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Goblet cell density and distribution in cats with clinically and histologically normal conjunctiva

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Abstract

Objective The aim of this study was to evaluate goblet cell density (GCD) and distribution in cats without clinical evidence of ocular surface disease and without histologic evidence of conjunctival disease.

Animals studied Fourteen Domestic Shorthair cats euthanized for reasons unrelated to this study.

Procedures Before euthanasia, cats were verified using slit-lamp biomicroscopy and fluorescein staining to be free of eyelid or ocular surface abnormalities. Immediately after euthanasia, bilateral conjunctival specimens including third eyelid (TEL) were collected, routinely processed, and stained with periodic acid–Schiff and hematoxylin and eosin. Thirteen conjunctival regions were identified. For each region, GCD was expressed as the percentage of goblet cells/200 basal epithelial cells.

Results Mean GCD ranged widely by region: anterior surface of the TEL = 48.8%, fornicial regions = 47.0%, palpebral regions = 38.5%, bulbar regions = 19.6%, and posterior surface of the TEL = 12.6%. The anterior surface of the TEL had significantly higher GCD than did the bulbar and the palpebral regions, but not the fornicial regions. Bulbar conjunctiva had significantly lower GCD than did all other conjunctival regions except the posterior surface of the TEL. No significant difference was noted between GCD of male versus female cats, dorsal versus ventral regions, or lateral versus medial regions.

Conclusions Although conjunctival GCD ranged widely by region, the anterior surface of the TEL appears to be an excellent location for assessing conjunctival goblet cells in cats because this area has high GCD and is more readily accessible than is the palpebral, fornicial, or bulbar conjunctiva.

Key Words: cat, conjunctiva, goblet cell, third eyelid

INTRODUCTION

Conjunctival goblet cells are specialized cells intercalated in conjunctival epithelium. They synthesize and secrete mucins onto the ocular surface, whose major functions are to stabilize the tear film, provide lubrication, and protect against microbial invasion and desiccation of the underlying epithelium.[1] Clinical evaluation of conjunctival goblet cells is important as it provides an indirect method of assessing pre-ocular mucins. It has long been recognized that changes in conjunctival goblet cell density (GCD) are a sensitive indicator of ocular surface disease,[1–3] likely superior to direct evaluation of pre-ocular mucin content.[4]

Density and distribution of conjunctival goblet cells have been described in dogs,[5] horses,[6] chinchillas,[7] guinea pigs,[8] rabbits,[9] rats,[10] nonhuman primates,[11] and humans.[12–14] However, to the authors' knowledge, little information is available regarding GCD and distribution in cats. This is particularly important as there is growing evidence of the link between the loss of conjunctival goblet cells and many commonly diagnosed feline ocular surface diseases such as conjunctivitis,[15] corneal ulceration,[16] and corneal sequestrum.[16,17] In addition, cats

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experimentally infected with feline herpesvirus have a marked and protracted reduction in conjunctival GCD and associated reduction in tear film stability, both of which persist after cats have apparently clinically recovered.[18]

Therefore, the purpose of the present study was to evaluate the density and distribution of conjunctival goblet cells in cats without clinical evidence of ocular surface disease and without histologic evidence of conjunctival disease. These normative data will guide future studies evaluating diseased feline eyes and will inform comparative discussions regarding the ocular surface in health and disease in many species.

MATERIAL AND METHODS

Animals

Samples were collected from cats euthanized for reasons unrelated to the study, including chronic kidney disease, urethral obstruction, pulmonary hypertension, pulmonary carcinoma, cardiomyopathy, or rodenticide toxicity. Prior to euthanasia, each cat underwent slit-lamp biomicroscopy (Kowa SL-15 biomicroscope, Kowa Company Ltd., Tokyo, Japan) of the ocular adnexa and anterior segment, followed by fluorescein staining of the cornea and conjunctiva (Flu-Glo, Akorn Inc., Buffalo Grove, IL). Cats were excluded if any abnormality involving the eyelids or ocular surface was detected.

Conjunctival sample collection and preparation

Following euthanasia, both eyes of each cat were enucleated using the transpalpebral technique ensuring that a perimeter of at least 10 mm of periocular skin was excised along with the globe (Fig. 1). Using the long posterior ciliary arteries and palpebral canthi as landmarks, the adnexal tissue was divided into dorsal and ventral sections by making two incisions perpendicular to the eyelid margins—one each from the lateral and medial canthi to the adjacent region of the corneoscleral limbus. The ventral adnexa included the third eyelid. The dorsal and ventral adnexa were then each reflected away from the globe, a 360° limbal peritomy was performed to separate them from the globe, and they were each laid flat on a piece of cardboard and immersed in 10% neutral buffered formalin for histologic processing. All samples were collected within 1 h of euthanasia, with most samples collected within the first 20 min, and care was taken during all tissue manipulations to minimize damage to the conjunctival surface.

Following fixation, the dorsal and ventral adnexa were each sectioned so as to separate them into medial and lateral halves, thus dividing the adnexa into four quadrants: dorsolateral, dorsomedial, ventrolateral, and ventromedial. Each of these four quadrants was then halved in the same plane as the original incision; the ventromedial quadrant was sectioned so as to also bisect the third eyelid. One half of each quadrant was routinely processed and embedded in paraffin. The cut edge of each halved quadrant was sectioned at 4 μm and mounted on glass slides. Samples were stained with hematoxylin and eosin (H&E) and periodic acid–Schiff (PAS) techniques and evaluated with light microscopy.

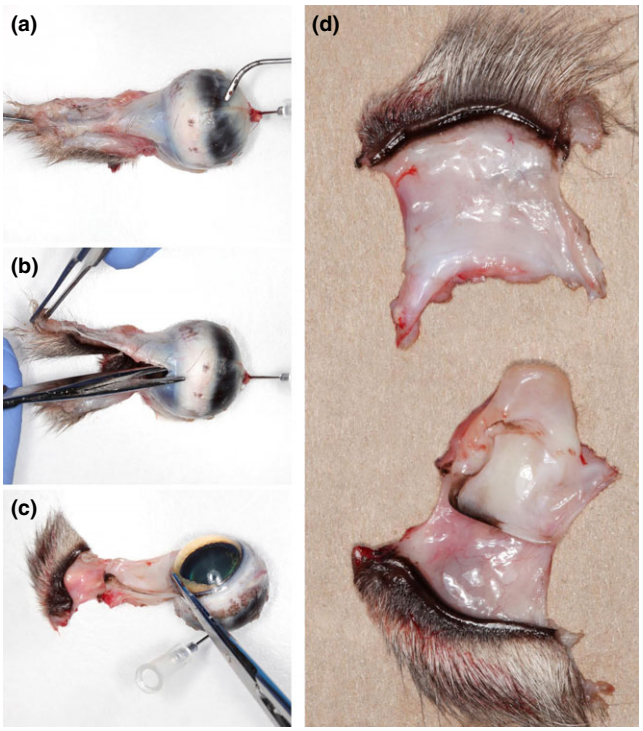

Figure 1. Surgical collection of the feline conjunctiva. (a) Each globe and its adnexa was removed using a standard transpalpebral enucleation technique. The globe was stabilized by a needle placed through the optic nerve. The long posterior ciliary arteries (one of which is indicated with the metal cannula) served as landmarks for the lateral and medial aspects of the globe. (b) Stevens tenotomy scissors were used to incise the conjunctiva from the medial and lateral canthi to the nearest point of the corneoscleral limbus. (c) The adnexa was separated from the globe via a 360° peritomy performed immediately adjacent to the corneoscleral limbus. (d) Dorsal and ventral blocks of adnexa were laid flat on cardboard with the conjunctival surface up and immersed in formalin. The ventral adnexal block included the third eyelid.

Goblet cell counts

Prior to the assessment of GCD, H&E- and PAS-stained sections were assessed histologically, and the samples were excluded if they could not be evaluated due to tangential sectioning of the conjunctival epithelium or if conjunctival or eyelid inflammation was graded as moderate, marked, or generalized using a published scale.[18] If the inflammation was graded as mild and localized, the sample was not discarded but goblet cell quantification was not performed in areas overlying or immediately adjacent to the inflammatory cells.

For the purposes of describing goblet cell distribution, the conjunctiva was divided into 13 conjunctival regions

for each eye. These were palpebral, fornicial, and bulbar regions of the dorsomedial, dorsolateral, and ventrolateral quadrants; palpebral and fornicial regions only of the ventromedial quadrant (due to the presence of the third eyelid); and anterior and posterior surfaces of the third eyelid. The following histologic criteria were established to identify each region in all cats consistently (Fig. 2):

(1) *Palpebral region* - The count was started from the first goblet cell noted after the eyelid margin.
(2) *Fornicial region* - The fornix was typically easily identified by a consistent and distinct fold in the conjunctiva. In samples without a distinct fold, the fornix was defined by the junction of the distinctive dense subconjunctival connective tissue of the tarsal plate with the looser, less eosinophilic connective tissue of the bulbar conjunctiva.
(3) *Bulbar region* - The count was initiated at the limbal cut edge of each conjunctival sample.
(4) *Third eyelid* - The count was initiated on the anterior and posterior faces in an area approximately equidistant from the fornix to the tip of the third eyelid using the third eyelid cartilage as a guide.

Using these tissue landmarks, a single histologic section was selected from each quadrant for goblet cell quantification. The section was selected so as to avoid technical artifacts such as tissue tearing, tangential sectioning, and areas with anything graded as greater than mild inflammation. In some specimens, 1 or more additional sections were required to provide slides without such artifacts. Using 400× magnification, the nuclei of 200 consecutive basal conjunctival epithelial cells were counted manually. The number of goblet cells present among these 200 basal epithelial cells was also counted, and GCD was expressed as a percentage of basal epithelial cells. Data from all conjunctival regions of the eye were excluded if assessment of GCD was not possible in over half (≥7/13) of the conjunctival regions evaluated.

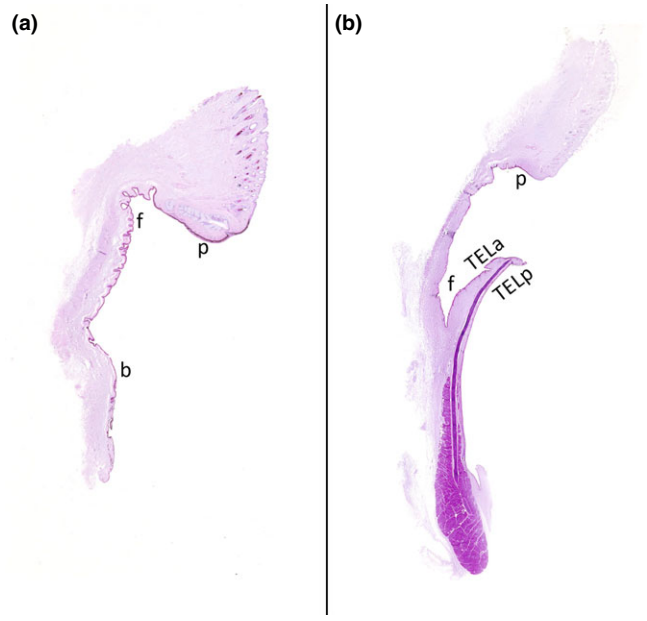

Figure 2. Low-magnification photomicrographs depicting representative dorsal (a) and ventral (b) sections of conjunctiva from a cat without clinical evidence of ocular surface disease and without histologic evidence of conjunctival disease. p = palpebral conjunctiva; f = fornicial conjunctiva; b = bulbar conjunctiva; TELa = anterior surface of third eyelid; TELp = posterior surface of third eyelid. PAS stain.

Statistical analysis

Normality of the data was assessed with Shapiro–Wilk test. Non-normally distributed data are presented as median and central range (25–75th percentile), while normally distributed data are presented as mean and standard deviation (SD). Mann–Whitney rank-sum test was used to evaluate the differences in GCD between male and female cats, dorsal and ventral regions, and lateral and medial regions. An ANOVA model for repeated measures was used to analyze the differences in GCD among the five conjunctival regions (palpebral, fornicial, bulbar, anterior surface of the third eyelid, and posterior surface of the third eyelid). The assumption of equal variance for GCD across regions was tested with Brown–Forsythe test. A P-value of < 0.05 was considered significant for all analyses.

RESULTS

Fourteen Domestic Shorthair cats met the clinical entry criteria; seven were castrated males and seven were spayed females with a mean ± SD (range) age of 8.0 ± 4.1 (2–14) years. Based upon evaluation of H & E-stained sections, all conjunctival regions were excluded from seven eyes of seven cats because of processing artifacts resulting in an inability to count goblet cells in at least half of the conjunctival regions. Six of the seven eyes excluded were harvested during the first half of the study period. Therefore, 21 eyes from 14 cats met all clinical and histologic inclusion criteria and underwent goblet cell quantification (Table 1).

Goblet cells were easily identified in all samples assessed due to the presence of PAS-positive intracellular material and often because of their distinctive shape (Fig. 3). Considering all data collectively and irrespective of region, no significant difference was detected between median (25–75th percentile) GCD of male [36 (21–50) %] and female [37 (20–53) %] cats ($P = 0.668$). Similarly, no significant difference was detected between dorsal [35 (20–52) %] and ventral [43 (29–51) %] regions ($P = 0.112$) or between lateral [37 (24–50) %] and medial [38 (24-52) %] regions ($P = 0.494$). However, mean ± SD GCD across the 13 specific conjunctival regions varied widely (Fig. 3, Fig. 4, Table 1), from 12.6 ± 8.9% (posterior surface of the third eyelid) to 49.5 ± 14.8% (ventromedial fornix). Mean ± SD GCD on the posterior surface of the third eyelid (12.6 ± 8.9%) was the lowest of all

regions, whereas mean ± SD GCD on the anterior surface of the third eyelid (48.8 ± 15.8%) was the second highest value recorded; this difference was significant ($P < 0.001$). Considering bulbar, palpebral, and fornicial regions irrespective of quadrant, mean ± SD GCD was significantly higher in fornicial (47.0 ± 13.7%) than in palpebral (38.5 ± 13.0%) or bulbar (19.6 ± 13.4%) conjunctivae ($P < 0.001$ for both). Mean ± SD GCD was significantly lower ($P < 0.001$) in bulbar conjunctiva (19.6 ± 13.4%) than in all other regions except the posterior surface of the third eyelid (12.6 ± 8.9%; $P = 0.314$). Mean ± SD GCD was significantly higher on the anterior surface of the third eyelid (48.8 ± 15.8%) than in the bulbar regions ($P < 0.001$) and the palpebral regions ($P = 0.026$), but not the fornicial regions ($P = 0.986$).

Table 1. Mean, standard deviation, and range of conjunctival goblet cell density (GCD) for 13 conjunctival areas from 21 eyes of 14 cats without clinical evidence of ocular surface disease and without histologic evidence of conjunctival disease. In each area, GCD was expressed as a percentage of epithelial cells based upon a count of 200 consecutive basal epithelial cells

Region	Number of samples examined	Goblet cell density	
		Mean ± SD (%)	Range (%)
Dorsolateral			
Palpebral	20	38.0 ± 14.2	13.5–67
Fornicial	20	48.2 ± 11.8	27–65
Bulbar	20	22.7 ± 13.8	2.5–48.5
Palpebral	20	37.4 ± 13.2	16.5–57
Dorsomedial			
Fornicial	20	45.4 ± 16.6	8.5–64.5
Bulbar	17	14.0 ± 8.9	1–35
Palpebral	19	37.9 ± 12.6	18–3.5
Ventrolateral			
Fornicial	17	45.1 ± 11.7	29–66
Bulbar	14	21.1 ± 15.9	2.5–51.5
Ventromedial			
Palpebral	16	41.0 ± 12.6	18.5–62.5
Fornicial	17	49.5 ± 14.8	19.5–75.5
Third eyelid			
Anterior surface	18	48.8 ± 15.8	7.5–69.5
Posterior surface	19	12.6 ± 8.9	1–28.5

DISCUSSION

Previous studies have described feline GCD in a limited number of cats and for only the palpebral,[16] fornicial,[17,18] or third eyelid[19] conjunctiva. To the authors' knowledge, the present study provides for the first time normative data for GCD and distribution throughout all conjunctival regions in cats without clinical evidence of ocular surface disease and without histologic evidence of conjunctival disease. As a result, we were able to demonstrate approximately fourfold variation in GCD across the 13 conjunctival regions we defined, with the following trend in GCD: anterior surface of the third eyelid ≈ fornicial regions > palpebral regions > bulbar regions ≈ posterior surface of the third eyelid. Earlier reports also revealed that the conjunctival fornix is very rich in goblet cells in dogs,[5] cats,[17] and horses,[6] but not in chinchillas[7] or guinea pigs.[8] Our finding that the anterior surface of the third

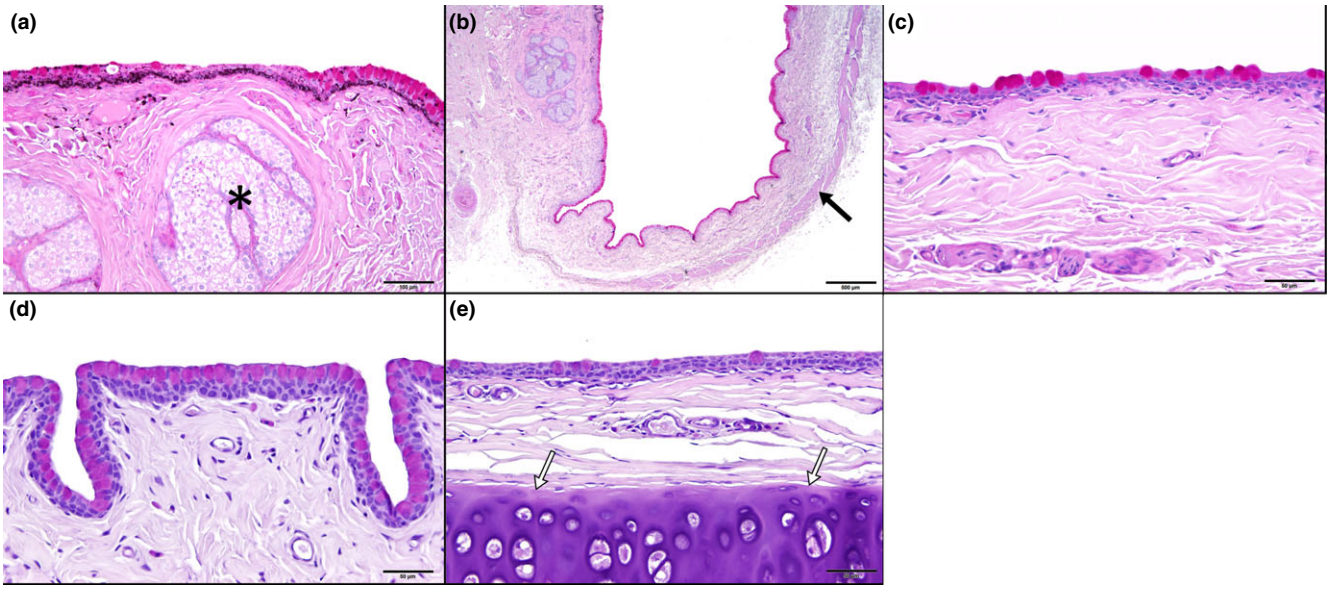

Figure 3. Representative photomicrographs of sections of 5 conjunctival regions stained with the PAS technique. Goblet cells are stained magenta. (a) palpebral conjunctiva. A meibomian gland (*) is present in this section (100× magnification, bar = 100 μm). (b) fornicial conjunctiva. Mueller's muscle (black arrow) is present in this section (20× magnification, bar = 500 μm). (c) bulbar conjunctiva (200× magnification, bar = 50 μm). (d) anterior surface of the third eyelid (200× magnification, bar = 50 μm). (e) palpebral surface of the third eyelid. The third eyelid cartilage (white arrows) is present in this section (200× magnification, bar = 50 μm).

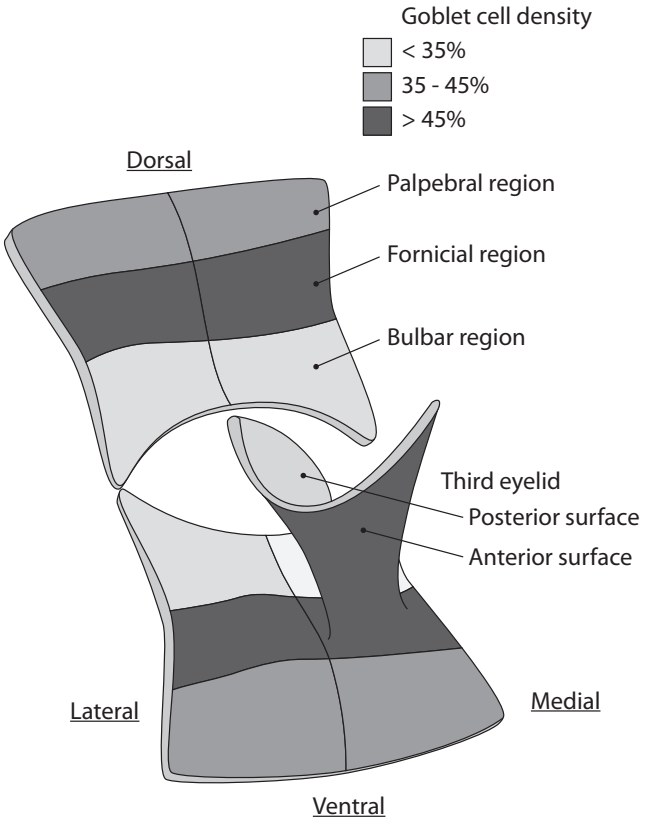

Figure 4. Schematic representation of the feline conjunctiva (laid flat as in Figure 1(d)), showing conjunctival goblet cell density (GCD) for each of 13 areas evaluated in 21 eyes of 14 cats without clinical evidence of ocular surface disease and without histologic evidence of conjunctival disease. Increasing shade intensity represents increased GCD expressed as a percentage of epithelial cells based upon a count of 200 consecutive basal epithelial cells. Counts were not performed for the ventromedial bulbar conjunctiva (shown in white). Goblet cell density of the fornicial conjunctiva and anterior surface of the third eyelid (>45%) was significantly greater than GCD of the palpebral conjunctiva (35–45%), which was significantly greater than GCD of the bulbar conjunctiva and posterior aspect of the third eyelid (<35%).

eyelid has high GCD was also reported in dogs[20] and cats.[19] Interestingly, a study conducted in humans revealed that the highest GCD is found on the plica semilunaris - a vestigial remnant of the nictitating membrane.[13] In the present study, GCD was notably lower in the bulbar conjunctival region than in other regions in cats. This has also been noted in dogs,[5] horses,[6] chinchillas,[7] and guinea pigs,[8] although feline bulbar conjunctival GCD (14–22.7%) tended to be higher than that reported for these other species.

It is interesting to postulate why GCD varies so widely among conjunctival regions and species. Some have suggested that the degree of surface hydration is important, with more hydrated areas having a higher GCD than those that are more prone to desiccation.[5,13] This theory might help explain why GCD of bulbar conjunctiva is greater in cats compared to other species, because the tight palpebral fissure of cats reduces exposure and desiccation of this area. The same theory could also explain the high GCD found in the ventromedial conjunctival fornix of cats in the present study, as well as in dogs[5] and horses,[6] because this region is where tears accumulate before being drained through the nasolacrimal puncta and is therefore likely well hydrated. However, the hydration theory would not support our finding of high GCD on the anterior surface of the third eyelid, especially in comparison with its more protected posterior surface. An alternate explanation for the difference in GCD between both surfaces of the third eyelid and for the high GCD in the conjunctival fornices may involve goblet cell morphogenesis. Studies in several species have demonstrated that genesis of conjunctival goblet cells occurs first in the fornix and then extends to palpebral and bulbar conjunctiva,[10,21,22] likely due to rich vascularization in the fornicial area that is believed to stimulate goblet cell differentiation.[23] Although not described for the nictitating membrane, perhaps goblet cell morphogenesis is greater in the conjunctival fornix anterior to the third eyelid than it is in the fornix posterior to it.

Comparison among studies must take into account the various methods employed in sampling and counting goblet cells. In the present study, the whole conjunctiva was collected en bloc and trimmed in parasagittal sections. Then, during goblet cell quantification, only basal epithelial cells were counted to establish GCD. In contrast, Moore and co-authors used a punch biopsy for each conjunctival area assessed, and all epithelial cells were counted for evaluation of GCD.[5] A likely advantage of the methodology from the present study is the ability to assess GCD over multiple conjunctival regions on one glass slide, thus improving consistency between specimens and making the process more rapid and cost-effective. This also generally enabled us to find sections without artifact for counting; however, this was not possible in all conjunctival regions. Importantly, the majority (6/7) of the eyes excluded from GCD evaluation due to artifact were collected in the first half of the study, suggesting that our sampling and processing techniques improved over time. In particular, we found it valuable to lay the conjunctival tissue flat and under slight tension on cardboard before immersion in formalin. In the present study, fluorescein dye was used as part of antemortem ophthalmic examination; we believe its effect on GCD would be minimal as globes were enucleated within 30 min of using fluorescein dye.

Our study utilized cats that were euthanized for various systemic illnesses, and one could argue that the normative GCD and distribution data we provide here should be verified in healthy cats. However, we believe this choice in cats had minimal impact on our findings because we included samples from clinically and histologically normal conjunctivae only.

In conclusion, GCD in cats free of conjunctival disease ranges widely by region, but tends to be highest in fornicial regions and on the anterior surface of the third eyelid. Based on the data we present here, the anterior surface of the third eyelid would be an excellent location for clinically assessing GCD because this area is more readily accessible than is the palpebral, fornicial, or bulbar conjunctiva due to the tight palpebral fissure of cats. Additionally, the high GCD in this region of cats with clinically and histologically normal conjunctiva may permit the detection of a large range of goblet cell deficiencies in cats with various ocular surface diseases. Future studies should compare our results with those from cats with various ocular surface diseases and should consider techniques such as conjunctival impression cytology that provide a less traumatic manner of assessing GCD.[24–26]

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