Tear production, intraocular pressure and conjunctival microbiota, cytology and histology of New Zealand rabbits (Oryctolagus cuniculus)

Arianne P. Oriá*, Deusdete C. Gomes Junior, Elane A. Arraes, Alessandra Estrela-Lima, Melissa H. Pinna, Íris D.S. Meneses and Emanoel F. Martins Filho


The purpose of this study was to establish reference values for selected ophthalmic diagnostic tests in New Zealand rabbits (Oryctolagus cuniculus). A total of 22 adult male rabbits were used. The ophthalmic tests included evaluation of tear production with Schirmer tear test 1 (STT1) and Endodontic absorbent paper point tear test (EAPPTT) using two different commercial brand materials. Applanation tonometry, Culture of the conjunctival bacterial flora, conjunctival cytology and conjunctival histology were also performed. Mean (±SD) for STT1, EAPPTTa, EAPPTTb and IOP was 7.27±2.51 mm/min, 12.43±1.69 mm/min, 15.24±2.07 mm/min, 12.89±2.80 mm Hg, respectively. Staphylococcus epidermidis, Staphylococcus sp. and Bacillus sp. were predominant. The cytological evaluation revealed the presence columnar epithelial cells, superficial squamous keratinized cells, lymphocytes, heterophils, red blood cells, mucus and bacteria. The histological analysis revealed a stratified epithelium, characterized by the presence of columnar epithelial cells with a large number of goblet cells. The reported data can be used for therapeutic or experimental purposes.

INDEX TERMS: Tear production, intraocular pressure, conjunctival microbiota, New Zealand rabbits, Oryctolagus cuniculus, lagomorphs, ophthalmology, Schirmer tear test, tonometry, endodontic paper point.

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Setor de Clínica Médica Veterinária, Escola de Medicina Veterinária e Zootecnia, Universidade Federal da Bahia (UFBA), Av. Adhemar de Barros s/n, Salvador, BA 40170-110, Brazil. *Corresponding author: arianeoria@ufba.br

Programa de Pós-Graduação em Ciência Animal nos Trópicos, Escola de Medicina Veterinária e Zootecnia, UFBA, Salvador, BA.

Médica Veterinária autônoma, Rua Jayme Sapollnik 1183, Torre B, Apto 905, Imbuí, Salvador, BA 41720-075.

Programa de Pós-Graduação em Cirurgia Veterinária, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (Unesp), Via de Acesso Prof. Paulo Donato Castellane s/n, Jaboticabal, SP 14884-900, Brazil.

RESUMO.- [Produção lacrimal, pressão intraocular, microbiota, citologia e histologia conjuntival de coelhos Nova Zelândia.] O objetivo deste estudo foi estabelecer va-
INTRODUCTION

The domestic rabbit (Oryctolagus cuniculus) has been commonly used on scientific research (Wagner and Fehr 2007). In the field of experimental ophthalmology they are used in tests of new medication, surgical procedures and compatibility of biomaterials to be used in humans (Oría et al. 2006, Werner et al. 2006, Kowalski et al. 2012).

Reports of glaucoma in rabbits date back to 1963 when Kolker et al. reported that glaucoma occurred spontaneously. Kneeper et al. (1978) reported that glaucoma could be induced by corticosteroids. This has been investigated and proven that young animals show a greater predisposition (Yi et al. 2012). For several years rabbits have been used in experimental studies for treatment of human glaucoma. Furthermore, when raised as domestic animals, they occasionally present eye disorders that need clinical or surgical treatment (Keller et al. 2007, Florin et al. 2009) due to occurrence of cataract, conjunctivitis, keratitis, eyelid laceration and dacyrocystitis (Jeong et al. 2005).

Thereby, the study’s objective was to evaluate the ophthalmic parameters in clinically healthy adult rabbits, aiming to establish standards to be used on an experimental or therapeutic basis.

MATERIALS AND METHODS

Twenty-two healthy New Zealand 1 to 2-year-old male rabbits (Oryctolagus cuniculus), weighing 2.5 to 3.3 kg, obtained from the Sector of Rabbit Breeding, Recôncavo Federal University, Bahia, were used in this investigation.

The research protocols were approved by the Ethics Committee of the School of Veterinary Medicine and Zootechny of the Federal University of Bahia. The bioethical guidelines of the Association for Research in Vision and Ophthalmology (National Institutes of Health, Publication No. 85-23: Revised, 1985) for the use of animals in vision research were followed.

The rabbits were kept in a bioterium in individual cages during the entire study period with water and appropriate diet ad libitum. After collection of the ophthalmic data a complete physical examination was performed and the anterior ocular structures of all rabbits were evaluated using a transilluminator, a binocular magnifying loupe (3x) and fluorescein dye (Ophthalmos®). Ribeirão Preto, São Paulo, Brazil. All ophthalmic data was collected by the same investigator (AO) with the exception of Endodontic absobent paper point test (STT1, EAPPTT) conducted by (DCGJ). All tests were performed between 8-10am and only gentle physical restraint was used to immobilize the animals for data acquisition. Special attention was paid to avoid applying any pressure in the neck region in order to prevent iatrogenic IOP alterations.

Data was collected from two groups. In group 1 (n=12), STT1, sampling of conjunctival bacterial flora, tonometry, conjunctival cytology and conjunctival histology were performed. In group 2 (n=10), EAPPTT was conducted.

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RESULTS

The data collected for STT, EAPPTT<sub>a</sub>, EAPPTT<sub>b</sub> and IOP was normally distributed, based on the Shapiro-Wilk normality test (p=0.162, 0.819, 0.819 and 0.274 respectively). Descriptive statistics of the results of these tests are presented in Table 1.

There were no significant differences between left and right eyes for STT (p=0.208), EAPPTT<sub>a</sub> (p=0.986), EAPPTT<sub>b</sub> (p=0.816) and IOP (p=0.793) (Paired Student t-tests) and there was no significant correlation between EAPPTT<sub>a</sub> and EAPPTT<sub>b</sub> using Pearson’s correlation test (p=0.172 r=-0.475).

A total of 8 types of Gram-positive bacteria and 1 type of Gram-negative bacteria were isolated and are presented in Table 2. In 83.3% (10/12) of animals, both eyes yielded positive culture results; 16.7% (2/12) of animals yielded positive culture in one eye.

Macroscopically, no conjunctival trauma was observed with either sampling method. Columnar epithelial cells, superficial squamous keratinized cells, lymphocytes, heterophils and red blood cells were the main cell populations observed (Fig.2). Bacteria and mucus filaments were also observed. Slides obtained using a barren interdental brush showed greater cellularity than those obtained with the moistened cotton swab (Fig.3).

Histology of the conjunctiva revealed stratified epithelium, characterized by the presence of layers of columnar epithelial cells. Large numbers of goblet cells were observed in the epithelium. A vascularized lamina propria formed by loose conjunctival tissue was observed and clusters of lymphoid cells were also seen (Fig.4).

DISCUSSION

Rodents are increasingly seen as pet species and thus the ophthalmic diseases may be presented to veterinarians in general practice (Williams 2007). They are also still considered and used as experimental model in researches (Oriá 2006). In the scientific literature there are scarce information concerning the baseline values for the routine ophthalmic diagnostic tests in rabbits. Besides, there are small but significant anatomical and physiological differences between the eyes of dogs and cats and those of rabbits (Williams 2007). Therefore the study of ophthalmic parameters becomes of great importance and should be considered for different species.

In this study, bacterial growth was identified in 91.7% of the ocular samples (22/24). Similar result was found
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by Cooper et al. (2001) (83%) in assessing the conjunctival microbiota of domestic rabbits. Out of the 33 strains isolated, 93.9% were Gram positive and 6.1% were Gram negative (see Table 2). Studies conducted on other species such as dogs 73.23% (Santos 2009) and 86.2% (Oliveira 2010); several domestic and wild species with 81% (Santos 2011) also presented a predominance of Gram positive bacteria.

The *Staphylococcus* genus had been found on high frequency in rabbits (57%) by others authors as Cooper et al. (2001); Kudirkienė (2006) in dogs (55%) and Santos (2011) in several species (46.8%). According to Shimamura (2008) the microorganisms of this genus are widely distributed in nature, as part of mammals and birds natural microbiota of the skin and mucosa. Haghkhah et al. (2005) and Oliveira et al. (2010) found high frequency for *Staphylococcus epidermidis* and *Bacillus* sp. in the conjunctival microbiota of healthy dogs, as observed in this study. According to Belletato (1981) the *Staphylococcus epidermidis* species is commonly found in the conjunctival fornix. *Enterobacter cloacae* was the only Gram negative bacteria isolated. According to Spinelli (2010) these microorganisms are present in healthy eyes, however, in small number, as noticed.

The STT1 has a low cost, is easy to perform and is also used to confirm CCS (low values) or to correlate with signs of irritation (high values) in veterinary practice. CCS naturally acquired has not been reported in rabbits; therefore, STT1 in rabbits has greater utility in the second case (Abrams et al. 1990, Koç et al. 2005).

Koç et al. (2005) determined that the mean value for STT1 in clinically normal rabbits was 8.1±3.4mm/min. The mean values for STT1 in New Zealand and Angora rabbits breeds and Mixed breed rabbits were of 7.9±3.6, 7.2±2.9 and 9.1±3.3mm/min, respectively. These data are similar to those found in this study (7.3±2.5mm/min), however, inferior results were described by Abrams et al. (1990) (5.30±2.96mm/min).

EAPPTT is widely used in dentistry during endodontic procedures, due to its high absorption properties. However its use in measuring the aqueous component of the tear film is not yet wide-spread (Lange et al. 2012). Relatively to its use, the EAPPTT revealed a greater insertion into the conjunctival fornix and produced smaller discomfort for the animal when compared to STT1. It was easy to use, and the strip was readily inserted by one person. There was small disperse of values in the comparison of the two brands of EAPPTT, however, it is noteworthy that the value from the tear production measurement with one of the brands can’t be compared to the values of the other one, once there is no overlap of the confidence interval. There are some possible explanations for this variance in the results concerning the two brands and these include the differences in the strips raw materials and its absorbance properties.

The mean IOP obtained in healthy adults rabbits with the rebound tonometer -Tonovet was 10.2±1.3mmHg (Albuquerque et al. 2008) and 9.5±2.6mmHg (Pereira 2010) and with the Tono-Pen Avia was 15.4±2.2mmHg (Pereira 2010). The values obtained in this study suggest that the IOP with the Tono-Pen XL presents intermediate values between the two other types of tonometers tested.

Relative to the collection methods, the samples obtained with the barren interdental brush presented superior number of cells and cellular diversity compared to the ones obtained with moisturized cotton swabs, as remarked by other studies (Willis et al. 1997, Borges 2012). Similarly the samples obtained by swab presented higher frequency in collecting mucus and bacteria concerning the ones taken.
by brushes, what can be related to the absorption capacity of the cotton.

The normal conjunctival epithelium is characterized by groups of epithelial cells and some individual cells (Rito 2009). The morphologic characteristics of the cells depend on the depth of the cytological collecting, and all cell layers can be found at the same sample. The small amount of keratinized epithelial cells found with the brush method suggests a skin contamination since this cellular type is not present in the conjunctiva. This could be the consequence of the brush’s bigger contact surface when compared to the swab during sample collection. Various types of lymphoid cells were found in the conjunctival cytology, however, some authors indicated that, in animals with no clinical signs of conjunctivitis, little emphasis should be given to this cellular type (Bolzan et al. 2005). The presence of intact erythrocytes in samples collected through exfoliation with the brush, suggests that micro vascular lesions could have occurred, but without immediate or late clinical changes as we didn’t observe any macroscopic conjunctival trauma or blood on the brush following sampling.

The stratified columnar epithelium is more commonly found in the conjunctival fornix and at the eyelid margin (tarsal region) the epithelium becomes stratified squamous as well as at the bulbar conjunctiva (Kuehnel 2003). This explains the obtaining of squamous and columnar cells in the conjunctival cytology where the sampling area was the entire conjunctiva compared to histology where the sampling area was the conjunctival fornix.

The columnar non-keratinized stratified epithelium observed in the rabbit’s conjunctiva was similar to the histological description of dogs (Samuelson 2007) and horses (Aughey & Frye 2001). The lamina propria, located in the subepithelial tissue, comprises a highly vascularized loose connective tissue, where clusters of lymphoid cells can be found (Samuelson 2007), as observed in this study.

**CONCLUSION**

The values found in this study can guide veterinarians that work with leporines, either for therapeutic or experimental purposes.

**REFERENCES**


