



ESCOLA UNIVERSITÁRIA VASCO DA GAMA

MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

**PRIMARY CORNEAL ENDOTHELIAL DEGENERATION IN DOGS
RETROSPECTIVE STUDY OF 30 CASES**

Rita Cacho Miranda
Coimbra, julho de 2019



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Aluna do Mestrado Integrado em Medicina Veterinária

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List of acronyms and abbreviations

BK - Bullous Keratopathy

BP - Blood pressure

CED - Corneal Endothelial Degeneration / Dystrophy

CXL - Cross-Linking

DBD - Diamond Burr Debridement

FECD - Fuchs Endothelial Corneal Dystrophy

KCS - Keratoconjunctivitis sicca

PCED - Primary Corneal Endothelial Degeneration

PK - Penetrating Keratoplasty

PPM - Persistent Pupillary Membranes

SK - Superficial Keratotomy

SQK - Superficial Grid Keratotomy

TK -Thermokeratoplasty

VBC -Visual Behavior changes

PRIMARY CORNEAL ENDOTHELIAL DEGENERATION IN DOGS

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Abstract

Primary corneal endothelial degeneration (CED) is mainly an age-related condition, characterized by a slow progression, that presents a breed predisposition in different canine breeds like Chihuahuas, Boston Terriers and Dachshunds, and is more common in females. Several surgical procedures are described to decrease the degree of corneal oedema and to help with the resolution of the ulcers.

The objective of this study was to conduct a retrospective investigation of canine CED, in order to characterize the clinical presentation of the disease, to evaluate potential risk factors and to analyse response to different surgical approaches.

Thirty dogs with CED presented to the ROWE Referrals Eye Clinic, Bradley Stoke, United Kingdom, between January 2016 and March 2019, with ages ranging from 3 to 14 years (mean 9.8 ± 2.7 years), were enrolled in this research. The studied population was composed mainly by females (n=19; 63.3%), and included dogs from 11 different breeds, with Shih-Tzus (n=7, 23.3%), Boston Terriers (n=4, 13.3%), Italian Spinones (n=4, 13.3%), and Jack Russel Terriers (n=3, 10.0%) being the most represented; and two cross-breed dogs. Most dogs presented a diffuse (46.7%, n =14) or focal corneal oedema (30.0%, n= 9). Twenty-four dogs were submitted to surgical procedures, mainly Diamond burr (n=11, 45.8%) and grid keratotomy (n=5; 20.1%). According to the tutor's perception, the Gunderson flap and corneal transplant were the surgical techniques with a better visual outcome.

Keywords

Bullae, Canine, Cornea, Corneal endothelial dystrophy, Corneal Surgery, Corneal Ulcer, Endothelium

Resumo

A degeneração primária do endotélio corneal (CED) é uma condição de progressão lenta, relacionada maioritariamente com a idade do animal, que apresenta uma predisposição para certas raças caninas como Chihuahuas, Boston Terriers e Dachshunds, sendo mais comum nas fêmeas. Estão descritos vários procedimentos cirúrgicos para tentar diminuir o grau de edema corneal e auxiliar na resolução das úlceras da córnea.

O objetivo deste estudo foi realizar um estudo retrospectivo sobre a degeneração do endotélio corneal em cães, com a finalidade de caracterizar a sua apresentação clínica, avaliar potenciais fatores de risco e analisar a resposta a diferentes abordagens cirúrgicas.

Trinta cães diagnosticados com CED, com idades entre os 3 e os 14 anos (média $9,8 \pm 2,7$), foram selecionados a partir da base de dados do ROWE Referrals Eye Clinic, Bradley Stoke, Reino Unido, no período relativo a Janeiro de 2016 a Março de 2019, e incluídos neste estudo. A população estudada foi composta principalmente por fêmeas ($n=19$; 63,3%) e incluiu cães de 11 raças diferentes, sendo as mais representadas o Shih-Tzu ($n = 7$, 23,3%), o Boston Terrier ($n = 4$, 13,3%), o Italian Spinone ($n = 4$, 13,3%) e o Jack Russel Terriers ($n = 3$, 10,0%); e dois cães de raça indeterminada. No exame oftalmológico a maioria dos cães apresentou edema corneal difuso (46,7%, $n=14$) ou focal (30,0%, $n=9$).

Vinte e quatro cães foram submetidos a procedimentos cirúrgicos, principalmente desbridamento com caneta de ponta de diamante ($n=11$, 45.8%) e queratotomia em grelha ($n=5$; 20.1%). De acordo com a perceção do tutor o transplante de córnea e o flap de Gundersen foram as técnicas cirúrgicas que resultaram num melhor resultado visual.

Palavras-chave

Bullae, Canino, Cirurgia corneal, Córnea, Degeneração do endotélio corneal, Endotélio, Úlcera da córnea

1. Background

The eye is a complex structure composed by three main layers. The outer layer is the fibrous tunic of the eye, and is composed by the sclera and the cornea; the intermediate layer is designated uvea, and comprises the iris, the ciliary body and the choroid; and the inner layer is the neural tunic, and is composed by the retina (Gould, D., & Mclellan, 2014; Maggs, D., Miller, P., & Ofri, 2008). A normal cornea is completely transparent despite featuring several layers: the epithelium, the stroma (which is composed of numerous layers of collagen membranes, mostly type I collagen), the descemet's membrane and the endothelium (Maggs, D., Miller, P., & Ofri, 2008). The endothelium presents a single layer of cells, with Na⁺/K⁺ ATPase enzymes that actively pump Na⁺ and Cl⁻ to the anterior chamber maintaining the cornea in a state of relative dehydration, which contributes to the alignment of the collagen fibres in the stroma (Gould, D., & Mclellan, 2014). This is essential for the transparency of the cornea and the visual ability (Maggs, D., Miller, P., & Ofri, 2008). The corneal epithelium also contributes to the maintenance of this state of relative dehydration, although to a lesser extent than the endothelium (Samuel *et al.*, 2018).

Physiologically, the canine endothelium presents a cellular density of 2815.18 ± 212.59 cells/mm², with a reduced regeneration capacity (Strom *et al.*, 2016). As the number of endothelial cells decrease with age, the remaining cells increase their size and create more ATPase channels to maintain the transparency and visual capacity of the eye (Inomata, Mashaghi, Hong, Nakao, & Dana, 2017). Canine corneal endothelial degeneration (CED) or dystrophy occurs when the endothelial cell density decrease to less than 500 to 800 cells/mm², and the endothelium loses the ability to keep the aqueous humour out of the stroma, which progressively leads to corneal oedema (Gould, D., & Mclellan, 2014). The corneal oedema can be focal, extensive or diffuse and ultimately can lead to severe vision impairment (Sanchez, Dawson, Matas Riera, & Escanilla, 2016). In advanced stages, the oedema makes the cornea fragile leading to formation of sub epithelial bullae, also designated bullous keratopathy (BK). These pockets of fluid can erupt and form non-healing superficial ulcers on the cornea surface that cause discomfort and pain (Bayley, Read, & Gates, 2018; Pot *et al.*, 2013).

The canine primary corneal endothelial degeneration (PCED) is an age-related slow progressive condition, that appears mainly in geriatric dogs, however, can also be a congenital problem that manifests in younger animals, especially in small breeds like Chihuahua, Boston Terrier and Dachshund (Bayley *et al.*, 2018). Primary corneal endothelial degeneration is also frequent in German shorthaired and wirehaired pointers (Shull, Reilly, Davis, Murphy, & Thomasy, 2017). A gender predisposition is also reported, being more frequent in females (K. Gelatt, 2008). Canine CED can also appear secondary to keratitis, glaucoma, uveitis, intraocular surgeries, trauma and lens luxation (Bayley *et al.*, 2018; Gould, D., & Mclellan, 2014).

Due to several similarities and to the similar heritable genetic basis, CED in Boston Terriers was compared with Fuchs endothelial corneal dystrophy (FECD) in humans, which is an ocular disorder characterized by a dysfunction on the cornea that ultimately results in corneal oedema (Aldave, Han, & Frausto, 2013; Thomasy *et al.*, 2016).

Despite the various treatment options described for CED, none is reported to be curative. In the early stages, CED is normally managed with administration of topical osmotic agents, being the ophthalmic solution of sodium chloride at 5% (NaCl 5%) the most commonly used (Knezovic, Dekaris, & Gabric, 2006; Samuel *et al.*, 2018). Recent studies (Knezovic *et al.*, 2006; Samuel *et al.*, 2018; Schiavo & Bentley, 2000) compared the efficacy of NaCl 5% with other osmotic agents like dextran, glycerine, polysaccharide, sodium sulfacetamide and a hypolipidemic agent (CGS 24565), but the ophthalmic solution of NaCl 5% proved to be the most effective, the only agent that decreased the corneal thickness, and to be relatively innocuous to the eye.

In the advanced stages of PCED, when Bullous Keratopathy (BK) develops, several treatments were reported, varying in the degree of invasion. Such treatments include simple cotton bud debridement, diamond burr debridement (DBD) or placement of contact lens (Gosling, Labelle, & Breaux, 2012). The described surgical procedures also include superficial keratotomy (SK), superficial grid keratotomy (SGK), thermokeratoplasty (TK), gundersen flap and penetrating keratoplasty (PK). Nevertheless, a definitive treatment was not described (Bayley *et al.*, 2018; Famose, 2016; Gosling *et al.*, 2012; Michau, Gilger, Maggio, & Davidson, 2003; Wu *et al.*, 2018). The cross-linking (CXL) is a relatively new procedure, which consists in the irrigation of the cornea with riboflavin and exposure to ultraviolet light, has been successfully used to increase the patient comfort in cases of BK, but the results are temporary (Famose, 2016; Pot *et al.*, 2013). Several authors have been testing the use of CXL protocols associated with other surgical procedures, such as superficial keratectomy, to increase the efficacy of treatment (Kim, Ji, Naoaki Takiyama, Bae, & Kim, 2018). A recent case report about the use of endothelial keratoplasty, the goldstandart technic in humans, in a canine patient with CED showed that this technique could be a potential option for a definitive treatment for dogs affected with this disease (Armour *et al.*, 2019).

This study aimed to conduct a retrospective investigation of canine CED, in order to contribute to the characterization of the clinical presentation of the disease, to evaluate potential risk factors and to analyse response to different surgical approaches.

2. Materials and methods

This retrospective study was approved by the Scientific Council of Vasco da Gama University School, Coimbra, Portugal.

2.1. Animals

Dogs presented to the ROWE Referrals Eye Clinic, Bradley Stoke, United Kingdom, between January 2016 and March 2019, diagnosed with a primary CED were enrolled in the study. Animals with a previous history of intraocular surgical procedure such as cataract surgery, dogs that had any type of ocular penetrating injury, dogs with persistent pupillary membranes (PPM) that could cause endothelium damage and dogs that presented other systemic diseases or ophthalmic lesions that could cause corneal oedema such as uveitis, glaucoma, melting or deep ulceration, toxic reaction, mature or hyper mature cataracts, diabetes *mellitus*, and systemic neoplasia were excluded from this research (Kirk N. Gelatt, 2008; Sansom & Labrue, 2012). All tutors signed a written informed consent to allow the use of clinical data from their dogs for research purposes.

Parameters related with identification and characterization of the animals enrolled in the study included breed, gender, and age. The motive for consultation was also recorded and subcategorized in eye colour changes, light sensibility and visual behaviour changes.

2.2. Ophthalmic examination

All dogs were submitted to a full bilateral ophthalmic examination, performed by a veterinary surgeon certificated in veterinary ophthalmology by the Royal College of Veterinary Surgeons. Examination included Schirmer tear test (Merck MSD animal Health, USA), neuro-ophthalmic examination (visual tests, dazzle reflex and pupillary light reflex), slit-lamp examination (SL-17, Kowa, Japan), tonometry (tonovet plus, Icare, Finland), indirect ophthalmoscopy (All Pupil II, Keller, UK), and fluorescein test when clinically indicated (fluorescein sodium 1 % w/v, Brausch & Lomb).

The degree of corneal oedema was classified in focal (when less than 40% of the corneal surface was affected), extensive (when the oedema covered between 40 to 80% of the cornea) and diffuse (when more than 80% of the cornea was affected). Presence / absence of corneal bullae and ulcers was also classified in three categories: no bullae or ulcers, presence of one or several bullae, and presence of ulcers (dogs with bullae but also corneal ulcers were all placed in the ulcers group). Other ocular problems were also analysed, including incipient cataracts (involving less than 15% of the lens volume), eyelid mass, entropion, nuclear sclerosis, iris atrophy, vitreous prolapse and dry eye. The time between CED diagnosis and the need of surgical treatment was categorized in less than one year, between one and three years and more than three years.

2.3. Surgical treatment

In this study, different surgical techniques were evaluated, including DBD, SGK, TKP, Gundersen flap and PK. The different surgical procedures performed were selected based on clinical indications and tutors' consent. All the techniques were performed according with procedures previously described (Kirk N. Gelatt & Gelatt, 2011; Gilger, 2013; Gould, D., & Mclellan, 2014; Lacerda *et al.*, 2016; Miller, 2010).

After surgery, all complications including fibrosis, grid lines delayed healing, persistency of bullae or ulcers and bacterial infection were recorded. In addition, the tutor's perception of the visual outcome of the surgery was also assessed, and classified in three categories: no improvement in vision, moderate improvement in vision and marked improvement in vision after surgery.

Postoperative medication protocol included topical administration of substitute tear with sodium hyaluronate (every 6 hours), heterologous serum (every 6 hours), sodium chloride 5 % (every 6 hours), ofloxacin or chloramphenicol (every 6 hours) and in some cases oral administration of meloxicam (0.1 mg/kg, every 24 hours, for 8 days). Other postoperative care consisted of daily full ophthalmologic examination during the hospitalization period. Follow ups were set at one, two and four weeks after surgery, and then every 6 months.

2.4. Statistical analysis

The various parameters were analysed with the SAS program (SAS Institute Inc., 2017), through PROC FREQ, in order to estimate counts and frequencies. Associations between parameters were determined by Independence Tests performed with the Chi-square. Several contingency tables were defined to study the relationship between each pair of parameters (categorical variables) in which the frequencies of the categories of one of the variables were estimated relative to the categories of another.

3. Results

Thirty dogs with primary CED were enrolled in this research. The studied population comprised 19 females (63.3%) and 11 males (36.7%), with ages ranging from 3 to 14 years (mean 9.8 ± 2.7 years). The affected dogs were of different breeds, with Shih-Tzus (n=7, 23.3%), Boston Terriers (n=4, 13.3%), Italian Spinones (n=4, 13.3%), and Jack Russel Terriers (n=3, 10.0%) being the most represented. Complete characterization of the canine population included in the study is presented in table 1.

Fourteen dogs presented a diffuse corneal oedema (46.7%), nine a focal corneal oedema (30.0%) and seven an extensive corneal oedema (23.3%). Ten dogs had no corneal ulceration (33.3%), four had bullae (13.3%) and 16 presented ulcers (53.3%). The most common symptom first noticed by tutors was eye colour change (n =20; 66.7%).

In the studied population, 24 dogs (80%) had concomitant ocular lesions, including 19 dogs with nuclear sclerosis, 10 dogs with iris atrophy, 10 dogs with incipient cataracts, eight dogs with eyelid masses, one dog with entropion, four dogs with dry eye and one dog presented vitreous prolapse

As shown in table 2, none of the dogs with focal corneal oedema presented corneal bullae or ulcers, while all dogs with diffuse corneal oedema presented corneal ulcers

TABLE 1. Characterization of the canine population enrolled in the study.

	<i>n (%)</i>	<i>Age (years)</i> <i>mean ± SD</i> <i>[min-max]</i>	<i>Male: female ratio</i>	<i>Motive for</i> <i>consultation</i>	<i>Stage of Corneal</i> <i>oedema</i>
Shi-Tzu	7 (23.3)	10.3 ± 2.21 [8-14]	2:5 (29% - 71%)	Colour changes:4 Photophobia: 0 Visual B. C.:3	Focal:1 Extensive:2 Diffuse: 4
Boston Terrier	4 (13.3)	6.0 ± 3.74 [3-11]	1:3 (25% - 75%)	Colour changes:3 Photophobia: 1 Visual B. C.:0	Focal:0 Extensive:4 Diffuse:0
Italian Spinone	4 (13.3)	11.0 ± 2.16 [9-14]	2:2 (50% - 50%)	Colour changes:4 Photophobia: 0 Visual B. C.:0	Focal:2 Extensive:0 Diffuse:2
Jack Russel Terrier	3 (10.0)	12.0 ± 2.65 [9-14]	2 :1 (66.6% – 33.3%)	Colour changes:1 Photophobia: 1 Visual B. C.:1	Focal:2 Extensive:0 Diffuse:1
Dalmatian	2 (6.7)	12.0 ± 0 [12-12]	0:2 (0%-100%)	Colour changes:1 Photophobia: 0 Visual B. C.:1	Focal:0 Extensive:0 Diffuse:2
German Wirehaired Pointer	2 (6.7)	9.5 ± 0.71 [9-10]	1:1 (50% - 50%)	Colour changes:1 Photophobia: 0 Photophobia :1	Focal:0 Extensive:0 Diffuse:2
Springer Spaniel	2 (6.7)	9.5 ± 2.83 [9-10]	0: 2 (0% – 100%)	Colour changes:1 Photophobia: 0 Visual B. C.:1	Focal:1 Extensive:1 Diffuse:0
Border Collie	1 (3.3)	12	0: 1 (0% – 100%)	Colour changes:0 Photophobia: 1 Visual B. C.:0	Focal:0 Extensive:0 Diffuse:1
Bichon Frise	1 (3.3)	11	0: 1 (0% – 100%)	Colour changes:1 Photophobia: 0 Visual B. C.:0	Focal:0 Extensive:0 Diffuse:1
Patterdale	1 (3.3)	9	1:0 (100% - 0%)	Colour changes:1 Photophobia: 0 Visual B. C.:0	Focal:1 Extensive:0 Diffuse:0
Basset Hound	1 (3.3)	10	0: 1 (0% – 100%)	Colour changes:1 Photophobia: 0 Visual B. C.:0	Focal:1 Extensive:0 Diffuse:0
Cross Breed	2 (6.7)	7.0 ± 0 [5-9]	1:1 (50% - 50%)	Colour changes:2 Photophobia: 0 Visual B. C.:0	Focal:2 Extensive:0 Diffuse:0
Total	30	9.8 ± 2.7 [3-14]	19 females (63.3%) and 11 males (36.7%)	Colour changes:20 Photophobia: 4 Visual B. C.:6	Focal:8 Extensive:7 Diffuse:15

(B.C, behaviour change; S.D.: Standard Deviation)

TABLE 2. Comparison of the degree of corneal oedema with the presence (n, %) of ulcers or bullae as observed in the first ophthalmic examination.

	No Ulcer/ Bullae (n, %)	Bullae (n, %)	Ulcers (n, %)	Total (n, %)
Focal	9 (30.0%)	0	0	9 (30.0%)
Extensive	1 (3.3%)	4 (13.3%)	2 (6.7%)	7 (23.3%)
Diffuse	0	0	14 (46.7%)	14 (46.6%)
Total (n, %)	10 (33.3%)	4 (13.3%)	16 (53.3%)	30

A total of 24 surgeries were performed, being the Diamond burr technique the most frequently performed (n=11, 45.8%). Five dogs were submitted to grid keratotomy (20.1%), three to Gundersen flap (12.3%), four submitted to thermokeratoplasty (16.7%) and one dog to a full-thickened corneal transplant (4.2%). Table 3 displays the tutor's perception on visual outcome after surgery considering the type of surgery performed.

Postoperative complications were detected in 15 surgeries (62.5%) including one dog with fibrosis formation (n=1) after a GK surgery, bacterial grow (n=1) after a Diamond burr surgery, grid lines delayed healing (n=1) after GK surgery and bullae or ulcers still present after surgeries (n=12), namely in eight dogs after diamond burr debridement, in two dogs after Grid Keratotomy and in two dogs after Gundersen flap.

TABLE 3. Tutors' perception on visual outcome after surgery according with different surgical procedures.

	No improvement in vision	Moderate improvement in vision	Marked improvement in vision
Diamond burr	6 (25.0%)	4 (16.6%)	1 (4.2%)
Grid keratotomy	4 (16.7%)	1 (4.2%)	0 (0.0%)
Gunderson flap	0 (0.0%)	1 (4.2%)	2 (8.3%)
Corneal transplant	0 (0.0%)	0 (0.0%)	1 (4.2%)
Thermokeratoplasty	1 (4.2%)	2 (8.3%)	1 (4.2%)

4. Discussion

The present study describes the clinical features and surgical outcomes of dogs with PCED presented to a reference ophthalmologic veterinary hospital in the United Kingdom during a period of approximately two and a half years.

In our study, a higher frequency of CED was observed in females than in males, suggesting a female predisposition also described in previous studies (K. Gelatt, 2008; Gould, D., & Mclellan, 2014; Maggs, D., Miller, P., & Ofri, 2008; Wu *et al.*, 2018). In our study, Shih-Tzu was the most commonly affected breed with CED. Chihuahuas, Boston Terriers and Dachshunds are frequently described as predisposed to CED in different studies (K. Gelatt, 2008; Gould, D., & Mclellan, 2014; Maggs, D., Miller, P., & Ofri, 2008). Although not reported as predisposed to PCED in most studies, a recent investigation reported Shih-Tzus as dogs commonly affected by CED (Kellam *et al.*, 2018). The results observed in our study are in line with this later study, and suggest that Shih-Tzu might also be a canine breed with predisposition to CED.

In this study, affected Boston Terriers were younger than the dogs of the other breeds, mean 6.0 years $\pm$ 3.74 years vs mean 9.8 years $\pm$ 2.7 years. As previously stated, PCED is frequently an aged related disease, being more frequent in geriatric dogs. However, canine CED might also be presented as a congenital disease, mainly in small canine breeds such as Boston Terriers (Bayley *et al.*, 2018; Kirk n. Gelatt, 2008; Thomasy *et al.*, 2016).

Regarding the clinical symptoms first perceived by the tutors, the most common was the change in the eye's colour, which is normal considering that the main clinical sign of canine CED is corneal oedema that originates a bluish appearance of the eye. It is also noticeable that problems such as light sensibility/photophobia, that can indicate presence of ulcers, only occurred in extensive and diffuse stages of corneal oedema. This suggests that ulcers tend to appear in the later stages of CED in which greater areas of the cornea are affected by oedema. Light sensibility can also result from the increased scattering of the light secondary to the corneal oedema (Kirk n. Gelatt, 2008). This theory is exposed in table 2 where it is showed that in cases of focal corneal oedema no bullae or ulcers were found, and in cases where the oedema has become diffuse all dogs presented corneal bullae and ulcers. Visual impairment was only reported in cases of diffuse corneal oedema. In this disease stage, the fluid accumulation in the stroma cause disarrangement of the collagen fibres, resulting in decreased visual capacity (Thomasy *et al.*, 2016).

The most frequently observed concomitant ocular lesion was nuclear sclerosis, followed by iris atrophy and incipient cataracts. All these lesions are considered frequent among senior dogs (Bellows *et al.*, 2015). Indeed, the majority of the dogs in the present study were older than 7 years old (mean of 9.8 $\pm$ 2.7 years).

Regarding the surgical procedures, none of the techniques showed to be curative in all cases (table 3). According to previous studies (Lacerda *et al.*, 2016; Shull *et al.*, 2017) the gold standard procedure in

canine CED is corneal transplant. Nevertheless, it is rarely performed due to several reasons, namely the difficulty of the procedure, the lack of appropriate donor tissue, the high risk of complications and the high cost of the surgery (Shull *et al.*, 2017; Siu, Young, & Jhanji, 2014). Considering the visual outcome, the surgery with more consistent results in the present study was the Gundersen flap. Our results are in agreement with previous studies (Horikawa *et al.*, 2016; Siu *et al.*, 2014) that described significant improvement in corneal oedema associated with conjunctival flaps. Furthermore, it is especially useful in cases where corneal transplants are not available as it circumvents the need of donor tissue by using autologous conjunctiva.

Diamond burr and grid keratotomy are primarily used to temporarily treat the ulcers rather than as an attempt to decrease the corneal oedema. This fact justifies the least favourable perception of the tutors regarding the visual outcome. According to Wu *et al.* (2018) no significant differences were found between Diamond burr and grid keratotomy regarding the healing outcomes of the corneal ulcers. Nevertheless, Diamond burr and grid keratotomy presents a number of advantages, namely they are less expensive, they are simple procedures, and a full general anaesthetics is not always required (Spertus, Brown, & Giuliano, 2017).

Thermokeratoplasty was reported to improve the quality of life of the animal by creating a slight scarred cornea in order to prevent corneal ulcer and bullae formation, however, the improvement in the visual capacity was reported to be low (Micheu *et al.*, 2003). Accordingly, it was observed in the present study that the majority of the tutor's dogs subjected to this surgery only noticed small improvements in the visual capacity of the animal.

This study presented several limitations, namely the reduced number of animals in some categories, which hindered statistical analysis. Nevertheless, the descriptive analysis of the data is important considering that CED severely affects the visual capacity of the dog, and that the superficial corneal ulcers are painful and affect the quality of life of the animal. Moreover, measurements of the corneal thickness were not performed, and the evaluation of the corneal oedema degree was only based on the ophthalmic examination.

5. Conclusions

Our results suggest that canine CED is more prevalent in females and in geriatric animals. However, in Boston Terriers, the disease appears to manifest in younger ages than in other breeds. The breed more commonly affected was Shih-Tzu, followed by Boston Terrier and Italian Spinone. Our results suggest a predisposition to CED in Shih-Tzus.

Colour changes were the clinical symptom more commonly perceived by the tutors, followed by light sensibility and visual behaviour changes. Regarding the surgical procedures, none of the techniques showed to be curative in all cases. In our study, the surgical procedures with more consistent results in visual outcome according to the tutor's perception were the Gundersen flap and corneal transplant.

6. References

- Aldave, A., Han, J., & Frausto, R. (2013). *Genetics of the corneal endothelial dystrophies: an evidence-based review*. 84, 109–119. <https://doi.org/10.1111/cge.12191>
- Bayley, K. D., Read, R. A., & Gates, M. C. (2018). Superficial keratectomy as a treatment for non-healing corneal ulceration associated with primary corneal endothelial degeneration. *Veterinary Ophthalmology*, 1–8. <https://doi.org/10.1111/vop.12617>
- Bellows, J., Colitz, C. M. H., Daristotle, L., Ingram, D. K., Lepine, A., Marks, S. L., ... Tomlinson, J. (2015). Common physical and functional changes associated with aging in dogs. *Journal of the American Veterinary Medical Association*, 246(1), 67–75. <https://doi.org/10.2460/javma.246.1.67>
- Famose, F. (2016). Evaluation of accelerated corneal collagen cross-linking for the treatment of bullous keratopathy in eight dogs (10 eyes). *Veterinary Ophthalmology* (2016), 19(3), 250–255. <https://doi.org/10.1111/vop.12280>
- Gelatt, Kirk N., & Gelatt, J. P. (2011). *Veterinary Ophthalmic Surgery*. Gainesville, USA: Saunders Elsevier.
- Gelatt, K. (2008). *Essentials of Veterinary Ophthalmology* (W.- Blackwell, ed.). Iowa, USA.
- Gilger, B. . (2013). Diseases and surgery of the canine cornea and sclera. *Veterinary Ophthalmology*, 690–752.
- Gosling, A. A., Labelle, A. L., & Breaux, C. B. (2012). Management of spontaneous chronic corneal epithelial defects (SCCEDs) in dogs with diamond burr debridement and placement of a bandage contact lens. *Veterinary Ophthalmology*, 1–6. <https://doi.org/10.1111/j.1463-5224.2012.01026.x>
- Gould, D., & Mclellan, G. (2014). *BSAVA Manual of Canine and Feline Ophthalmology* (third edit; BSAVA., ed.). Gloucester, England.
- Horikawa, T., Thomasy, S. M., Stanley, A. A., Calderon, A. S., Li, J., Linton, L. L., & Murphy, C. J. (2016). Superficial Keratectomy and Conjunctival Advancement Hood Flap (SKCAHF) for the Management of Bullous Keratopathy. *Cornea*, 35(10), 1295–1304. <https://doi.org/10.1097/ico.0000000000000966>
- Inomata, T., Mashaghi, A., Hong, J., Nakao, T., & Dana, R. (2017). Scaling and maintenance of corneal thickness during aging. *PLoS ONE*, 12(10), 1–11. <https://doi.org/10.1371/journal.pone.0185694>
- Kim, J., Ji, D.-B., Naoaki Takiyama, J., Bae, J., & Kim, M.-S. (2018). Corneal collagen cross-linking following superficial keratectomy as treatment for corneal endothelial cell dystrophy in dogs: Preliminary clinical study. *Veterinary Ophthalmology*, 1–8. <https://doi.org/10.1111/vop.12611>
- Knezovic, I., Dekaris, I., & Gabric, N. (2006). Therapeutic efficacy of 5% NaCl hypertonic solution in patients with bullous keratopathy. *Collegium Antropologicum*, 30(2), 405–408.
- Lacerda, R. P., Pe~ Na Gimenez, M. T., Laguna, F., Costa, D., Ios, J. R., Leiva, M., & Leiva, M. (2016). Corneal grafting for the treatment of full-thickness corneal defects in dogs: a review of 50 cases. *Veterinary Ophthalmology*, 1–10. <https://doi.org/10.1111/vop.12392>
- Maggs, D., Miller, P., & Ofri, R. (2008). *Slatter's Fundamentals of Veterinary Ophthalmology* (: Elsevier Inc, ed.). Missouri.
- Michau, T. M., Gilger, B. C., Maggio, F., & Davidson, M. G. (2003). Use of thermokeratoplasty for

- treatment of ulcerative keratitis and bullous keratopathy secondary to corneal endothelial disease in dogs: 13 cases (1994-2001). *JAVMA*, 222(5), 607–612. Retrieved from <http://cyber.sci-hub.tw/MTAuMjQ2MC9qYXZtYS4yMDAzMjlyMi42MDc=/10.2460%40javma.2003.222.607.pdf>
- Miller, W. (2010). keratoleptynsis for treatment of canine corneal endothelial cell dystrophy. *Proceedings of the 41st Annueal Metting of the American College of Veterinary Ophthalmologists*. san diego.
- Pot, S. A., Gallh , N. S., Walser-Reinhardt, L., Hafezi, F., Spiess, B. M., & Pot, S. A. (2013). Treatment of bullous keratopathy with corneal collagen cross-linking in two dogs. *Veterinary Ophthalmology*, 18(2), 168–173. <https://doi.org/10.1111/vop.12137>
- Samuel, M., Thomasy, S. M., Calderon, A. S., Kass, P. H., Collins, K., Christopher, J., ... Murphy, C. J. (2018). Effects of 5% sodium chloride ophthalmic ointment on thickness and morphology of the normal canine cornea. *Veterinary Ophthalmology*, 1–9. <https://doi.org/10.1111/vop.12582>
- Sanchez, R. F., Dawson, C., Matas Riera, M., & Escanilla, N. (2016). Preliminary results of a prospective study of inter- and intra-user variability of the Royal Veterinary College corneal clarity score (RVC-CCS) for use in veterinary practice. *Veterinary Ophthalmology*, 19(4), 313–318. <https://doi.org/10.1111/vop.12307>
- Sansom, J., & Labruy , J. (2012). Penetrating ocular gunshot injury in a Labrador Retriever. *Veterinary Ophthalmology*, 15(2), 115–122. <https://doi.org/10.1111/j.1463-5224.2011.00941.x>
- Schiavo, D. M., & Bentley, P. (2000). Ocular Changes in Beagle Dogs following Oral Administration of CGS 24565, a Potential Hypolipidemic Agent. *TOXICOLOGICAL SCIENCES*, 55, 189–194.
- Shull, O. R., Reilly, C. M., Davis, L. B., Murphy, C. J., & Thomasy, S. M. (2017). *Phenotypic Characterization of Corneal Endothelial Dystrophy in German Shorthaired and Wirehaired Pointers Using In Vivo Advanced Corneal Imaging and Histopathology*. Retrieved from www.corneajrnl.com
- Siu, G. D. J. Y., Young, A. L., & Jhanji, V. (2014). Alternatives to corneal transplantation for the management of bullous keratopathy. *Current Opinion in Ophthalmology*, 25(4), 347–352. <https://doi.org/10.1097/ICU.0000000000000062>
- Spertus, C. B., Brown, J. M., & Giuliano, E. A. (2017). Diamond burr debridement vs. grid keratotomy in canine SCCED with scanning electron microscopy diamond burr tip analysis. *Veterinary Ophthalmology*, 20(6), 505–513. <https://doi.org/10.1111/vop.12460>
- Strom, A. R., Cort s, D. E., Thomasy, S. M., Kass, P. H., Mannis, M. J., & Murphy, C. J. (2016). In vivo ocular imaging of the cornea of the normal female laboratory beagle using confocal microscopy. *Veterinary Ophthalmology*, 19(1), 63–67. <https://doi.org/10.1111/vop.12264>
- Thomasy, S. M., Cortes, D. E., Hoehn, A. L., Calderon, A. C., Li, J. Y., & Murphy, C. J. (2016). In Vivo Imaging of Corneal Endothelial Dystrophy in Boston Terriers: A Spontaneous, Canine Model for Fuchs' Endothelial Corneal Dystrophy. *Ophthalmol Vis Sc*, 57, 495–503. <https://doi.org/10.1167/iovs.15-18885>
- Wu, D., Smith, S. M., Stine, J. M., Michau, T. M., Thomas, J., Miller, R., ... Freeman, S. (2018). Treatment of spontaneous chronic corneal epithelial defects (SCCEDs) with diamond burr debridement vs combination diamond burr debridement and superficial grid keratotomy. *Veterinary Ophthalmology*, 1–10. <https://doi.org/10.1111/vop.12556>

7.Attachments