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# COMPARATIVE OF 10 CASE REPORTS OF DOGS WITH EVAPORATIVE DRY EYE

María Simó Vesperinas, Francisco Simó Domenech

# ABOUT THE AUTHORS:

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Veterinarian María Simó graduated in Veterinary Medicine from the Autonomous University of Barcelona (UAB) in 2016. She then completed 2 years of internship at Canis Hospital Veterinari in Girona and upon completion, she moved to England to work as an emergency veterinarian at Vets Now Hospital Manchester, while also taking the three-year BSAVA Postgraduate Certificate in Small Animal Ophthalmology. She has completed the ACVO Basic Science Course. Since 2020, she works as an ophthalmologist at the ophthalmology referral center Instituto Veterinario Oftalmológico (IVO) located in Barcelona.

## Francisco Simó Domenech

Veterinarian Paco Simó graduated in Veterinary Medicine from the University of Zaragoza in 1986. Since his professional beginnings he has been passionate about ophthalmology, completing his training with postgraduate degrees in ophthalmology at the Autonomous University of Barcelona (UAB) and l'École Vétérinaire de Toulouse (ENVT).
He has completed residencies at the Veterinary Faculty of Toulouse, with Dr. Marc Simon in Paris and at the Ophthalmology Service of Long Island Veterinary Specialists in New York. Since 1990 he has concentrated on veterinary ocular microsurgery. He was the first veterinarian to perform cataract surgery by phacoemulsification and to place an intraocular lens in a dog in Catalonia.
In 2008 he founded the ophthalmology reference center Instituto Veterinario Oftalmológico (IVO) located in Barcelona, where he is still the medical director. From IVO, Paco Simó is committed to excellence and innovation based on a broad knowledge of the latest trends in human ophthalmology and its applications to the particularities of the animal eye.

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# 1. INTRODUCTION

Keratoconjunctivitis sicca (KCS) is an inflammatory disease of the ocular surface (cornea and conjunctiva) secondary to a deficiency of one of the layers of the tear film [1] and has a prevalence in dogs of 0.4% according to a recent study by the BSAVA (British Small Animal Veterinary Association) [2].

The tear film in dogs consists of three layers: The innermost layer contains mucin, which is secreted by conjunctival goblet cells and whose function is to provide a smooth corneal surface to enhance tear film spreading [3]. The middle layer is the aqueous layer, secreted by the lacrimal glands and responsible for the main metabolic and defensive functions of the tear [4]. Finally, the outermost layer is the lipid layer, secreted by the Meibomian glands and having the main function of limiting evaporation, improving the adhesion of the tear film to the cornea and providing the necessary surface tension to prevent tear overflow [1].

Several different types of KCS can be identified depending on the tear film layer that is affected, although in any of them, the key pathological mechanism of the pathology will be tear hyperosmolarity, which will damage the ocular surface both directly and indirectly by causing an inflammatory process on the ocular surface. When there is a reduction in the production of the aqueous component of the tear film under normal evaporation conditions, this is called aqueous-deficient dry eye (ADDE), whereas if there is excessive tear evaporation in the presence of a functional lacrimal gland, this is called evaporative dry eye (EDE) [5]. However, on many occasions where there is tear gland dysfunction with reduced tear secretion, we will find in these patients a hybrid form in which both features of aqueous deficiency and increased evaporation will be present [5]. In the dog, the evaporative form can be seen in any breed, although it will be more common in brachiocephalic animals with lagophthalmos (inability to fully close the eyelid) or in dogs where a deficiency in the lipid layer of the tear film results in increased evaporative loss [6]. Meibomian gland dysfunctions as a result of marginal blepharitis, blepharoconjunctivitis, Meibomianitis and dermatological diseases are the main causes that have been associated in both dogs and humans for the evaporative form of the disease [7].

Even though it is relatively common, EDE often goes unnoticed in general practices, because the results of the Schirmer test (STT-1) are usually within normal values and the tests recommended for its diagnosis, such as the Tear Break-Up Time (TBUT) or the OSA-VET® (Ocular Surface Analyzer for Veterinary Medicine) [1], are less widely used in non-specialised centres. As a result, a large number of patients suffering from this variant of the disease do not receive adequate and effective treatment, worsening their symptoms and quality of life.

The purpose of this paper is to compile and compare the results obtained in various ophthalmological tests in 10 dogs diagnosed with EDE to assess the effectiveness of the treatments implemented, including the inclusion of an orally administered food supplement rich in omega-3 acids, Lactoferrin and antioxidants (Lacrimalis, Dr+Vet by Böthmen Pharma®), to improve the quality and production of tears one month and two months after the start of the therapeutic protocol.

# 2. MATERIALS AND METHODS

## 2.1 Animals

The selected patients had blepharospasm, epiphora, increased serous secretions compared to normal, or a combination of these symptoms at the first visit, and in order to be included in the present paper, they had to have Schirmer's test values (STT-1) higher than 10mm/min and to have a confirmed diagnosis of EDE at the Instituto Veterinario Oftalmológico (IVO) (Barcelona, Spain). A total of 10 dogs were selected (6 females and 4 males, 20 eyes), 5 patients were of brachiocephalic breeds and the other 5 patients were of various non-brachiocephalic breeds. The mean age of all patients was 5 years (between 1 and 15 years). The exclusion criteria was the use of corticosteroids and other drugs that could influence the correct performance of the diagnostic tests, as well as the presence of corneal ulceration.

## 2.2 Treatment

The treatment prescribed to the study patients was as follows:

- Ocular lubricants and moisturizers (VisuXL®, Lubristil LIPID®, Matrix Ocular 3®, Recugel®) three times a day.
- A nutraceutical composed of omega-3 fatty acids, Lactoferrin, vitamins (C and E) and minerals (Zinc and Copper) (Lacrimalis 30 tablets of 750mg of Dr+Vet by Böthmen Pharma,) at a dose of 1 tablet/10Kg of BW every 24 hours.
- Three patients (13, 14 and 20) received Tacrolimus at different concentrations (0.1%, 0.03%, 0.05%) BID or TID, at the discretion of the veterinary clinicians.

## 2.3 Protocol

It was decided to follow up each patient for 2 months from the first visit (Day 0), performing the following tests in order:

| 1st Visit (Day 0) | 2nd Visit (Day 30) | 3rd Visit (Day 60) |
|---|---|---|
| Schirmer's test (STT-1)<br>OSA-VET®<br>• Interferometry<br>• Meniscometry<br>• Non-Invasive Break-up time (NIBUT)<br>• Meibografy<br>Fluorescein eye stain<br>Break-up time (TBUT)<br>Lissamine green test<br>Impression cytology | Schirmer's test (STT-1)<br>Fluorescein eye stain<br>Break-up time (TBUT)<br>Lissamine green test | Schirmer's test (STT-1)<br>OSA-VET®<br>• Interferometry<br>• Meniscometry<br>• Non-Invasive Break-up time (NIBUT)<br>• Meibografy<br>Fluorescein eye stain<br>Break-up time (TBUT)<br>Lissamine green test<br>Impression cytology |

**Table 1**. Protocol of diagnostic tests performed in the study.

To avoid possible interference of the treatment with the performance of diagnostic tests, the owners of the 10 dogs were instructed to stop local drop treatments at least 12 hours before attending the clinic for examination.

## 2.4 Diagnostic tests

### 2.4.1 SCHIRMER'S TEST (STT-1)

It was performed on both eyes in all dogs (20 samples in total) at all visits (day 0, 30 and 60). Standard STT-1 strips (Tear Touch Blu, Madhu Intruments Pvt) were used and placed in the lateral third of the lower conjunctival sac of the left and right eye of each dog for 60 seconds

and the results noted.

#### 2.4.2 OSA-VET®

- Interferometry: One sample per eye per patient was collected at all visits (20 samples per visit) using an ocular surface analyser (OSA-VET®, SBM Sistemi, Torino, Italy) at the 1st visit and at the 3rd visit. Twenty seconds of video was recorded in each eye and the images were used to estimate the pattern and thickness of the lipid layer of the tear film according to the human medicine grading scale recommended by the manufacturer and adapted for veterinary use: Grade 0 "almost complete absence of the aqueous phase", Grade 1 (15-30 nm) "barely visible homogeneous mesh pattern", Grade 2 (31-60 nm) "more compact mesh pattern with grey waves and occasionally some shades of colour can be observed", Grade 3 (61-100 nm) "a mesh with waves where interference fringes with some colours are observed" and Grade 4 (over 100 nm) "waves with many colours" [8].
- Meniscometry: Meniscometry measures the height of the tear meniscus (TMH). The tear meniscus in humans contains 75% to 90% of the total tear volume. In the study it was measured in both eyes of each patient (20 samples in total) at the 1st visit (day 0) and at the 3rd visit (day 60) using software that processes the selected interferometric image, 3 seconds after the patient blinks. The assessment of TMH with OSA-VET® is based on the interferometric reflection pattern in the space between the lower eyelid and the cornea.
- NIBUT: This is the time elapsed between a blink and the appearance of a discontinuity in the tear film and was measured in both the right and left eye (20 samples in total) using an OSA-VET® ocular surface analyser.
- Meibography: This was performed in both eyes of each patient (20 samples in total) at the 1st visit and at the 3rd visit, using OSA-VET®. Its function is to define the percentage of tissue that does not contain Meibomian glands and its morphology. The morphological changes were classified into 4 grades with a colour scale, as marked by the manufacturer: Loss between 0 and 25% (green), loss between 25 and 50% (yellow), loss between 50 and 75% (orange) and loss between 75 and 100% (red) [9].

#### 2.4.3 FLUORESCEIN EYE STAIN TEST

It was performed to assess corneal integrity in all visits during the course of the study. A drop of saline was applied to 1 standard strip impregnated with Fluorescein (Fluoro Touch, Madhu Instruments Pvt) and approached to lightly touch the bulbar conjunctiva of the right eye and left eye of each of the patients (one from the left eye and one from the right eye, 20 samples in total), allowing them to blink and without rinsing off the excess staining debris. Subsequently, the light was turned off and observed by slit lamp using the cobalt blue filter. In order to objectively quantify the results, the eye was divided into 4 quadrants (Punctata 1-4) and the number of quadrants in which fluorescein-stained punctate lesions were observed was quantified.

#### 2.4.4 TBUT

It was evaluated in both eyes of each patient (20 samples in total) by microscopy using a cobalt blue filter after applying fluorescein eye stain.

#### 2.4.5 LISSAMINE GREEN TEST

It was performed to complement the evaluation of corneal integrity. Lissamine Green strips (Madhu Intruments Pvt) were used, and the same procedure explained above to perform the fluorescein test was repeated.

#### 2.4.6 IMPRESSION CYTOLOGY

One sample per eye and patient (20 samples in total) was collected at the 1st and 3rd visit, using filter paper with 0.22-micron pores of micropore-type cellulose acetate. The paper was rested on the conjunctival surface for approximately 10 seconds and then immediately fixed with 96% ethanol until stained with periodic acid Schiff (PAS) and haematoxylin and dehydrated in ethanol. The paper was then placed in Xylol to complete dehydration and rinse the filter paper. Finally, it was fixed on a slide so that the sample could be studied. Microscopic evaluation of the cytological samples was performed using the Nelson and Adams 4-grade scale (Grade 0-4) based on the morphology of epithelial and goblet cells.

# 3. RESULTS

## 3.1 SCHIRMER'S TEST (STT-1)

Table 2 shows that the value of the AVERAGE parameter has a reduction of 2mm in the 3rd visit compared to the 1st visit. Furthermore, when analyzing the results according to race (brachiocephalic eyes vs. non-brachiocephalic eyes) and age (eyes of patients aged 0-4 years vs. eyes of patients older than 4 years), it is evident that in the case of the value of the parameter >4 years, there is a reduction of 3mm between the 1st and 3rd visit, while in the 0-4 years, it remains the same in all visits (STT-1 of 16mm).

## 3.2 INTERFEROMETRY

As shown in Graph 1, the value of the parameter AVERAGE increases from Grade 1 at the 1st visit to Grade 2 at the 3rd visit. Only in the case of the parameter value >4 years, no improvement in Interferometry is observed.

## 3.3 MENISCOMETRY

Table 2 shows the results of the Meniscometry test. A reduction of 0.07mm of the AVERAGE parameter value is observed between the 1st and 3rd visit. The value of the No Braq. parameter is maintained (there is only an increase of 0.01mm between visits), which is far from the value of the Braq. parameter that decreases 0.13mm between the 1st and 3rd visit.

## 3.4 NIBUT

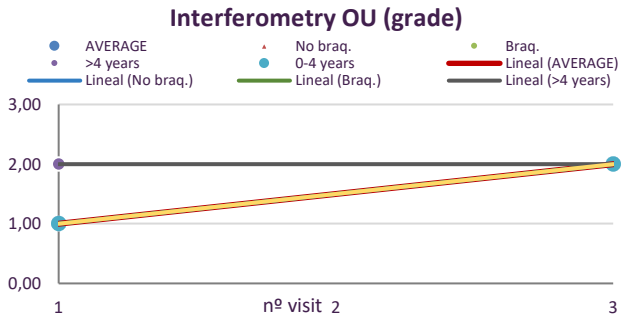
Graph 2 shows the results of the NIBUT test, in which an increase in the AVERAGE parameter value of 1 second was observed at the 3rd visit compared to the 1st visit. In the case of the >4 years parameter, no improvement was observed during the course of the study.

## 3.5 MEIBOGRAPHY

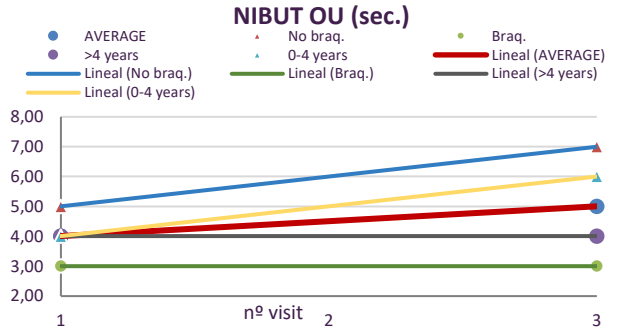
Table 2 shows that the value of the AVERAGE parameter

| PARAMETERS | AVERAGE | | | No braq. | | | Braq. | | | >4 years | | | 0-4 years | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nº VISIT | 1ª | 2ª | 3ª | 1ª | 2ª | 3ª | 1ª | 2ª | 3ª | 1ª | 2ª | 3ª | 1ª | 2ª | 3ª |
| STT-1 (mm) | 17 | 16 | 15 | 16 | 15 | 15 | 18 | 18 | 16 | 18 | 17 | 15 | 16 | 16 | 16 |
| Interferometry (grade) | 1 | - | 2 | 1 | - | 2 | 1 | - | 2 | 2 | - | 2 | 1 | - | 2 |
| Meniscometry (mm) | 0,53 | - | 0,46 | 0,44 | - | 0,31 | 0,62 | - | 0,63 | 0,6 | - | 0,56 | 0,48 | - | 0,41 |
| NIBUT (sec.) | 4 | - | 5 | 5 | - | 7 | 3 | - | 3 | 4 | - | 4 | 4 | - | 6 |
| Meibography (%) | 14 | - | 17 | 14 | - | 18 | 15 | - | 15 | 11 | - | 17 | 17 | - | 17 |
| Fluorescein stain (grade) | 3 | 3 | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 3 | 2 |
| TBUT (sec.) | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Lissamine Green test | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| Impression Cytology (grade) | 2 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 1 | 2 | - | 1 |

**Table 2.** Averages of the value of the different parameters evaluated during the course of the study in the 1st, 2nd and 3rd visit of the total eyes or patients (AVERAGE), of the non-brachiocephalic eyes or patients alone (No braq.), of the brachiocephalic eyes or patients alone (Braq.), of the eyes or patients older than 4 years alone (>4 years) and of the eyes or patients between 0 and 4 years alone (0-4 years).




**Graph 1**. Interferometry value trend (in Degrees) at the 1st and 3rd visit of: Of the total eyes of patients in the study (AVERAGE), of only those eyes of non-brachiocephalic patients (No braq.), of the eyes of brachiocephalic patients (Braq.), of the eyes of patients older than 4 years (>4 years) and of the eyes of patients aged 0-4 years (0-4 years).




**Graph 2**. Trend of NIBUT values (in seconds) at the 1st and 3rd visit of: All eyes of the study patients (AVERAGE), of the eyes of only those non-brachiocephalic patients (No braq.), of the eyes of brachiocephalic patients (Braq.), of the eyes of patients older than 4 years (>4 years) and of the eyes of patients between 0 and 4 years (0-4 years).

increased by 3% at the 3rd visit compared to the 1st visit. The No braq. parameter worsens by 4% (from 14% at the 1st visit to 18% at the 3rd visit), while Braq. remains at 15% during the 2 months. On the other hand, the 0-4 years parameter is maintained (at 17%) while the >4 years parameter worsens by 6% at the 3rd visit (17%) compared to the 1st visit (11%).

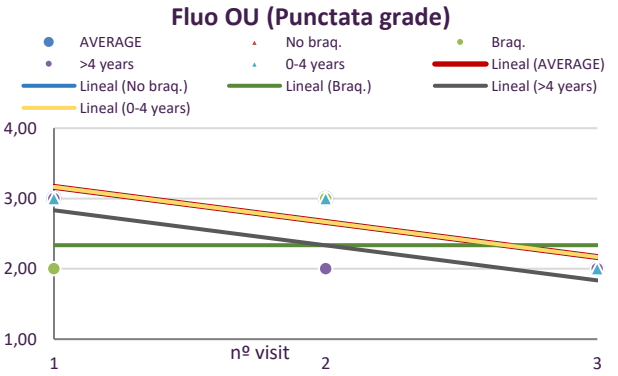
## 3.6 FLUORESCEIN EYE STAIN TEST

In Table 3, it can be seen that in the 1st visit, only 45% of the eyes had values of Punctata 1 and 2 (Grade 1, 0%; Grade 2, 45%), while in the 3rd visit, 80% of the eyes studied presented values of Punctata 1 and 2 (Grade 1, 30%; Grade 2, 50%).

In addition, as shown in Graph 3, there is a generalized progressive improvement in the Punctata grade, with a reduction in the AVERAGE parameter value at the 2nd visit and even more accentuated at the 3rd visit. Both the results of the 0-4, >4 years and No Braq. parameters were reduced by 1 grade of Punctata between the 1st and 3rd visit. On the other hand, in the case of the Braq. parameter, there was no improvement (it remained at Grade 2).

| PUNCTATA GRADE | 1st Visit | 2nd Visit | 3rd Visit |
|---|---|---|---|
| 1 | 0 | 1 | 6 |
| 2 | 8 | 9 | 10 |
| 3 | 5 | 8 | 2 |
| 4 | 6 | 2 | 2 |

**Table 3**. Number of eyes, classified according to their Punctata value (in grades) at the 1st, 2nd and 3rd visit.




**Graph 3**. Trend of NIBUT values (in seconds) at the 1st and 3rd visit of: All eyes of the study patients (AVERAGE), of the eyes of only those non-brachiocephalic patients (No braq.), of the eyes of brachiocephalic patients (Braq.), of the eyes of patients older than 4 years (>4 años) and of the eyes of patients between 0 and 4 years (0-4 años).

## 3.7 TBUT

Table 2 shows that there was an improvement in the AVERAGE parameter value of 1 second between the 1st and 3rd visit, but when analyzed by groups, the Braq. and 0-4 years parameters did not change during the course of the study.

Table 4 shows that in 14/20 of the eyes studied (70%) there was no improvement in the BUT value and it remained at 0 at all visits. In 6/20 of the eyes studied (30%) there was a slight increase in the BUT value of between 1 to 3 seconds.

## 3.8 LISSAMINE GREEN STAIN

As can be seen in Table 2, the Lysamine green test result was negative in all patients at all visits.

| BUT (sec.) | 1st Visit | 2nd Visit | 3rd Visit |
|---|---|---|---|
| 0 | 18 | 18 | 14 |
| 1 | 0 | 1 | 1 |
| 1,5 | 2 | 0 | 0 |
| 2 | 0 | 1 | 4 |
| 3 | 0 | 0 | 1 |

**Table 4**. Number of eyes, classified according to their BUT value (in seconds) at the 1st, 2nd and 3rd visit.

## 3.9 IMPRESSION CYTOLOGY (CIC)

Table 2 shows a 1 grade improvement in impression cytology at the 3rd visit (Grade 1) compared to the 1st visit (Grade 2) for all parameters.

| Grades | 1st Visit | 2nd Visit | 3rd Visit |
|---|---|---|---|
| 0 | 0 | - | 2 |
| 1 | 2 | - | 3 |
| 2 | 3 | - | 5 |
| 3 | 5 | - | 0 |
| 4 | 0 | - | 0 |

**Table 5**. Number of patients, classified according to their grade on impression cytology at the 1st visit and 3rd visit.

Furthermore, as can be seen in Table 5, at the first visit there were no patients in Grade 0 and 50% of the patients had Grade 3, while at the third visit there were two patients in Grade 0 and no patients in Grade 3.

# 4. DISCUSSION

In reference to the treatment chosen, it was decided to proceed with topical moisturizers and lubricants as tear replacements and with an oral nutraceutical rich in omega-3 fatty acids, Lactoferrin, vitamins C and E, Zinc and Copper (Lacrimalis, Dr+Vet by Böthmen Pharma®) at the doses recommended by the manufacturer. In some of the patients, the topical immunosuppressant Tacrolimus was also administered at different concentrations, in an attempt to reduce corneal pigmentation and help improve tear production and its quality in three of the patients (one that suffered a worsening in its condition in the 2nd visit, and two with STT-1 values of 12mm/min on the 1st visit in both of their right eyes). The use of antioxidants such as Zinc and vitamins C and E, in addition to omega-3 fatty acids has been studied extensively in human medicine, with improvements seen in ophthalmological tests BUT, STT-1, CIC, as well as in tear production and stability, improving symptoms in patients with EDE, probably by helping to reduce reactive oxygen species (ROS) in the tear in the case of antioxidants [10, 11, 12] and in the case of Omega-3s, by helping to reduce tear osmolarity and inflammation on the ocular surface and Meibomian glands, as well as altering the composition of tear secretions in patients with chronic blepharitis or Meibomian gland disease [12, 13, 14].

On the other hand, it has been shown in some studies with human subjects with KCS of different aetiologies that the tear concentration of Lactoferrin is reduced compared to patients without KCS, with a significant negative correlation between dry eye and tear Lactoferrin levels [15, 16, 17]. Lactoferrin may help to improve tear volume and EDE symptoms by exhibiting anti-inflammatory, immunomodulatory, antioxidant and antimicrobial properties on the ocular surface (due to its iron chelating effect and its ability to directly interact with pathogens on the ocular surface [18]) [12, 15, 17, 19]. Another study in mice [19] showed that it could also have an anti-inflammatory effect on the lacrimal gland.

In veterinary medicine, although there are fewer scientific references evaluating the efficacy of the ingredients contained in the nutraceutical used in the present paper, it has been shown that the use of omega-3 fatty acids and antioxidants as adjuvants to immunosuppressive drug therapy in dogs with ADDE improved clinical symptoms, TBUT, STT-1 and cytology results compared to those prescribed topical drug therapy alone [20, 21]. A study in rabbits with induced KCS showed that administration of omega-3 fatty acids improved STT-1 and fluorescein test results compared to the control group, the possible mechanism of action being its anti-inflammatory effect on the cornea, conjunctiva and lacrimal glands [22].

In the ophthalmological tests performed in this study, CIC, more commonly studied and used in human ophthalmology and applied to veterinary medicine, helps us both to assess the progression of the pathology at the level of the ocular surface and to predict the efficacy of the treatment prescribed to the patients. Our results indicated that there was an increase in the presence of goblet cells at the end of the study and a reduction in epithelial cell metaplasia, so that from the joint assessment of the number of goblet cells together with epithelial cell metaplasia, which is associated with a reduction in the severity of dry eye syndrome symptoms in human medicine [23] and consequently in veterinary medicine, we can affirm that there was an improvement in the values of this test at the end of the study.

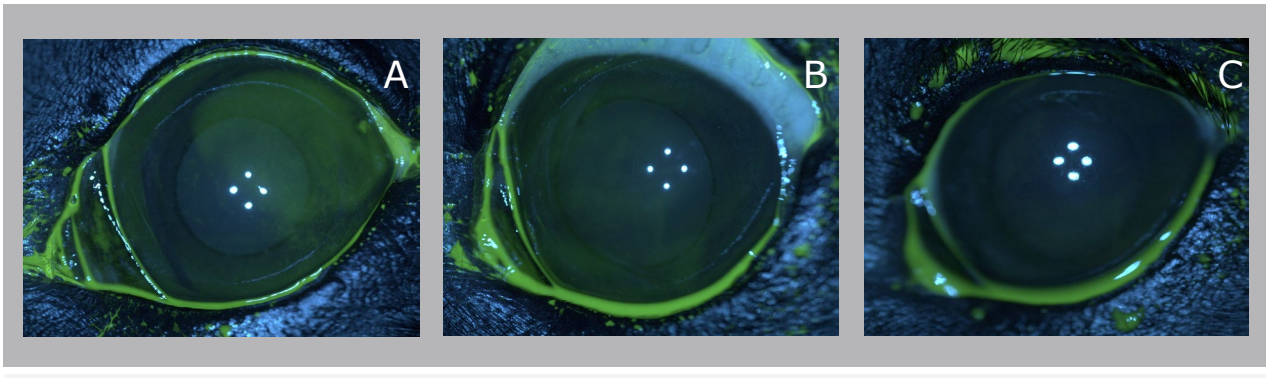



**Figure 1**. Images of the left eye of Doberman patient number 16 during the fluorescein test, diagnosed with quantitative keratoconjunctivitis sicca at the Instituto Veterinario Oftalmológico (IVO): (A) On the first visit on day 0, (B) on the 2nd visit on day 30, (C) on the 3rd visit on day 60.

In the case of the fluorescein test, which allows us to evaluate the ocular surface and appreciate the de-epithelialised areas of the cornea, we also saw a clear improvement in the Punctata value (shown in degrees) of the average value of all the eyes of the patients as a whole (AVERAGE), but when we separated them into groups according to their race (brachiocephalic vs. brachiocephalic), it was found that in the case of the brachiocephalic race (Braq.), there was no improvement in this parameter at the 3rd visit compared to the 1st. This could probably be due to the fact that they might need a longer treatment time than only the 2 months of the study duration, or that, due to the low number of patients in the study, when there is a marked worsening of one of them (patient 14), the values are mostly altered. In any case, the prominent eyeballs of brachiocephalic dogs with dry eye disease, as well as the presence of lagophthalmos typical of these breeds, increase the likelihood of developing corneal ulcers. Indeed, it has been shown that even in the absence of corneal ulceration, the corneo-conjunctival epithelial barrier can be disrupted in brachiocephalic dogs with KCS, demonstrated by the presence of punctate patterns on the cornea during fluorescein staining [24].

When looking at the results of the two tests assessing tear production, both a slight reduction in the average STT-1 value and the average TMH (Meniscometry) value were observed, the reasoning behind this reduction in tear production could be that because of the hyperosmolarity of tears and epithelial lesions, stimulation is generated in the corneal nerve endings, increasing the frequency of blinking and potentially increasing the compensatory reflex in tear secretion. This compensatory secretion is more likely in EDE, where tear gland function is usually normal [5]. In the case of STT-1, it was found that while those of non-brachiocephalic race follow the same pattern of slight reduction of the TMH value (from 0.44mm at the 1st visit to 0.31mm at the 3rd visit), in brachiocephalic breeds the average value at the 1st visit did not vary much from the 3rd visit (0.62mm at the 1st visit and 0.63mm at the 3rd visit). In fact, taking into account that the TMH values estimated to be normal in dogs are 0.53 ± 0.11 mm [25], the brachiocephalic patients maintained higher values, which could be due to the ocular conformation of this type of dog and to the greater difficulty of tear drainage, since many of them have a hidden lower tear orifice, especially those who also have a more pronounced medial canthal entropion in the lower eyelid. Finally, macroblepharon, infrequent blinking and lagophthalmos could also influence the value, according to the authors' experience.

When analyzing the two measures of tear Break-up time, TBUT and NIBUT, the same trend of slight increase (increase of 1 second on AVERAGE) of the two values was evident at the end of the study at 60 days.

It is also important to note that NIBUT values are generally always higher than TBUT values, as the activation and deactivation of the timer by the clinician performing the test will always add time to the total test time. Tear Break-up time is one of the most relevant parameters for the diagnosis and follow-up of EDE cases. In the eyes of brachiocephalic patients, it was observed that there was no improvement in the mean NIBUT value and that only 10% of the eyes improved their mean TBUT value at the 3rd visit compared to the 1st visit.

The absence of a more marked increase in BUT and NIBUT after completion of the study, considering that normal BUT in dogs is 20 seconds or more [26], could be due to the short duration of the study, as improvement in tear quality is often difficult and requires a long treatment time. In the case of the lack of response in the eyes of brachiocephalic patients, in addition to the previous point, there is the fact that these are breeds predisposed to have lower BUT than non-brachiocephalic breeds [27].

Referring to Interferometry, which evaluates the density and thickness of the lipid layer of the tear, an improvement of 1 grade was observed in the average of all patients (AVERAGE), but when separated by age, it was seen that those >4 years old did not show any change during the study (they remained at Grade 2).

It should be noted, therefore, that these patients initially started with a higher degree of interferometry than those aged 0-4 years (initial average Grade 1) and that one of the patients in this group (patient number 14) suffered a worsening of the disease during the course of the study, which, taking into account the low number of patients, could have a more important influence on the results of the tests.

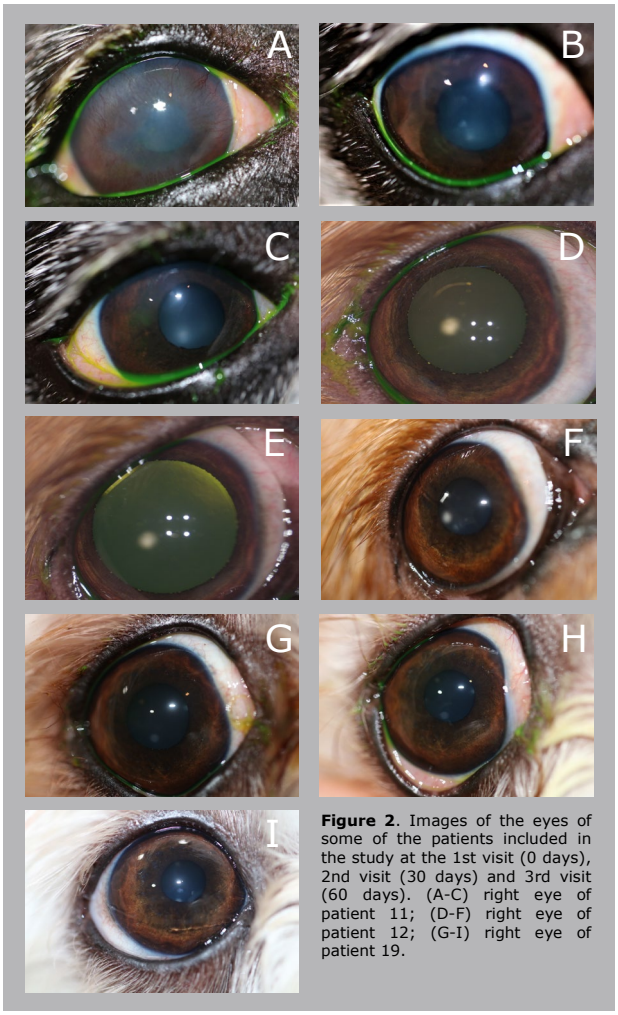



**Figure 2**. Images of the eyes of some of the patients included in the study at the 1st visit (0 days), 2nd visit (30 days) and 3rd visit (60 days). (A-C) right eye of patient 11; (D-F) right eye of patient 12; (G-I) right eye of patient 19.

Finally, when evaluating the results of Meibography, there was a very slight increase in AVERAGE in the 3rd visit (17%) compared to the 1st visit (14%). Although this result could be seen as negative, in the clinical context it is necessary to evaluate all the tests together (the rest of the results being generally positive), in addition to the fact that the authors' experience indicates that, on many occasions, since it is a value that shows the percentage of tissue in the absence of Meibomian glands (atrophy), this progresses together with the pathology, even when effective treatment for the pathology has been established. The variance of results when separating by age and race could be explained by the low number of participants in the study.

# 5. CONCLUSION

The results obtained show an improvement in the results of the CIC, in the degree of Interferometry, an increase of 1 second in both NIBUT and TBUT and a slight reduction in tear production as indicated by STT-1 and Meniscometry, probably due to the reduction of the compensatory reflex of tear production due to hyperosmolarity. So it can be said that in this series of cases, by applying an effective treatment based on topical ocular moisturisers and lubricants in addition to the oral administration of the Lacrimalis food supplement, there was a symptomatic improvement and the results of the ophthalmological tests carried out after two months were clinically significant. However, the present document is a compilation and comparison of 10 clinical cases, so a randomised, double-blind, controlled clinical study with a larger number of participants would be necessary to demonstrate the efficacy of the treatment, as well as the potential relationship of age and/or race with the variance of the results.

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