, vascular retinopathy, exudative retinal detachment and optic neuropathy. (18-20)

Lana et al (2004) reported that 76.5% of their 51 patients receiving radiation therapy of tumors of the nose, experienced acute ocular effects, varying from mild to severe. Follow-up of 39 of these patients showed that 36% had late ocular side effects, i.e. dry eye, cataract formation, keratitis, or corneal vascularization. (21) In the following, the complications of irradiation in different parts of the eye and adnexa will be discussed in more detail.

Ocular adnexa and anterior segment

Hair loss occurs in humans with conventionally fractionated (1.5-2 Gy per fraction) radiation therapy with cumulative doses over 20 Gy, but has also been reported with 10 Gy delivered over three days. In humans several predisposing factors for skin reaction to irradiation have been determined, i.e. male gender, increasing age, and increasing sun exposure. When high doses are used (>50Gy in conventional fractionation) several other problems can occur, i.e. moist desquamation, secondary infection, and consequential long-term scarring as a result of nonhealing ulceration. Scarring can lead to ectropion or entropion. (8)
Acute conjunctivitis is a common complication in human and simian patients receiving 30 Gy or more. Secondary infections may occur. Damage to the tear producing cells can lead to transient or permanent keratoconjunctivitis sicca. (8,11)
Corneal edema and in severe cases corneal ulceration can develop due to keratoconjunctivitis sicca, entropion and ectropion. After hair loss, the hairs can grow back in an altered position, leading to trichiasis or distichiasis, which also can lead to damage to the cornea. (8)
Transient early iritis can develop after a single dose of 10 Gy or more, but is rare. In humans, neovascular glaucoma may occur as a late complication, and has been reported in up to 20% of eyes treated with radiotherapy. (8,22)

Lens

The function of the lens is to focus light on the retina. This is only possible when the lens is transparent. (23) The lens is predominantly composed of soluble proteins and is a highly transparent tissue. In mammalian lenses four types of these proteins have been identified, α-crystallin, β_H-crystallin, β_L-crystallin and γ-crystallin. (24) During terminal differentiation of lens fiber cells, programmed elimination of cytoplasmic organelles occurs. This process contributes to the transparency of lens tissue. The underlying molecular mechanisms are not fully understood yet. (23,25) Organelle breakdown in the lens shows similarities with apoptosis and fiber cells express most or all components of the cell death apparatus. The process of organelle breakdown can be considered as a form of “attenuated” cell death. (23) An

important factor involved in this process is a family of cysteine proteases called caspases. (23,26,27) In 1984 Chylack and in 1991 Medvedovsky and Worgul established a threshold value in human and mice of 2.0 Gy to cause noxious effects of lens crystallin, and apoptosis in which again caspases play a central role. (26-29) However, Ainsbury et al. state in 2009 that in human changes in the lens due to irradiation can be found at doses as low as 0.5 Gy, and that it may be more correct to speak of a linear model, instead of a threshold model. (30)

In rats, radiation causes development of free radicals, which causes lens crystallin oxidation with loss of clarity. This loss of clarity is called cataract. (31) This type of radiation damage manifests first at the anterior surface of the lens. (10,32) Besides irradiation, many other confounding risk factors can contribute to the development of lens opacities in humans, such as age, diabetes mellitus and the systemic use of corticosteroids. (8,30,33-36) Although the amount of research that has been done in this area is enormous, the exact mechanisms of radiation induced cataractogenesis are still not completely understood. (30)

Depending on the anatomical location in the lens, three forms of cataract can be distinguished: (i) cortical, i.e. in the lens cortex; (ii) nuclear, i.e. in the central portion of the lens; and (iii) posterior sub-capsular (PSC), i.e. in the back surface of the lens beneath the lens capsule. Ionizing radiation induces mainly posterior sub-capsular and cortical changes, in contrast to age-related cataract, which in humans is mainly located in the nuclear region and diabetic cataract, which in humans is mainly found in the cortical region. (30,34,35,37)

Species differences in time between exposure and development of cataract were documented in 1956 by Von Sallmann et al. His research demonstrated that nine out of 11 guinea pigs showed radiation-induced lesions of the lens about two months after exposure, followed by mice, which showed changes of the lens after three months. More dispersion in time was seen in cats, which developed lens changes after two to four months. Only one out of 12 dogs developed cataract after four months. (13)

Research of Stadler and Nash demonstrated a minimal time of 279 days between exposure to irradiation and development of cataract in mice. In this research much lower radiation doses were used, which could be the explanation to the different outcome compared to the research from Von Sallmann et al. in 1956. (12)

At dose levels of 40 Gy or more a 62-100% incidence of cataract in humans has been described in literature. Therefore in the past, radiation induced cataract has been considered a serious complication threatening vision. However, since the development of surgical treatment visual acuity can be restored without significant complications and is radiation induced cataract no longer considered a severe complication. (22)

Retina

In the retina, no acute complications of radiation therapy have been reported following irradiation of the eyes. Radiation retinopathy is a late complication which can lead to blindness and can be seen in humans after irradiation of ocular, orbital, periorbital, nasopharyngeal and cranial area. (8,38,39) It has also been demonstrated in monkeys following irradiation of the head and whole-body irradiation. (11)

The retinopathic effects of irradiation were first described by Stallard in 1933 and 1936. (40,41) Stallard described in humans retinal hemorrhages and exudates in a circinate pattern followed by the development of optic disc edema, vascular cuffing, and ultimately optic nerve atrophy. (38) The period between exposure to radiation and the clinical occurrence of vascular changes reportedly varies from

three weeks to seven years, but is mostly seen after six months to three years. (8,38,42)
Research of Takeda et al (1999) showed an incidence of late retinal complications of 38.1% in human patients irradiated with 50 Gy or more. (22)
Radiation retinopathy is characterized by slowly progressive microangiopathic decompensation with focal loss of capillary endothelial cells and pericytes and manifests as microaneurysms, telangiectasia, hard exudates (lipid breakdown products that are left behind after localized edema resolves), cotton wool spots (accumulations of axoplasmic material within the nerve fiber layer), and neovascularization. These changes can also be found in patients with diabetes, hypertension, or leukemia. (8,22,39)

Optic nerve

In the optic nerve, no acute complications of radiation therapy have been reported following irradiation of the eyes. Radiation induced optic neuropathy is a rare late complication, first described in 1961 by Forrest, and can lead to complete loss of vision in the affected eye. The latent period before the onset of optic neuropathy in humans ranges from 3 months to 8 years, but optic neuropathy is mostly seen at 18 months after radiation therapy. (8,43)

In summary, a variety of acute and late ophthalmic complications of irradiation of the head can be found.
This retrospective and prospective study will focus on ophthalmic complications of radiation therapy of tumors of the head in dogs and cats treated at the Utrecht University Clinic for Companion Animals. Findings will be compared with reported ophthalmic complications in veterinary literature. The focus of this research is to find an answer to the following questions:

- Which ophthalmic complications of radiation therapy of tumors of the head in dogs and cats are described in literature?
- Which of the ophthalmic complications described in literature have been seen in dogs and cats treated with irradiation for tumors of the head at the University Clinic for Companion Animals in Utrecht, The Netherlands?
- Did ophthalmic complications occur in these patients that haven't been described in literature?
- Which preventive measures can be taken to reduce the chance of ophthalmic complications of radiation therapy of tumors of the head in dogs and cats, and are these measures applicable at the Utrecht University Clinic for Companion Animals?

The hypotheses for this study are the following:

- Late complications are less frequent in cats than in dogs due to their shorter survival following radiation therapy of tumors of the head.
- Dogs and cats that have received radiation therapy of the head don't show late complications until at least one year after therapy.
- When the eye is in the trajectory of radiation, it is not possible to use preventive measures like protective shields.

Material and methods

Since April 2010 a radiation bunker with a linear accelerator has been available at the University Clinic for Companion Animals in Utrecht, The Netherlands.
The study consisted of a retrospective part and a prospective part.
For the retrospective part of the study, patient files of dogs and cats that received radiotherapy of the head at the Utrecht University Clinic for Companion Animals in The Netherlands between April 2010 and March 2014 were retrieved from the administration program Vetware. After obtaining the owner's informed consent, a questionnaire was performed by telephone and the patients that were still alive were invited for a full ophthalmological

examination. The aim of the retrospective study was to give an impression of the late ophthalmic complications found in the animals irradiated in this clinic.
For the prospective part of the study, patients which received radiation therapy between March and July 2014 and participated in this study (with the informed consent of their owners) were examined ophthalmologically before the course of radiation therapy, at the last day of the treatment course and at approximately three weeks after the last dose of irradiation.
The choice for these examination points are based on the periods Farrely and McEntee (2003) pointed out. They stated that the acute side effects can be first observed during radiation treatment, progress for the first seven to fourteen days after the end of radiation treatment, and may persist for four weeks. (44) Late side effects become visible months to years after the end of radiation therapy, at least in humans. (38,42) The aim of the prospective part of the study mainly was to give an impression of the acute ophthalmic complications found in the animals irradiated in this clinic.

Procedure for radiation therapy in the head area

In all patients a computed tomographic exam (CT scan) was performed. A dental print was used for precise and repeatable positioning. The dental print was placed in a custom-made frame which contained small lead pellets. These lead pellets emerged as small dots on the image and were used as a base line from which the exact position of the tumor was calculated by a computer.
The margins of the tumor, brains and eyes were marked in the images of the CT scan. The trajectory of the radiation was added to the image, to visualize the tissues that would receive radiation. The computer calculated the exact dose of radiation for each tissue. This way the ideal trajectory of radiation was calculated.
During radiation therapy the patient was in ventral recumbency and the position of the head was secured with the earlier mentioned frame and dental print (Figure 1).

Protection of healthy tissues of the head was attempted by the use of MLC (Multi Leaf Collimator) for patients irradiated with photons, or cerrobend blocks for patients irradiated with electrons (Figure 2).

Protocols for anesthesia differed per animal, but preferably included dexmedetomidine because of the possibility to use an antagonist after the therapy session to reduce total anesthesia time.

Fig 1. (**a** and **b**) Dental print fixed on frame used for positioning. (**c**) Patient positioned using the dental print and frame. Notice the green laser lights, used for exact positioning.

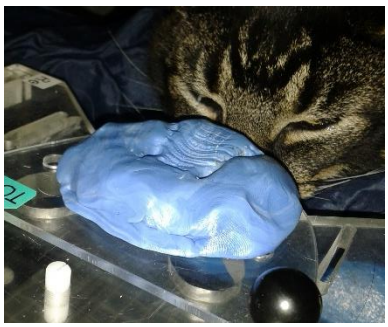

Fig 1.a

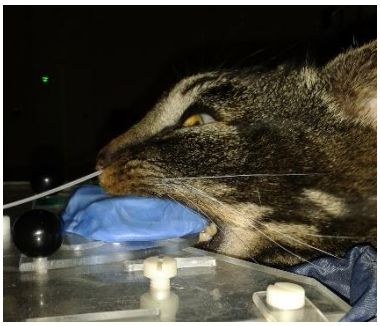

Fig 1.b

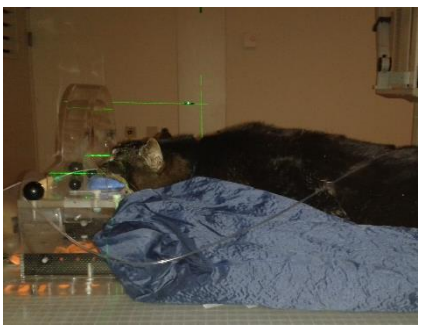

Fig 1.c

Fig 2. A multi-leaf collimator (MLC) assists in the shaping of the radiation beam. In this photograph the MLC is removed from the head of the treatment delivery machine (a linear accelerator). Data from the 3D treatment plan is used to shape the radiation fields with the MLC. Source: http://www.phoenix5.org/Infolink/Michalski/Part2.html, June 23th 2014.

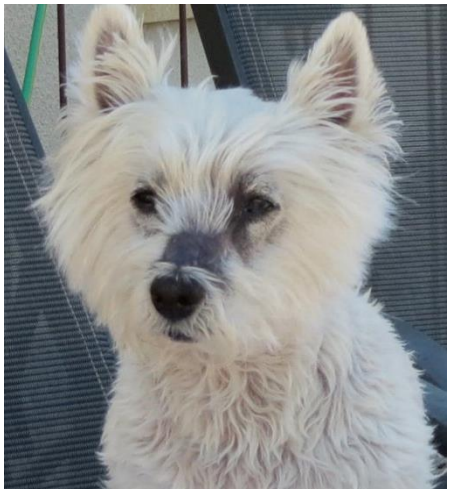

Fig 3. Hair loss and pigmentation of the skin around the eyes and on the bridge of the nose in a dog, two months after irradiation for a nasal carcinoma.

Results

Patients

A total of fifteen patients (thirteen dogs and two cats) were included in the study. Their breeds, ages, tumor location, tumor type, minimum and maximum radiation dose to the eyes and total radiation dose are summarized in Table 1. All, except one, received photon irradiation. Only patient *D* received electron irradiation.

A questionnaire was taken from the owners or caretakers of all fifteen patients. Eight of these patients (seven dogs and one cat) were available for the clinical part of the study (four canine patients for the prospective, and two dogs and one cat for the retrospective study). One of these seven canine patients was euthanized during the course of radiation therapy and was therefore only examined prior to the first radiation fraction. The patient files of three of the seven remaining cases contained information on an ophthalmic examination. This information was included in the clinical part of this study. The files of three dogs and one cat did not contain any information on an ophthalmic examination and unfortunately these patients were not able to visit the clinic for ophthalmic examination during the research period.

Table 2 shows a checkup timeline with all ophthalmic examination moments of the patients included in this study.

Questionnaire

A total of fifteen questionnaires were completed. The questionnaire consisted of a list of symptoms that can be noticed by the owner of patients with ocular problems. Also a list of diseases and medications which can influence the ocular symptoms was added to the questionnaire.
Only the most notable findings will be discussed here. The complete table with the examined variables can be found in appendixes 1.1 and 1.2.

Epiphora was noted in two out of fifteen patients (13%) prior to radiation treatment (patient *F* and *O*). Patient *F* showed epiphora in both eyes, patient *O* only in the left eye. Epiphora during the therapy course was reported by four out of fifteen owners (27%, patients *F*, *H*, *I* and *O*), in patient *O* still only the left eye was

affected, while in the other three patients both eyes were affected.
Four owners reported hair loss around the eyes during treatment (27%, patients *A*, *C*, *I and J*), which only persisted after the treatment course in patient *I* (Figures 3 and 4).
Only the owner of patient *D* (7%) reported palpebral erythema of the right eye during treatment. This did not persist after treatment.
One of the owners (patient *C*) reported bilateral conjunctival hyperemia prior to radiation treatment. However, three out of fifteen owners (20%, patients *B*, *E* and *I*) reported this during treatment. In only one patient (patient *I*) the hyperemia of the conjunctivae persisted in both eyes for three months after the treatment course.
Turbidity of the lens was noted in two patients before, during and after treatment (patient *A* and *L*). No changes in severity were reported.
Two owners reported loss of vision. Patient *M* lost vision in the left eye a few weeks after radiation treatment. The owner did not consult a veterinarian for this problem, so the cause was not identified. The second owner reported loss of vision in both eyes in patient *I* 15 weeks after irradiation. The cause of loss of vision in this dog was retinopathy.

None of the owners reported entropion, hyphema, mydriasis, hypermetric gate or anxiety.

Five owners visited a veterinarian prior to radiation treatment because of ocular problems, three of them visited the veterinarian after radiation again with ocular problems. A fourth patient was presented to a veterinarian with ocular problems after radiation therapy. Reasons for consulting the veterinarian prior to radiation therapy were mild corneal injury due to a foreign body (n=1/5), conjunctivitis (n=1/5), protrusion of the nictitating membrane (n=1/5), ectropion (n=1/5), and keratoconjunctivitis sicca (n=1/5).

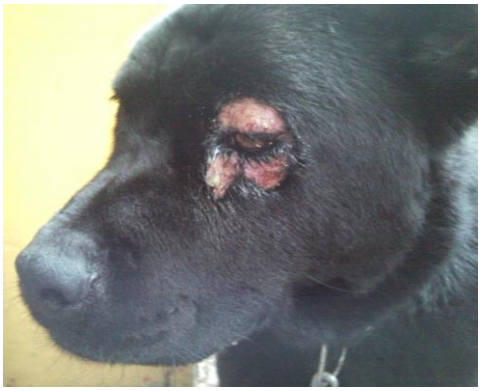

Fig 4. Hair loss, swelling, erythema and ulceration of the skin around the eyes in a dog, three weeks after irradiation for a nasal tumor.

Reasons for consulting the veterinarian after irradiation were bilateral conjunctivitis (n=1/4), loss of vision (n=1/4), keratoconjunctivitis sicca (n=3/4), and blepharitis (n=1/4).

Concurrent health issues, unrelated to the cranial neoplasia or the radiation therapy, were reported by the owners of four patients: One patient was diagnosed with otitis media in the past, but no clinical signs were present in the research period.
Two owners reported their dogs as being intolerant to certain components in food. The ophthalmic clinical signs found in these patients during the study period were not attributed to the food intolerance, because both dogs were successfully managed with a hypoallergenic diet and no clinical signs of intolerance or allergy were seen in these patients.
Lupus erythematosus was diagnosed in one patient. No signs of uveitis were found in this patient. Only some crusts (dried exudate) at the medial canthus of both eyes were present.
None of the patients showed clinical signs which could be related to the medication they received.

Patient	Breed	Age (years)	Tumor location	Tumor type	Minimum and maximum radiation dose OD (cGy)	Minimum and maximum radiation dose OS (cGy)	Total radiation dose (cGy)
Dogs							
A	Mixed	15	Nasal cavity (right)	Sarcoma	125 – 3775	162 – 2327	4800
B	Labrador retriever	8	Nasal cavity (left)	Carcinoma	2246 – 2624	1500 – 4642	4800
C	Saluki	10	Nasal cavity (right)	Adenocarcinoma	2070 – 4680	1965 – 4030	5120
D	Beagle	8	Nasal cavity (right)	Mastocytoma	-*	-*	2000
E	Weimaraner	11	Nasal cavity (bilateral, suspect brain involvement)	-	2000 – 4300	2000 – 4300	4800
F	Norwegian elkhound	8	Nasal cavity (left)	Chondrosarcoma	2459 – 4000	2459 – 5000	5440
G	Mixed	11	Brain	-	12 – 100	12 – 100	Discontinued
H	Mixed	6 †	Nasal cavity (left)	-	-**	-**	4800
I	Chow chow	10 †	Nasal cavity (left)	Adenocarcinoma	-**	-**	3600
J	West Highland White terrier	11	Nasal cavity (left)	Carcinoma	720 – 2479	913 – 4100	4800
K	Golden retriever	7 †	Brain	Meningioma	335 – 2579	335 – 3025	4800
L	Border collie	10	Nasal cavity (left)	Adenocarcinoma	550 – 848	550 – 1163	4800
M	Labrador retriever	9 †	Processus coronoideus mandibula (left)	Multilobular osteochondrosarcoma	0 – 0	72 – 3307	4000
Cats							
N	Mixed	15	Nasal cavity (medial, suspect brain involvement)	Adenocarcinoma	414 – 4712	414 – 3260	4800
O	European shorthair	6	Nasal cavity (left)	Osteosarcoma	1900 – 3885	1900 – 5543	5120

Table 1. Summary patients

* This patient was irradiated with electrons, therefore no exact dose could be calculated.
** Due to a system crash of the computers at the Oncology section, exact radiation data of patients irradiated before January 2013 were lost.

Ophthalmic examination

The complete table with the examined variables can be found in appendix 2.

Pre-therapy examination

A total of three patients (patient *D, F* and *G*) were examined ophthalmologically before the start of the course of radiation treatment. All three patients had normal tear production as assessed with the Schirmer Tear Test, ranging from 15 to 19 mm measured in one minute (reference range for dogs: 13-25 mm in one minute, and for cats: 10-20 mm in one minute).
The intraocular pressure (IOP), as measured with a rebound tonometer (TonoVet®, Icare, Helsinki, Finland), was also within normal limits, with values ranging from 15 to 20 mmHg (reference range: 15-25 mmHg). None of the patients bumped into any of the obstacles during the obstacle test in light and in dark, neither before nor after administration of tropicamide eye drops (Tropicamide Minims 0.5%, Bausch & Lomb, Kingston-upon-Thames, UK). Patient *D* had bilateral distichiasis, which was more prominent in the left eye. Two patients (*D* and *F*) had tear stains. Patient *D* had a tear stain only in the right eye, and patient *F* in both eyes with a crusty appearance (this patient was suffering lupus erythematosus, which could also be the cause of the crusts).
Corneal edema was seen in both eyes of one patient (*F*) prior to radiation treatment.
One patient (*D*) showed mild atrophy of the iris.
Cataract was present in both eyes in patients *F* and *G*.
Focal hyperreflectivity of the tapetal area was seen in the left eye of patient *F*.

First post-therapy examination

The first post-therapy examination took place at the last day of the treatment course or one day later and was performed in six dogs (patient *A, C, E, F, H* and *J*).

Tear production was measured in only two (E and *F*) out of these six patients and was 16 mm/min (OD) and 15 mm/min (OS) in one patient (*E*) and 23 mm/min (OD) and 22 mm/min (OS) in the other patient (*F*). The patient with 23 and 22 mm/min showed epiphora and the conjunctiva was hyperemic and swollen in both eyes.
Also the IOP (OD and OS, respectively) was only measured in these two patients and results were 16 and 14 mmHg in patient *E*, and 12 and 12 mmHg in patient *F*.

Five patients underwent a full ophthalmic examination and of the sixth patient (*J*) only an incomplete examination form was found in the patient file in Vetware.

None of the five patients bumped into any of the obstacles during the obstacle test in light and in dark, neither before nor after administration of tropicamide eye drops.
Patient *J* showed blepharospasm and purulent discharge in both eyes. The conjunctiva of both eyes was markedly hyperemic.
Epiphora and tear stains were seen in three of six patients (*F, H* and *J*). In patient *H* only the left eye was affected, which in this dog was also the side of the tumor. The other two patients had both eyes affected, with patient *J* having purulent discharge and patient *F* having severe serous discharge.
One patient (*J*) had bilaterally swollen eyelids. It is not known whether these abnormalities were present in this patient prior to irradiation.
Three out of six patients (*E*, *F* and *J*) showed hyperemia and swelling of the palpebral conjunctiva in both eyes. Only patient *E* showed bilateral hyperemia of the nictitating membrane as well.

Table 2. Checkup timeline

Ophthalmic examination moments per patient. Green: ophthalmic examination. Red: no ophthalmic examination in patient administration and not available for ophthalmic examination during the research period.

Patient	Pre-therapy	Post-therapy (days after last radiation fraction)																																																				
		Acute complications												Late complications																																								
		D0-6	D7-13	D14-20	D21-27	D28-34	D35-41	D42-48	D49-55	D56-62	D63-69	D70-76	D77-83	D84-90	D91-97	D98-104	D105-111	D112-118	D119-125	D126-132	D133-139	D140-146	D147-153	D154-160	D161-167	D168-174	D175-181	D182-188	D189-195	D196-202	D203-209	D210-216	D217-223	D224-230	D231-237	D238-244	D245-251	D252-258	D259-265	D266-272	D273-279	D280-286	D287-293	D294-300	D301-307	D308-314	D315-321	D322-328	D329-335	D336-342	D343-349	D350-356	D357-364	D365- ∞
A		Green			Green																																														Green			
B						Green														Green																																		
C		Green			Green																																																	
D	Green			Green																																																		
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L	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
M	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red
N[1]							Green																																															
O[1]	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red

[1]cat

Second post-therapy examination

The second post-therapy check took place at two to four weeks after the last day of treatment. In this period the acute complications should be maximally visible and the largest group, eight patients (patient *A-E, H, J* and *N*), was seen during this check.

Tear production was measured in seven of eight patients (*A-E, J* and *N*) and ranged from 6 to 20 mm/min. The tear production had not been measured before in the patient with the lowest tear production (6 and 12 mm/min for OD and OS respectively), so the onset of low tear production as related to radiation therapy and the progression remain unresolved.
The IOP was measured in only four patients and ranged from 9 to 15 mmHg.

None of the patients bumped into any of the obstacles during the obstacle test in light and in dark, neither before nor after administration of tropicamide eye drops.

Changes in the orbital bones were seen in only one patient on the left side of the head, blocking drainage of tears and causing epiphora on the left side, which was also the side of the tumor (patient *H*). Severe epiphora of the left eye was also seen in patient *N* and moderate epiphora was seen in both eyes in three more patients (*A, E* and *J*).
Patient *D* had bilateral distichiasis, with the left eye more severely affected (same patient as described in pre-treatment examination).
Abnormalities of the eyelids and lid margins were seen in both eyes in two patients (patient *C* and *J*), with patient *J* having a severely affected left eye. The patient with the severely affected left eye had only moderately swollen eyelids in the previous post-treatment check, so some progression had occurred in just three weeks. The other patient showed bilateral irregularities, some depigmentation and moderate swelling of the lid margins.

Patients *C* and *D* showed hyperemia, swelling and follicles of the conjunctiva in both eyes, and in patients *H* and *J* only the left eye was affected, with in patient *H* conjunctival erythema and swelling and involvement of the nictitating membrane, and in patient *J* only conjunctival erythema. Both patient *H* and *J* were irradiated on their left side. Patient *A* showed conjunctival hyperpigmentation and swelling. In patient *E* some follicles and mild swelling had been seen. Patient *N* showed only mildly swollen conjunctiva. That makes seven out of eight patients (88%) with inflammatory changes of the mucous membranes of the eyes.
Mild exophthalmus was seen in the right eye of patient *E*. This abnormality was not noted on the previous examination form, but the patient was difficult to examine due to uncooperative behavior. According to the owner, the exophthalmus had decreased during radiation treatment, so it should have been present before treatment.
Patient *J* showed episcleral vascular injection in the left eye, but this patient had not been examined before, so progression in time could not be evaluated. This patient also showed a scattered reflection image of the cornea in both eyes.
Five out of eight patients showed mild corneal edema, in patient *B* only the left eye was affected, in patient *C* only the right eye and in three patients (*A, D* and *N*) both eyes were affected. Patients *B* and *C* showed corneal edema ipsilateral to the irradiated side.
In two patients (*A* and *D*) atrophy of the iris was present in both eyes. One of these patients (*A*) had several persistent pupillary membranes.
Cataract was seen in only the right eye of patient *C*, which was also the irradiated side.
Patient *C* also showed vitreal liquifaction in both eyes, which had not been noted three weeks earlier.

Third post-therapy examination

Only three patients (*A, B* and *I*) were seen at a third post-therapy check. Patients *A* and *B* were invited for a check in relation to this research (344 and 127 days after treatment), and patient *I* came 105 days after the last radiation treatment for clinical signs of keratoconjunctivitis sicca and loss of vision.

Patient *A* came to the clinic almost a year after the last radiation fraction. Three weeks after radiation treatment (second post-therapy examination) this patient had a tear production of 6 and 12 mm/min. The first six weeks after diagnosis of KCS, the owners had treated the patient with cyclosporine A 0.2% eye ointment (Optimmune canis, MSD Animal Health, Boxmeer, The Netherlands) twice daily on both eyes, and for a short period with chloramphenicol eye ointment (CAF, CEVA Sante Animale B.V., Naaldwijk, The Netherlands) thrice daily on both eyes. No treatment was given during the rest of the year. At the third post-therapy examination, twelve weeks after the second post-therapy examination, the tear production had increased to 14 and 19 mm/min for OD and OS respectively.
The IOP was 14 and 11 mmHg for OD and OS respectively. No prior measurements had been performed. Entropion and trichiasis were present in the left eye. Epiphora and corneal edema were found in both eyes. In both eyes hyperpigmentation of the conjunctiva was seen. The iris of both eyes showed mild atrophy and darker non-elevated spots. Cataract had developed in both eyes in the twelve weeks since the previous ophthalmic examination. Both eyes showed petechia in the retina, which had not been present at prior ophthalmic examinations.

Patient *B* came 127 days after treatment. The tear production was 16 and 18 mm/min for OD and OS respectively, compared to 18 and 20 mm/min four weeks after the last therapy session. The IOP was 14 and 16 mmHg, compared to 9 and 14 mmHg at the previous checkup. Many follicles were present in the conjunctiva of both eyes. The corneal edema seen during the previous examination had completely disappeared. No other abnormalities were found in this patient.

Patient *I* was irradiated for an adenocarcinoma in the left nasal cavity, and came 105 days after the last radiation treatment for clinical signs of keratoconjunctivitis sicca and loss of vision. This patient had not been examined ophthalmologically before, so no comparison could be made with previous clinical presentation. The appointment at the clinic was made at the owners' own initiative, which makes it plausible that these clinical signs had not been visible or noticed before. Tear production was 15 and 5 mm/min for OD and OS, respectively. The IOP was not measured. During the obstacle test prior to administration of tropicamide drops it was very clear that the patient did not see the obstacles, neither in bright nor in dim light. After administration of tropicamide drops in the eyes, the results of the obstacle test were not different from prior to administration. Epiphora was present in both eyes, with the left eye more severely affected than the right eye. Both eyes showed hyperemia and swelling of the conjunctiva including the nictitating membrane. Corneal edema was present in both eyes, with again the left eye more severely affected. Both eyes showed complete absence of both the direct and the indirect pupillary light reflexes. Severe retinal hemorrhages were present in the left eye and a complete retinal detachment in the right eye.

None of the following abnormalities were found during the complete research period: abnormalities in size of the mandibular lymph nodes and muscles of mastication; ectopic cilia; abnormal palpebral reflexes;

ectropion; strabismus; enophthalmos; changes in retropulsion of the globe; microphthalmos; phthisis bulbi; buphthalmos; abnormal color of the sclera; positive fluorescein stain; abnormal shape or depth of the anterior chamber; turbidity of the anterior chamber; abnormal shape of the pupil; irregular surface or increased thickness of the iris; colobomata of the iris; abnormal size, shape or position of the lens; asteroid hyalosis; vitreal strands; abnormal color, shape, border or prominence of the optic nerve head; atrophy of the retinal vasculature; hyporeflective tapetal area.

Discussion

In the underlying study, both acute and late complications of radiation therapy of tumors of the head in dogs and cats were found.

In the ophthalmic examination directly after the therapy course, only some acute reactions were seen. The acute reactions mainly consisted of hyperemia of the conjunctiva and epiphora. Some progression was noted at the second post-therapy checkup, two to four weeks after the first post-therapy checkup. Five out of eight patients showed corneal edema, epiphora and conjunctival hyperemia. One patient showed moderately swollen eyelids in the first post-treatment check, and one severely swollen eyelid in the second post-treatment check. One patient with no vitreal liquefaction in the first post-treatment check, had developed moderate vitreal liquefaction by the second check.
Acute reactions were mostly seen in both eyes, but more severely at the irradiated side.

The three patients which were seen months after the treatment course showed several late complications. Two of these three patients were diagnosed with keratoconjunctivitis sicca. An additional third patient was diagnosed with keratoconjunctivitis sicca at the second post-treatment examination, but this patient wasn't seen for a third checkup. One patient was diagnosed with keratoconjunctivitis sicca prior to radiation therapy (at four years of age). This latter patient was a West Highland White terrier, a breed known to be predisposed for developing keratoconjunctivitis sicca.
One patient completely lost vision and showed retinal abnormalities in both eyes, with retinal hemorrhages in the left eye and a complete retinal detachment in the right eye. One of the other patients also showed retinal hemorrhages. One different patient showed only mild changes with some conjunctival follicles in both eyes and no retinal changes in both eyes.
Cataract was seen in some patients, but did not show any progression.
No relation between irradiated side and occurrence and severity of late complications could be made, due to the low number of patients.

Keratoconjunctivitis sicca may result from general anesthesia. Research in rats has demonstrated that keratoconjunctivitis sicca can develop following exposure to one single anesthetic dose of xylazine and ketamine. A combination of pentobarbital and ketamine does not cause significant effect on the eye. (45) The use of the alpha-2 adrenoreceptor agonist xylazine has nowadays been largely superseded by (dex)medetomidine. Keratoconjunctivitis sicca due to repeated administration of dexmedetomidine is not mentioned in the medication information leaflet, but a significant decrease in tear production following sedation using medetomidine has been reported in dogs, with a duration of up to 15 minutes postreversal or up to 2 hours postanaesthesia (46,47). At the Utrecht University Clinic for Companion Animals dexmedetomidine is the anesthetic of first choice for sedation of patients without circulatory disease because of the possibility to use an

antagonist after the therapy session to reduce total anesthesia time.

During the research period some fluctuations in median tear production and median IOP was seen. Research of Berger and King demonstrated that fluctuations in Schirmer tear test values in dogs occur on a daily and weekly basis. (48) Hsiao et al reports daily fluctuations of IOP of 4-6 mmHg in healthy children. (49) The fluctuations in individual patients and variation between patients might be interpreted as normal fluctuations and variation, because no particular trend was visible in the collected data on tear production and IOP.

When the questionnaires of the ophthalmic examined patients are compared to the findings of the ophthalmic examination, the most notable fact is that owners often did not see clinical signs, while ophthalmic examination revealed some clinical signs which had apparently been missed by the owner. This could mean that some ophthalmic examination findings are early signs of complications of the radiotherapy, but not clinically relevant yet. A different explanation could be that owners miss some complications, or misinterpret what they see in their own pets.

In this short research period, it would be possible to report the acute side effects in the newly applied patients. It is unlikely to find any late ophthalmic complications in these patients. It is possible to find late complications in the patients from the retrospective part of this research. However, not all of these patients were ophthalmologically examined. Therefore, the late complications can only be reliably found in the animals available for clinical exam and knowledge about the acute complications depends on the information available in the administration program Vetware. The information derived from the questionnaire must be interpreted with care, because owners are not likely to be as perceptive as an ophthalmic specialist and some clinical signs may have been overlooked or interpreted differently. Especially impairment of vision is a symptom often noticed at a late stage of disease process.

The first late complications were seen in a canine patient at 105 days after the last day of irradiation. This means that the hypothesis that dogs don't show late complications until at least one year after therapy, can be rejected. No cats were ophthalmologically examined for late complications, so no conclusions regarding late complications could be drawn.

One of the aims in the design of this study was to let all the ophthalmic examinations be performed by one ophthalmologist (Dr. S.C. Djajadiningrat-Laanen), to reduce the chance on any observer bias and differences in documentation or interpretation of clinical presentation. For organizational reasons it was not possible to achieve this goal. Some patients were examined by a different ophthalmologist (Prof.Dr. M.H. Boevé or Drs. A.P.M. van Schaik-Verboven). Therefore, a total of three ophthalmologists have been involved in this research and although unlikely, some differences in interpretation of clinical presentation may have occurred.
The research student was present at all ophthalmic examinations performed by the ophthalmologists, in order to standardize the documentation of observations as much as possible.

The researchers tried to plan the appointments for the second post-treatment checkup at three weeks after the last day of irradiation. This was not achieved in all patients for organizational reasons. Patients visited the clinic for this check at 18 to 28 days after the last treatment session. The maximally visible acute complications may have been missed in some patients because of too early or too late an examination.

Many articles used in the introduction of this article should be interpreted with care, because these were written for human medicine or on experiments performed on laboratory animals. There may be species-to-species variation, i.e. in the molecular characteristics underlying the degradation process in the lens. (27)

Several articles regarding measures to prevent complications of radiation therapy in humans can be found.
In humans, skin toxicity can be reduced by the use of smaller fractions, longer treatment schedules, and smaller treatment volumes. Lacrimal gland shielding is only recommended when tumor control will not be compromised. Prophylactic nasolacrimal duct intubation with silicon tubing has been proven to decrease the risk of epiphora. (8,50)
The risk of cataract formation can be reduced in humans by using customized lens shields and lens-sparing radiation techniques, and by fully fractionated or even hyperfractionated radiation therapy schedules. Also IMRT (intensity modulated radiotherapy) may be used to reduce cataractogenesis. (8,24,51-53) The customized lens shields are specially mounted lead discs on plastic shells fitted to the conjunctival sac. (54)
Research of Belkacémi et al. (1996 and 1998) showed that administration of heparin to prevent veno-occlusive disease (VOD) of the liver, appeared to have a protective role in development of cataract in humans. Systemic heparin therapy was given against VOD at a dose of 1 mg/kg in 24 hours by i.v. infusion. This protective effect of heparin on cataractogenesis had not been described before. The crystalline lens has no blood supply, but i.v. administered heparin might reach the lens through the aqueous humor. Its protective effect remains intriguing. It is known that heparin causes a dose-dependent inhibition of *in vivo* cell proliferation from the germinative zone, but the exact cataractogenesis protective mechanism is not fully understood yet. (8,24,52,55,56) However, in a more recent study of van Kempen-Harteveld et al., the administration of heparin increased cataract formation compared with patients not treated with heparin. (57)
A fully fractionated radiation schedule, irradiating the smallest possible volume of eye tissues, usage of a shield block, and excluding of the macula from the high-dose region if possible, is advised for reduction of clinically significant retinopathy and optic neuropathy in humans. (8,22,54)
All aforementioned literature on preventive measures described research in humans. The extent to which these preventive measures, such as customized lens shields, are applicable in dogs and cats, is debatable. However, not many patients showed development or progression of cataract (1/8 at second post-therapy examination and 1/3 at third post-therapy examination) during this research. The cataract in patient *C* was not symmetrical and on the same side as the tumor, which suggests that it is a possible complication of irradiation of the head. Patient *A* showed bilateral cataracts, which could also be the result of other factors, such as age. The findings of this preliminary study suggest that cataract is not a common complication. Hence they do not warrant the use of additional measures to prevent cataractogenesis, although future studies may prove otherwise.
The applicability of heparin as a measure for prevention of cataractogenesis is debatable. The exact mechanism is not fully understood yet, and contradictory reports on its effect can be found in literature. More research needs to be done on this subject to clarify the working mechanism, effect, dosage and best administration route.

One of the hypotheses of the research was that late complications are less frequent in

cats than in dogs due to their shorter survival following radiation therapy of tumors of the head. Because of the low numbers of cats included in this study, no comparison between dogs and cats could be made.

The side where the tumor was located, did not develop ocular complications more often than the other side. So no relation between side of the tumor and side of the ophthalmic complications has been seen during this research.

The patients receiving the highest doses of irradiation did not show more complications than the patients receiving the lower doses. However, differences between doses received by the patients were not big (μ=4549 cGy, σ=862 and median is 4800 cGy). Therefore, not many differences were expected.
The left and right eye received nearly the same dose in most patients. Therefore, no relation between laterality of neoplasia and of ocular complications could be demonstrated. The patient whose right eye received no irradiation, did not show any ocular complications on the right side, but did show loss of vision on the left side.

Conclusions

During the research period, several ophthalmic complications of radiation therapy of tumors of the head in dogs and cats have been seen in the fifteen patients treated at the Utrecht University Clinic for Companion Animals in The Netherlands. The acute complications seen during the research period consisted of conjunctivitis-related clinical signs, such as epiphora, conjunctival hyperemia and conjunctival swelling.
Late complications included loss of vision due to retinopathy in one dog, and keratoconjunctivitis sicca in three dogs.
All complications that were found during the research period had already been described in literature, so no new complications were identified.

Based on the information collected in this preliminary study, the addition of preventive measures described in human literature to the current preventive measures at the clinic in Utrecht does not seem applicable at this time point. The exact mechanism of heparin as a measure for prevention of cataractogenesis is not fully understood yet, and contradiction on its effect can be found in literature. More research needs to be done on this subject to clarify the working mechanism, dosage and best administration route. Not many patients developed cataract during this study. Two out of three patients developed retinopathy, so some progression regarding preventive measures can be gained here.
The University Clinic in Utrecht uses fractionated irradiation programs and MLC shielding. Based on the information gained during this research, this should be sufficient to minimize the occurrence and severity of ophthalmic complications of radiation therapy of tumors of the head in dogs and cats. More patients should be examined to increase the reliability of the information about the acute and late ophthalmic complications seen in dogs and cats, and further advice should be based on this broader information.
It is recommended to include an ophthalmic examination at three weeks after radiation therapy for acute complications, and at three to four months after radiation therapy for late complications, in the follow-up protocol of patients receiving radiation therapy of tumors of the head. The owners should be informed about the complications that can be seen after irradiation and about the timeframe in which these complications may be expected.

Taken into account the length of the period between exposure to radiation and the clinical occurrence of late complications such as retinopathy, this research was only

an inventory study. Further research with more patients and longer follow-up times is necessary to determine precisely which acute and late complications occur in the University Clinic for Companion Animals in Utrecht, The Netherlands.

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Appendix 1.1 Table questionnaire

Symptom	Before therapy	During therapy	After therapy
Eye rubbing	1 (15) right eye 2 (15) both eyes	3 (15) both eyes	1 (14) right eye 1 (14) left eye 2 (14) both eyes
Blinking	1 (15) left eye	- (15)	1 (14) both eyes
Blepharospasm	1 (15) left eye	1 (15) both eyes	1 (14) left eye
Photophobia	- (15)	- (15)	1 (14) both eyes
Tear stain	1 (15) left eye 1 (15) both eyes	2 (15) both eyes	1 (14) both eyes
Epiphora	1 (15) left eye 1 (15) both eyes	1 (15) left eye 3 (15) both eyes	1 (14) left eye 2 (14) both eyes
Purulent discharge	3 (15) both eyes	2 (15) both eyes	1 (14) right eye 3 (14) both eyes
Exophthalmos	1 (15) right eye 2 (15) left eye	1 (15) right eye 1 (15) left eye	1 (14) right eye 1 (14) left eye
Enophthalmos	- (15)	1 (15) right eye 1 (15) left eye	1 (14) right eye
Protrusion nictitating membrane	1 (15) right eye	1 (15) right eye	1 (14) both eyes
Reduced corneal transparency	1 (15) right eye 1 (15) left eye	- (15)	- (14)
Erythema eyelid skin	- (15)	2 (15) both eyes 1 (15) right eye	1 (14) both eyes
Periocular hair loss	- (15)	2 (15) right eye 2 (15) both eyes	1 (14) both eyes
Erythema of the eyelids	- (15)	1 (15) right eye	- (14)
Conjunctival hyperemia	1 (15) both eyes	3 (15) both eyes	1 (14) both eyes
Ectropion	1 (15) both eyes	- (15)	1 (14) left eye 1 (14) both eyes
Entropion	- (15)	- (15)	- (14)
Swollen eyelids	- (15)	1 (15) both eyes	1 (14) both eyes
Swollen conjunctiva	- (15)	1 (15) both eyes	1 (14) both eyes
Scleral hyperemia	1 (15) right eye 1 (15) both eyes	- (15)	- (14)
Intraocular hemorrhage	- (15)	- (15)	- (14)
Turbidity of the lens	2 (15) both eyes	2 (15) both eyes	2 (14) both eyes
Mydriasis	- (15)	- (15)	- (14)
Vision loss	- (15)	1 (15) left eye 1 (15) both eyes	1 (14) left eye 1 (14) both eyes
Decreased orientation	- (15)	1 (15)	1 (14)
Hypermetric gate	- (15)	- (15)	- (14)
Insecurity	1 (15)	2 (15)	1 (14)
Anxiety	- (15)	- (15)	- (14)
Bumping into obstacles	- (15)	1 (15) left eye 2 (15) both eyes	1 (14) left eye 1 (14) both eyes

Appendix 1.2 Table contributing factors questionnaire

	Yes
Veterinary care before treatment	5(15)
Veterinary care after treatment	4(15)
Diabetes mellitus	- (15)
Systemic arterial hypertension	- (15)
Ocular foreign body	1 (15) left eye
Clotting disorder	- (15)
Malignant lymphoma	- (15)
Otitis media	1 (15)
Allergy (food, surroundings)	2 (15)
Canine Distemper	- (13)
Hypothyroidism	- (15)
Lens luxation	- (15)
Uveitis	- (15)
Infectious hepatitis	- (13)
Ehrlichia/Rickettsia/Babesia	- (15)
Leishmania	- (15)
Toxoplasmosis	- (15)
Dirofilaria	- (15)
Lupus erythematosus	1(15)
Uveodermatological syndrome	- (15)
Feline Herpes virus infection	- (2)
FIP/FIV/FeLV	- (2)
Phenazopyridine	- (15)
Etodolac	- (15)
Sulpha derivates	- (15)
Atropine	- (15)
Longterm antibiotics	1 (15)
Longterm corticosteroids	1 (15)
Longterm topical anesthesia	- (15)
Enrofloxacin	- (2)

Appendix 2. Table clinical study

Symptom	Pre therapy	0 weeks post therapy	2-4 weeks post therapy	17-52 weeks post therapy
STT (mm/min)	OD 2x18, 1x19 (3) OS 1x15,16,19 (3)	OD 1x16,23 (2) OS 1x15,22 (2)	OD1x6,11,15, en 2x17,18 (7) OS 1x12,14, 2x15, 1x17,18,20 (7)	OD1x14,15,16 (3) OS 1x5,18,19 (3)
IOP (mmHg)	OD 1x15,17,18 (3) OS 1x15,19,20 (3)	OD 1x12, 1x16 (2) OS 1x12, 1x14 (2)	OD 1x9,10,13,15 (4) OS 1x11,12 en 2x14 (4)	OD 1x14,16 (2) OS 1x11,16 (2)
Obstacle light pre mydriatic	- (3)	- (6)	- (7)	1 (3) severe
Obstacle dark pre mydriatic	- (3)	- (6)	- (7)	1 (3) severe
Abnormal position head	- (3)	- (5)	- (8)	- (3)
Blepharospasm	- (3)	1 (6) OU present	- (8)	- (3)
Enlarged mandibular lymph nodes	- (3)	- (5)	- (8)	- (3)
Atrophy of the muscles of mastication	- (3)	- (5)	- (8)	- (3)
Changes of the (peri-) orbital bone	- (3)	- (5)	1 (8) OS present	- (3)
Trichiasis	- (3)	- (5)	- (8)	1 (3) OS present
Distichiasis	1 (3) OD present, OS severe	- (5)	1 (8) OD present, OS severe	- (3)
Ectopic cilia	- (3)	- (5)	- (8)	- (3)
Epiphora/tear stain	1 (3) OD present 1 (3) OU present	1 (6) OS present 1 (6) OU present 1 (6) OU severe	1 (8) OS present 1 (8) OS severe 3 (8) OU present	1 (3) OD present, OS severe
Abnormal palpebral reflex	- (3)	- (5)	- (8)	- (3)
Irregularities/swelling lid margin	- (3)	1 (6) OU present	1 (8) OU present 1 (8) OD present, OS severe	- (3)
Entropion	- (3)	- (5)	- (8)	1 (3) OS present
Ectropion	- (3)	- (5)	- (8)	- (3)
Hyperemia conjunctiva palpebral side	- (3)	3 (6) OU present	2 (8) OS present 1 (8) OU present	1 (3) OU present
Hyperemia conjunctiva bulbar side	- (3)	2 (6) OU present	2 (8) OS present 2 (8) OU present	1 (3) OU present
Hyperemia nict. membr.	- (3)	1 (5) OU present	1 (8) OS present	1 (3) OU present

Other abnormalities conjunctiva palpebral side	- (3)	3 (5) OU present	2 (8) OS present 4 (8) OU present	1 (3) OD severe, OS present
Other abnormalities conjunctiva bulbar side	- (3)	3 (5) OU present	1 (8) OS present 1 (8) OD severe, OS present	1 (3) OU present
Other abnormalities nict. membr.	- (3)	- (4)	- (8)	- (3)
Abnormal scleral conjunctivae	- (3)	- (5)	- (8)	- (3)
Strabismus	- (3)	- (5)	- (8)	- (3)
Exophthalmos	- (3)	- (5)	1 (8) OD present	- (3)
Enophthalmos	- (3)	- (5)	- (8)	- (3)
Decreased retropulsion globe	- (3)	- (5)	- (8)	- (3)
Increased retropulsion globe	- (3)	- (5)	- (8)	- (3)
Microphthalmos	- (3)	- (5)	- (8)	- (3)
Phtisis bulbi	- (3)	- (5)	- (8)	- (3)
Buphthalmos	- (3)	- (5)	- (8)	- (3)
Abnormal color sclera	- (3)	- (5)	- (8)	- (3)
Abnormal vasculature sclera	- (3)	- (5)	1 (8) OS present	- (3)
Other abnormalities sclera	- (3)	- (5)	- (8)	- (3)
Abnormal reflection cornea	- (3)	- (5)	1 (8) OU present	- (3)
Decreased transparency cornea	1 (3) OU present	- (5)	1 (8) OS present 1 (8) OD present 3 (8) OU present	1 (3) OU present 1 (3) OD present, OS severe
Positive fluorescein stain	- (3)	- (5)	- (8)	- (3)
Abnormal shape/depth anterior chamber	- (3)	- (5)	- (8)	- (3)
Turbidity anterior chamber	- (3)	- (5)	- (8)	- (3)
Abnormal pupil shape	- (3)	- (5)	- (8)	- (3)
Abnormal direct pupillary light reflex	- (3)	- (5)	- (8)	1 (3) OU severe
Abnormal indirect pupillary light reflex	- (3)	- (5)	- (8)	1 (3) OU severe
Abnormal color iris	- (3)	- (5)	- (8)	1 (3) OU present
Irregular iridal surface	- (3)	- (5)	- (8)	- (3)
Abnormal thickness iris	- (3)	- (5)	- (8)	- (3)
Colobomata iris	- (3)	- (5)	- (8)	- (3)
Iridal atrophy	1 (3) OS present	- (5)	2 (8) OU present	1 (3) OU present
Persistent pupillary membranes	- (3)	- (5)	1 (8) OU present	1 (3) OS present
Nuclear sclerosis	1 (3) OU present	1 (5) OU present	6 (8) OU present	1 (3) OU severe
Cataract	2 (3) OU	- (5)	1 (8) OD present	1 (3) OU present

	present			
Abnormal size/shape lens	- (3)	- (5)	- (8)	- (3)
Abnormal position lens	- (3)	- (5)	- (8)	- (3)
Asteroid hyalosis vitreous body	- (3)	- (5)	- (8)	- (3)
Vitreal strands	- (3)	- (5)	- (8)	- (3)
Vitreal liquifaction	- (3)	- (5)	1 (8) OU present	- (3)
Abnormal color optic nerve head	- (3)	- (5)	- (8)	- (3)
Abnormal shape optic nerve head	- (3)	- (5)	- (8)	- (3)
Abnormal border optic nerve head	- (3)	- (5)	- (8)	- (3)
Abnormal prominence optic nerve head	- (3)	- (5)	- (8)	- (3)
Abnormal tortuosity retinal vasculature	1 (3) OU present	- (5)	- (8)	- (3)
Atrophy retinal vasculature	- (3)	- (5)	- (8)	- (3)
Hyperreflective tapetal area	1 (3) OS present	- (5)	- (8)	- (3)
Hyporeflective tapetal area	- (3)	- (5)	- (8)	- (3)
Retinal hemorrhage	- (3)	- (5)	- (8)	1 (3) OS severe
Retinal detachment	- (3)	- (5)	- (8)	1 (3) OD severe
Obstacle course light post mydriatic	- (3)	- (6)	- (7)	1 (3) severe
Obstacle course dark post mydriatic	- (3)	- (6)	- (7)	1 (3) severe