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ORIGINAL ARTICLE

Effect of topical application of 0.5% proparacaine on corneal culture results from 33 dogs, 12 cats, and 19 horses with spontaneously arising ulcerative keratitis

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Abstract

Objective: To investigate the effect of topically applied proparacaine on bacterial and fungal culture results and to compare cytologic and culture results in patients with ulcerative keratitis.

Procedure: Corneal samples were collected from 33 dogs, 19 horses, and 12 cats with spontaneously arising ulcerative keratitis. Samples for bacterial (dogs, cats, horses) and fungal (horses) cultures were collected prior to and following application of 0.5% proparacaine or saline. All patients then received a topical anesthetic, and samples were collected for cytology. Frequency of cultivatable bacteria before (Swab 1) and after (Swab 2) application of proparacaine or saline was compared using Fisher's exact test. Homogeneity of culture and cytology results was assessed using McNemar's test.

Results: No difference was detected in number of animals from which bacteria were isolated from Swab 1 or Swab 2 for proparacaine (21/37 and 17/37, respectively) or saline (10/27 and 12/27, respectively). Small numbers prevented analysis of fungal culture results in horses between Swab 1 and Swab 2 for proparacaine (2/12 and 1/12, respectively) or saline (both, 1/8). Bacteria were isolated from 10 of 20 horses and detected cytologically in 3 of these; fungi were isolated from 3 of 20 horses and detected cytologically in 2 of these. Bacteria were detected more frequently using culture (31/64) than cytology (19/64).

Conclusion: Proparacaine did not significantly alter bacterial or fungal culture results in cats, dogs, or horses; however, clinical significance warrants investigation. Culture and cytology provided complementary data; both should be performed to maximize organism detection in patients with ulcerative keratitis.

KEYWORDS

bacteria, corneal culture, cytology, fungi, proparacaine

1 | INTRODUCTION

Topical ophthalmic anesthetic agents are frequently used to minimize patient discomfort when collecting corneal

samples for culture or cytologic assessment. However, it is possible that these anesthetic agents, or the inactive ingredients and preservatives with which they are compounded, inhibit or kill microorganisms; and that, this effect may

not be uniform for all microorganisms. Therefore, collection of corneal samples for microbiological assessment has sometimes been recommended without topical application of an anesthetic.¹ However, minimal but sometimes conflicting data regarding such antimicrobial effects have been reported. One in vitro study investigated the antibacterial activity of 0.125%, 0.25%, and 0.50% solutions of proparacaine and tetracaine along with the preservatives—EDTA and benzalkonium chloride.² There were no zones of growth inhibition for *Pseudomonas aeruginosa* or *Staphylococcus aureus* after 24 hours of incubation, suggesting that neither proparacaine or tetracaine interfered with growth of these bacteria in culture media. By contrast, another study suggested that proparacaine did inhibit bacterial growth, but in a selective manner.¹ Specifically, proparacaine strongly inhibited the growth of *S. aureus* at all concentrations tested (0.125%, 0.25%, and 0.5%), and inhibited growth of *P. aeruginosa* at 0.25% and 0.5%, but not at 0.125%. In a third study, minimal effects of topically applied proparacaine on bacterial culture results were noted in samples collected from dogs with suspected bacterial keratitis or conjunctivitis.³ To the authors' knowledge, there are no in vitro or in vivo studies evaluating the effect of proparacaine on fungal culture in any veterinary species.

Bacterial or fungal keratitis resistant to such empirical treatment is painful and can result in globe rupture, vision loss, or both. Culture results can provide critical information for clinical management of and prognostication for patients with infectious keratitis. Therefore, this study was designed to investigate whether topical application of proparacaine before collection of microbiological samples was associated with a change in the number or type of bacteria cultured from corneal samples collected from dogs, cats, or horses, and in the number and type of fungi cultured from corneal samples collected from horses, all of which had spontaneously arising corneal ulcers believed to be infected.

2 | METHODS

2.1 | Study design

Each patient enrolled in the study was randomly assigned to receive proparacaine (treatment) or saline (control). For each enrolled patient, the eye affected with a corneal ulcer was sampled once prior to (Swab 1), and again after (Swab 2), instillation of the proparacaine or saline solution. Thus, the study represented a 2 × 2 design, with topical solution (proparacaine or saline) varying between subjects, and sampling [Swab 1 (collected before test solution instillation) and Swab 2 (collected after test solution instillation)] varying within subjects.

2.2 | Patient selection

Owners of canine, feline, or equine patients diagnosed with a spontaneously occurring corneal ulcer suspected to be infected were invited to participate in this study at the time of initial presentation of the patient. Criteria for suspecting bacterial or fungal infection included any combination of corneal stromal loss, malacia, or white blood cell infiltration, or purulent ocular discharge. Animals of all ages, breeds, gender, and disease duration were eligible for enrollment. Topical or systemic administration of any drugs prior to presentation did not exclude a patient from participation. This study was approved by the Institutional Animal Care and Use Committee of University of California, Davis. Written consent from owners or agents was obtained prior to study participation.

2.3 | Preparation of topical solutions

Commercially available, preserved, 0.05% proparacaine solution (Akorn Pharmaceuticals, Lake Forest, IL, USA) was used as the test solution. Nonpreserved, 0.9% sterile saline (OCuSOFT, Inc., Richmond, TX, USA) was used as the control solution. Using sterile technique in a biosafety cabinet, 0.2 mL aliquots of proparacaine or saline were placed into sterile 3-mL plastic dropper bottles (Steri-Dropper, EPS, Inc., Ivyland, PA, USA) for use in dogs and cats, or into sterile 2-mL multidose vials (Sterile silver capped vial; Greer Laboratories, Lenoir, NC, USA) for use in horses. For each container type, proparacaine and saline aliquots were sequentially numbered in randomized order based upon numbers generated by commercial software (Research Randomizer Version 4.0, Urbaniak, G. C., & Plous, S. (2013). Retrieved on June 22, 2014, from <http://www.randomizer.org/>). Each numbered aliquot was then assigned in ascending numerical order to a single patient based upon the order of patient enrollment in the study. Prior to use, all aliquots were stored at 4°C for not more than 2 weeks—the maximum period for which proparacaine has been confirmed to retain efficacy.⁴

2.4 | Clinical procedures

Type, duration, and frequency of treatment with any antibiotic or antifungal drugs applied topically prior to referral were recorded. All animals underwent ophthalmic examination consisting of slit-lamp biomicroscopy and rebound (dogs and cats) or applanation (horses) tonometry. The ulcerated cornea of all patients then was sampled using a microbiological swab (Transporter Single Transport Swab, Healthlink, Florida, USA) prior to (Swab 1) and two minutes following (Swab 2) application of a single drop (dogs

and cats) or 0.1 mL (horses) of proparacaine or saline. The timing of Swab 2 was based upon the reported time for proparacaine to produce corneal anesthesia.⁵ For dogs and cats, but not horses, the attending clinician (who was masked as to treatment group) subjectively graded as mild, moderate, or severe the patient's response to collection of Swab 1 using, collectively, degree of blepharospasm, head movement, vocalization, and aggressive behavior. Response to swabbing was not assessed in horses because the extent of their blepharospasm made this impossible. As a result, most were sedated and an auriculopalpebral nerve block was administered in many rendering the assessment unreliable. To ensure a uniform swabbing technique, all participating clinicians were trained in a standardized method of sample collection prior to the start of the study. Briefly, beginning at the dorsal rim of the ulcer, swabs were passed four times around the ulcer circumference in a counter-clockwise direction, always ensuring that the swab was kept in constant contact with the cornea. To further ensure uniformity of swabbing technique among species and between the two test compounds, and consistent implementation of the semiquantitative scoring system for patient response to sample collection, 60 of 64 samples were collected by one clinician (SGE).

Following collection of Swab 2, 0.05% proparacaine (dogs and cats) or 0.5% tetracaine (horses; Bausch + Lomb, Bridgewater, NJ, USA) was applied to the ocular surface of all patients. Tetracaine rather than proparacaine was used in horses based on its reportedly superior corneal anesthesia in this species.⁶ Following anesthetic administration, a sample for cytologic assessment was collected by passing a cytology brush (dogs and cats; Cytosoft, Medical Packaging Corporation, Camarillo CA, USA) or the blunt end of a Bard-Parker scalpel blade (horses) four times over the area of most dense stromal white blood cell infiltrate adjacent to the ulcer always ensuring that the ulcer base was not touched. Finally, to ensure sterility of the proparacaine and saline, each aliquot was cultured by placing onto a swab (Swab 3) one drop (for dogs and cats) or 0.1 mL (for horses) of the solution remaining in each patient's multidosed vial. All clients, attending veterinarians, veterinary technicians, and veterinary students were masked as to treatment group.

2.5 | Microbiologic and cytologic assessment

For each patient, Swab 1 (collected prior to instillation of topicals), Swab 2 (collected after instillation of topicals), and Swab 3 (the proparacaine or saline solution) were submitted for microbiologic assessment, and the corneal cytobrush (dogs and cats) or scalpel blade (horses) sample was submitted for cytologic assessment at the University of California Davis Veterinary Medical Teaching Hospital

Clinical Laboratories where all staff were masked as to treatment group, swab source, and swab number. All three swabs from each patient were submitted for aerobic bacterial culture and susceptibility testing. For horses, all three swabs also were submitted for fungal culture. For bacterial isolation, swabs were used to inoculate 5% defibrinated sheep blood and MacConkey agars (Hardy Diagnostics, Santa Maria, CA, USA). After streaking the sheep blood agar for isolation, a staphylococcal feeder streak was added to assist with isolation of fastidious bacteria. Incubation for bacterial organisms was performed at 35°C in 5% CO₂. Plates were incubated for a total of 5 days prior to calling a result as negative. For fungal isolation, swabs were inoculated onto inhibitory mold agar (Hardy Diagnostics) and incubated at 30°C under room air for a total of 4 weeks. To better look at microbial diversity, bacteria isolated were assigned to one of seven groups (*Staphylococcus* spp., *Streptococcus* spp., Gram-positive rods, enterics, nonenterics, *Pseudomonas* spp., and beta-hemolytic *Streptococcus* spp.) and fungi were assigned to one of three groups (*Aspergillus* spp., *Fusarium* spp., or "other").

2.6 | Data analysis

Outcomes measured for each swab were as follows: (a) frequency (%) of positive bacterial and fungal cultures (chi-square or Fisher's exact test); and (b) number of individual cultured bacterial or fungal species that were preselected [bacteria (0-7) and fungi (0-3)]. In addition, the presence of bacteria and fungi cultured from each swab was compared with the presence of organisms seen on cytologic assessment, and the frequency (%) of concordant and discordant results was reported. For comparison of gender and age, a probability test and Mann-Whitney test were used, respectively. For dogs and cats, a perceived qualitative pain score was assigned during collection of Swab 1 by the following criteria—no pain: patient allowed culture to be obtained with no vocalization or effort to blink or pull head away; moderate pain: patient attempted to blink and pull head away; and severe pain: patient attempted to blink, and continued to keep eye shut after culture obtained, pulled head away, and vocalized, tried to bite. A chi-square test was used to test for correlations in perceived pain and skull type. A Fisher's exact test was used to test for differences in the number of positive culture results between Swab 1 and Swab 2 for both the proparacaine and saline groups. McNemars' chi-square test was used to test for discordance in results of culture and cytology. All tests were performed using computerized statistical software (StatXact 11, Cytel Software Corporation, Cambridge, MA, USA). For all assessments, a *P*-value \leq 0.05 was considered significant.

3 | RESULTS

A total of 33 dogs, 12 cats, and 19 horses (64 animals) were enrolled in the study. Among the 33 dogs, there were 19 neutered males and 14 spayed females with a median (range) age of 8.2 (2-14) years. Breeds represented included Shih Tzu ($n = 12$), Pug (8), Boston Terrier (5), Maltese Terrier (4), and one each of Labrador Retriever, Border Terrier, Boxer, and Golden Retriever. Among the 12 cats, there were three neutered males and nine spayed females with a median (range) age of 7.8 (1-12) years. Breeds represented included Domestic Short-haired (seven), Persian (two), and Siamese (three). The majority of dogs (26/33) but only two of 12 cats were brachycephalic. Among the 19 horses, there were 10 geldings, eight mares, and one filly ranging in age from 1 month to 27 years; median = 14.7 years. Breeds represented included Quarter Horse (eight), Arabian (five), Thoroughbred (three), and one each of Connemara, Tennessee Walking Horse, and Warmblood. Thirty-seven of the 64 animals (16 dogs, seven cats, and 14 horses) had received topically applied antibiotics for a median (range) period of 7 (1-42) days prior to study enrollment. Ulcer size and depth varied widely among patients.

Nineteen dogs, seven cats, and 11 horses (37 animals) were randomized to receive proparacaine, and 14 dogs, five cats, and eight horses (27 animals) were assigned to receive saline. Following randomization, males and females were equally distributed between the saline and proparacaine groups ($P \leq 0.05$). However, median; range ages (years) of dogs (7.5; 0.33-12), cats (5; 3-10), and horses (10.5; 0.08-20) enrolled in the saline group were each significantly less than median; range age (years) of those enrolled in the proparacaine group (dogs: 9.5; 2-14, cats: 10; 5-15, and horses 17; 1-27; $P = 0.05, 0.02, 0.05$, respectively). Brachycephalic dogs were assigned to the proparacaine group ($n = 16$) or saline group (10) in proportions not significantly different from one another ($P > 0.05$). One brachycephalic cat was assigned to the proparacaine group and 1 to the saline group. During collection of Swab 1 (ie, prior to application of any topical agents), 25 of 26 brachycephalic dogs showed no overt signs of discomfort. The remaining brachycephalic dog showed signs consistent with moderate discomfort. At the same time point, the seven nonbrachycephalic dogs showed signs consistent with no overt discomfort ($n = 2$), moderate discomfort ($n = 2$), or marked discomfort ($n = 3$). The proportion of dogs exhibiting signs of discomfort during collection of Swab 1 tended to be greater ($P = 0.06$) for those with a nonbrachycephalic skull shape than for those considered brachycephalic. Neither of the two brachycephalic cats showed evidence of overt discomfort during collection of Swab 1; however, signs of moderate ($n = 9$) or marked ($n = 1$) discomfort

were noted at this time point in the 10 nonbrachycephalic cats. Due to the low number of cats enrolled, statistical comparison of skull type and perceived pain during collection of Swab 1 was not possible.

Considering data from dogs, cats, and horses collectively, no organisms were ever cultured from Swab 3, suggesting that differences between culture results of Swabs 1 and 2 were not due to contamination of the proparacaine or saline aliquots. By contrast, a broad diversity of bacteria was isolated from Swabs 1 and 2 in all 3 species (Figure 1). Considering Swabs 1 and 2 collectively, a single *Staphylococcus* species was isolated on 18 occasions from nine dogs, seven cats, and two horses, a single *Streptococcus* spp. was isolated on 16 occasions from five dogs, two cats, and one horse, and two *Streptococcus* spp. were isolated from one dog. A single nonenteric spp. was cultured

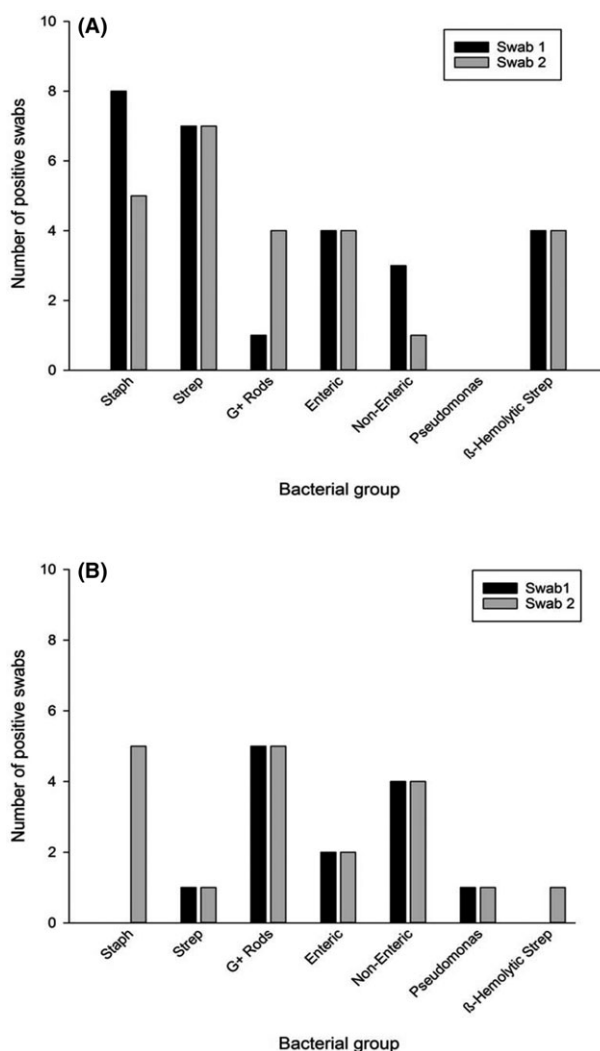


FIGURE 1 Diversity of bacterial species cultured before (Swab 1) or after (Swab 2) corneal application of proparacaine (A) or saline (B) in 33 dogs, 12 cats, and 19 horses with spontaneously arising ulcerative keratitis. All bacteria identified were assigned to one of 7 groups.

on 12 occasions in six dogs, two cats, and one horse; no individual had more than one nonenteric bacterial species isolated. Gram-positive rods were isolated on 10 occasions from three dogs, two cats, and two horses. Two or three species of Gram-positive rods were isolated from one cat and one horse, respectively. A single enteric bacterial species was isolated on six occasions from three dogs; three enteric species were isolated from two dogs each. Enteric bacterial species were never isolated from cats or horses. A single beta-hemolytic *Streptococcus* spp. was isolated on nine occasions from two dogs and three horses, and a single *Pseudomonas* spp. was isolated on two occasions from one horse. *Enterococcus* spp. were not isolated from any animals in this study. Due to low numbers within each bacterial group, statistical analysis of differences among bacterial species isolated from dogs, cats, and horses was not possible.

Bacterial culture results for Swabs 1 and 2 for proparacaine or saline are presented in Table 1. Topical application of proparacaine did not significantly alter the frequency of positive bacterial cultures in dogs, cats, or horses when each species (patient) was considered individually ($P = 0.39-1.00$), or when all species were considered jointly ($P = 0.49$). However, analysis of individual patients in which culture results of Swabs 1 and 2 did differ revealed some findings of potential clinical importance. Specifically, a *Staphylococcus* species was cultured before but not after topical application of proparacaine in one horse, one dog, and one cat; however, none of the *Staphylococcus* spp. were resistant to any antibiotic tested. Likewise, a nonenteric species was no longer detectable by culture following topical application of proparacaine in one horse and one dog. By contrast, cultures became positive in one eye following application of proparacaine. Analysis of the diversity of bacteria cultured before and after application of proparacaine was performed by assigning all identified bacteria to one of seven groups (Figure 1A).

Topical application of saline did not significantly alter the frequency of positive bacterial cultures in dogs, cats, or

TABLE 1 Frequency of positive corneal bacterial culture results in 33 dogs, 12 cats, and 19 horses with spontaneously arising ulcerative keratitis. Cultures were performed on corneal swabs collected prior to (Swab 1) or following (Swab 2) application of proparacaine or saline (control).

	Proparacaine			Saline		
	Swab 1	Swab 2	P-value	Swab 1	Swab 2	P-value
Dogs	11/19	11/19	0.74	6/14	6/14	0.70
Cats	3/7	2/7	1.00	1/5	3/5	0.52
Horses	7/11	4/11	0.39	3/8	3/8	1.00
Total	21/37	17/37	0.49	10/27	12/27	0.78

horses when each species was considered individually ($P = 0.52-1.00$), or when all species were considered jointly ($P = 0.78$). Although the bacterial species cultured before and after topical application of saline differed in some patients, culture results did not become negative after application of saline in any dog, cat, or horse. By contrast, cultures became positive in 6 eyes following application of saline. *Staphylococcus* spp. were isolated after application of saline in two dogs, one horse, and two cats, and beta-hemolytic *Streptococcus* sp. was isolated after application of proparacaine in one horse. Analysis of the diversity of bacteria isolated before and after application of saline was performed by assigning all identified bacteria into one of seven broad groups (Figure 1B).

Fungal species were isolated from five swabs collected from three horses; in each case, only one fungal species was isolated per swab. One *Aspergillus* spp. was cultured on both Swabs 1 and 2 from one horse receiving saline and one receiving proparacaine. A *Mucor* sp. was cultured on Swab 1 but not Swab 2 for the third horse, which was in the proparacaine group. As a result of the low number of positive fungal cultures, no statistical comparisons were possible. Coinfection with bacteria and fungus was not detected in any horse in this study.

Considering patients of all species and from both treatment groups jointly, bacteria were detected significantly less often by use of cytologic examination (19 animals) than by culture of Swab 1 (31 animals; $P = 0.03$) or Swab

TABLE 2 Concordance between results of cytologic assessment (all species) and bacterial (all species) or fungal (horses only) culture in 33 dogs, 12 cats, and 19 horses with spontaneously arising ulcerative keratitis.

		Organism detected by culture	
		Yes	No
Organism detected cytologically	Yes	Dog: 12	Dog: 1
		Cat: 2	Cat: 0
		Horse (bacteria): 3	Horse (bacteria): 0
		Horse (fungus): 2	Horse (fungus): 2
	No	Dog: 9	Dog: 11
		Cat: 4	Cat: 6
		Horse (bacteria): 7	Horse (bacteria): 9
		Horse (fungus): 1	Horse (fungus): 14

2 (29 animals; $P = 0.005$) (Table 2). By contrast, considering both treatment groups jointly, a significant difference was not detected in the number of horses from which fungi were detected cytologically ($n = 4$) or by culture of Swab 1 (three horses; $P = 0.69$), or Swab 2 (two horses; $P = 0.22$). Considering data from Swabs 1 and 2 jointly for all species, bacteria were detected cytologically in 12 of 19 animals receiving proparacaine and 7/19 of animals receiving saline. Fungi were detected cytologically in only four of 19 horses; fungus could not be isolated from either swab in 2 of these—one that received proparacaine and one that received saline, and was detected in both Swabs 1 and 2 in the remaining 2.

4 | DISCUSSION

This study describes the effect of proparacaine on bacterial and fungal isolation rates in 33 dogs, 12 cats, and 19 horses. Limitations of this study included the lack of age-matched controls resulting in an older group of animals assigned to the proparacaine group. While older animals may have an increased risk for developing degenerative disorders of the cornea that can lead to ulceration, age should not affect a topical solution's intrinsic ability to retard growth of a cultured organism. Results of the current study indicate that a topical anesthetic, 0.5% proparacaine, does not exert a statistically detectable antibacterial effect on bacterial culture results of corneal swabs collected from canine, feline, or equine patients with spontaneously arising ulcerative keratitis. While a bacteriostatic/bactericidal effect of proparacaine was not detected when considering all bacterial species and all three host species collectively, consideration of individual patients revealed fewer patients in which *Staphylococcus* spp. were isolated following application of proparacaine but not following application of saline. This finding is in concordance with results from a study in humans where topically applied 0.5% proparacaine strongly inhibited the growth of *S. aureus* (as well as *P. aeruginosa*).² A similar effect was not seen in the present study for *Pseudomonas* spp.; however, this species was isolated from both Swab 1 and Swab 2 in only one horse in the saline group. In another study,⁷ the number of human eyes from which conjunctival cultures yielded bacteria was reduced from 76 to 4 after proparacaine was used. This effect was not seen in the present study, in which results are in broad agreement with those from a veterinary study which reported minimal effects of a topical anesthetic on corneal culture results.³

Benzalkonium chloride is a commonly used preservative in many ophthalmic preparations, including topical proparacaine. Its disinfectant properties⁸ are alleged to be one reason

topical anesthetics might be expected to alter culture results. However, an in vitro study revealed that preservative-free proxymetacaine reduced growth of *Staphylococcus epidermidis* and that preservative-free 1% tetracaine along with 4% oxybuprocaine-inhibited *S. epidermidis*, *S. aureus*, *P. aeruginosa*, and *Streptococcus pneumoniae* growth.⁹ Therefore, it appears that preservatives may not be the sole cause for inhibition; and that, the anesthetic agents themselves may exert a direct antibacterial action. For example, topical anesthetics, specifically ion channel blockers like proparacaine, decrease bacterial growth through at least two proposed mechanisms.¹⁰ First, these compounds inhibit molecules required for bacterial respiratory chain reactions and energy production for efflux activity. Second, treatment with ion channel blockers increases phagosome acidification and induces phagosomal hydrolase expression within the host cell, thereby restricting bacterial growth.¹⁰ Results from studies assessing human samples⁹ and results from the present study suggest that proparacaine may have an inhibitory effect on specific bacterial species, particularly *Staphylococcus* spp.

While data from the present study suggested that proparacaine did not inhibit bacterial growth in a statistically significant manner, it is important also to consider whether any clinically significant alterations arose. *Staphylococcus* spp. could no longer be cultured after application of proparacaine in one horse, one dog, and one cat in the present study. All three of these bacterial isolates were susceptible to all tested antibiotics, and all of these three patients had an epithelialized wound and no evidence of infiltrate within 25 days of topical treatment with antimicrobials. While waiting for finalized culture and susceptibility results in patients with suspected bacterial keratitis, it is the authors' practice to institute topical therapy with two topical antibiotics selected to provide broad-spectrum bactericidal activity against known corneal pathogens. Typically, this would include cefazolin (a first-generation cephalosporin, with presumptive efficacy against many Gram-positive organisms and anaerobes), and a second-generation fluoroquinolone with presumptive activity against many Gram-negative and aerobic corneal pathogens. It is possible but unlikely that staphylococci would be resistant to this combination of medications; therefore, management of these patients would likely not be altered, even in the face of a negative culture. However, methicillin-resistant *Staphylococcus* was not isolated from any animal in the present series. With a larger population, it is possible that a resistant organism may have been isolated and that failure to identify this organism due to prior use of proparacaine could have affected the management of such an individual. One study revealed that 21 of 71 (24%) of cultures obtained from dogs with bacterial keratitis detected methicillin-resistant *Staphylococcus* spp. and that

≥80% of these resistant isolates were susceptible to only amikacin, chloramphenicol, and gentamicin.¹¹ In another study,¹² 49 isolates of *Staphylococcus pseudintermedius* that were resistant to oxacillin (n = 16) and were *mecA*-positive were also resistant to ciprofloxacin (40.8%), ofloxacin (38.8%), enrofloxacin (38.8%), levofloxacin (34.7%), or moxifloxacin (4.1%).

The apparent differences in the species of bacterial organisms isolated after proparacaine was placed on the ocular surface were not assessed statistically and so should be interpreted cautiously. This apparently selective inhibitory effect of proparacaine on streptococci spp. is in agreement with an in vitro study comparing zones of inhibition between proparacaine, tetracaine, and cocaine.² It is possible that *Staphylococcus* spp. may be more susceptible than are other bacteria to the preservative or the described mechanisms of ion channel blockers.

In the present study, three groups of organisms were isolated in greater numbers after application of saline or proparacaine than before instillation of these solutions. Gram-positive rods were isolated from a greater number of corneas after application of proparacaine, and *Staphylococcus* and beta-hemolytic *Streptococci* spp. were isolated from a greater number of corneas after application of saline. It is conceivable that the topically applied solution “floated” bacteria from the corneal surface, leading to an increase in positive cultures. However, it is unclear why this phenomenon would be seen in certain bacterial species vs others. Perhaps bacterial mass, adherence factors, or tropism for epithelium may play a role. It is also likely that these reflect statistically nonsignificant changes resulting from the low numbers compared.

Low numbers prevented statistical analysis of the effect of proparacaine on fungal isolation, but only one fungal species was isolated per swab. Based on the very low numbers reported herein, it is possible that proparacaine may inhibit fungal isolation as *Mucor* spp. became nonculturable after application of proparacaine in one horse; however, *Aspergillus* spp. remained culturable after application of saline or proparacaine in one horse each. None of the horses in this study had coinfections with both bacterial and fungal organisms, which is not consistent with findings that report mixed bacterial and fungal infections in as many as 20%-33% of horses.¹³⁻¹⁵

Microbial culture and cytologic evaluation, when used together, maximize identification of causative pathogens, especially in animals receiving antimicrobial treatment;¹⁶ therefore, both diagnostic tests should be used to tailor treatment and reduce risk of vision impairment in animals. This is supported by data from the current study, in which culture and cytology results complemented each other, although cytology was positive more often than culture, detecting corneal fungal organisms, with 15% and 10% of

horses having a positive fungal culture before and after application of either proparacaine or saline, while 25% of horses were positive for fungus cytologically. While these results may reflect the low numbers we report, it is also possible that fungal organisms may exfoliate more readily than do bacterial organisms, further supporting that culture and cytology should be used as complementary tests.

Finally, in our study population, 26 of 33 canine patients were brachycephalic. Brachycephalic dogs have exophthalmos and lagophthalmos, as well as decreased corneal sensitivity,¹⁷ all of which likely make them more susceptible to corneal ulceration. It is therefore not surprising that brachycephalic breeds formed the majority of canine patients in our study.

In conclusion, data from the current study indicate that proparacaine does not significantly affect isolation rates of bacteria and, given its anesthetic effects, the authors recommend its use before obtaining corneal culture samples to help ensure patient comfort and aid sampling. Corneal culture and cytology should be used together to maximize diagnostic yield, and corneal cytology may be more likely to reflect a positive test result in the case of fungal infections compared with culture in the horse.

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