PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
MESTRADO EM CIÊNCIA ANIMAL

FELIPE FRANCO NASCIMENTO

COMPARAÇÃO ENTRE O TESTE DE MENISCOMETRIA DE TIRA E OUTROS TESTES OFTÁLMICOS EM CÃES COM OLHOS NORMAIS E COM CERATOCONJUNTIVITE SECA

Presidente Prudente - SP
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Dissertação apresentada a Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade do Oeste Paulista, como parte dos requisitos para obtenção do título de Mestre – Área de concentração: Fisiopatologia e Saúde Animal

Orientadora:
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Presidente Prudente, 04 de abril de 2019.

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RESUMO

Comparação entre o teste de meniscometria de tira e outros testes oftálmicos em cães com olhos normais e com ceratoconjuntivite seca

O objetivo do presente estudo foi correlacionar o teste de meniscometria de tira (TMT) com o teste lacrimal de Schirmer 1 (TLS-1), o teste de ruptura do filme lacrimal (TRFL) e o teste de lissamina verde (TLV), em cães com olhos normais e com ceratoconjuntivite seca (CCS). Foram avaliados 138 olhos de 69 cães, sendo 78 olhos olhos normais, e 60 olhos diagnosticados com CCS. Foi realizado primeiramente o exame padrão TLS-1 para alocar os cães no grupo normal ou grupo CCS, e ato contínuo TMT, TRFL e TLV . Os resultados em média e desvio padrão do grupo normal foram: TLS-1 23,68±4,25, TMT 9,43±1,59, TRFL 23,06±6,95 e TLV 7 olhos positivos e do grupo CCS foram: TLS 6,20±4,45, TMT 3,02±2,52; TRFL 11,62±4,53 e TLV 14 olhos positivos. Houve uma correlação positiva entre os testes de TMT com TLS e TRFL e negativa entre TMT e TLV, sendo observada correlação forte e positiva entre TMT e TLS ($r=0,87$, $p<0,001$), moderada entre TMT e TRFL ($r=0,61$, $p<0,001$) e muito baixa entre TMT e TLV ($r=-0,17$, $p<0,001$). Considerou-se melhor ponto de corte do TMT o valor 7,5 mm/5seg segundo a Curva ROC, demonstrando 100% de sensibilidade e 91,7 % de especificidade. O presente estudo forneceu os valores de TMT em olhos normais e com CCS na espécie canina, demonstrando alta sensibilidade e especificidade comparado ao TLS, com um significativo poder preditivo para diagnóstico de CCS, podendo vir a substituir futuramente o TLS.

Palavras-chave: ceratoconjuntivite seca, cães, meniscometria de tira, teste de Schirmer, teste de ruptura do filme lacrimal, teste de lissamina verde.

ABSTRACT

Comparison between the strip meniscometry test and other ophthalmic tests in dogs with normal eyes and with keratoconjunctivitis sicca

The objective of the present study was correlate the strip meniscometry test (SMT) with the Schirmer tear test 1 (STT-1), the tear film break up test (TBUT), and the lissamine green test (LGT) in dogs with normal eyes and with dry keratoconjunctivitis (KCS). Were evaluated 138 eyes from 69 dogs, including 78 normal eyes and 60 eyes diagnosed with KCS. The tests were performed in the following sequence: the STT-1 was used to allocate the dogs to the normal group or the KCS group, followed by the SMT, TBUT, and LGT. The results in means and standard deviations for the normal group were: 23.68±4.25 (STT-1), 9.43±1.59 (SMT), and 23.06±6.95 (TBUT); LGT 7 positive eyes; and for the KCS group were: 6.20±4.45 (STT-1), 3.02±2.52 (SMT), and 11.62±4.53 (TBUT); LGT 14 positive eyes. There was a positive correlation between the SMT and the STT-1 and TBUT, as well as a negative correlation between the SMT and LGT. The correlation was very high between the SMT and STT-1 ($r=0.87$, $p<0.001$), moderate between the SMT and TBUT ($r=0.61$, $p<0.001$) and very low between the SMT and LGT ($r=-0.17$, $p<0.001$). The cut-off considered for the SMT was 7.5 mm/5 sec according to the ROC curve, presenting a sensitivity of 100% and specificity of 91.7%. This study provided SMT values in normal and KCS eyes for dogs, revealing high sensitivity and specificity compared with the STT-1 and a good predictive power for the diagnosis of KCS, showing that in the future, the SMT could replace the STT-1.

Keywords: dogs, keratoconjunctivitis sicca, lissamine green test, Schirmer tear test, strip meniscometry test, tear film breakup test.

LISTA DE SIGLAS

ARVO – Association for Research in Vision and Ophthalmology
CAPES – Coordination of Improvement of Higher Level Personnel
CEUA – Ethical Committee on Animal Use
FT – Fluorescein Test
KCS – Keratoconjunctivitis Sicca
LGT – Lissamine Green Test
PRT – Pupillary Reflex Test
PRT – Phenol Red Test
RBT – Rose Bengal Test
ROC – Receiver Operator Characteristic Curve
SD – Standard Deviations
STT – Schirmer Tear Test
SMT – Strip Meniscometry Test
TBUT – Tear Film Break up Test
UNOESTE – Universidade do Oeste Paulista

SUMÁRIO

1 ARTIGO CIENTÍFICO

Correlation between the strip meniscometry test and other ophthalmic tests in dogs with normal eyes and with keratoconjunctivitis sicca

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Running Title: Strip meniscometry test normal and KCS eyes dogs

ABSTRACT

Objective To correlate the strip meniscometry test (SMT) with the Schirmer tear test 1 (STT-1), the tear film break up test (TBUT), and the lissamine green test (LGT) in dogs with normal eyes and dogs diagnosed with keratoconjunctivitis sicca (KCS) *Animals* 138 eyes from 69 dogs, including 78 normal eyes and 60 eyes diagnosed with KCS.

Procedures The tests were performed in the following sequence: the STT-1 was used to allocate the dogs to the normal group or the KCS group, followed by the SMT, TBUT, and LGT.

Results The means and standard deviations for the normal group were: 23.68±4.25 (STT-1), 9.43±1.59 (SMT), and 23.06±6.95 (TBUT); LGT 7 positive eyes; and for the KCS group were: 6.20±4.45 (STT-1), 3.02±2.52 (SMT), and 11.62±4.53 (TBUT); LGT 14 positive eyes. There was a positive Pearson's correlation between the SMT and the STT-1 and TBUT, as well as a negative correlation between the SMT and LGT. The correlation was very high between the SMT and STT-1 ($r=0.87$, $p<0.001$), moderate between the SMT and TBUT ($r=0.61$, $p<0.001$) and very low between the SMT and LGT ($r=-0.17$, $p<0.001$). The cut-off considered for the SMT was 7.5 mm/5 sec according to the ROC curve, presenting a sensitivity of 100% and specificity of 91.7%.

Conclusion This study provided SMT values in normal and KCS eyes for dogs, revealing high sensitivity and specificity compared with the STT-1 and a good predictive power for the diagnosis of KCS, suggesting that in the future, the SMT could replace the STT-1.

Keywords: dogs, keratoconjunctivitis sicca, lissamine green test, Schirmer tear test, strip meniscometry test, tear film breakup test.

INTRODUCTION

Keratoconjunctivitis sicca (KCS), also known as dry eye syndrome, is a chronic inflammatory disease that is usually immune-mediated and is commonly diagnosed in both dogs and humans. It results in an insufficient production of the aqueous layer of the tear film (quantitative deficiency) or in excessive tear evaporation (qualitative deficiency) due to the inadequate production of the lipid or mucin layer, which diminishes the protective function of the tear. [1-5]

The diagnosis is based on the clinical signs and on the results of specific ophthalmologic tests. The golden standard test for measuring the quantitative of the tear in dogsis the Schirmer tear test (STT). However, other tests may be employed in addition to the STT to detect tear disorders, including the tear film breakup test (TBUT), the phenol red test (PRT), the Rose Bengal test (RBT) and the lissamine green test (LGT), as well as conjunctival cytology to assess goblet cells, mucin production, lipid production and the function of the meibomian glands. [1,3,6-9]

The STT consists of a semiquantitative technique that aims at determining the aqueous production of the precorneal tear film. It is conducted using commercially available filter paper strips and may be conducted with or without local anesthesia; it is termed the Schirmer tear test 1 (STT-1) in cases where sensations in the cornea, including the stimulation caused by the test's paper strips in the conjunctival sac, are preserved, and it is termed the Schirmer tear test 2 (STT-2) in cases where local anesthesia is applied, which results in lower values.[6,7] STT-1 is the most commonly applied version and is popular for diagnosing and verifying the progression of keratoconjunctivitis sicca in dogs, although it is not recommended to be the sole exclusion criteria for KCS since it only measures aqueous production and does not assess

possible mucin and lipid deficiencies in the precorneal tear film.[3,6,7] In addition, other factors, such as age, sex, and time of the day, may affect the Schirmer tear test. [10]

The TBUT is a qualitative test used to assess evaporative dry eye disease and to detect deficiencies in the mucin and lipid layers of the tear film by assessing the ability of the surface of the cornea to retain a homogeneous tear coating, which shows stability in the tear film. [6,11]. Ocular surface staining, such as in the LGT and RBT, is also an important auxiliary test in the diagnosis of KCS. Lissamine green is an important synthetically produced organic dye that stains damaged and devitalized cells in the conjunctiva and in the corneal epithelium; it is similar to Rose Bengal dye but without the adverse effects caused by Rose Bengal dye, such as discomfort and burning sensations. [11-14]

Diagnostic tests for KCS should combine high precision, good sensitivity and practicality.[12-14] Therefore, in humans, the need to develop a faster, less invasive and easier to use method to assess the volume of tears has led researchers to develop a simple and innovative method called the strip meniscometry test (SMT).[15,16] The SMT was recently introduced in veterinary medicine to measure tear volume in a simple, fast (5 sec) and less invasive method (the tip of the strip is projected to touch only the lower tear meniscus instead of inserting it in the conjunctival sac as in the Schirmer tear test) that has been recognized as a promising technique, particularly in screening and diagnosing disorders of the ocular surface, such as KCS. Another advantage of the SMT is the lower volume of tears absorbed by the strips, which allows other tests to be conducted shortly afterward. [17-19]

It has also been reported that the tear meniscus contains 75 to 90% of the aqueous tear volume, which is directly correlated with the tear secretion rate.[15] In humans, it was shown that using the SMT in tandem with other ocular surface tests promoted higher specificity than using the SMT alone. [16]

This study aims at assessing the SMT and its results in relation to the STT-1, TBUT and LGT in normal eyes and in eyes diagnosed with KCS and determining its cut-off, sensitivity and specificity according to the ROC curve.

MATERIALS AND METHODS

Animals

The study was conducted according to the standards of animal experimentation of the UNOESTE Ethical Committee on Animal Use (protocol # 3895) and was conducted according to the ARVO Guidelines (*Association for Research in Vision and Ophthalmology for the use of animals in ophthalmic and visual research)*.

To determine the minimum sample size required to estimate the mean tear production in the SMT, we used the formula described by Pagano et al. (2004), with a standard deviation value based on the results obtained by Rajaei et al. (2018). Based on these parameters, we concluded that a minimum of 50 normal eyes and 50 KCS eyes would be needed. The actual sample sizes used in the study were larger to improve reliability, and a total of 138 eyes from 69 dogs were assessed, including 78 normal eyes and 60 eyes diagnosed with KCS. There were 29 male and 40 female dogs included in the study with ages between 3 months and 16 years and an average weight of 11.10±7.66 kg, being them: French Bulldog (16), Yorkshire Terrier (5), Miniature Pinscher (1), Crossbreed (15), Boxer (1), Poodle (6), Lhasa Apso (9), Chihuahua (1), Rottweiler (1), Maltes (2), Pekingese (1), Shih-tzu (4), Bulldog Campeiro (1), English Cocker Spaniel (3) Chow Chow (1) English Bulldog (2). All animals included in the study were attended at the ambulatory of Ophthalmology Department of the Veterinary Hospital of UNOESTE, Presidente Prudente, SP, Brazil.

Ophthalmologic Examinations

All dogs included in the study had their ocular clinical signs assessed with a portable slit lamp (SL-15, Kowa, Japan) and underwent a pupillary reflex test (PRT) with a punctiform light, an ophthalmoscopy of the fundus of the eye with an ophthalmoscope (Pocket Jr®, Welch Allyn, USA) and rebound tonometry with a Tonovet® tonometer (Icare, Finland) to verify the intraocular pressure. After these examinations, specific ophthalmic examinations were conducted to diagnose KCS and assess the ocular surface in the following order: the STT-1, SMT, TBUT, fluorescein test (FT) for staining corneal ulcers and LGT. Inclusion criteria for the study were as follows: for healthy eyes, STT-1 $\geq$ 15 mm/min and normal ophthalmic exams; for eyes with clinical signs of KCS, STT-1 < 15 mm/min. The exclusion criteria were increased intraocular pressure, negative pupillary reflex and abnormalities in the fundus of the eye.

The STT-1 was conducted by introducing 0.5 cm of the filter paper strip (Schirmer Tear Test Ophthalmos®, Brazil) to the conjunctival sac for one minute. The paper strip was then removed, and the wet area was immediately measured (ignoring the 0.5 cm). Animals were considered positive for KCS when the STT-1 < 15 mm/min, and they were considered healthy when the STT-1 $\geq$ 15 mm/min with normal results in the ophthalmic exams, and clinical signs.[6]

The SMT was conducted by placing the tip of the paper strip (I-Tear®, Canada) at the edge of the lower tear meniscus for 5 sec (Fig. 1) with open eyes, without touching the ocular surface and observing when tears enter the ridge and turn blue (when they come in contact with the natural blue dye at the tip of the strip) without expanding onto the hydrophobic pellicle to the sides. After the time is up, the value obtained is immediately recorded. [15, 17-20]

The TBUT was conducted by applying 1 drop of 1% fluorescein eye drops (Allergan, Brazil), and after two blinks, the eyelid was held open manually, and the cornea was observed with a portable slit lamp (Kowa- SL15, Japan) with cobalt blue illumination. The tear film breakup time (appearance of the first dry spots on the cornea) was measured in seconds, and values ≥ 20 sec were considered normal.[3,6]

The FT was conducted to detect irregularities or ulcers in the cornea using 1 drop of 1% fluorescein eye drops (Allergan, Brazil) and then washing the eye with saline solution. [3,6]

The LGT was conducted by holding the lissamine green strip (Ophthalmos®, Brazil) in contact with the tear meniscus of the lower conjunctival sac for 2 minutes. [10, 11] The results were categorized as follows: 0 for nonstained eyes and 1 for eyes stained with lissamine green. [6,11]

STATISTICAL ANALYSIS

The relationships between the variables STT-1, SMT, TBUT and LGT were assessed using Pearson's correlation test. To determine the accuracy of the SMT in comparison with the STT-1, a ROC curve (receiver operating characteristic curve) was plotted. The area under the curve was estimated through the confidence interval for validation (difference from 0.5). From this curve, the cut-off point was determined as the point with higher sensitivity and specificity values. All calculations were conducted in R software with a significance level of 5%. [22]

RESULTS

The means ± standard deviations and amplitudes (maximum and minimum values) of the ophthalmic examination results are described in Table 1. In the FT, 127 eyes presented negative results, and 11 presented positive results, all of which belonged to the KCS group.

Of these, 8 were classified as superficial ulcers (STT-1 results between 10 and 14 mm/min) and 3 as stromal ulcers (STT-1 results below 10 mm/min).

The correlations between the ophthalmic tests (STT-1, SMT, TBUT and LGT) obtained through Pearson's correlation coefficient are described in Table 2. The ROC curve showing the cut-off value for the SMT is shown in Fig. 2.

DISCUSSION

This is the first study in dogs to assess the SMT and to compare it with the main ophthalmic tests for the diagnosis of KCS, including the standard STT-1, the TBUT and the LGT. In addition, this is the first time a study assessed the cut-off value for eyes with KCS, which is an important parameter in establishing the diagnosis of KCS with this new technique.

In Pearson's correlation analysis, we observed that all tests are correlated, especially the STT-1 and SMT, which showed a very high correlation. There was a negative and weak correlation with the LGT, revealing that the number of cells stained by lissamine green decreases as the results of the tests (STT-1, SMT, and TBUT) increase.

There was also a correlation between the lower values for the STT-1 and the higher number of corneas stained with fluorescein, with the more severe ulcers observed presenting lower STT-1 scores.

The area under the ROC curve showed that the SMT had good predictive power considering that the cut-off presented an improved performance of 7.5 mm, with a sensitivity of 100% and a specificity of 91.7%. However, the test was unable to correctly classify all healthy animals, with a false-positive rate of 8.3%.

A study conducted by Ishikawa et al. (2018) defined an ideal cut-off for the SMT of ≤5 mm in humans, resulting in a sensitivity of 70.6% and a specificity of 84.6%.[22] In this study,

the test had a sensitivity of 100% and a specificity of 91.7%, showing that the SMT has better diagnostic performance in dogs than in humans and has efficiency and safety when diagnosing dry eye syndrome in dogs.

STT-1 has been used in both humans and animals as the gold standard for the quantitative diagnosis of KCS by measuring the volume of the aqueous portion of the tear film. However, the test requires that patients endure the discomfort caused by inserting the paper strip in the eye, which characterizes the technique as invasive.[12-16] The SMT is a noninvasive diagnostic tool for KCS that can be performed in 5 sec without inducing the lacrimation reflex. However, the technique requires higher precision in comparison with the STT-1 because it does not touch the cornea or the conjunctiva during the examination.[16,17]

This study did not observe any signs of discomfort or pain during the examination, corroborating the observations of previous studies, in which most dogs tolerated the procedure well.[17] In a study conducted with 333 soldiers, [23] the authors reported that only one soldier (0.003%) complained about discomfort after an SMT, but the patient had conjunctivitis and presented with ocular irritation.

Combining the SMT with other lacrimal function tests, particularly the TBUT, results in acceptable sensitivity and specificity, which is valuable in the assessment of ocular surface diseases.[16] Assessing the stability of the tear film with the TBUT is difficult and requires training.[22] This study noted difficulties with the animals blinking their third eyelid during the examination, but with training and repeating the test on those animals, we were able to obtain reliable values.

Despite the difficulties faced when conducting the TBUT, it is still an important examination due to its relevance in the diagnosis of KCS.[11,24] This study showed that the mean and standard deviation values for the TBUT were 23.06 ± 6.95 sec (14-44) on normal

eyes and 11.62 ± 4.53 (3-20) in KCS eyes, showing that, as observed by Seyer et al. (2018), the TBUT values decrease as the severity of the corneal surface disease increases. [24]

Another study[17] observed mean and standard deviation values for the SMT and STT-1 of 9.66 ±.2.15 (mm/5 sec) and 15.10 ± 3.06 (mm/min), respectively, in healthy dogs, 10.50 ± 0.7 (mm/5 sec) and 11.00±1.41 (mm/min), respectively, in healthy cats, and 4.72 ± 1.20 (mm /5 sec) and 4.22 ± 2.47 (mm/min), respectively, in healthy rabbits. The SMT values reported in another study with healthy dogs[17] are similar to those observed in this study (9.43 ± 1.59 mm/5 sec), as is the case for other animals, such as cats and humans. Similar studies in humans[23] reported SMT values of 6.4 ± 2.0 (mm/5 sec) in healthy eyes and 4.8 ± 1.6 (mm/5 sec) in KCS eyes. A study in capuchin monkeys obtained median and semi-interquartile range SMT values of 7.5 ± 1.85 for the right eye and 8.5 ± 1.62 for the left eye.[18] The values observed in humans with KCS are also similar to the results observed in this study (3.02 ± 2.52 mm/5 sec). These findings are in line with the observations of Lee et al. (2017), who concluded that the measurements of the tear meniscus were significantly lower in patients with KCS than in healthy patients.[19]

Therefore, the SMT is an exam that is fast and easy to conduct in dogs, with distinct values in healthy and KCS eyes and presenting excellent sensitivity and specificity compared with the STT-1, to which it presented a high correlation. In addition, SMT values below 7.5 mm/5 sec may be indicative of keratoconjunctivitis sicca, showing that in the future, the SMT could replace the STT-1.

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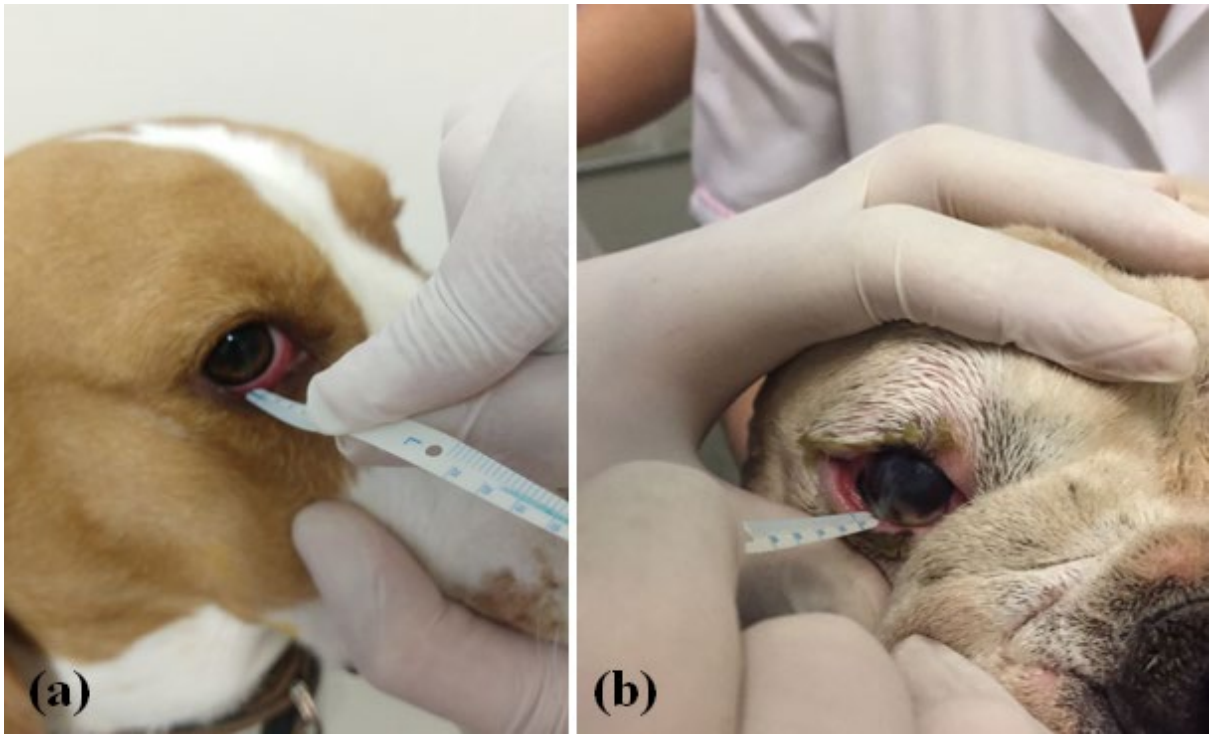


Figure 1. (a) Strip meniscometry test on a dog with a normal eye; (b) Strip meniscometry test on a dog with keratoconjunctivitis sicca.

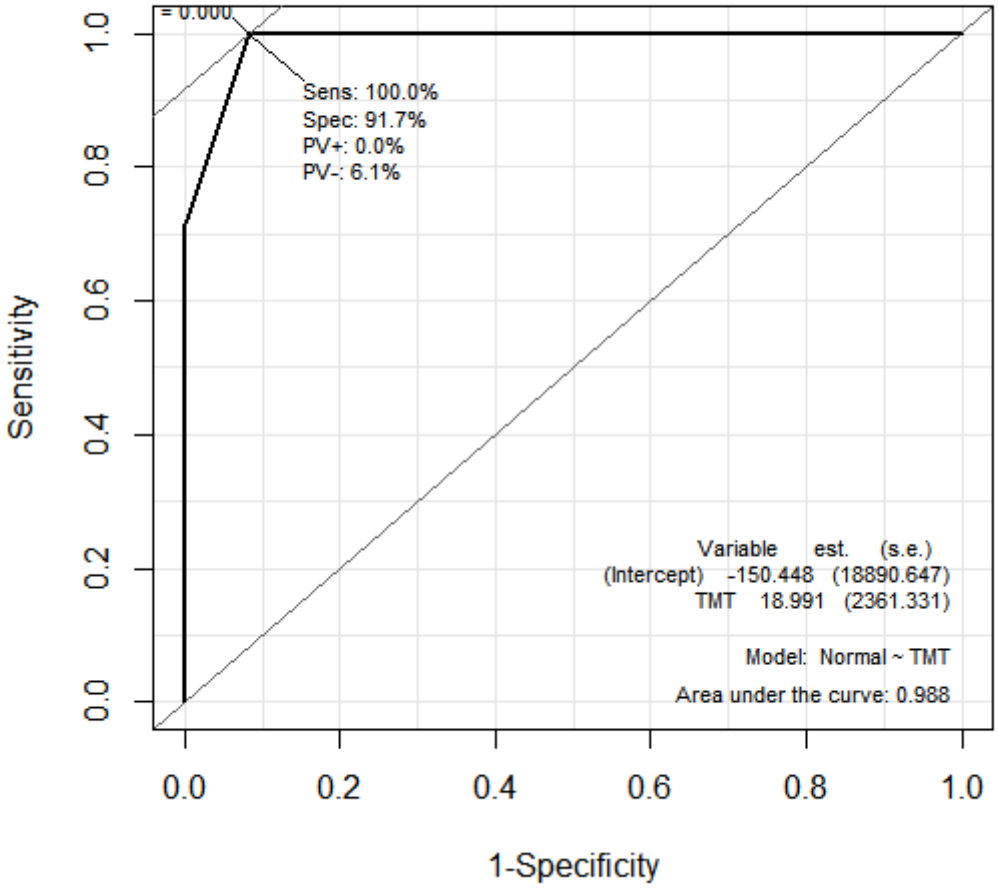


Figure 2. ROC curve showing the cut-off value for the strip meniscometry test, the sensitivity, the specificity and the area under the curve.

Table 1. Means ± standard deviations and amplitudes (maximum and minimum values) obtained from the Schirmer tear test 1 (STT-1) in mm/min, the strip meniscometry test (SMT) in mm/5 sec, and the tear film breakup test (TBUT) in seconds, as well as the results of the lissamine green test (LGT).

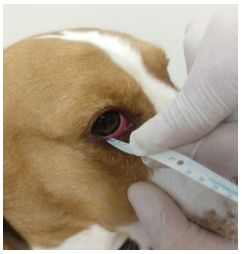
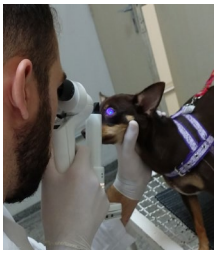
	Specific Ophthalmic Tests			
	STT-1	SMT	TBUT	LGT
Groups				
NORMAL EYES (n=78)	23.68±4.25 (15-31)	9.43±1.59 (8-14)	23.06±6.95 (14-44)	7 positive eyes
KCS EYES (n=60)	6.20±4.45 (0-14)	3.02±2.52 (0-7)	11.62±4.53 (3-20)	14 positive eyes

Table 2. Correlation between different ophthalmic tests obtained through Pearson's correlation coefficient.

Correlation	r*	IC95%	p**
SMT x STT-1	0.87	0.82 a 0.91	< 0.001
STT-1 x TBUT	0.67	0.56 a 0.75	< 0.001
SMT x TBUT	0.61	0.49 a 0.70	< 0.001
SMT x LGT	-0.17	-0.33 a -0.003	0.046
STT-1x LGT	-0.18	-0.33 a -0.012	0.036
TBUT x LGT	-0.20	-0,35 a -0,03	0.019

*Pearson's linear correlation coefficient; 95% CI = Estimation of r through an interval with 95% confidence; **significance level for the hypothesis that r differs significantly from zero. r = 0.9 to 1.0 (-0.9 to -1) = very high correlation; r = 0.7 to 0.9 (-0.7 to -0.9) = high correlation; r = 0.5 to 0.7 (-0.5 to -0.7) = moderate correlation; r = 0.3 to 0.5 (-0.3 to -0.5) = low correlation; r = 0 to 0.3 (0 to -0.30) = very low correlation.[24]

ANEXO 1- GUIDELINES FOR PUBLICATION IN VETERINARY OPHTHALMOLOGY JOURNAL

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