

Tear Film Osmolarity in Atopic Dogs with Allergic Conjunctivitis

Ana Mafalda Lourenço, Maria Guadalupe Megre and Esmeralda Delgado*

CIISA, Faculty of Veterinary Medicine, University of Lisbon

***Corresponding author:** Esmeralda Delgado, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisboa, Portugal, Tel: 00 351 21 3652893; Fax: 00 351 21 3652822, Email: esmeralda@fmv.ulisboa.pt

Published Date: November 03, 2017

ABSTRACT

Introduction: Tear film osmolarity is a crucial parameter to evaluate tear's quality. It represents cations and anions concentration in the tear's aqueous portion. Hyperosmolarity has been described in human literature as a primary marker of tear film integrity.

Objectives: To evaluate tear film osmolarity in atopic dogs with allergic conjunctivitis.

Procedures: Atopic dogs with allergic conjunctivitis (n=20) and a control group (n=20) were included in the study. All animals underwent dermatological exam, including Canine Atopic Dermatitis Extent and Severity Index (**CADESI-03**) classification and ophthalmic examination to assess clinical signs of allergic conjunctivitis. Chemosis, pruritus, epiphora, conjunctival hyperemia, ocular discharge and corneal disease were rated from 0 to 3. Tear film osmolarity was evaluated using Tear Lab system® (Produlab, Lisbon, Portugal). Statistics was performed with Graph Pad Instat (Mann-Whitney test and linear regression tests).

Results: Tear film osmolarity was 320.8 ± 9.6 mOSms/L in the control and 340.3 ± 21.6 mOSms/L in the atopic group, this difference being statistically significant ($p < 0.0001$). No significant differences were found between osmolarities of both eyes from the same animal in

the control ($p=0.524$) or atopic groups ($p=0.123$). Tear film osmolarity in atopic dogs exhibited a positive correlation with CADESI values ($P < 0.0001$) but no correlation was found with allergic conjunctivitis score ($P = 0.8385$).

Conclusions: Tear film osmolarity showed to be increased in atopic dogs with allergic conjunctivitis. This parameter may prove useful for diagnosis and assessment of response to therapy. Dogs may be a potential model for humans with similar conditions.

Keywords: Tear film osmolarity, Allergic conjunctivitis, Canine atopic dermatitis, Hiperosmolarity, Model

INTRODUCTION

Allergic conjunctivitis remains a diagnostic challenge. The eye is an ideal place for type 1 hypersensitivity allergic reactions. In humans with allergic conjunctivitis high levels of IgE are found in blood and tears [1] and conjunctival cytology usually shows eosinophils, basophils or mast cells. The majority of dogs (60%) which suffer from atopic dermatitis, a disease with an estimated prevalence of about 10-20%, also exhibit symptoms of allergic conjunctivitis [2]. There is mounting evidence that allergic conjunctivitis is underestimated in dogs. Nevertheless, it is an exclusion diagnosis. No simple and effective diagnostic test is available in replacement [2]. It is known that anything that causes ocular surface inflammation, whether an infectious agent or an allergy, can lead to alteration of the tear film.

Osmolarity is a basic and essential aspect of physiologic homeostasis in body fluids. Small deviations in osmolarity activate physiological mechanisms to return that variable to its set point. The body is usually able to regulate osmolarity of body fluids within very narrow limits through various mechanisms of osmo-regulation, such as the compensation and correction of fluid volume and the oils, lipids, proteins and mucins that make up the tear. Hyperosmolarity of any body fluid, including tear fluid, indicates a disorder in the ability to maintain homeostasis and is a basic indication of a physiological disorder. Tear film osmolarity corresponds to the concentration of cat ions (sodium, potassium, calcium, magnesium, iron and copper) and anions (chloride, bicarbonates and phosphates) dissolved in the aqueous portion of tears. In humans it is a useful parameter for assessing tear film quality [3]. In fact, tear hyperosmolarity is regarded as the central mechanism causing ocular surface inflammation, damage, and symptoms, and the initiation of compensatory events in dry eye disease [3].

Elevated tear osmolarity, a hallmark of DE, is also frequently encountered by immunoallergologists as a clinical finding in Man. When left unchecked, hyperosmolar tears in dry eye disease early stage will lead to damage of the cornea and conjunctiva, evident in later stage disease [4].

Dogs are a species very prone to cutaneous and ocular allergies. Striking similarities between the disease in Man and in dogs have been highlighted in recent years [5,6]. Therefore, it is likely

that dogs with allergic conjunctivitis also present elevated tear osmolarity. In this prospective study we aimed to evaluate for the first time tear film osmolarity in atopic dogs with allergic conjunctivitis and to compare our findings with those described for man.

MATERIAL AND METHODS

This was a prospective study conducted in a control group of healthy dogs with no signs of atopy or conjunctivitis (n=20) and in a group of atopic dogs with symptoms of allergic conjunctivitis (n=20). The study was performed at the Teaching Hospital of the Faculty of Veterinary Medicine, University of Lisbon, after Ethical Committee approval. All the owners gave informed written consent for the inclusion of their dogs in the study.

Dermatological Exam

For the group of atopic dogs, diagnosis of cAD was made according to standard criteria [7]. In summary, combination of (1) compatible history; (2) fulfillment of clinical criteria strongly associated with the disease; (3) exclusion of other pruritic skin diseases, specifically: no response to a minimum 8-week diet trial with either a home-cooked or commercial hydrolyzed protein diet and water only to eliminate the possibility of an adverse food reaction; no response to a veterinary approved flea control regimen for at least 8 weeks; sarcoptic mange excluded by trial therapy and/or negative serology. In addition, the occurrence of at least one positive sensitization by intra dermal test (IDT) reaction performed and interpreted according to accepted criteria that agreed well with the patient's history was required. No anti-inflammatory medication (topical or systemic) was given for at least 3 weeks prior to examination.

Additionally, the atopic group was assessed according to the CADESI-03 scale. This is a validated evaluation of clinical lesions (erythema, excoriation, lichenification and self-induced alopecia) at 62 anatomical sites, each measured from 0 (normal) to 5 (most severe) [8]. Proposed intervals by the International Task Force on Canine Atopic Dermatitis for CADESI-03 are: remission: 0-15; mild AD: 16-59; moderate AD: 60-119; and severe AD: ≥ 120 [25].

Ophthalmological Exam

A complete ophthalmic exam was performed in all dogs. The investigator was blinded to dog's group allocation. This examination included:

(i) Firstly, tear film osmolarity evaluation using Tear Lab Osmolarity System® (Tear Lab Corp., San Diego, E.U.A.) was performed before any drop instillation. This equipment is described as a "lab-on-a-chip" system that uses a 50 nL tear sample in order to measure the osmolarity of the tear. The system is non-invasive, user friendly and provides a result in less than 1 minute (Figure 1). Briefly, the investigator positioned the tip of the Pen just above the lateral aspect of lower eyelid. Subsequently, the Pen was gently lowered until the bottom of the tip touched the thin line of moisture between the eyelid and the eye. After a successful tear collection, the pen

was immediately docked into the Reader. The test result (mOSms/L) was displayed within a few seconds.



Figure 1: Sample collection for tear film osmolarity measurement in a patient.

(ii) Tear production test assessment using Schirmer I Tear Test (STT, Dina strips Schirmer-Plus, Luneau SAS, Chartres, France) strips to exclude dry eye disease. Reference values were between 15 and 25 mm/minute [9].

(iii) Slit lamp bio microscopy using Kowa SL 15 (Tokyo, Japan) which was performed in a darkened room. Ocular signs of allergic conjunctivitis were evaluated, including conjunctival hyperaemia, chemosis, epiphora, ocular discharge, pruritus and corneal involvement, and graded 0 to 3 according to severity.

(iv) “Tear Break-Up Time” evaluation after instillation of a drop of sterile fluorescein staining (Fluorescein, Haag-Streit international, Köniz, Switzerland) in the cornea and observation of time to rupture of the tear film using the blue cobalt filter from the slit lamp to exclude qualitative dry eye disease. Normal values were considered above 20 sec [10].

(iv) Intraocular pressure (**IOP**) measurement by applanation tonometry (Tono-Pen XL, Medtronic Solan, USA) following instillation of a drop of topical anesthetic (0.4% Oxibuprocaine,

Anestocil®, Oftalder, Oeiras, Portugal). Reference values were between 15 mm Hg and 25 mm Hg [9]. Measurements were repeated three times. The values were considered valid when the coefficient of variance was 5% and the mean value was calculated.

(vi) Funduscopy performed in a darkened room by indirect ophthalmoscopy (Heine Ómega 500, Herrsching, Germany) after pupil dilation with tropicamide (Tropicamide 1%, Tropicil Top®, Edol, Linda-a-Velha, Portugal).

Additionally, owners were asked to evaluate their dog's pruritus according to the following classification: (0) no ocular pruritus; (1) mild ocular pruritus; (2) moderate ocular pruritus; (3) severe ocular pruritus. Signs of ocular pruritus were defined as ocular squinting, blepharospasm, attempts to scratch or rub their eyes with the paws or against objects and periocular alopecia as a result of self-inflicted trauma.

The final clinical score for the severity of the ocular signs resulted from sum of the individual scores (conjunctival hyperemia, chemosis, epiphora, ocular discharge, and corneal involvement and ocular pruritus) varying from 0 to 18.

Statistical Analysis

Descriptive statistics were calculated for sample description and characterization. CADESI-03 and ocular score values for the test group are shown as median \pm standard deviation.

Statistics was performed with GraphPad Instat using Mann-Whitney non-parametric test and linear regression for data correlation.

The following parameters were analyzed: tear film osmolarity for control and atopic groups; right and left eye osmolarity in both groups; median CADESI-03 values and allergic conjunctivitis score. The correlations studied were (1) Tear film osmolarity in atopic dogs with atopic dermatitis severity and (2) Tear film osmolarity in atopic dogs with allergic conjunctivitis score.

RESULTS

Tear film osmolarity showed means values of $320,8 \pm 9,6$ mOsm/L in the control group and $340,3 \pm 21,6$ mOsm/L in the atopic group. Osmolarity values of both groups showed a statistically significant difference ($P < 0,0001$ Mann-Whitney test) (Figure 2).

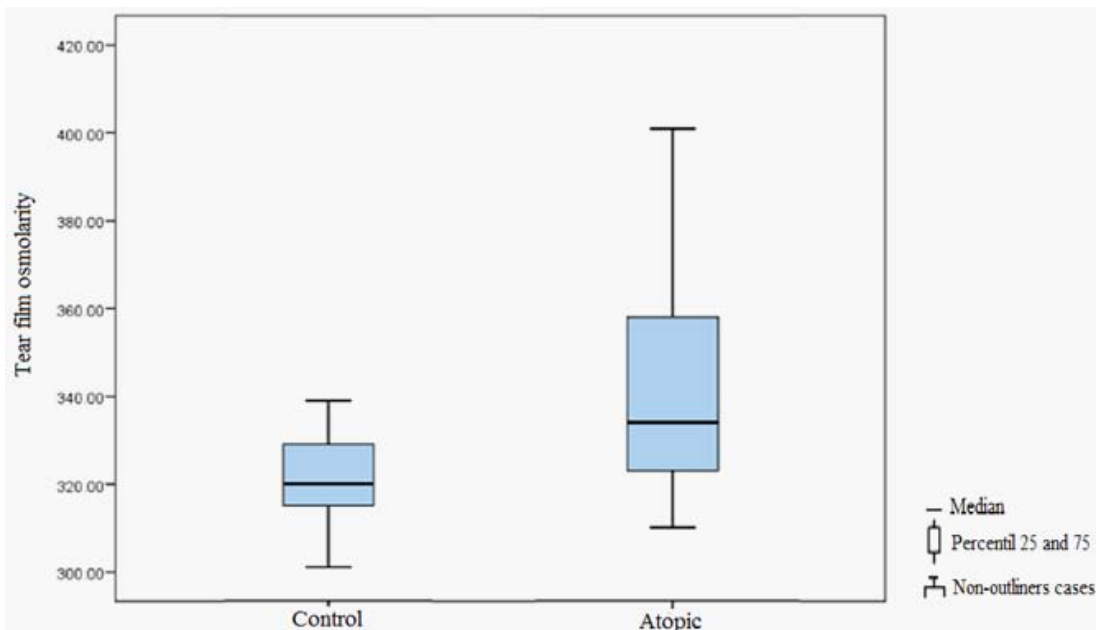
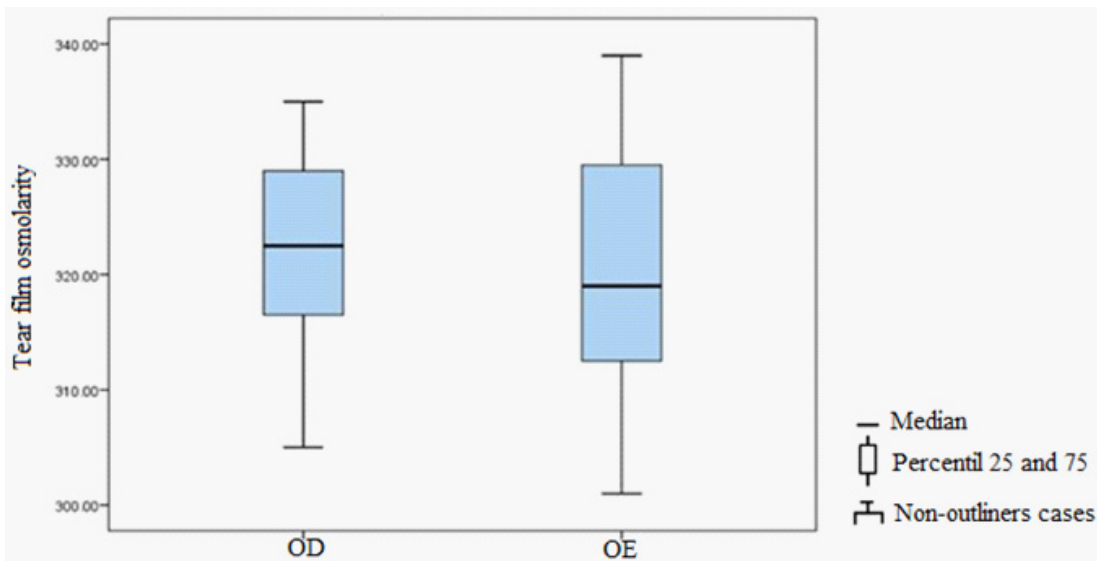


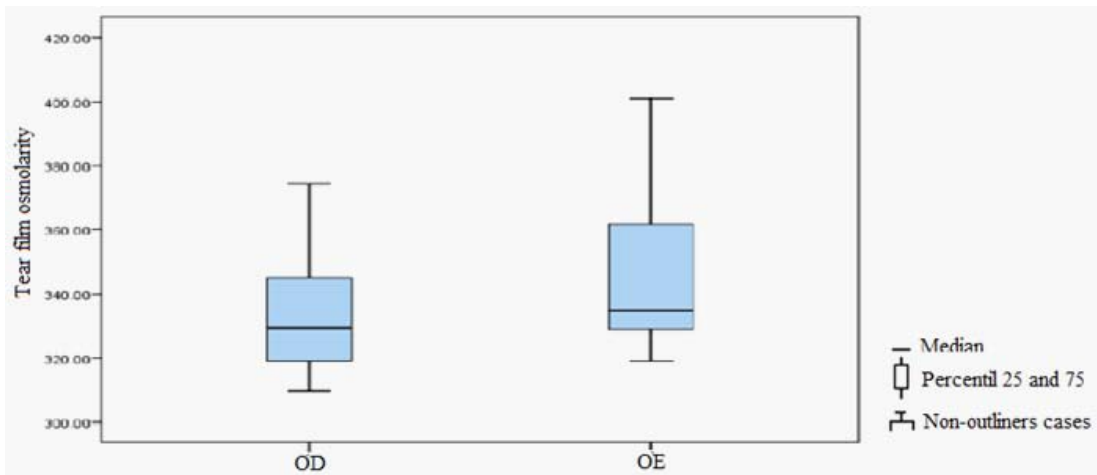
Figure 2: Tear film osmolarity was 320.8 ± 9.6 mOsm/L in the control group and 340.3 ± 21.6 mOsm/L in the atopic group, this difference being statistically significant ($P < 0.0001$).

No significant differences were found between osmolarities of both eyes from the same animal in the control ($P = 0.524$, Mann-Whitney test) (Figure 3) or in atopic group ($P = 0.123$, Mann-Whitney test) (Figure 4). In the atopic subjects, mean CADESI-03 value was $180,1 \pm 66,50$, and allergic conjunctivitis score was $8,2 \pm 2,36$.



| | Right eye (OD) | Left eye (OE) |
|--------------|----------------|---------------|
| Median | 322,5 mOsm/L | 319 mOsm/L |
| Percentil 25 | 316,75 mOsm/L | 313,25 mOsm/L |
| Percentil 75 | 329 mOsm/L | 328,75 mOsm/L |
| Maximum | 335 mOsm/L | 339 mOsm/L |
| Minimum | 305 mOsm/L | 301 mOsm/L |

Figure 3: No significant differences were found between osmolarities of both eyes from the same animal in the control group ($P = 0.5244$).



| | Right Eye (OD) | Left eye (OE) |
|--------------|----------------|---------------|
| Median | 329,5 mOsm/L | 335 mOsm/L |
| Percentil 25 | 319,5 mOsm/L | 329,5 mOsm/L |
| Percentil 75 | 343,5 mOsm/L | 361,5 mOsm/L |
| Maximum | 374 mOsm/L | 401 mOsm/L |
| Minimum | 310 mOsm/L | 319 mOsm/L |

Figure 4: No significant differences were found between osmolarities of both eyes from the same animal in the atopic group ($P = 0.123$).

In atopic patients CADESI-03 values were 180.1 ± 66.50 and allergic conjunctivitis score was 8.2 ± 2.36 . Tear film osmolarity in atopic dogs exhibited a positive correlation with atopic dermatitis severity [CADESI-03 values ($P < 0.0001$)] (Figure 5) but no correlation was found with allergic conjunctivitis score ($P = 0.8385$) (Figure 6).

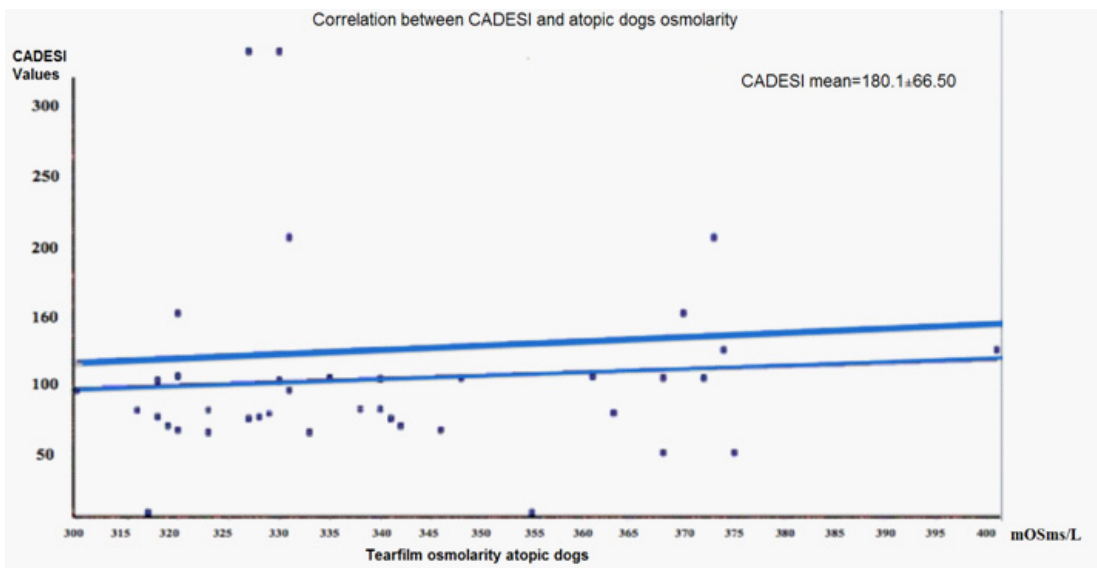


Figure 5: In atopic patients CADESI values were 180.1 ± 66.50 . Tear film osmolarity in atopic dogs exhibited a Positive correlation with CADESI values ($P < 0.0001$).

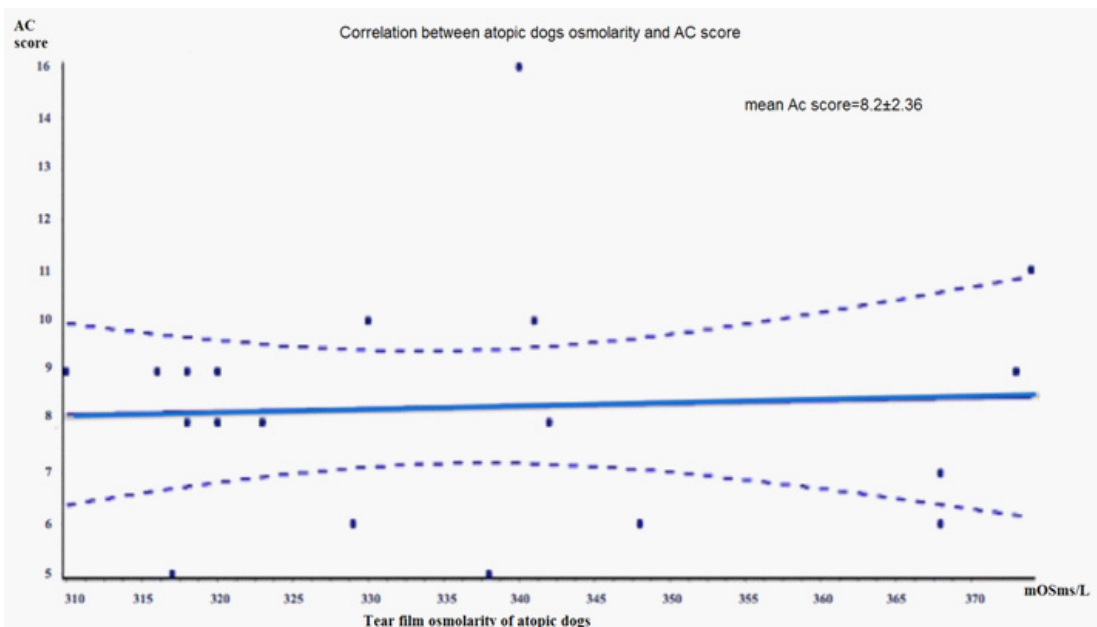


Figure 6: In atopic patients allergic conjunctivitis score was 8.2 ± 2.36 . No correlation was found between tear film osmolarity and allergic conjunctivitis score ($P = 0.8385$).

DISCUSSION

Tears play an essential role in maintaining ocular surface integrity, protecting against microbial challenge and preserving visual acuity. In allergic conjunctivitis there is apoptosis of calciform cells which leads to changes in the mucin layer and mucus production. Inflammation of the Meibomian glands produces changes in the lipidic layer. Through a compensatory mechanism there is an increase in aqueous tear film production plus an increase in evaporation, which provokes changes in the water layer. Humans with allergic conjunctivitis present with tear film hyperosmolarity (normal value is 290 mOsm/L) due to increased evaporation, which leads to a saltier solution. Tear osmolarity is the best single metric both to diagnose and classify dry eye disease. Inter-eye variability is a characteristic of dry eye not seen in normal subjects [4].

Concerning veterinary medicine, there are very few studies concerning this parameter. In a recent study, tear-film osmolarity of normal cats was found to be 328.5 ± 17.94 mOsm/L and in cats with conjunctivitis it was 325.0 ± 24.84 mOsm/L [11]. In normal dogs, tear-film osmolarity varied from a minimum value of 303 to a maximum of 400 mOsm/L, with a median of 355.50 mOsm/L, which was less uniform than in humans [12]. These values will also present variations depending on the exposure to a more or less desiccating environment [13].

In man allergic conjunctivitis is a cause of hyperosmolarity. Striking similarities between the disease in Man and in dogs have been highlighted in recent years [5]. Therefore, it is likely that dogs with allergic conjunctivitis also present elevated tear film osmolarity.

In our study tear film osmolarity was increased in atopic dogs suffering from allergic conjunctivitis.

The positive correlation found with severity of skin signs might be related to chronicity of the disease, which explains the saltier tear film, so an association was found between severe or older forms of atopy and hyperosmolarity. No correlation was found between hyperosmolarity and severity of allergic conjunctivitis, although this correlation should be subject to further studies in the future.

Tear film osmolarity in atopic dogs seems to be a promising tool to help in diagnosis of allergic conjunctivitis as well as in evaluation of treatment effectiveness. Dogs can also be considered as a potential model for humans with similar conditions.

ACKNOWLEDGEMENTS

To Produlab (Portugal) for allowing us to use their equipment.

SUPPORT

UID/CVT/00276/2013.

References

1. Bielory L. Allergic and immunologic disorders of the eye. Part II: Ocular allergy. *J Allergy Clin Immunol.* 2000; 106: 1019-1032.
2. Lourenço-Martins AM, Delgado E, Neto I, Peleteiro MC, Morais-Almeida M, et al. Allergic conjunctivitis and conjunctival provocation tests in atopic dogs. *Veterinary Ophthalmology.* 2011; 14: 248-256.
3. Lemp MA, Baudouin C, Baum J, Dogru M, Foulks GN, et al. The definition and classification of Dry Eye Diseases: Report of the definition and classification Subcommittee of the International Dry Eye Workshop. *The Ocular Surface.* 2007; 5: 75-92.
4. Lemp MA, Bron AJ, Baudouin C, Benítez Del Castillo JM, et al. Tear osmolarity in the diagnosis and management of Dry Eye Disease. *American Journal of Ophthalmology.* 2011; 151: 792-798.
5. Marsella R, Girolomoni G. Canine models of atopic dermatitis: a useful tool with untapped potential. *J Invest Dermatol.* 2009; 129: 2351-2357.
6. Marsella R, Olivry T, Carlotti DN. International Task Force on Canine Atopic Dermatitis. Current evidence of skin barrier dysfunction in human and canine atopic dermatitis. *Vet Dermatol.* 2011; 22: 239-248.
7. DeBoer DJ, Hillier A. The ACVD task force on canine atopic dermatitis (XV): fundamental concepts in clinical diagnosis. *Veterinary Immunology and Immunopathology.* 2001; 81: 271-276.
8. Olivry T, Marsella R, Iwasaki T, Mueller R. Validation of CADESI-03, a severity scale for clinical trials enrolling dogs with atopic dermatitis. *Veterinary Dermatology.* 2007; 18: 78-86.
9. Gelatt K. Ophthalmic examination and diagnostic procedures. In: *Essentials of Veterinary Ophthalmology.* Lippincott Williams and Wilkins, Philadelphia, 2000: 1-26.
10. Moore CP. Diseases and surgery of the lacrimal secretory system. In Gelatt KN. (ed.) *Veterinary Ophthalmology.* (3rd ed) Lippincott Williams & Wilkins. 1999; 583-607.
11. Davis K, Townsend W. Tear-film osmolarity in normal cats and cats with conjunctivitis. *Veterinary Ophthalmology.* 2011; 14: 54-59.
12. Korth RME, Romkes G, Eule JC. Tear film osmolarity as a diagnostic tool in small animal and equine medicine? [abstract]. Abstracts: European Society of Veterinary Ophthalmologists, Dublin- Ireland, 13-16 May. *Veterinary Ophthalmology.* 2010; 13: 348-351.
13. Abusharha AA, Pearce EI. The effect of low humidity on the human tear film. *Cornea.* 2013; 32: 429-434.