Corneal sequestrum in a dog with chronic unilateral keratoconjunctivitis sicca

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**Case Description**—A 14-year-old 8.2-kg (18.04-lb) castrated male Cairn Terrier with chronic keratoconjunctivitis sicca in the left eye was evaluated because of severe blepharospasm and a black plaque of 3 weeks’ duration.

**Clinical Findings**—Abnormalities of the left eye included a decreased Schirmer tear test value and the presence of a brownish-black plaque in the center of the cornea. The plaque was surrounded by fibrovascular tissue except at the medial aspect where there was mild malacia of the adjacent corneal stroma.

**Treatment and Outcome**—The plaque was removed by superficial keratectomy, and a conjunctival graft was performed. Histologic evaluation of the plaque and surrounding cornea revealed ulceration, stromal necrosis, and chronic supplicative keratitis with fibrosis and neovascularization. Evaluation of plaque sections that were stained with Gram and Von-Kossa stains yielded negative results for bacteria and mineralization, respectively; examination of sections stained with periodic acid–Schiff stain revealed multiple intracytoplasmic inclusions in macrophages. Virus isolation and a PCR assay for canine herpesvirus yielded negative results. Transmission electron microscopy revealed collagen disruption with interspersed macrophages and apoptotic keratocytes; no viral particles or evidence of other infectious agents was observed. The graft healed without complication and was trimmed 2 weeks after surgery. Four months after surgery, the Schirmer tear test value remained decreased from reference limits despite topical tacrolimus treatment, and pigmentary keratopathy was present surrounding the graft.

**Clinical Relevance**—Corneal sequestra are rare in species other than cats. In this dog, it was possible that chronic keratoconjunctivitis sicca might have contributed to the development of the corneal sequestrum. (*J Am Vet Med Assoc* 2013;243:1751–1755)

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A 14-year-old 8.2-kg (18.04-lb) castrated male Cairn Terrier was examined by the ophthalmology service at the Foster Hospital for Small Animals at the Cummings School of Veterinary Medicine at Tufts University because of blepharospasm and a brownish-black plaque in the left eye along with severe bilateral masticatory muscle atrophy that was noticed this week by the referring veterinarian. The patient had a history of KCS in the left eye of unknown duration, which had been sporadically treated with topical administration of 0.2% cyclosporine and diffusely edematous. The menace response was normal in the right eye and absent in the left eye. Palpebral, dazzle, and pupillary light reflexes were normal in both eyes. The Schirmer tear test value was 18 mm/60 seconds (reference limits, 18.64 ± 4.47 mm/60 seconds) for the right eye and 12 mm/60 seconds for the left eye. Applanation tonometry results were 18 and 20 mm Hg (reference limits, 18.7 ± 5.5 mm Hg) for the right and left eyes, respectively. The right eye had a pulverulent nuclear cataract and nuclear sclerosis. The fundic examination of the right eye was unremarkable. Detailed intraocular examination of the left eye was limited because of the corneal plaque and edema.

Results for the remainder of the physical examination were unremarkable except for the severe bilateral...

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**ABBREVIATIONS**

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>KCS</td>
<td>Keratoconjunctivitis sicca</td>
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<td>PAS</td>
<td>Periodic acid–Schiff</td>
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<td>TEM</td>
<td>Transmission electron microscopy</td>
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masticatory muscle atrophy. The range of motion of the temporomandibular joints was considered normal. Results of a CBC, serum biochemical analysis, and urinalysis were all within reference limits. Results for evaluation of thoracic radiographs were unremarkable.

The patient was anesthetized, and a superficial lamellar keratectomy, which included approximately one third to one half of the corneal depth, was performed on the left eye to remove the plaque. Tissue specimens were submitted for bacterial and fungal cultures, canine herpesvirus isolation and PCR assay, histologic evaluation, and TEM. A bulbar conjunctival hood graft was then performed on the left eye to facilitate corneal healing. The dog recovered from anesthesia uneventfully.

Postsurgical treatment consisted of topical administration in the left eye of 0.3% tobramycin solution (q 6 h), 1% tropicamide solution (q 8 h), 0.2% cyclosporine ointment (q 12 h), and hyaluronic acid ophthalmic drops (q 6 h) oral administration of carprofene (2.2 mg/kg [1 mg/lb], q 12 h for 4 days) and doxycycline (100 mg, q 24 h for 10 d). One week after surgery, topical administration of 0.02% compounded tacrolimus in corn oil (q 6 h) and oral administration of carprofene (2.2 mg/kg [1 mg/lb], q 12 h for 4 days) and doxycycline (100 mg, q 24 h for 10 d). One week after surgery, topical administration of 0.02% compounded tacrolimus in corn oil (q 6 h) was initiated and topical administration of cyclosporine and tropicamide was discontinued. Bacterial culture of the plaque specimen resulted in growth of Streptococcus hemolyticus, which was resistant to amino-glycosides and susceptible to erythromycin; thus, topical administration of tobramycin was discontinued and topical administration of erythromycin ointment (q 8 h for 3 weeks) was initiated.

Two weeks after surgery, the bulbar conjunctival hood graft was trimmed. The patient was reexamined 4 months after surgery. The graft had completely healed; it was locally surrounded by pigmentary keratopathy and there was no evidence of recurrence of the plaque (Figure 1). The left eye had regained a menace response, although it was subjectively considered decreased. The Schirmer tear test value for the left eye was 5 mm/60 seconds. The tacrolimus and hyaluronic acid drops had been administered in the left eye throughout the 4-month follow-up period, and their continued administration was recommended for the life of the patient. After this examination, the patient was lost to follow-up.

Histologic examination of the excised tissues revealed that the surface of the plaque was composed of hyalinized, laminated, acellular material (approx 150 µm thick), which was consistent with exposed and desiccated stromal collagen (Figure 2). In the tissue sections examined, the corneal epithelium was entirely absent. The subjacent corneal stroma contained neutrophils, pyknotic nuclear debris, and macrophages with variable amounts of PAS-positive cytoplasmic inclusions (Figure 3). Numerous prominent immature capillaries lined by plump endothelial cells and fibrosis replaced the normal corneal stroma. Evaluation of Von Kossa-, PAS-, and Gram-stained tissue sections revealed no evidence of mineralization, fungal organisms, or bacteria, respectively. Evaluation of tissue sections with TEM revealed macrophages separated by disordered collagen fibrils, a paucity of keratocytes, and regions of electron-dense material considered to be cellular remnants (Figure 4). Keratocytes were characterized by a shrunken cellular morphology and multiple intracytoplasmic vacuoles, consistent with apoptosis. Virus isolation and PCR assay for canine herpesvirus yielded negative results. Fungal culture also yielded negative results. The histologic diagnosis was a corneal sequestrum.
Historically, corneal sequestra were considered unique to cats. However, since 1994, there have been multiple case reports that describe corneal sequestra in horses and 1 report that describes a dog with a corneal sequestrum, which was associated with an eyelid neoplasm. The dog of the present report had a history of chronic KCS prior to the diagnosis of the corneal sequestrum.

In cats, corneal sequestra are characterized as ill-defined or discrete focal light brown to black plaques within the corneal stroma, which usually extend to the corneal surface where they may be encircled by an area of superficial corneal ulceration or fibrovascular reaction. These plaques represent a focal area of stromal collagen degeneration and necrosis. Corneal vascularization, corneal edema, and WBC infiltration into the underlying stromal tissue are clinicopathologic findings often associated with corneal sequestra. Signs of pain are often associated with corneal sequestra, and affected cats typically have varying degrees of blepharospasm, epiphora, and photophobia. Over time, most sequestrum plaques will spontaneously slough from the cornea. However, because of the unpredictable length of time required for a corneal sequestrum to slough from the cornea, surgical removal of the sequestrum plaque by keratectomy alone or keratectomy followed by one of various grafting procedures is the preferred method of treatment to alleviate the discomfort of affected animals. Also, although rare, if the sequestrum is thick, allowing it to slough naturally from the cornea may result in a ruptured globe; therefore, surgery is recommended for a more favorable and predictable outcome.

Light microscopy and TEM have been used to evaluate the ultrastructural composition of corneal sequestra in cats. Characteristics of these lesions include ulceration of the axial area of the corneal epithelium with attenuated or abnormal peripheral epithelial cells that extend to or partially cover the edge of the sequestra. The surfaces of sequestra consist of hyalinized acellular material consistent with exposed and desiccated stromal collagen, remnants of dead keratocytes, and disarranged collagen fibrils. In some keratocytes, the chromatin is clumped and marginated and the cytoplasm is shrunken, which is characteristic of apoptosis. Infiltration by various inflammatory cells, including neutrophils, monocytes, macrophages, lymphocytes, and plasma cells, is frequently observed at the plaque periphery and beneath areas of stromal necrosis are fibroblasts and endothelial cells, which are associated with fibrosis and neovascularization.

In dogs, dematiaceous fungi can infect the cornea and cause a centralized black lesion with peripheral edema and superficial vascularization, which resembles a corneal sequestrum clinically. However, the dark color of those lesions is caused by pigmentation of fungal hyphae, and those lesions can be easily differentiated from a corneal sequestrum histologically because of the presence of diffuse septated fungal organisms that infiltrate the corneal stroma. One of the authors (SP) of the present report has occasionally observed anterior axial corneal degeneration and necrosis in dogs with buphthalmia or following implantation of intraocular prostheses. In dogs with corneal sequestra, brown discoloration of the cornea is considered rare.

The pathogenesis of corneal sequestra in cats has not been elucidated, although corneal sequestra have been associated with several other corneal disorders including chronic irritation and ulceration, trauma, exp-
Sure keratopathy, and feline herpesvirus infection, and as a sequel to treatment with topical corticosteroids or grid keratotomy. However, the cause of a corneal sequestrum is often defined as idiopathic, and some breeds such as Persians, Colorpoints, Himalayans, Siamese, and American Shorthair seem to be predisposed to the condition. Histologic evaluation of the corneal sequestrum excised from the dog of this report revealed findings similar to those observed in corneal sequestra of cats, which consisted of a superficial plaque composed of denatured collagen with evidence of noninflammatory kerocyte apoptosis, subjacent chronic active keratitis, neovascularization, and fibrosis. For the dog of this report, no evidence of a bacterial, viral, or fungal pathogen was detected as the cause of the corneal sequestrum. The number of S. hemolyticus colonies that grew during bacterial culture was low, and the presence of this organism was considered a superficial opportunistic infection. Within the corneal stroma, there was a severe pyogranulomatous reaction and necrosis characterized by macrophages with variable amounts of PAS-positive cytoplasmic inclusions, which could have been phagocytized cellular debris. When tissue specimens were stained with Von Kossa stain, no evidence of mineralization was detected in the macrophage inclusions or the surrounding tissues. This finding was unique because in dogs, corneal stromal degeneration and sequestration is generally associated with mineralization.

Interestingly, many of the breeds of cats that are predisposed to the development of corneal sequestra are brachycephalic and lagophthalmic and they generally develop corneal sequestra bilaterally, which is hypothesized to be the result of tear film abnormalities. In brachycephalic cats, sensitivity of the axial area of the cornea is significantly lower than that in domestic shorthair cats, although age and sex differences have been reported. Decreased corneal sensitivity can result in a blinking rate that is lower than usual and dryness of the axial area of the cornea because of tear evaporation or changes in tissue homeostasis. However, results of another study indicate that there is no significant difference in the number of conjunctival goblet cells, qualitative tear film abnormalities, and tear film break-up time between cats with and without corneal sequestra.

The dog of the present report had bilateral masticatory muscle atrophy, which was believed to be the result of its advanced age and was not investigated further. It is possible that dysfunction of the trigeminal nerve may have caused the masticatory muscle atrophy as well as decreased corneal sensitivity. Corneal aesthesiometry was not performed in this dog, and the contralateral eye did not have any corneal abnormalities, so trigeminal nerve dysfunction was considered unlikely in this case.

Herpesvirus infection has been associated with necrotic keratitis in humans and cats, although there are conflicting results regarding the quantity of herpesvirus DNA detected between cats with and without corneal sequestra. Keratitis associated with canine herpesvirus infection is rare and has only been described in 2 dogs, neither of which had necrotic keratitis.

For the dog of this report, we hypothesize that the corneal sequestrum was associated with chronic KCS in the affected eye, although, to our knowledge, this would be the first such case, despite the fact that many dogs are affected by KCS. A corneal sequestrum and KCS have been concurrently diagnosed in a horse. In contrast to the cat breeds that are predisposed to developing corneal sequestra, the affected eye of the dog in this report was enophthalmic; however, the tear film was abnormal and the corneal area involved was band shaped, which was suggestive of exposure keratitis or keratopathy. We postulate that multiple factors (such as chronic irritation, dryness, age, decreased amount or rate of blinking) might have contributed to the development of the sequestrum. The investigators of another case report of a dog with a corneal sequestrum hypothesized that the sequestrum was the result of chronic superficial corneal erosion and irritation caused by a palpebral mass.

The superficial keratectomy performed on the dog of this report resulted in the removal of the sequestrum and approximately one-third to one-half of the anterior corneal stroma. Because of the relatively large size of the corneal defect, a bulbar conjunctival hood graft was performed to provide tectonic and vascular support. Conjunctival grafts or corneoconjunctival transposition often facilitates successful outcomes following radical keratectomy. However, the rate of corneal sequestrum recurrence did not differ between cats that had only a superficial keratectomy performed and cats that had a superficial keratectomy performed followed by a conjunctival graft. Additionally, none of the cats with corneal sequestra that had a corneoconjunctival transposition or complete excision of the affected cornea performed a recurrence of the lesion. Similarly, the dog of this report had a successful outcome without recurrence of the corneal sequestrum. At the last examination 4 months after the keratectomy and conjunctival graft, the corneal defect of the affected eye was covered with graft and fibrotic tissue, the surrounding cornea was transparent, and the menace response in that eye had returned. To our knowledge, this is the first report of a corneal sequestrum associated with KCS in a dog. A corneal sequestrum, although rare, should be included as a differential diagnosis for dogs with blepharospasm and brownish-black corneal plaques, ulceration, and edema.

References
