LACRIMAL PRODUCTION OF CATS: SCHIRMER TEAR TEST, PHENOL RED THREAD TEAR TEST AND ENDODONTIC ABSORBENT PAPER POINT

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ABSTRACT

The lacrimal production measurement is part of the ophthalmic semiotécnique in clinical routine of small animals and the Schirmer Tear Test (STT) is the most used tool for lacrimal production assessment in cats. There are few studies in the literature using alternative methods that aim optimizing the results and reduce stress of manipulation or discomfort caused by the test. Thus, the aim of this study was to determine the values of lacrimal production in cats through Schirmer tear test type-1 (STT1), Phenol red thread tear test (PRTT) and the Endodontic absorbent paper point tear test (EAPPTT). Twenty two cats, eleven males and eleven females, castrated, without evidence of clinical and ophthalmic disorder and assessed in domiciliary environment were used in this experiment. STT1 caused the major discomfort, followed by EAPPTT and PRTT. There were no significant differences between right and left eye and gender in all tests. The mean ($\pm$ SD) STT1 value was 16.77 ± 5.10 mm/min., PRTT value was 27.95 ± 4.79 mm/15 sec. and EAPPTT value was 13.64 ± 2.67 mm/15 sec. The results obtained may help in the diagnosis of quantitative disorders in the aqueous portion of the tear film, through alternative tests, as well as in the guiding for selecting the most suitable test.

KEYWORDS: Feline, eye, STT, PRTT, EAPPTT

AVALIAÇÃO DA PRODUÇÃO LACRIMAL EM GATOS ATRAVÉS DO TESTE LACRIMAL DE SCHIRMER, TESTE LACRIMAL DE VERMELHO FENOL E TIRA ENDODONTICA DE PAPEL ABSORVENTE

RESUMO

A mensuração da produção lacrimal compõe a semiotécnica oftálmica de rotina na clínica de pequenos animais e em gatos o Teste lacrimal de Schirmer é o mais utilizado para a aferição da produção lacrimal. Escassos são os estudos com a utilização de métodos alternativos que visem otimizar os resultados e reduzir o estresse a manipulação ou o desconforto ocasionado. Desta forma, este estudo

objetivou determinar os valores da produção lacrimal em gatos através dos Testes lacrimal de Schirmer tipo-1 (TLS1), Teste Lacrimal vermelho fenol (TLVF) e Tira endodôntica de papel absorvente (TEPA). Foram utilizados 22 felinos, 11 machos e 11 fêmeas, castrados, sem alterações clínicas e oftálmicas, avaliados em ambiente domiciliar. O TLS1 ocasionou o maior desconforto, sequenciado pelo TEPA e o TLVF. Não foram encontradas diferenças significativas entre o olho direito e esquerdo e entre os gêneros para todos os testes. A média e o desvio-padrão para TLS1 foi de $16,77 \pm 5,10$ mm/min, TLVF foi $27,95 \pm 4,79$ mm/15s e a TEPA foi $13,64 \pm 2,67$ mm/15s. Os resultados obtidos poderão auxiliar no diagnóstico de alterações quantitativas da porção aquosa do filme lacrimal através de testes alternativos, tal como no norteammento para a escolha do teste mais adequado.

PALAVRAS-CHAVE: Felino, olho, TLS, TLVF, TEPA

INTRODUCTION

The tear provides homeostasis to the eye bulb surface by removing dirtiness and microorganisms, besides lubricate, nourish and oxygenate the cornea. The tear film is stratified into three layers. The outer oily layer contains fatty acid esters and cholesterol provided by meibomian glands and sebaceous glands of Zeis, reducing evaporation of aqueous portion. The middle aqueous layer is secreted by the lacrimal gland and the gland of the third eyelid, mainly composed by inorganic salts, glucose, urea, proteins, glycoproteins and biopolymers. The inner layer is composed by mucin, which is produced by the conjunctival goblet cells and assists in setting the aqueous layer to the corneal surface (MANTELLI, 2013; MILLER, 2013; SEBBAG et al., 2015).

Abnormalities associated with reduction of tear production are less frequent in cats than in dogs. In this specie, discomfort is the most frequently sign demonstrated. The use of general anesthesia (GHAFFARI et al., 2012), topical instillation of mydriatics and cycloplegics (WIESER et al., 2013), the corneal sequestrations (CULLEN et al., 1999), conjunctivitis and systemic diseases such as the feline respiratory disease complex (LIM et al., 2009), may damage the tear film in quality and quantity. In addition, the stress of manipulation for ophthalmic evaluation can lead to disorders in tear production, tending to reduction (GHAFFARI et al., 2012).

Schirmer tear test (STT) is the most quantitative method used for measurement of basal and reflex tear secretion (STT1). It is a standardized paper strip placed into the lower conjunctival sac for 60 minutes and the length of the wetted paper is measured using the millimeter scale on the strip (GHAFFARI et al., 2010, FEATHERSTONE & HEINRICH, 2013, WIESER et al., 2013). The normality values in cats are between 14.3 ± 4.7 mm/min and 16.92 ± 5.73 mm/min (FEATHERSTONE & HEINRICH, 2013).

The Phenol red thread tear test (PRTT) has been used as an alternative (FEATHERSTONE & HEINRICH, 2013; SEBBAG et al., 2015), in which the end of the cotton thread, impregnated with phenol red, is placed into the lower conjunctival sac for 15 seconds (HOLT et al., 2006). The measurement of the wetted portion is made immediately after this period using a scale. The pigment indicator is sensitive to tear normal pH and, after contact, it can present a yellow color (pH = 6.6), red (pH = 8.0) or magenta, when pH is greater than 8.1 (HIDA et al., 2005).

Recently, the tear production has been evaluated with the Endodontic absorbent paper point tear test (EAPPTT), originally used by odontology due to its absorption capacity (YOSHINO et al., 2013). When adapted to ophthalmology, the

EAPPTT end is inserted into the lower conjunctival sac and after 60 seconds, the wetted portion is measured with a scale (LANGE et al., 2012).

The aim of this study is to establish reference values, correlations, of lacrimal production in cats, with use of Schirmer tear test (STT1), Phenol red thread tear test (PRTT) and Endodontic absorbent paper point tear test (EAPPTT).

MATERIAL AND METHODS

The research protocols were approved by the Ethics and Animal Welfare Committee of School of Veterinary Medicine and Zootechny of Federal University of Bahia (protocol n. 24/2012). In addition, were followed the bioethical guidelines recommended by Association for Research in Vision and ophthalmology – ARVO (National Institutes of Health Publications N. 85-23: Revised 1985) on the use of animals for research.

The study was performed by the Ophthalmology Service of Veterinary Hospital of Federal University of Bahia. Twenty-two mongrel cats (*Felis silvestres catus*) were selected, 11 males and 11 females, adults, castrated, without systemic disease observed by physical examination and no use of ocular or systemic medication. Ophthalmic semiotecnikue with ocular and periorcular evaluation was performed, using 3X magnification lens and transilluminator.

STT1, PRTT and EAPPTT were conducted, respectively, with 20 minutes intervals bilaterally. For data collection, all animals were evaluated in domiciliary environment, by the same team of three professionals and the owner, in order to reduce the stress. Temperature and humidity were measured during assessment (Thermo-hygrometer MT-241®, Minipa, São Paulo, Brazil). STT1 was initially performed, followed by PRTT and EAPPTT, during morning shift.

Schirmer tear test

To perform the STT1, absorbent millimeter paper strips (Tear Flo tear test strips®, Oasis Medical, California, EUA) were used. The strips were placed into the lower conjunctival sac for 60 seconds (Figure 1A).

Phenol red thread tear test

The PRTT (Zone-Quick®, Oasis Medical, California, EUA) was performed by insertion of a thread into the lower conjunctival sac (Figure 1B) during 15 seconds. Immediately after removing the thread, the measurement was made using a digital caliper (Mitutoyo, São Paulo, Brazil).

Endodontic absorbent paper point tear test

The EAPPTT point (Roeko color - Color size 30, Langenau, Germany) was inserted into the lower conjunctival sac (Figure 1C). The procedure was adapted for 15 seconds, once the strip was completely moistened when remained for 60 seconds (ORIÁ et al., 2014). After this time, the paper point was removed and measured immediately using a digital caliper (Mitutoyo, São Paulo, Brazil).

FIGURE 1 - Lacrimal production measurement in cats (A) Schirmer tear test (B) Phenol red thread tear test (C) Endodontic absorbent paper point tear test.

Statistical analysis

The statistical analysis was performed by IBM® SPSS® program, version 20.0, for operational system Windows® (IBM Corporation, Somers, NY). The quantitative data were evaluated using Shapiro-Wilk normality test. Unpaired Mann-Whitney test and Wilcoxon test were used, respectively, for data comparison among STT1, PRTT, EAPPTT, gender, and between right and left eyes. Spearman correlation test was used to verify association between variables. The significance level in this study was 5%.

RESULTS

The mean environmental temperature during the procedures was 32.2°C (32° - 32.4°C) and mean humidity was 63% (59 – 68%).

The age of animals ranged from 120 to 204 months. During the procedures, some discomfort was noted, as blepharospasm or attempt to remove the strip or thread, mainly the STT1, which presented the highest discomfort, followed by EAPPTT and PRTT. The variables are summarized in table 1.

TABLE 1 - Results of diagnostic tests performed in eyes of 22 healthy cats, 11 males and 11 females.

Parameter	Mean value	Standard-Deviation	Confidence interval
STT1(mm/min)	16.77	5.10	15.22 - 18.32
PRTT (mm/15'')	27.95	4.79	26.49 - 29.41
EAPPTT (mm/15'')	13.64	2.67	12.0 - 14.45

STT1: Schirmer tear test 1; PRTT: Phenol red thread tear test; EAPPTT: Endodontic absorbent paper point tear test.

The data for STT1 (p value= 0.299) and PRTT (p value= 0.332) presented a normal distribution according Shapiro-Wilk test, while EAPPTT were not normally distributed (p value= 0.008). No significant differences were found for the mean values of right and left eye between males (p value= 0.539) and females (p value= 0.914) for STT1; males (p value= 0.999) and females (p value= 0.519) for PRTT and males (p value= 0.310) and females (p value= 0.637) for EAPPTT. There wasn't significant difference for gender to STT1 (p value= 0.629), PRTT (p value= 0.227) and EAPPTT (p value= 0.135). A significant moderate correlation was found (p value= 0.004; p = 0.421) among PRTT and STT1. There wasn't significant correlation

between STT1 and EAPPTT (p value= 0.466; p= 0.113) and between PRTT and EAPPTT (p value= 0.874; p= 0.250).

DISCUSSION

Studies assessing tear production and composition in cats has been carried out with the objective of establishing an appropriate methodology (SEBBAG et al., 2015). Similarly, to the present study, the discomfort caused by the tests in rabbits, comparing STT and EAPPTT, was higher with STT (LIMA et al., 2014; ORIÁ et al., 2014a). Perhaps, the highest discomfort with this test may be related to the longer execution time (60 seconds) and the larger contact area of the strip with the cornea.

The mean $\pm$ SD of STT1 value was 16.77 ± 5.10 mm/min that is in agreement with Sebbag et al. (2015) findings of 9 to 34mm/min in healthy cats. The present findings are also in accordance with the literature that presents normality values ranged from 14.3 ± 4.7 mm/min to 16.92 ± 5.73 mm/min in cats and dogs (GUM & MACKAY, 2013; FEATHERSTONE & HEINRICH, 2013). Due to possibility of stress during ophthalmic evaluation, discrepant values should be interpreted in association with clinical manifestations (FEATHERSTONE & HEINRICH, 2013).

The mean $\pm$ SD of PRTT value was 27.95 ± 4.79 mm/15s which was higher than the values obtained for cats by Brown et al. (1997) ($\pm$ SD 23.04 ± 2.23 mm/15s). This difference can be attributed to climatic differences. However, the values from this study are in agreement with SEBBAG et al. (2015) who found values between 15 to 37mm / 15s in healthy cats.

According to TROST et al. (2007), studying guinea pigs, the PRTT is used when the animal has a reduced basal or reflex tear production, besides it is relatively easy to handling. However, in the present study, some complications to perform the test was observed, especially by the excessive flexibility of the thread, which makes difficult the insertion in the inner surface of the lower eyelid and can lead to an increased tearing due to the protective reflex. The insertion difficulty was also reported by Holt et al. (2006) in birds.

The EAPPTT has values described in several species of animals like marmosets (LANGE et al., 2012), rats (LANGE et al., 2014), broad-snouted (ORIÁ et al., 2015a) and tortoise (ORIÁ et al., 2015b), however its use in domestic animals is not found. The mean $\pm$ SD for EAPPTT values in this study was 13.64 ± 2.67 mm/15s that is similar to turtles (15.9 ± 0.7) in 15 seconds (LANGE et al., 2014).

Due to the rigidity of the strip, the EAPPTT is an easy to perform test, which leads to a lower stress condition, with good performance in reduced time. In addition, LIMA et al. (2014) stated that this test leads to a minimal change in corneal sensitivity in rabbits, suggesting their efficacy. The EAPPTT is a versatile test that can be used in animals with small or large eyes (small or large palpebral fissures) with deep or shallower conjunctival sacs with greater or lesser tear production because the test duration can be adjusted to 60 or 15 seconds (LANGE et al., 2012; LANGE et al., 2014; ORIÁ et al., 2015b).

Comparing results of the three tear production tests employed in this study, the mean value of tear production was lower using EAPPTT (see Table 1). There are several possible explanations for this difference, including differences in strip width and absorbance properties (ORIÁ et al., 2015). Furthermore, the EAPPTT strip promotes minimal corneal irritation or alteration in corneal touch threshold (LIMA et al., 2014) that could result in a lesser reflex tear secretion.

CONCLUSION

The determination and comparison of normal values of STT1, PRTT and EAPPTT will serve as a basis to guide the clinical treatment of cats in order to detect ophthalmic disorders related to the aqueous fraction of the tear film. In addition, considering the EAPPTT an easy to handling test, accurate, with low cost and short period of execution in cats that diminish the stress, it could be considered a feasible alternative to be used as an ophthalmic evaluation routine basis in cats in a near future.

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