Open Veterinary Journal, **(2024), Vol. 14(3): 879-884**
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: *10.5455/OVJ.2024.v14.i3.15*

Submitted: 01/01/2024 Accepted: 25/02/2024 Published: 31/03/2024

Assessment of lipid layer patterns in domestic dogs and rabbits: an observational study

Essam S. Almutleb, Gamal A. El-Hiti*, Abdulmalik N. Alshulayyil, Abdullah D. Alghamdi, Meznah S. Almutairi, Mashaaer A. Baashen, Basal H. Altoaimi, Saud A. Alanazi and Ali M. Masmali

Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

Abstract
Background: Maintaining a stable tear film is crucial for having healthy human and animal vision. Animals are expected to have thicker lipid layers than humans due to living in high-temperature and humid environments.
Aim: The study aimed to evaluate the lipid layer patterns (LLPs) in Arabian dogs and rabbits using a non-invasive, practical, and easy-to-use device and compare them to humans with healthy eyes.
Methods: The study included 75 domestic Arabian dogs (42 males and 33 females; mean ± SD = 6.1 ± 12.7 months) and 75 rabbits (37 males and 38 females; mean ± SD = 3.1 ± 3.4 months). In addition, 75 individuals with healthy eyes (39 males and 36 females; mean ± SD = 25.7 ± 5.0 years) were included for comparison. EASYTEAR View+ assessed the LLP in each animal's and individual's right eye.
Results: The median LLP grades significantly differed between dogs and humans (Mann–Whitney U test, $p < 0.001$). Similarly, the LLP grades differed significantly between rabbits and humans (Mann-Whitney U test, $p < 0.001$). No significant difference (Mann–Whitney U test) in the LLP grades between dogs and rabbits was found. The analysis indicated that most dogs had either an A (34.7%) or a B grade (37.3%). Similarly, rabbits had predominantly A or 1 (46.7%) and B (30.7%) grades. On the other hand, humans had predominantly D (53.3%) and E (30.7%) grades.
Conclusion: The EASYTEAR View+ has been employed to assess LLP in dogs and rabbits, and the measurements were compared to those of humans with normal ocular health. Dogs and rabbits have thinner lipid layers than healthy humans.
Keywords: Dogs, Rabbits, Tear film, Lipid layer patterns, Dry eye.

Introduction

The tear film has a role in maintaining the health of the eyes of both humans and animals (Corsi *et al*., 2022). It is the primary means of refracting light, lubricating, protecting, and smoothing the ocular surface. In addition, it has a vital role in preserving the cornea's health. Alterations in the quality and quantity of the tear film can increase the risk of corneal ulceration, infectious diseases, discomfort, irritation, and dryness (Nakamura *et al*., 2004). The tear film comprises a lipid layer and a mucoaqueous phase, with the latter making up most of the tear volume (Bron *et al*., 2023). Animals' tear film status has been evaluated using various techniques that assess tear film quality, quantity, and stability. The most used test to detect ocular disorders in veterinary practice is the Schirmer measurement. However, other techniques such as osmolarity, tear meniscus height, and tear ferning tests are employed (Iwashita *et al*., 2023).

Previous studies have shown that dogs and rabbits have a higher prevalence of ocular surface diseases (Czerwinski, 2019; Maggio, 2019). As a result, various studies have been carried out to examine the lacrimal parameters in rabbits (Biricik *et al*., 2005; Ghaffari *et al*., 2009; Kaswan *et al*., 1990; Kaswan *et al*., 1998; Rajaei *et al*., 2016; Whittaker 2015). The tear osmolarity has been evaluated among animals such as dogs (Brito *et al*., 2022; Lamkin *et al*., 2020; Sebbag *et al*., 2017; Brito *et al*., 2021), rabbits (Lantyer-Araujo *et al*., 2020), and others (Davis and Townsend, 2011; Korth *et al*., 2010).
The lipid content found in tears plays a vital role in tear film stabilization and prevents excessive tear evaporation (Viñas *et al*., 2019). The tear film's lipid layer is very thin, and its thickness can be measured using interferometry (Yokoi *et al*., 1996). This method provides information about the quality and quantity of lipids in the tear film, which can help identify the presence of dry eye syndrome (Arita *et al*., 2016).

***Corresponding Author:** Gamal A. El-Hiti. Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia. Email: *gelhiti@ksu.edu.sa*

The lipid content is reduced in older male dogs with meibomian gland dysfunction, resulting in a thinner lipid layer (Arita *et al*., 2016). The lacrimal meniscus has been measured in animals (e.g., dogs, rabbits, and cats) using a strip meniscometry tool (Kovaļčuka *et al*., 2021; Miyasaka *et al*., 2019; Oria *et al*., 2019; Rajaei *et al*., 2018). Meibomian glands secrete the lipids in tear film and can be visualized *in vivo* using meibography (Arita *et al*., 2018). The secretion of the meibomian gland is a thick, creamy-white discharge that contains polar and non-polar lipids. At normal body temperature, the lipids turn to fluid and cover the mucoaqueous phase in each blink, reducing tear evaporation. In addition, it lowers the surface tension of the ocular surface.

Studies have examined rabbits in medical research (Eom *et al*., 2018; Jester *et al*., 2015) and dogs with various eye and skin disorders (Kitamura *et al*., 2019; Sartori and Peruccio, 2020). They are standard experimental models to investigate ocular diseases (Zernii *et al*., 2016). They are accessible, easy to work with, and have visual responses similar to humans (Zhou *et al*., 2007). The tear film in laboratory rabbits and dogs has been assessed using various techniques. However, EASYTEAR View+ has never been used to determine the lipids layer patterns in domestic dogs and rabbits. Recently, we have shown that EASYTEAR View+ can be used as a single device to assess the tear film parameters among smokers and subjects with a high body mass index (BMI) and healthy eyes (Fagehi *et al*., 2022). Therefore, the study aimed to evaluate the patterns and thickness of the lipid layer among domestic dogs and rabbits using a portable device.

Materials and Methods

Dogs and rabbits

This study examined 75 domestic dogs (42 males and 33 females; mean ± SD = 6.1 ± 12.7 months) and 75 rabbits (37 males and 38 females; mean ± SD = 3.1 ± 3.4 months). The animals used in this study were chosen randomly from a farm 120 km east of Riyadh. Only healthy animals without any ocular disorders or diseases were selected. The IRB approved the study (King Saud University; E-21-6474) based on the guidance for the care and use of laboratory animals. The subjects were treated based on the principles of the Helsinki Declaration.

Healthy eye subjects

The study included 75 individuals with healthy eyes (39 males and 36 females; mean ± SD = 25.7 ± 5.0 years) were included for comparison. The ocular surface disease index (OSDI) was utilized to detect the presence of ocular irradiation and inflammation due to dry eyes. Participants completed the OSDI, and those with a score above 13 were not included (Schiffman *et al*., 2000). The assessment was conducted at the Clinics of the College of Applied Medical Sciences, Male Campus in Riyadh. Before performing the research, the participants were given a written consent form and asked to sign it, indicating their informed consent to participate. This ensures that the participants were fully aware of the study's purpose, procedures, and risks and gave their voluntary agreement to take part.

EASYTEAR view+

The lipid layer patterns (LLPs) were evaluated on the right eye of each participant using EASYTEAR View+, with a 5-minute interval between assessments. Two independent examiners took the measurements thrice, recording the average value.

LLP

Each subject was assigned a grade (A, B, C, D, or E) based on their LLP (Garcia-Resua *et al*., 2017). The lipid layer thickness (LLT) was the highest for grade E (90–140 nm) and the lowest for grade A (13–15 nm). For statistical analysis, the traditional grades A, B, C, D, and E were replaced with numerical ones as 1, 2, 3, 4, and 5, respectively. This change was previously reported and implemented to ensure accurate and efficient analysis of the data. By translating the traditional grades into numerical values, the data were better suited for statistical analysis and allowed for more precise conclusions (Fagehi *et al*., 2022).

Statistical analysis

The Statistical Package for the Social Sciences software (version 22; IBM Software, Armonk, NY, USA) was used to analyze the data. The Kolmogorov–Smirnov test (with a significance level of $p < 0.05$) was conducted to determine that the data did not follow a normal distribution. Therefore, the Mann–Whitney U test was used to analyze the data in both groups. The association between different parameters was tested using Spearman's correlation coefficient (r) (Cohen, 1988). The Wilcoxon signed-rank test was used within the same group to investigate significant parameter differences. Median and Interquartile ranges represented the mean scores.

Ethical approval

Before performing the research, the participants were given a written consent form and asked to sign it, indicating their informed consent to participate. This ensures that the participants were fully aware of the study's purpose, procedures, and risks and gave their voluntary agreement to take part.

Results

The median LLP scores in dogs, rabbits, and humans were 2(2), 2(1), and 4(1), respectively. The median LLP grades significantly differed between dogs and humans (Mann–Whitney U test, $p < 0.001$). Similarly, the LLP grades differed significantly between rabbits and humans (Mann–Whitney U test, $p < 0.001$). No significant difference (Mann–Whitney U test) in the LLP grades between dogs and rabbits was found. Please refer to Figure 1 for the side-by-side boxplots that show the LLP in dogs, rabbits, and healthy-eyed humans.

According to the LLP analysis, the most common grades for dogs were A or 1 (*N* = 26, 34.7%) and B or 2 (*N* = 28, 37.3%). The most common grades for rabbits were A or 1 (*N* = 35, 46.7%) and B or 2 (*N* = 23, 30.7%). However, the most common grades for humans were D or 4 (*N* = 40, 53.3%) and E or 5 (*N* = 23, 30.7%). Figure 2 shows the LLP grades for dogs, rabbits, and humans with healthy eyes. A weak correlation (Spearman's rank correlation coefficient; $r = 0.271$, $p = 0.030$) was found between rabbits' age and LLP grades.

Discussion

The current study reported for the first time the use of EASYTEAR view+ to assess the LLP in dogs and rabbits. The results revealed that dogs [2(2)] and rabbits [2(1)] have significantly thinner lipid layers than humans with healthy eyes [4(1)]. The EASYTEAR

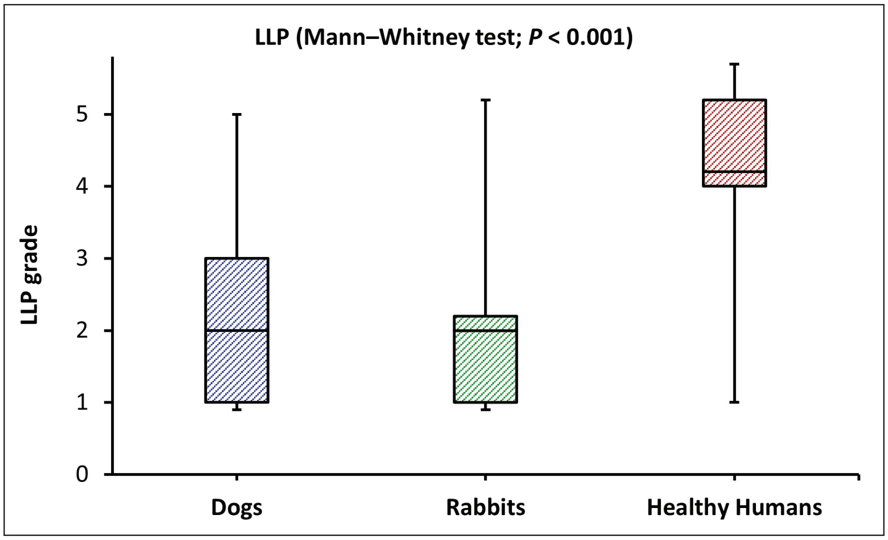

Fig. 1. Side-by-side boxplots of the LLP scores in dogs, rabbits, and healthy-eye humans. The grades are categorized as follows: grade A or 1 (showing a grayish appearance, with LLT = 13–15 nm), grade B or 2 (more compact with LLT = 30–50 nm), grade C or 3 (gray waves with LLT = 50–80 nm), grade D or 4 (dense white-blue layer, with LLT = approximately 80 nm), and grade E or 5 (showing variable colors, with LLT = 90–140 nm)

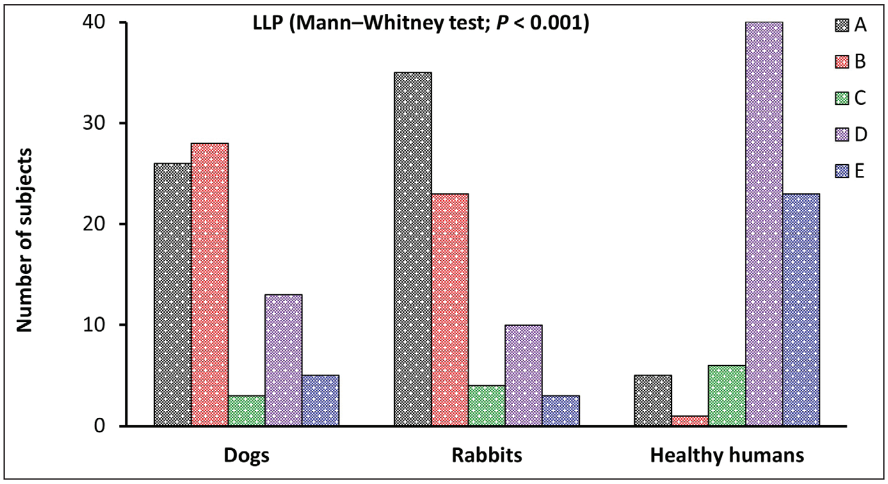

Fig. 2. The LLP in dogs, rabbits, and healthy-eyed humans. The grades are categorized as follows: grade A or 1 (showing a grayish appearance, with LLT = 13–15 nm), grade B or 2 (more compact with LLT = 30–50 nm), grade C or 3 (gray waves with LLT = 50–80 nm), grade D or 4 (dense white-blue layer, with LLT = approximately 80 nm), and grade E or 5 (showing variable colors, with LLT = 90–140 nm).

view+ can be a helpful tool for evaluating the LLP and LLT in animals. The difference in LLP in humans and animals could be due to variations in tear protein among them (Davidson *et al*., 1994). The tear proteins were highly concentrated in horses (13.7 ± 4.0 mg/ml) and dogs (10.2 ± 3.5 mg/ml). On the other hand, the protein concentration was low in the tears of dogs (2.6 ± 1.0 mg/ml) and cows (5.8 ± 2.2 mg/ml) (Davidson *et al*., 1994). In humans, tear protein concentration is much higher than that recorded for animals (de Souza *et al*., 2006). The dogs and the human meibum demonstrated were similar. In contrast, the rabbits' meibum differs entirely from humans (Butovich *et al*., 2012). Rabbits are comparable to humans based on tear biochemistry, osmolarity measurements, and TF grades. Moreover, the biochemical features of rabbits and humans are very similar (Lantyer-Araujo *et al*., 2020).

The LLP average grade based on the five-point (Grade 0 to 4) grading scale in rabbits (2 ± 1) using an ocular surface analyzer was similar to that obtained from the current study (Corsi *et al*., 2022). A variation in lipid layer grades has been seen among female and male rabbits. Grade 1 was seen in females (46%), while grade 2 was observed in males (50%) (Corsi *et al*., 2022). The variation could be due to the effect of hormonal changes on the meibomian gland function (Bron *et al*., 2004).

The LLP in smokers and individuals with high BMI measured using EASYTEAR view+ was similar to those found in dogs and rabbits and much lower than in humans with healthy eyes. Subjects with healthy eyes had a significantly higher median LLP score [4 (1)] than smokers [2.4 (1.0)] and high BMI subjects [2 (1.3)] (Fagehi *et al*., 2022). Subjects with a high BMI typically had grade B in LLP (30–50 nm LLT, more compact), and healthy eye subjects had grade D (around 80 nm, dense white–blue layer). On the other hand, smokers had grade C (50–80 nm, gray waves) (Fagehi *et al*., 2022).

The tear film osmolarity in dogs with keratoconjunctivitis sicca has improved after 45 days of using cyclosporine (1%) with oral mucosa transplantation based on IPen measurements (Brito *et al*., 2021). However, such a study included only a few dogs, and the environmental conditions (e.g., temperature and humidity) were never controlled. In addition, IPen generated errors during the measurements if it came into contact with the tissues of the eye surface in dogs. The average tear film osmolarity was 315.3 ± 6.2 mOsm/l in dogs (Brito *et al*., 2022) compared to 337.5 ± 27.1 in rabbits (Corsi *et al*., 2022).

The average scores of Schirmer test 1 in rabbits varied and ranged from 4.9 ± 3.4 to 9.9 ± 3.4 mm/minute (Corsi et al., 2022; Rajaei *et al*., 2016; Whittaker 2015; Abrams *et al*., 1990). These large variations could be due to the different breeds and emotional states of doges, environmental conditions, and types of Schirmer strips used (Yoon *et al*., 2020).

The TF test was used to evaluate the tear film in dogs (Oriá *et al*., 2018; Williams and Hewitt, 2017). The TF grades in healthy doges were mainly (78%) of types I and II, indicative of normal eyes. On the other hand, doges with keratoconjunctivitis sicca showed only TF grades of types III and IV (100%), indicating dry eyes in all of them. In addition, the Schirmer test score was lower in dogs with keratoconjunctivitis sicca (6.2 ± 3.5 mm/minute) than in the control group (6.2 ± 5.6 mm/minute) (Williams and Hewitt, 2017).

No single test can be used to assess tear film in animals (Iwashita *et al*., 2023). The correlation between measurements of different tear film parameters is poor. Each tet assess a different paratmeter. Therefore, several devices and tools should be used. Diagnosing dry eye in animals is challenging, and non-invasive instruments are advantageous and more comfortable for animals.

The study's limitations are attributed to using one test to evaluate the tear film parameters. Further research must encompass diverse animal species of varying sizes across various locations in Saudi Arabia. In addition, multiple tests, such as tear evaporation, tear ferning, and osmolarity tests, are employed to appraise the tear film parameters in animals.

In conclusion, the EASYTEAR View+ has been employed to assess LLP in dogs and rabbits, and the measurements were compared to those of humans with normal ocular health. Dogs and rabbits have thinner lipid layers than healthy humans.

Acknowledgment

The authors acknowledge the research received from the Researchers Supporting Project (number RSP2024R404), King Saud University, Riyadh, Saudi Arabia.

Conflict of interest

The authors declare that there is no conflict of interest.

Author contributions

Essam S. Almutleb: Software, validation, formal analysis, funding acquisition, and draft preparation. Gamal A. El-Hiti: Conceptualization, methodology, investigation, funding acquisition, project administration, supervision, draft preparation, review, and editing. Abdulmalik N. Alshulayyil and Abdullah D. Alghamdi: Investigation. Meznah S. Almutairi: Software, validation, formal analysis, and draft preparation. Mashaaer A. Baashen: Software, validation, formal analysis, and draft preparation. Basal H. Altoaimi: Software, validation, formal analysis, and draft preparation. Saud A. Alanazi: review and editing. Ali M. Masmali: review and editing.

Funding

King Saud University (RSP2023R404).

Data availability

All data supporting the findings of this study are available within the manuscript.

References

Abrams, K.L., Brooks, D.E., Funk, R.S. and Theran, P. (1990). Evaluation of the Schirmer tear test in clinically normal rabbits. Am. J. Vet. Res. 51, 191–193.

Arita, R. (2018). Meibography: a Japanese perspective. Invest. Ophthalmol. Vis. Sci. 59, DES48–DE55.

Arita, R., Morishige, N., Fujii, T., Fukuoka, S., Chung, J.L., Seo, K.Y. and Itoh, K. (2016). Tear interferometric patterns reflect clinical tear dynamics in dry eye patients. Invest. Ophthalmol. Vis. Sci. 57, 3928–3934.

Biricik, H.S., Oğuz, H., Sindak, N., Gürkan, T. and Hayat, A. (2005). Evaluation of the Schirmer and phenol red thread tests for measuring tear secretion in rabbits. Vet. Rec. 156, 485–487.

Brito, F.L.C., Kuner, A., Voitena, J.N. and Marinho, T.O.C. (2021). Oral mucosa transplantation may improve tear film osmolarity in dogs with keratoconjunctivitis sicca—A preliminary study. Arq. Bras. Med. Vet. Zootec. 73, 1278–1286.

Brito, F.L.C., Voitena, J.N., Marinho, T.O.C., Moore, B.A. and Montiani-Ferreira, F. (2022). Assessment of tear film osmolarity using the IPen® vet osmometer in pug and Shih-Tzu dogs with and without keratoconjunctivitis sicca. Vet. Ophthalmol. 25, 219–224.

Bron, A.J., de Paiva, C.S., Chauhan, S.K., Bonini, S., Gabison, E.E., Jain, S., Knop, E., Markoulli, M., Ogawa, Y., Perez, V., Uchino, Y., Yokoi, N., Zoukhri, D. and Sullivan, D.A. (2023). TFOS DEWS II pathophysiology report. Ocul. Surf. 15, 438–510.

Bron, A.J., Tiffany, J.M., Gouveia, S.M., Yokoi, N. and Voon, L.W. (2004). Functional aspects of the tear film lipid layer. Exp. Eye Res. 78, 347–360.

Butovich, I.A., Lu, H., McMahon, A. and Eule, J.C. (2012). Toward an animal model of the human tear film: biochemical comparison of the mouse, canine, rabbit, and human meibomian lipidomes. Invest. Ophthalmol. Vis. Sci. 53, 6881–6896.

Cohen, J. (1988). Statistical power analysis for the behavioral sciences. Hillsdale, NJ: Lawrence Erlbaum Associates.

Corsi, F., Arteaga, K., Corsi, F., Masi, M. Cattaneo, A., Selleri, P., Crasta, M., Peruccio, C. and Guandalin, A. (2022). Clinical parameters obtained during tear film examination in domestic rabbits. BMC Vet. Res. 18, 398.

Czerwinski, S. (2019). Medical and surgical management of ocular surface disease in exotic animals, An issue of veterinary clinics of North America: exotic animal Practice, Ebook, Volume 22. Elsevier, New York, NY, USA.

Davidson, H.J., Blanchard, G.L. and Montgomery, P.C. (1994). Comparisons of tear proteins in the cow, horse, dog and rabbit. Adv. Exp. Med. Biol. 350, 331–334.

Davis, K. and Townsend, W. (2011). Tear-film osmolarity in normal cats and cats with conjunctivitis. Vet. Ophthalmol. 14, 54–59.

de Souza, G.A., Lyris MF Godoy, L.M.G. and Mann, M. (2006). Identification of 491 proteins in the tear fluid proteome reveals a large number of proteases and protease inhibitors. Genome Biol. 7, R72.

Eom, Y., Han, J.Y., Kang, B., Hwang, H.S., Lee, H.K., Kim, H.M. and Song J.H. (2018). Meibomian glands and ocular surface changes after closure of Meibomian gland orifices in rabbits. Cornea 37, 218–226.

Fagehi, R., El-Hiti, G.A., Almojalli, A., Alzuhairi, F.S., Alanazi, M.A., Masmali, A.M. and Almubrad, A.M. (2022). Assessment of tear film parameters in smokers and subjects with a high body mass index. Optom. Vis. Sci. 99, 358–362.

Garcia-Resua, C., Pena-Verdeal, H., Giraldez, M.J. and Yebra-Pimentel, E. (2017). Clinical performance of an objective methodology to categorize tear film lipid layer patterns. Proc SPIE 10453, T1–8.

Ghaffari, M.S., Moghaddassi, A.P. and Bokaie, S. (2009). Effects of intramuscular acepromazine and diazepam on tear production in rabbits. Vet. Rec. 164, 147–148.

Iwashita, H., Sebbag, L., Leonard, B.C. and Saito, A. (2023). A review of diagnostic tests for qualitative and quantitative tear film deficiency in dogs. Vet. Ophthalmol. 26(Suppl. 1), 5–15.

Jester, J.V., Parfitt, G.J. and Brown, D.J. (2015). Meibomian gland dysfunction: Hyperkeratinization or atrophy? BMC Ophthalmol. 15, 156.

Kaswan, R., Pappas, C., Wall, K. and Hirsh, S.G. (1998). Survey of canine tear deficiency in veterinary practice. Adv. Exp. Med. Biol. 438, 931–939.

Kaswan, R.L. and Salisbury, M.A. (1990). A new perspective on canine keratoconjunctivitis sicca. Treatment with ophthalmic cyclosporine. Vet. Clin. North Am. Small. Anim. Pract. 20, 583–613.

Kitamura, Y., Maehara, S., Nakade, T., Miwa, Y., Arita, R., Iwashita, H. and Saito A. (2019). Assessment of meibomian gland morphology by noncontact infrared meibography in Shih Tzu dogs with or without keratoconjunctivitis sicca. Vet. Ophthalmol. 22, 744–750.

Korth, R.M.E., Romkes, G. and Eule, J.C. (2010). Tear film osmolarity as a diagnostic tool in small animal and equine medicine? Vet. Ophthalmol. 13, 348–351.

Kovaļčuka, L., Šarpio, L. and Mālniece, A. (2021). Schirmer tear test and strip meniscometry in healthy cats. Open Vet. J. 11, 695–699.

Lamkin, I.D., Zimmerman, K.L., Smith Fleming, K.M. and Martins, B.C. (2020). Osmolarity of basal and reflex tears of normal dogs. Vet. Ophthalmol. 23, 747–453.

Lantyer-Araujo, N.L., Lacerda, A.J., Mendonça, M.A., da Silva, A.P.S.M., Dórea Neto, F.A., Portela, R.D.

and Oria A.P. (2020). Rabbit as an animal model for ocular surface disease, tear osmolarity, electrolyte, and tear ferning profiles. Optom. Vis. Sci. 97, 847–851.

Maggio, F. (2019). Ocular surface disease in dogs part 1: Aetiopathogenesis and clinical signs. Companion Anim. 24, 240–245.

Miyasaka, K., Kazama, Y., Iwashita, H., Wakaiki, S. and Saito, A. (2019). A novel strip meniscometry method for measuring aqueous tear volume in dogs: clinical correlations with the Schirmer tear and phenol red thread tests. Vet. Ophthalmol. 22, 864–871.

Nakamura, S., Okada, S., Umeda, Y. and Saito, F. (2004). Development of a rabbit model of tear film instability and evaluation of viscosity of artificial tear preparations. Cornea 23, 390–397.

Oriá, A.P., Raposo, A.S.S., Araújo, N.L.L.C., Lima, F.B. and Masmali, A.M. (2018). Tear ferning test in healthy dogs. Vet Ophthalmol. 21, 391–398.

Oria, A.P., Santos Raposo, A.C., da Silva Cardoso de Brito, V.J., Lantyer Cordeiro de Araujo, N.L., Souza Oliveira, M.M. and Barros Albuquerque, I.M. (2019). Tear meniscometry test in wild animals. Cienc. Rural 49, e20181002.

Rajaei, S.M., Ansari Mood, M., Asadi, F., Rajabian, M.R. and Aghajanpour, L. (2018). Strip meniscometry in dogs, cats, and rabbits. Vet. Ophthalmol. 21, 210–213.

Rajaei, S.M., Rafiee, S.M., Ghaffari, M.S., Masouleh, M.N. and Jamshidian, M. (2016). Measurement of tear production in English angora and Dutch rabbits. J. Am. Assoc. Lab. Anim. Sci. 55, 221–223.

Sartori, R. and Peruccio, C. (2020). A case of sebaceous adenitis and concurrent Meibomian gland dysfunction in a dog. Vet. Sci. 7, 37.

Schiffman, R.M., Christianson, M.D., Jacobsen, G., Hirsch, J.D. and Reis, B.L. (2000). Reliability and validity of the ocular surface disease index. Arch. Ophthalmol. 118, 615–621.

Sebbag, L., Park, S.A., Kass, P.H., Maggs, D.J., Attar, M. and Murphy, C.J. (2017). Assessment of tear film osmolarity using the TearLab™ osmometer in normal dogs and dogs with keratoconjunctivitis sicca. Vet. Ophthalmol. 20, 357–364.

Viñas, M., Maggio, F., D'Anna, N., Rabozzi, R. and Peruccio, C. (2019). Meibomian gland dysfunction (MGD), as diagnosed by non-contact infrared meibography, in dogs with ocular surface disorders (OSD): a retrospective study. BMC Vet. Res. 15, 443.

Whittaker, A.L. and Williams, D.L. (2015). Evaluation of lacrimation characteristics in clinically Normal New Zealand white rabbits by using the Schirmer tear test I. J. Am. Assoc. Lab. Anim. Sci. 54, 783–787.

Williams, D. and Hewitt, H. (2017). Tear ferning in normal dogs and dogs with keratoconjunctivitis sicca. Open Vet. J. 7, 268–272.

Yokoi, N., Takehisa, Y. and Kinoshita, S. (1996). Correlation of tear lipid layer interference patterns with the diagnosis and severity of dry eye. Am. J. Ophthalmol. 122, 818–824.

Yoon, A., Liu, C.C., Carter, R.T. and Lewin, A.C. (2020). Environmental relative humidity affects Schirmer tear test results in normal dogs. Vet. Ophthalmol. 23, 923–926.

Zernii, E.Y., Baksheeva, V.E., Iomdina, E.N., Averina, A.A., Permyakov, S.E., Philippov, P.P., Zamyatnin, AA., Senin, II., (2016). Rabbit models of ocular diseases: new relevance for classical approaches. CNS Neurol. Disord. Drug Targets 15, 267–291.

Zhou, L., Beuerman, R.W., Huang, L., Barathi, A., Foo, Y.H., Li, S.F., Chew, F.T. and Tan, D. (2007). D. Proteomic analysis of rabbit tear fluid: defensin levels after an experimental corneal wound are correlated to wound closure. Proteomics 7, 3194–206.