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Conjunctival and nasal microbiota evaluation of dogs submitted to dacryocystorhinostomy

Avaliação da microbiota conjuntival e nasal de cães submetidos à dacriocistorrinostomia

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Highlights

Dacryocystorhinostomy is performed in dogs with nasolacrimal duct obstruction.
After dacryocystorhinostomy, there was a balance in the conjunctival microbiota.
Pseudomonas aeruginosa was found in the lacrimal system of dogs.
Nasal cavity bacteria did not increase after surgery in the long term.

Abstract

The purpose of this study was to evaluate changes in the conjunctival and nasal microbiota, in the long term, of dogs undergoing bilateral dacryocystorhinostomy. Twelve male and female dogs (23 eyes), aged between 1 and 10 years, were enrolled in the study, selected on the basis of presentation with epiphora and chromodacryorrhea for at least six months. Cultures of material obtained from the ocular conjunctiva and nasal sinus of all dogs were evaluated to determine the conjunctival and nasal microbiota pre-and postoperatively (at 60, 120, and 240 d). Preoperatively, gram-negative bacteria were identified in the conjunctival microbiota of 66.5% (n=8), while gram-positive bacteria were found in 33.3% (n=4). Throughout the clinical evolution, a balance was found between the presence of gram-positive and gram-negative bacteria in the conjunctival microbiota. Pure cultures of *Pseudomonas aeruginosa* (25%) and *Staphylococcus intermedius* (25%) were found most frequently. Regarding the conjunctival microbiota, we can conclude that in obstructive diseases, there is a predominance of gram-negative bacteria in the lacrimal system, notably *Pseudomonas aeruginosa.* This study did not observe an increase in bacterial counts in the nasal cavity through the new surgical pathway to the conjunctival sac.

Key words: Dacryocystorhinostomy. Dogs. Conjunctival microbiota. Nasal microbiota. Ocular microbiota.

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Resumo

O objetivo deste estudo foi avaliar a microbiota conjuntival e nasal, a longo prazo, de cães submetidos à dacriocistorrinostomia bilateral. Doze cães, machos e fêmeas (23 olhos), com idade entre 1 e 10 anos foram incluídos no estudo, previamente selecionados e agrupados apresentando epífora e cromodacriorreia há pelo menos seis meses. A cultura do material obtido da conjuntiva ocular e seios nasais de todos os cães foi avaliada, a fim de determinar a microbiota conjuntival e nasal de acordo com os momentos pré e pós-operatórios (60º, 120º e 240º dias). A microbiota conjuntival consistia em 66,5% (n=8) de bactérias gram-negativas e 33,30% (n=4) de bactérias gram-positivas antes do procedimento cirúrgico. Ao longo da evolução clínica, foi encontrado um equilíbrio entre a presença de bactérias Gram-positivas e Gram-negativas com agentes da microbiota conjuntival. *Pseudomonas aeruginosa* (25%) foi encontrada com maior frequência e *Staphylococcus intermedius* (25%) isoladamente. No que se refere à microbiota conjuntival, podemos concluir que nas doenças obstrutivas há predominância de bactérias Gram-negativas no sistema lacrimal, notadamente a *Pseudomonas aeruginosa.* Este estudo não mostrou aumento de bactérias na cavidade nasal através do neo-trajeto cirúrgico para o saco conjuntival.
Palavras-chave: Dacriocistorinostomia. Dogs. Microbiota conjuntival. Microbiota nasal. Microbiota ocular.

Introduction

In small animal clinical practice, there is a high incidence of nasolacrimal disease, with inadequate drainage of tears, which can lead to epiphora, chromodacryorrhea, conjunctivitis, and mucopurulent ocular discharge, causing the appearance of fistulas in the nasal canthi (Grahn & Sandmeyer, 2007; Slatter, 2005). These diseases are caused by congenital or acquired alterations in the lacrimal system and their identification is essential for treatment initiation (Wouk & Kleiner, 2003). At diagnosis, the Schirmer tear test and Jones test are essential for differentiating the signs of epiphora and tearing (Grahn & Sandmeyer, 2007). Additional tests, such as magnetic resonance imaging (MRI) and dacryocystorhinography, allow the exact location of the obstruction to be identified (Grahn & Sandmeyer, 2007). Recently, the successful use of an endoscope guided through the punctum for the diagnosis and treatment of nasolacrimal duct patency in a horse was described (Smith et al., 2023).

Dacryocystitis is characterized by inflammation of the lacrimal sac and often causes epiphora and chromodacryorrhea, which can be treated clinically (Lavach, 1998; Kleiner & Wouk, 2004; Slatter, 2005). Surgical therapy is indicated for total or partial stenosis of the nasolacrimal duct (Smith et al., 2023). The surgical techniques of dacryocystorhinostomy (DCR), conjunctival maxillary sinusostomy, and dacryobuccostomy (Grahn & Sandmeyer, 2007; Kleiner & Wouk, 2004; Lavach, 1998) have been described. Clinical treatment is indicated only for patients with partial obstruction of the nasolacrimal duct (Felchle & Urbanz, 2001; Giuliano et al., 2006) and consists of cannulation of the obstructed duct followed by vigorous washing with saline solution (Felchle & Urbanz, 2001).

Antibiotics as well as topical and systemic anti-inflammatory drugs should also be considered (Smith et al., 2023). Ductal patency can be maintained with the help of a polyethylene catheter, which should be kept in place for two to three weeks (Laforge, 1997). In animals with total obstruction, the duct cannot be cannulated and those with inflammation and obstruction caused by foreign bodies (Steinmetz et al., 2022) should undergo clinical therapy before undergoing other treatments (Grahn & Sandmeyer, 2007). In such cases, surgery should be considered to restore the lacrimal drainage (Smith et al., 2023).

DCR involves devising a new drainage path connecting the bottom of the conjunctival sac with the nasal cavity to restore the physiological drainage of tears (Giuliano et al., 2006; Grahn & Sandmeyer, 2007; Wouk & Kleiner, 2003). Conjunctivobuccostomy has the same purpose; however, a new path links the bottom of the sac with the oral cavity, where the soft and hard palates meet (Kleiner & Wouk, 2004). Laser DCR has been reported to successfully treat congenital obstruction of the nasolacrimal duct in humans (Grahn & Sandmeyer, 2007). However, there are few examples in the literature where both techniques are used, and they relate to cases in which the results were discouraging (Giuliano et al., 2006; Machado et al., 2008). As most disorders of the lacrimal apparatus do not endanger the visual acuity of patients, these diseases are underestimated by physicians. However, epiphora can cause constant wetting of the hairs in the nasal canthi of the eye bulb and pruritic dermatitis, which, coupled with dacryocystitis, can cause discomfort to the animal, followed by recurring conjunctivitis and ulcerative keratitis (Kleiner & Wouk, 2004). To date, the conjunctival and nasal microbiota in dogs subjected to DCR have not been described.

This study aimed to identify organisms that grow in the lacrimal sac of patients requiring DCR. We also investigated whether there was a correlation between the organisms cultured from the sac and from the conjunctiva and/or nasal cavity.

Materials and Methods

Ethical consideration

The study was carried out with the authorization of the animal tutors and the Committee of Ethics in Animal Experimentation of São Paulo State University (UNESP), Veterinary Medicine Faculty from Araçatuba-SP (Process n. 018663-08). Bioethical care was conducted according to the standards of the Association for Research in Vision and Ophthalmology - ARVO (National Institutes of Health Publications No 85-23: Revised 1985).

Animals

A total of 12 dogs, both male and female (23 eyes), aged between 1 and 10 years, were enrolled in the study and treated at the Veterinary Clinic *Cães e Gatos* in Sorocaba city (São Paulo State, Brazil). They were included based on presentation with epiphora and chromodacryorrhea for at least six months. The animals were subjected to an initial physical examination, followed by a routine eye check using Schirmer and Jones tests. The Schirmer test was performed using paper strips (Schirmer Test; Ophthalmos, São Paulo, Brazil); the Jones test was performed

by dyeing the ocular surface with fluorescein (Fluorescein Strips; Ophthalmos, São Paulo, Brazil), followed by measuring how long it took to move from the lacrimal drainage system to the nasal ostium and hard palate. A normal passage time, which was considered a positive test, was 5 min. Animals presenting with fistulas in the medial canthi (n=2) were subjected to dental examinations, which included radiographs of the oral cavity to identify the occurrence of a fistula in the fourth premolar. We were unable to perform dacryocystorhinography in the patients in the study, since this exam required an additional general anesthesia prior to the one required for the surgical procedure and was therefore not authorized by the owners. Other imaging tests, such as computed tomography (CT) and MRI, were also not performed, as they are not available due to their high costs; moreover, these examinations also require anesthesia. Thus, we chose to conduct only flushing of the nasolacrimal duct prior to the surgery. Before preparing the operative field, the lacrimal ducts of all the animals were cannulated so we could perform the test. We introduced 10 mL sterile saline into the duct under pressure until the liquid could be seen at the end of the nose. We also confirmed that there were no congenital diseases, such as agenesis of the lacrimal punctum or nasolacrimal duct.

Anesthesia and surgical procedures

With the consent of the owners, all animals underwent bilateral DCR, except for one patient in whom the procedure was unilateral (case #7). Patients underwent general inhalation anesthesia with morphine (Dimorf, 1mg/mL; Cristália, São Paulo, Brazil) at 0.2 mg/kg given intravenously (iv) as pre-anesthesia medication; general anesthesia was induced using propofol (Fresofol, 1%; Fresenius Kabi, Brazil) at 5mg/kg iv and maintained with isoflurane (Isoflorine; Cristália, São Paulo, Brazil) in a semi-open circuit. Animals were placed in lateral recumbency with the nose slightly tilted to facilitate the surgical procedure. After washing the eye area with sterile saline (sodium chloride 0.9%; Aster, Brazil), the lower nasal and conjunctival sacs were swabbed, and the material obtained was sent for analysis. During collection, care was taken not to touch the eyelashes and eyelid tarsus or to contaminate the samples. DCR was performed as described by Grahn and Sandmeyer (2007).

The postsurgical protocol included an Elizabethan collar for 15 days for all patients, enrofloxacin (Flotril 2.5%; Schering-Plough, Brazil) at 10 mg/kg a day (orally) for 10 days, and ofloxacin-based eye drops (Oflox; Allergan, Brazil) six times a day to prevent infection. For post-surgery comfort and pain control, patients also received meloxicam (Maxican 0.5 mg; Ouro Fino, Brazil) at 0.1 mg/kg (orally) and tramadol chlorhydrate (Dorless 12 mg; Agener União, São Paulo, Brazil) at 0.3 mg/kg (orally), every 24 hours; both for 5 days. For topical adjuvant therapy, patients were administered prednisolone acetate eye drops (Pred Fort; Allergan, São Paulo, Brazil) three times a day for 60 days to prevent cicatricial stenosis of the access route.

After 60 days, the patients were subjected to another general intravenous anesthesia using the same dose of propofol, and the tubes inserted and maintained in the created access routes were removed. Next, to evaluate patency, a catheter was inserted (peripheral intravenous catheter 22G; Embramac, Brazil) at the proximal end

(the lower lacrimal punctum), and the new duct was washed with a solution of 19.0 mL sterile saline plus 1.0 mL intravenous sodium fluorescein (Sodium Fluorescein 25%; Ophthalmos, São Paulo, Brazil).

Post-operative assessment

Over a period of 30 days, patients were assessed on a weekly basis, taking into consideration: blepharospasm, ocular secretions, and conjunctival hyperemia. The clinical parameters were graded in terms of quality and quantity as follows: (0) = absent; (1) = slight; (2) = moderate; (3) = moderately severe; and (4) = severe. The presence or absence of ulcerative keratitis and the positivity or negativity of the Jones test were also evaluated. The same parameters were evaluated on postoperative days 60, 120, and 240.

Nasal and conjunctival microbiota

Materials obtained from the conjunctiva and nasal sinus were cultured for all dogs included in the study to determine the conjunctival microbiota according to postoperative time. To that end, three samples were collected to isolate aerobic and anaerobic bacteria, and fungi. Samples were collected on M0 (before surgery), M60 (postoperative day 60), M120 (postoperative day 120), and M240 (postoperative day 240).

The swabs with the collected material were placed in Stuart transport medium (Transprov III; Newprov, Brazil) and stored at 2–8 ºC until processing, but never for more than 72 hours. Swabs collected for the purpose of isolating anaerobic bacteria were kept in thioglycolate transport medium (Himedia, China), and kept oxygen-free by the aspiration of residual air from the flask with the help of a sterile needle and syringe.

In the laboratory, the swabs collected for the purpose of identifying the aerobic bacteria were introduced in a Brain Heart Infusion broth (Oxoid, Hampshire, UK), for an incubation period of 24 h, at 37 ºC, and then pricked out in Petri dishes (JProlab, São José dos Pinhais, Paraná, Brazil) containing blood agar (Blood Agar Base; Oxoid, Hampshire, UK) and MacConkey agar (Oxoid, Hampshire, UK), and incubated at 37 ºC for 24 h.

Once the colonies grew, they were subjected to biochemical identification tests. After the initial identification of the bacteria, the size, morphology, and individual characteristics of each bacterial colony were examined using the following procedures: Gram staining, growth on MacConkey Agar, and catalase, oxidase, motility, and oxidation-fermentation tests (Carter & Chengappa, 1988; Laforge, 1997).

After preliminary identification of agents according to their type, the following biochemical tests were performed to identify their species: production of sulfuric acid, Simmon's citrate, coagulase, glucose, hemolysis, indole, urea, lysine ltd (lactose – trehalose – dextrose), mannitol salt agar, motility, and sensitivity to polymyxin and novobiocin (Carter & Chengappa, 1988)

To identify anaerobic bacteria, the swabs were placed in tubes containing blood agar at 37 ºC in an anaerobic jar for 24–48 h. Slides were prepared with developed colonies, which were later subjected to Gram staining. Colonies were subjected to biochemical tests (Laforge, 1997). The same

tests used for the preliminary identification and type of aerobic bacteria were used to analyze the isolated colonies.

To isolate the fungi, swabs were placed on Sabouraud agar medium (Agar Sabouraud Maltose; Himedia, China). Preliminary classification was performed by analyzing the morphology of the colonies and separating them according to the presence of hyphae, conidia, and the germinative layer. Slides were prepared from colonies that were stained using specific methods according to the techniques recommended by Carter and Chengappa (1988).

The following staining methods were used for the microscopic examination of fungi: cotton blue, Gram, methylene blue, Periodic Acid-Schiff (PAS), Giemsa, and silver salt impregnation (Barnett & Hunter, 1972; Larrone, 1995). After four examinations showing no traces of fungal growth, the tubes were classified as negative and discarded.

Results and Discussion

The study focused on 12 dogs of different breeds, sex, and age, of which 33.3% (n=4) were poodles, 25% (n=3) Maltese Terriers, 16.6% (n=2) West Highland White Terriers, 8.3% (n=1) Yorkshire Terrier, 8.3% (n=1) English cocker spaniel, and 8.3% (n=1) undefined breed; 66.6% (n=8) of dogs were male and 33.4% (n=4) were female. The mean patient age was 3.5 years.

General physical examination of all patients revealed no other clinical-systemic alterations apart from eye complaints. With regard to these complaints, after careful eye examination, it was observed that 100% (n=12) of patients presented with epiphora, 75% (n=9) presented with abundant mucopurulent secretions, 66.6% (n=8) presented with chromodacryorrhea, 33.3% (n=4) conjunctival hyperemia, 16.6% (n=2) medial canthus fistula, and 8.3% (n=1) presented with nuclear sclerosis of the lens.

The Schirmer test values for both eyes of the patients in this study varied between 15 and 25 mm/min. The Jones test was negative in 95.8% (n=23) of eyes evaluated and only 4.2% (n=1) showed a positive result.

Regarding the DCR procedure, obstacles were identified as soon as the cutaneous incision was made in the deeper tissue, where it reached the angular vein of the eye bulb. This caused intense and persistent bleeding, preventing further steps in the procedure. In this case, the bleeding stopped after temporary hemostasis was achieved with the help of a Halstead clamp. It should be noted that this occurred in the first four cases (17.3%), that were operated on. In the remaining eight patients (n=8), we dissected and isolated the vein to avoid this complication.

In all operated eyes (n=23), it was difficult to create a proximal orifice near the lower lacrimal punctum with the help of a lacrimal punctum dilator.

Severe blepharospasm was observed in 27.3% (n=3) of patients in the right eye seven days post-surgery. In these cases, this clinical condition was found in the three eyes presenting with ulcerative keratitis as a result of dehiscence where the catheter was fixed to the orifice, enabling the upper migration of the catheter to create friction with the corneal surface. Conjunctival secretion was

severe as early as the seventh day in 81.8% (n=9) of right eyes and 75% (n=9) of left eyes during the same period of observation. These clinical signs regressed over 60 days in both eyes and became slight by the end of the evaluation. There were no statistically significant differences between eyes and evaluation periods.

Conjunctival hyperemia presented as severe in the first evaluation period (7 days), regressed over the 60 post-surgery days, becoming absent in 81.8% (n=9) and 83.3% (n=10) of right and left eyes, respectively. The clinical sign was graded as slight at the final observation period in the remaining four eyes.

Ulcerative keratitis was observed on the seventh day in 27.3% (n=3) of right eyes and none of the left eyes. This was restricted to the lower nasal quadrant of the cornea and was confirmed by fluorescein staining. The ulcerative keratitis was observed in patients in whom the catheter migrated because of dehiscence of the conjunctival suture. In these cases, the patient underwent a new anesthesia to reposition the catheter, with subsequent suturing. Over time, the ulcerative keratitis resolved, local corticosteroids were discontinued, and ketorolac tromethamine (Acular; Allergan, São Paulo, Brazil) was administered every 8 h for 15 d. Since the cornea healed and the fluorescein staining test was negative, we again administered corticosteroids to control the stenosis.

The Jones test was positive in 54.5% (n=6) and 66.7% (n=8) of right and left eyes, respectively. Over time, this positivity decreased from postoperative day 15, to become negative in 90.9% (n=10) and 100% (n=12) of right and left eyes, respectively. After postoperative day 60 on, the Jones test was positive in 100% of the eyes. The catheters were removed during the evaluation period. However, on post-operative day 240, 36.4% (n=4) and 41.7% (n=5) of right and left eyes, respectively, tested negatively.

Table 1 shows the frequency of bacteria and fungi found in the conjunctival microbiota and nostrils of patients with obstructive diseases of the lacrimal system before being subjected to DCR. Prior to treatment (M0), conjunctival microbiota cultures showed gram-negative bacteria in 66.5% (n=8) and gram-positive bacteria in 33.30% (n=4). Among the gram-negative organisms, pure cultures of *Pseudomonas aeruginosa* were the most frequent (33.3%, n=4), although this bacterium was also present in association with Proteus mirabilis, *Malassezia pachydermatis* and *Enterococcus* sp. Among the gram-positive bacteria, pure cultures of *Staphylococcus intermedius* were found in 16% (n=2) of eyes, and in association with beta-hemolytic *Streptococcus* in 8.3% (n=1).

The identification of organisms presents in the nostril before surgery (M0) revealed a predominance of gram-positive bacteria, represented by *Staphylococcus aureus, Staphylococcus intermedius* and coagulase negative Staphylococcus in 66.7% (n=8) of patients, and growth of *Staphylococcus intermedius* in one patient in association with *Pseudomonas aeruginosa.*

Table 1
Bacteria in the ocular conjunctiva and nostril of 12 dogs at M0

Bacteria	Nasal cavity		Ocular conjunctiva	
	n	%	n	%
Enterococcus sp / Pseudomonas aeruginosa	1	8.3	0	0.0
Klebsiella oxytoca	0	0.0	1	8.3
Klebsiella pneumoniae	0	0.0	1	8.3
Malassezia pachydermatis / Pseudomonas sp / Proteus mirabilis	0	0.0	1	8.3
Pseudomonas aeruginosa	2	16.7	4	33.3
Pseudomonas aeruginosa / Enterococcus sp	0	0.0	1	8.3
Pseudomonas aeruginosa / Staphylococcus intermedius	1	8.3	0	0.0
Staphylococcus aureus	3	25.0	0	0.0
coagulase negative Staphylococcus	2	16.7	0	0.0
Staphylococcus intermedius	3	25.0	2	16.7
Staphylococcus intermedius / beta–hemolytic *Streptococcus*	0	0.0	1	8.3
Staphylococcus aureus / beta–hemolytic *Streptococcus*	0	0.0	1	8.3

Legend: n = number of cases; % = percentage.

Throughout the postoperative course, a balance was found between the presence of gram-positive and gram-negative bacteria. *Pseudomonas aeruginosa* (25%) and *Staphylococcus intermedius* (25%) were found most frequently. By post-operative day 240, the balance had changed, revealing a greater predominance of gram-positive bacteria (85%, n=9). *Staphylococcus intermedius* was isolated from 41.7% (n=5) of patients. Table 2 shows the distribution of the conjunctival microorganisms over 240 days in patients who underwent DCR.

Diseases of the lacrimal system often prevent proper drainage, resulting in epiphora, chromodacryorrhea, abundant mucopurulent ocular secretions, and recurrent conjunctivitis. In more severe cases, medial canthus fistulae can be observed (Grahn & Sandmeyer, 2007; Slatter, 2005). These signs were observed in the patients included in this study, of whom 100% (n=12) presented epiphora, 75% (n=9) with abundant mucopurulent ocular secretion, 66% (n=8) with chromodacryorrhea, 33% (n=4) conjunctival hyperemia, and two patients with medial canthus fistulas. In these cases, we chose to perform radiographic and clinical examinations of the oral cavity to exclude the presence of severe periodontal disease caused by a secondary fistula (Wouk et al., 1999).

The study focused on 12 dogs, of which 33.3% (n=4) were Poodles, 25% (n=3) Maltese Terriers, 16.6% (n=2), West Highland White Terriers, and one each of Yorkshire Terrier, English Cocker Spaniel, and no defined breed. Miniature and toy poodles as well as Maltese terriers are the most affected (Slatter, 2005). With regard to sex predisposition, Gussoni and Barros (2003)

reported that chromodacryorrhea does not depend on sex despite its low predominance among females. Our study revealed the same, with 66.6% (n=8) being male and 33.4% (n=4) females, although no other data were found in literature showing a possible relationship between the occurrence of this disease and the sex of the animals. In our study, age varied with presentation, with patients included in the study aged between 1 and 10 years (average: 3.5 years). It should be noted that the time of presentation of chromodacryorrhea was not determined; therefore, it could have occurred before data presentation.

Table 2
Bacteria in the ocular conjunctiva of 12 dogs preoperatively and during the postoperative period

Day	Bacteria	n	%
0	*Klebsiella oxytoca*	1	8.3
	Klebsiella pneumoniae	1	8.3
	Malassezia pachydermatis / Pseudomonas sp / Proteus mirabilis	1	8.3
	Pseudomonas aeruginosa	4	33.3
	Pseudomonas aeruginosa / Enterococcus sp	1	8.3
	Staphylococcus intermedius	2	16.7
	Staphylococcus intermedius / beta–hemolytic *Streptococcus*	1	8.3
	Staphylococcus aureus / beta–hemolytic *Streptococcus*	1	8.3
60	*Burkholderia cepacia*	1	8.3
	Enterobacter cloacae	1	8.3
	Pseudomonas aeruginosa	3	25.0
	Serratia marcescens	1	8.3
	Staphylococcus aureus	2	16.7
	Staphylococcus intermedius	3	25.0
	Staphylococcus intermedius / beta–hemolytic *Streptococcus*	1	8.3
120	*Acinetobacter sp*	1	8.3
	Burkholderia cepacia	1	8.3
	Enterobacter agglomerans	1	8.3
	Escherichia coli	1	8,3
	Escherichia coli / Enterococcus spp	1	8.3
	Pseudomonas aeruginosa	2	16.7
	Staphylococcus aureus	2	16.7
	Staphylococcus intermedius	3	25.0
240	*Pseudomonas aeruginosa*	2	16.7
	Serratia marcescens	1	8.3
	Staphylococcus aureus	3	25.0
	Staphylococcus intermedius	5	41.7
	Staphylococcus sp (coagulase negative)	1	8.3

Legend: n = number of cases; % = percentage.

The Schirmer test is used for the confirmation of epiphora, where the mean values varied between 15 and 25 mm/min, making it possible to differentiate it from the occurrence of tearing (Grahn & Sandmeyer, 2007; Kleiner & Wouk, 2004). The Schirmer's test should be the first test performed on animals with epiphora. This stimulates the production of reflex tears, and when the results exceed 25 mm/min, it is consistent with a diagnosis of tearing (Grahn & Sandmeyer, 2007). Tearing is the excessive production of tears triggered by a psychological stimulus in humans, or irritation to the eyes in animals, as in the case of keratitis, conjunctivitis, abnormal eyelids, glaucoma, and uveitis (Grahn & Sandmeyer, 2007; Kleiner & Wouk, 2004; Slatter, 2005). In the study patients, these diseases were excluded by thorough eye examination. All patients presented with obstructive disease of the lacrimal system.

The patency test of the nasolacrimal duct or the Jones test evaluates the integrity of the lacrimal apparatus after administering fluorescein to the eye bulb, registering the time it takes to travel through the lacrimal apparatus to the nostril or oral cavity, which normally takes up to 5 min. Factors, such as the amount of dye, time of tear production, and length of the nasolacrimal duct, can influence this time (Grahn & Sandmeyer, 2007; Slatter, 2005). In our study, 95.8% (n=23) of eyes evaluated tested negative, confirming the absence of patency in the nasolacrimal duct and confirming the presence of an obstructed drainage system. Only 1 eye (4.2%) tested positive. Since it was impossible to perform image exams, which would confirm the existence of patency of the duct and rule out the occurrence of congenital diseases of the lacrimal apparatus (Grahn & Sandmeyer, 2007), we performed, in all cases, the flushing test of the nasolacrimal duct without any problems and confirmed that all patients had only dacryocystitis. During the test, and in all patients, we encountered mechanical resistance when injecting the fluid, probably due to swelling resulting from inflammation and the presence of debris in the duct (Giuliano et al., 2006; Grahn & Sandmeyer, 2007; Slatter, 2005). The lack of similar studies in the literature motivated us to prospectively study patients with obstructive diseases of the lacrimal system who were undergoing DCR, to follow their postoperative course and to identify the conjunctival microbiota, and compare it with the organisms present in the mucosa of the nostrils. Previous descriptions refer only to case studies (Giuliano et al., 2006; Kleiner & Wouk, 2004; Ota et al., 2009; Wouk & Kleiner, 2003). Although the aim of therapies applicable to lacrimal system diseases is to remove obstructions, which can be achieved through clinical or surgical treatment, long-term clinical therapy presents unsatisfactory results.

Among the surgical techniques described, we highlight DCR and conjunctivobuccostomy (Grahn & Sandmeyer, 2007, Kleiner & Wouk, 2004; Machado et al., 2008; Nykamp et al., 2004; Slatter, 2005). Both conjunctivobuccostomy and conjunctivorhinostomy are associated with high postoperative complication rates. The connection with the oral cavity is prone to contamination, causing infections across the entire path covered by the catheter, in addition to the risk of obstruction of the catheter by food debris, recurring epiphora, and inflammatory obstructions in the long term (Machado et al., 2008). For these reasons, we chose to perform DCR in all

patients, given the lower rate of postoperative complications. However, there were some problems with this technique. In the first four patients that were operated on, it was found that when the cutaneous incision was made in deeper tissue, where it reached the angular vein of the eye bulb, a branch of the facial vein (Evans & De Lahunta, 2012), this caused intense and persistent bleeding, preventing further steps of the procedure from being performed. The bleeding stopped after temporary hemostasis using a hemostatic clamp. The same results were reported by Giuliano et al. (2006). Therefore, extra care was taken when making the incision. This did not occur in the other patients because we were careful when dissecting and isolating the vein in question.

A lacrimal punctum dilator was used in all cases to expand the existing lacrimal punctum. However, this was insufficient for sufficient expansion to insert the catheter. In all the patients, the orifice was expanded using a scissors to facilitate catheter introduction. This has also been reported by Grahn and Sandmeyer (2007) and Kleiner and Wouk (2004), who described the need to evert the tip of the catheter by heating it so that it could remain in place at the bottom of the conjunctival sac. However, used in this manner, it was found that the catheter could shift towards the ocular surface. Therefore, we sutured it near the orifice. Even so, dehiscence of stitches occurred, and the catheter migrated to one eye in three patients (27.3%). This led to the development of corneal ulcers in the first postoperative week. In view of this, we chose to suspend corticosteroids and replace them with a non-steroidal anti-inflammatory agent. After resolving the ulcerative keratitis, which took approximately 15 days on average, the patients were again administered corticosteroids to minimize the risk of stenosis in the orifice, which was also applied to the other patients.

Conjunctival stenosis of the orifice is a common complication in all surgical procedures for obstructive diseases (Felchle & Urbanz, 2001; Grahn & Sandmeyer, 2007; Kleiner & Wouk, 2004; Nykamp et al., 2004; Slatter, 2005; Wouk & Kleiner, 2003). To minimize this, we recommend the use of acetylsalicylic acid-based eye drops at 0.3%, given their antifibrotic and anti-inflammatory properties (Wouk et al., 1999). Since this drug for topical ocular use is not available in the market, we chose to use prednisolone acetate at 0.1%, which also hinders the progression of fibrosis to stenosis (Grahn & Sandmeyer, 2007; Slatter, 2005). The efficacy of this steroid drug, used until the catheter was removed (60 days) to control long-term postsurgical stenosis rin the late period (240 days), was 63.6% (n=7) and 58.3% (n=7) in the right and left eyes, respectively. However, these results were not statistically significant. Better clinical results could be achieved if the drug was used until the end of the postoperative observation period (240 days).

Blepharospasm was not present at the beginning in most of the eyes (86.4%, n=20) evaluated. It presented severely in three patients in the first week due to dehiscence of the stitches and later displacement of the catheter, causing ulcerative keratitis, suggesting a correlation with the stimulation of nerve endings of the epithelium and corneal stroma (Waring, 1998).

Bacteria are often found in the ocular conjunctiva of healthy dogs. Several studies

have isolated bacteria from these animals, with similar results. Gram-positive bacteria prevailed (McDonald & Watson, 1976; Wang et al., 2008).

Studies that have isolated bacteria from animals with obstructive diseases of the lacrimal ducts are rare. In our study, we analyzed the conjunctival microbiota of these animals, and the results showed the presence of gram-negative bacteria in 66.5% (n=8), notably *Pseudomonas aeruginosa* in 33.3% (n=4). This result is not in line with the literature; some studies have isolated bacteria from dogs with obstructive diseases, and gram-positive bacteria were most common (Santos et al., 2009). Studies on eyes with other ophthalmic diseases have also shown a predominance of gram-positive bacteria (Prado et al., 2005; Santos et al., 2009).

In the nostrils of animals with obstructive diseases of lacrimal ducts, we found gram-positive bacteria to be the most commonly identified preoperatively, being present in 66.7% of bacterial cultures, which has not been reported in the literature. The most commonly found bacteria were *Staphylococcus aureus* and *Staphylococcus intermedius*, both in 25% of cultures.

Preoperatively (day 0), gram-negative bacteria prevailed in the conjunctiva of the dogs, being found in 66.5% of cases. At day 60 of the postoperative period bacteriological examinations showed equal frequency of gram-positive and -negative bacteria in the conjunctiva; by day 120 gram-negative bacteria accounted for 58.3% of isolates. On day 240, gram-positive bacteria were the commonest isolates, at 75%, with *Staphylococcus intermedius* accounting for 41.7% of the total. As for the gram-negative bacteria, *Pseudomonas aeruginosa* was found in 16.7% of the isolates.

This change in the types of conjunctival bacteria found of the dogs, was because in the 240 days period the majority of the eyes (63.6%, n=7) no longer presented obstructive diseases of the lacrimal ducts and became healthy eyes. This result is very similar to those of other studies that evaluated the conjunctival microbiota in healthy eyes (McDonald & Watson, 1976; Santos et al., 2009).

Obstructive lacrimal duct diseases are common in small animals. Proper diagnosis and treatment are key differentiators for veterinarians. The present study has shown a practical and streamlined way to diagnose these diseases. Surgical treatment is the chosen procedure because, in most cases, clinical treatment is usually followed by a setback in a short period of time.

We chose the DCR technique described by Grahn and Sandmeyer (2007), in which the catheter placed at the bottom of the conjunctival sac was self-contained. However, in our opinion, this was insufficient to maintain it in place for the entire period required to create a path around it. However, we noted that no study has evaluated the possible changes caused by catheter displacement. Thus, in the present study, the catheter was sutured to the bottom of the conjunctival sac, which proved to be very efficient, since only 27.3% (n=3) of eyes in the 7-day and 15-day postoperative periods presented ulcerative keratitis, following suture rupture and catheter displacement. After the catheter was repositioned, and a new suture was placed, no other cases of ulceration were identified.

DCR was well tolerated by the animals. Blepharospasm was identified in only 27.3% (n=3) 7 days after surgery, and this was due to the presence of ulcerative keratitis.

The evaluation of conjunctival secretion showed a high incidence, 81.8% (n=9) and a score of 4 on post-operative day 7. The incidence and score decreased until 60 days after surgery 54.5% (n=6) and 27.3% (n=3) of eyes presented, respectively. This coincided with the removal of the catheter from the bottom of the conjunctival sac, leading us to conclude that irritation caused by the catheter was the most important factor underlying the presence of conjunctival secretion.

Conjunctival hyperemia demonstrated behavior similar to that of conjunctival secretion at the beginning of the evaluation, again showing that the presence of a catheter at the bottom of the conjunctival sac causes irritation to the eye, which in turn leads to conjunctival hyperemia.

A negative Jones test was found in 54.5% (n=6) on post-operative day 7, 72.7% (n=8) of eyes on postoperative day 15, and 100% at post-operative day 30. This increase in percentage of the negative Jones test is probably due to the obstruction of the catheter by debris. At 60 days after removing the catheter, a positive Jones test was found for 100% of the eyes; and at 120 and 240 days, 63.6% (n=7) were positive and 36.4% (n=4) negative.

These results show the efficacy of DCR in the correction of lacrimal duct obstruction, justifying the use of the technique instead of attempting to flush and clinically treat patients, as recommended in some studies, because this treatment has a high recurrence rate in a short period of time, which does not occur with the use of DCR. However, the biggest problem with using this technique is stenosis of the new path, following catheter removal. Corticosteroids were used in the postoperative period to inhibit healing, as described in the literature (Andrade et al., 2002). However, some studies have suggested the use of acetylsalicylic acid-based eye drops with excellent results, as in the study by Wouk et al. (1999), where acetylsalicylic acid inhibited the healing process in surgeries involving the drainage of glaucoma compared to the use of mitomycin.

To our knowledge, this is the first report to correlate conjunctival and nasal microbiota in dogs undergoing DCR. These findings are similar to those reported by Blicker and Buffam (1993), who investigated organisms that grow in the lacrimal sac of human patients requiring DCR and concluded that there was no clinically significant correlation.

Conclusion

Regarding the conjunctival microbiota, we can conclude that in obstructive diseases, the lacrimal system is dominated by gram-negative bacteria, namely *Pseudomonas aeruginosa.* This study did not show an increase in bacteria from the nasal cavity via the new surgical pathway to the conjunctival fundus in dogs that underwent a DCR.

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