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Comparison of corneal degeneration and calcific band keratopathy from 2000 to 2013 in 69 horses

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Abstract

Objective To compare signalment, presentation, treatment, and outcome in horses diagnosed with corneal degeneration (CD) or calcific band keratopathy (CBK) at a referral hospital.

Animals studied Sixty-nine horses (87 eyes) diagnosed with either CD or CBK.

Procedures Medical records of horses diagnosed with CD or CBK at the University of California-Davis Veterinary Medical Teaching Hospital (UCD-VMTH) between 2000 and 2013 were reviewed. Signalment, concurrent ophthalmic diagnoses, previous therapies, diagnostic tests, systemic diagnoses, treatment, follow-up, and outcomes were compared between horses diagnosed with CD or CBK. Age, breed, and gender were compared between the CD/CBK and UCD-VMTH populations.

Results Thirty-three horses (42 eyes) and 36 horses (45 eyes) were diagnosed with CD and CBK, respectively. Horses with CD or CBK were significantly older ($P < 0.001$) than the UCD-VMTH population with a median age of 16 or 18 years, respectively. Appaloosas were significantly overrepresented in the CD/CBK population (33%) in comparison with the UCD-VMTH population (1.8%, $P < 0.001$). Equine recurrent uveitis was concurrently diagnosed in 67% and 84% of horses with CD or CBK, respectively. Pituitary pars intermedia dysfunction (PPID) was diagnosed significantly less often in horses with CD vs. CBK ($P = 0.03$). Chemical chelation with ethylenediaminetetraacetic acid was performed significantly less frequently in horses diagnosed with CD (7.1%) vs. CBK (31.1% of eyes) ($P = 0.012$).

Conclusions Despite some differences, equine CD and CBK are relatively similar conditions and may represent a continuum of disease severity. Horses with PPID should be monitored closely for corneal disease including CBK.

Key Words: band keratopathy, cornea, degeneration, equine, mineralization, PPID

INTRODUCTION

Mineral and/or lipid deposition in the cornea causes loss of corneal transparency and can result in corneal ulceration with subsequent ocular discomfort. Such deposition is a defining component of at least 2 different disease entities, namely corneal degeneration (CD) and calcific band keratopathy (CBK). In horses and other species, CD and CBK are terms used to describe corneal mineral and/or lipid deposition in association with keratitis. In both instances, calcium hydroxyapatite is deposited immediately underlying or within the anterior basement membrane. With CD, mineral distribution is typically associated with a region of corneal trauma, such as ulceration or thermal injury, or is seen as an age-related change.[1,2] In CD, lipid (often cholesterol) deposition may occur along with or instead of calcium hydroxyapatite.[3] Calcific band keratopathy is a specific type of CD whereby the lesions are distributed in a horizontal band in the interpalpebral fissure and has been described in humans,[4] dogs,[5] cats,[6] rabbits,[7] an alpaca,[8] and horses.[7, 9] Systemic diseases, including hyperadrenocorticism and hypercalcemia secondary to

chronic renal failure, neoplasia, hyperparathyroidism, and hypervitaminosis D, have been associated with the development of CBK in veterinary species.[4–7] Ocular diseases, including chronic uveitis, keratoconjunctivitis sicca, glaucoma, and herpetic keratitis, as well as topical administration of corticosteroids are also thought to contribute to development of CBK in humans and animals.[4–7,9–11]

Patients affected with CD or CBK may be presented with similar clinical signs, including ocular discomfort and corneal ulceration, especially if mineral deposits disrupt epithelial adhesion to the underlying stroma. Additionally, human patients with CD or CBK note vision disturbance including glare.[11] Treatment options for veterinary patients affected with CD or CBK include superficial keratectomy, chemical chelation using 4% ethylenediaminetetraacetic acid (EDTA), diamond burr debridement, frequent topical application of EDTA ophthalmic gel, or some combination of these.[12,13] Additional therapies reported for humans with CD or CBK include phototherapeutic keratectomy, amniotic membrane transplantation, and keratoplasty.[11,14] Whenever possible, any predisposing or concurrent disease should also be treated.[7] However, treatment for mineral deposition often is not sought or initiated until complications, such as nonhealing or recurrent ulcerative keratitis, arise. Enucleation or euthanasia may be considered in refractory cases due to vision loss and/or ocular pain resulting from corneal disease or underlying intraocular pathology, such as equine recurrent uveitis (ERU).

Little is published regarding mineral deposition in the cornea of horses. To the authors' knowledge, the literature is limited to one article from 1993 describing CBK in 21 horses[7] and a review from 2012.[9] Little data exist regarding the definition of equine CD and CBK. Therefore, the purpose of this retrospective study was to compare the signalment, presentation, treatment, and outcome between horses ultimately diagnosed with CD or CBK at a single referral hospital. We hypothesized that CD and CBK are similar conditions in horses and that significant differences in signalment, presentation, treatment, and outcome would not be detected between the two groups.

MATERIALS AND METHODS

Retrospective study

Medical records of horses that were presented to the University of California, Davis William R. Pritchard Veterinary Medical Teaching Hospital (UCD-VMTH) from January 1, 2000 through November 1, 2013 were electronically searched for cases with a clinical diagnosis of CD or CBK. Search terms included 'calcific band keratopathy', 'band keratopathy', 'corneal degeneration', 'corneal mineralization', 'calcareous degeneration', and 'corneal dystrophy'. Following electronic retrieval, each record was individually reviewed by a single author (EHB) to ensure it met inclusion criteria, which included a clinical diagnosis of CD or CBK and a supporting description of corneal opacity with mineral and/or lipid deposition as part of the ophthalmic examination. Patients were placed in either the CD or CBK groups based on clinical diagnosis given and distribution of the mineral opacity described in the ophthalmic examination. Information retrieved from records of horses meeting the inclusion criteria included breed, age, gender, presence of ERU, concurrent ophthalmic diagnoses, prior or current treatment at time of referral and duration, medical treatments instituted, surgical procedures performed, and evidence of systemic disease. Systemic disease was considered present if there was a recent historical diagnosis stated or clinical diagnosis made at the time of presentation based on examination parameters and diagnostics performed. A clinical diagnosis of ERU was made if there were chronic, repeated bouts of uveitis. A single uveitic event secondary to trauma, keratitis, neoplasia, or surgical intervention was classified as nonrecurrent uveitis. Results of corneal cytology and histology were included. Cytologic samples were interpreted by clinical pathology residents and reviewed by diplomates of the American College of Veterinary Pathology (ACVP). Histologic samples were interpreted by a diplomate of the ACVP with expertise in ocular pathology (CMR). Corneal lesion descriptions were recorded, and photographs of the eyes were examined if available. Follow-up visits, time relative to initial examination, and outcomes were recorded.

Statistical analysis

Data for eyes with CD or CBK were compared, specifically: age, gender, breed distribution, presence of ERU, nonrecurrent uveitis, phthisis bulbi, cataract, ulcerative keratitis, squamous cell carcinoma (SCC), presence of pituitary pars intermedia dysfunction (PPID) or other systemic disease, topical use of corticosteroids, atropine, antibiotic, nonsteroidal anti-inflammatory drugs (NSAIDs), cyclosporine, ocular hypotensive medications, or EDTA gel; and whether interventions such as superficial keratectomy, chemical debridement with 4% EDTA, or enucleation were performed. Presence of glaucoma, lens luxation, corneal fibrosis, vitreous degeneration, chorioretinal scarring, or retinal detachment were recorded but not analyzed due to their low prevalence. Logistic regression with robust variance estimation (to account for clustering of eyes within individuals) was performed so as to evaluate associations between eyes affected with CD or CBK and concurrent ophthalmic diagnoses, prior topical therapy, treatment, systemic disease, and signalment (age, breed, gender). Because of the high proportion of Appaloosa horses diagnosed with CBK, horses were assigned to one of two categories for analysis by breed: non-Appaloosa or Appaloosa/Appaloosa cross. For analysis by age, horses were assigned to 1 of 5 categories: <5, 6–10, 11–15, 16–20, and >20 years. Horses were assigned as male or female, without consideration of

neuter status. Presence of PPID and other systemic disorders was evaluated using Fisher's exact test, treating the individual rather than the eye as the unit of observation.

All horses presented to the UCD-VMTH during the same study period as the study population formed the hospital reference population. Proportions of horses categorized by age, breed, or gender were each compared between the study and hospital populations with a chi-square test using an interactive chi-square calculator.[15] Observed to expected (O:E) ratios were determined using an exact Pearson chi-square test or an exact Kruskal–Wallis test for breed or age, respectively. For all analyses, a P value of <0.05 was considered significant. Data are presented as median (range); odds ratios (OR), and 95% confidence intervals (CI), or O:E ratios are shown for trends or significantly different results.

RESULTS

Signalment

Thirty-three horses were diagnosed with CD. Fifteen (45.4%) were affected in the right eye (OD), 9 (27.2%) in the left eye (OS), and 9 (27.2%) in both eyes (OU). Ten (30.3%) of the affected horses were mares, 22 (66.7%) were geldings, and 1 (3%) was a stallion. Median (range) age was 16 (1-27) years. Breeds most commonly affected were Appaloosa/Appaloosa cross (n = 12; 36.3% of horses), quarter horse (n = 8; 24.2%), Arabian/Arabian cross (n = 4; 12.1%), or thoroughbred (n = 3; 9%). Other breeds represented included warmblood/warmblood cross (n = 2), and mule, paint, Friesian, and a pony of unspecified breed (n = 1 each). Thirty-six horses were diagnosed with CBK. Fifteen (41.7%) were affected OD, 12 (33.3%) OS, and 9 (25%) OU. Fourteen (38.9%) of the affected horses were mares, and 22 (61.1%) were geldings. The median (range) age of horses affected with CBK was 18 (9–33) years. Breeds most commonly affected with CBK included Appaloosa/Appaloosa cross (n = 11; 30.6%), Arabian/Arabian cross (n = 8; 22.2%), quarter horse (n = 5; 13.9%), and warmblood/warmblood cross (n = 3; 8.3%). Other breeds affected were Pony of the Americas (n = 2), thoroughbred (n = 3), and Friesian, Icelandic pony, Clydesdale, and saddlebred (n = 1 each). A significant difference in breed (P = 0.459), gender (P = 0.645), or eye affected (P = 0.125) was not detected between the population with CD and those with CBK.

A significant difference between the proportion of males and females within the combined (CD and CBK) study population and the general equine population presented to the UCD-VMTH during the same time period was not detected (P = 0.16). Appaloosas comprised a significantly greater percentage of the combined CD and CBK study populations (33%) than the UCD-VMTH reference population (1.8%, P < 0.001) with an O:E ratio of 18. Although CD and CBK populations were each primarily composed of animals >15 years old, horses with CD tended to be younger than patients with CBK (P = 0.054; OR 3.02; CI 0.98–9.27). Horses within the combined CD and CBK study population were older than the reference UCD-VMTH equine population (P < 0.001; Fig. 1). Specifically, horses between 0 and 5 years of age were significantly underrepresented in the CD/CBK population, vs. the UCD-VMTH population (P < 0.05, O:E ratio = 0.081). By contrast, horses between 16 and 20 years of age or >20 years of age were significantly overrepresented in the CD/CBK population vs. the UCD-VMTH population (P < 0.05) with O:E ratios of 2.34 and 3.81, respectively.

Ocular disease

Other ophthalmic lesions noted at presentation included equine recurrent uveitis (ERU), nonrecurrent uveitis, cataracts, glaucoma, lens luxation, vitreous degeneration, phthisis bulbi, chorioretinal scarring, and retinal detachment (Table 1). However, complete examination of the anterior and posterior segments of the eye was obscured by corneal edema or infiltrate in 6 eyes diagnosed with CD and 4 eyes with CBK, and fundic examinations were not performed due to anterior segment and lenticular changes in 10 eyes diagnosed with CD and 19 with CBK. The prevalence of concurrent ophthalmic diagnoses did not differ between horses diagnosed with CD and those diagnosed with CBK; however, ERU tended to be diagnosed less commonly in horses with CD than in those with CBK (P = 0.071; OR 0.33; CI 0.10–1.10). All Appaloosa/Appaloosa crosses, independent of CD or CBK status, were concurrently diagnosed with ERU. Concurrent corneal findings noted during the presenting visit included ulcerative keratitis (Fig. 2), corneal fibrosis, or SCC (Table 2). Local strontium radiation therapy for suspected SCC occurred 7 months prior to diagnosis of CD in 1 eye. A corneoconjunctival mast cell tumor was excised

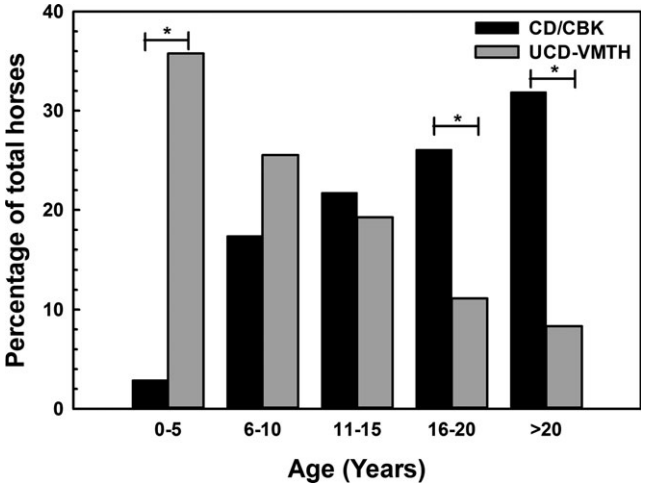

Figure 1. Horses diagnosed with corneal degeneration (CD) or calcific band keratopathy (CBK) were significantly older than the equine population at the UCD-VMTH during the same time period. *P < 0.05, exact Kruskal–Wallis test.

Table 1. Equine recurrent uveitis was commonly diagnosed concurrently in patients with corneal degeneration (CD) or calcific band keratopathy (CBK). Additional ophthalmic pathologies affecting eyes diagnosed during the same visit with CD or CBK, including changes commonly associated with chronic uveitis as well as corneal pathology. No significant differences between the two groups were noted for any concurrent ophthalmic pathologies as determined by logistic regression.

	Number of eyes (%)				
Concurrent ophthalmic diagnoses	CD (n = 42)	CBK (n = 45)	P-value	OR	CI
ERU	28 (66.7%)	38 (84.4%)	0.071	0.33	0.1–1.10
Nonrecurrent uveitis	0 (0%)	3 (6.7%)	N/A	N/A	N/A
Cataracts	14 (33.3%)	26 (57.8%)	0.107	N/A	N/A
Glaucoma	4 (9.5%)	7 (15.6%)	N/A	N/A	N/A
Lens luxation	3 (7.1%)	2 (4.4%)	N/A	N/A	N/A
Vitreous degeneration	4 (9.5%)	1 (2.2%)	N/A	N/A	N/A
Phthisis bulbi	3 (7.1%)	3 (6.7%)	0.944	N/A	N/A
Chorioretinal scar	3 (7.1%)	2 (4.4%)	N/A	N/A	N/A
Retinal detachment	0 (0%)	1 (2.2%)	N/A	N/A	N/A
Ulcerative keratitis	17 (40.5%)	26 (57.8%)	0.163	N/A	N/A
Corneal fibrosis	5 (11.9%)	0 (0%)	N/A	N/A	N/A
SCC	2 (4.8%)	0 (0%)	N/A	N/A	N/A

ERU = equine recurrent uveitis; SCC = squamous cell carcinoma; N/A = not assessed; OR = odds ratio; CI = 95% confidence interval; reported for close to significantly different values.

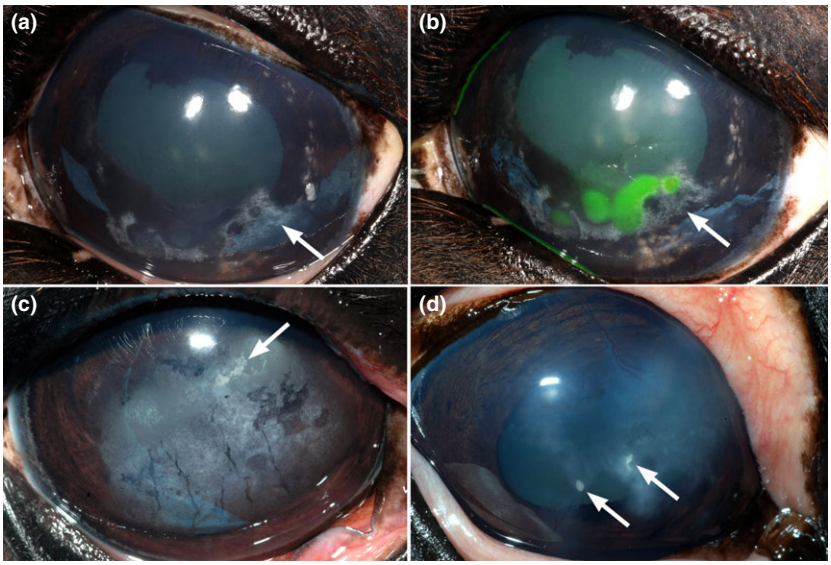

Figure 2. Severity of lesions due to corneal degeneration (CD) or calcific band keratopathy (CBK) varied among horses. (a & b) External photographs of the left eye of a 25-year-old quarter horse gelding with CBK and concurrent ERU revealing moderate mineral deposition within the anterior ventral cornea (a; arrow), and corneal fluorescein stain retention indicating ulceration in the regions of mineral deposition (b). (c) External photographs of the right eye of a 22-year-old Arabian gelding with CBK and a concurrent mature cataract and lens-induced uveitis revealing extensive diffuse corneal mineralization with a focal region of increased mineral density (arrow). (d) External photograph of the left eye of a 20-year-old quarter horse gelding with CD. Note the diffuse corneal edema with focal areas of lipid/mineral (arrows).

5 years prior to diagnosis of CBK in 1 eye of another horse.

Histologic and cytologic examination results

Histology was performed on 4 eyes diagnosed with CD; samples assessed included corneal biopsy (n = 1) or the enucleated globe (n = 3). Neither mineral nor lipid was definitively identified in any sample. The 3 enucleated globes had marked axial stromal malacia (n = 2) or chronic keratitis with stromal fibrosis (n = 1). The corneal sample obtained by superficial keratectomy revealed limbal SCC. Two eyes had been fixed in Bouin's fixative, and, when reviewed, 1 of these had histologic changes consistent with mineral, but the presence of mineral was not confirmed when these sections were stained with Von Kossa stain. Four horses with CD had cytology performed. Mineral or lipid was not identified in any sample; cytologic diagnoses were epithelial hyperplasia or

Table 2. Topical corticosteroids were frequently applied to eyes prior to diagnosis of corneal degeneration (CD) or calcific band keratopathy (CBK) in equine patients. Topical medications applied to eyes at the time of diagnosis of CD and CBK. No significant differences in topical treatment frequency were identified between the two groups as determined by logistic regression.

	Number of eyes (%)				
Topically applied medication	CD (n = 42)	CBK (n = 45)	P-value	OR	CI
Corticosteroid	19 (45.2%)	36 (80%)	0.059	0.34	0.11–1.04
Atropine	18 (42.9%)	30 (66.7%)	0.103	N/A	N/A
Antibiotic	22 (52.4%)	19 (42.2%)	0.292	N/A	N/A
NSAIDS	4 (9.5%)	4 (8.9%)	0.868	N/A	N/A
Cyclosporine	2 (4.7%)	3 (6.7%)	0.803	N/A	N/A
Ocular hypotensive agent	3 (7.1%)	3 (6.7%)	0.964	N/A	N/A

OR = odds ratio; CI = 95% confidence interval; N/A = not applicable.

reactivity (n = 3), purulent exudate (n = 1), or marked squamous cell proliferation suspicious for SCC (n = 1; SCC was confirmed histologically in this eye).

Histology was performed on 10 eyes diagnosed with CBK; samples assessed included corneal biopsy (n = 1) or the enucleated globe (n = 9). Corneal mineral deposition was confirmed in 7/10 (70%) of those sampled. Mineral was present in the corneal anterior basement membrane in all 7 eyes, with extension into the corneal stroma in 5/7 (71%) eyes and corneal epithelium in 1/7 (14%) eyes (Fig. 3). Of the remaining 3 eyes in which CBK was clinically diagnosed but not confirmed histologically, 2 were enucleated globes (1 performed 54 months after successful treatment for CBK with EDTA chelation and topical gel and 1 due to corneal rupture 2.5 months after diagnosis of CBK). The final histologic sample was from an eye diagnosed with CBK 0.5 months after corneoconjunctivectomy for a mast cell tumor. Cytologic examination was performed in 6 eyes with CBK and led to a cytologic diagnosis of normal epithelial cells/superficial sample (n = 2), epithelial hyperplasia (n = 3), or neutrophilic inflammation (n = 1). Mineral was not identified in any cytologic sample.

Topical treatment prior to diagnosis

Nineteen (45.2%) eyes with CD and 36 (80%) eyes with CBK had been topically treated with a corticosteroid prior to referral. Neomycin–polymyxin–dexamethasone (NPD) was the predominant formulation applied (n = 9, CD; n = 24, CBK). Other formulations included prednisolone acetate (n = 1, CBK), dexamethasone (n = 1, CBK), hydrocortisone (n = 2, CD), NPD and betamethasone (n = 1, CBK), NPD and prednisolone acetate (n = 2 CD; n = 1, CBK), bacitracin–neomycin–polymyxin–hydrocortisone and prednisolone acetate (n = 1, CD), NPD and subconjunctival triamcinolone (n = 1, CBK), or an unknown topically applied corticosteroid with subconjunctival triamcinolone (n = 1, CD). There was a trend toward fewer eyes with CD than those with CBK receiving topically administered corticosteroids prior to presentation (P = 0.059; OR 0.33; CI 0.11–1.04). Median (range) known duration of topical treatment with corticosteroids was not statistically different for the 22 horses with CD (4.4; 0.2–72 months) vs. the 30 horses with CBK (12; 0.7–144 months) (P = 0.239). However, the period of corticosteroid treatment was unspecified or intermittent in many horses from each group (CD, n = 10; CBK, n = 14). Other topical therapies used prior to presentation included atropine, antibiotics, NSAIDs, cyclosporine, or ocular hypotensive agents (Table 2); the number of horses receiving these medications did not significantly differ between eyes with CD or CBK (P > 0.05).

Treatment

Superficial keratectomy (SK), EDTA chemical chelation, and topical administration of EDTA gel were evaluated as treatments for eyes diagnosed with CD or CBK (Table 3). Topically administered EDTA gel was used in 6 eyes with CD (n = 2) or CBK (n = 4) for a median (range) period of 7 (6-8) or 1.7 (0.75–5) months, respectively. There was no significant difference in number of eyes with CD or CBK treated with topically administered EDTA gel (P = 0.464). Likewise, there was no significant difference in number of eyes with CD or CBK treated with SK (P = 0.609). However, chemical chelation with 4% EDTA was performed in significantly fewer patients diagnosed with CD than those diagnosed with CBK (P = 0.012; OR 0.17; CI 0.04–0.70). Sixteen (CD, n = 3; CBK, n = 13) eyes underwent EDTA chemical chelation once, and 1 eye with CBK required a second procedure 1.1 months after the first. Chelation procedures were performed under standing sedation in 14 eyes (CD, n = 2; CBK, n = 12); 1 eye with CD and 2 eyes with CBK were treated under general anesthesia for suprachoroidal cyclosporine implantation. Chelation was performed by applying topical anesthetic to the cornea, debriding corneal epithelium overlying the mineral deposits, and then applying cellulose sponge spears soaked in 4% EDTA to the cornea until mineral was visibly chelated and removed. Where reported in the medical records, the application of 4% EDTA took approximately 40–45 min to perform. The procedure

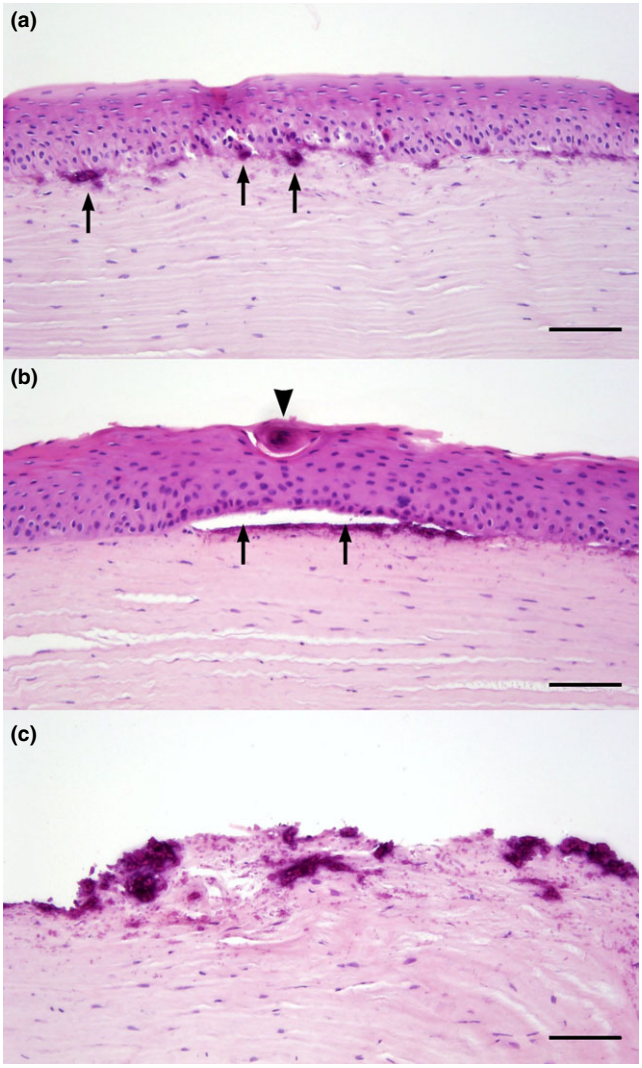

Figure 3. Histologic characteristics of calcific band keratopathy (CBK) in an enucleated eye from a 22-year-old Arabian gelding. Samples were fixed in formalin and stained with hematoxylin and eosin. Arrows show mineral deposition. Scale bars in all images are equivalent to 100 μm. (a) Photograph of mineral at the basement membrane (BM), with no disruption of the corneal epithelium. (b) Photograph of mineral at the basement membrane causing focal detachment of the corneal epithelium from the BM (large arrow) and secondary disorganization of surface corneal epithelium (arrowhead). (c) Photograph of mineral within the anterior stroma with absence of epithelium.

was deemed complete when the remaining corneal surface felt smooth under application of a cotton tipped applicator rather than gritty and when mineral was not apparent when evaluated with slit-lamp biomicroscopy. Two horses diagnosed with CBK underwent a single EDTA chelation followed by topical application of EDTA gel for 1.4 or 2 months, respectively. The affected eye of 1 of these horses was enucleated due to persistent ERU and secondary glaucoma 54 months after the diagnosis of CBK. The second horse was lost to follow-up after the single visit and treatment.

Enucleation tended to be less common in eyes diagnosed with CD ($n = 3$; 7.1%) compared to those diagnosed with CBK ($n = 11$; 24.4%) ($P = 0.074$; OR 0.27; CI 0.06–1.14). The primary reason for enucleation was uveitis with or without secondary glaucoma (CD, $n = 2$; CBK, $n = 11$). However, horses undergoing enucleation also had keratomalacia ($n = 1$; CD), recurrent corneal ulceration ($n = 1$; CBK), or corneal perforation secondary to bacterial keratitis ($n = 1$; CBK). Two eyes had received treatment for CBK approximately 7 or 54 months before enucleation. Median (range) time between diagnosis and enucleation was 0.2 (0.16–0.5) and 0.5 (0–144) months for eyes diagnosed with CD and CBK, respectively; horses with CD were significantly more likely to be enucleated earlier following diagnosis than horses with CBK ($P = 0.019$; OR 1.02; CI 1.00–1.04). No horses with CD were euthanized for ocular disease, whereas 2 horses with CBK were euthanized for reasons pertaining to ocular disease. One horse treated by enucleation in an eye affected with CBK was euthanized following severe uveitis in the contralateral eye, and the second horse with CBK was euthanized due to its age and owner-perceived ocular discomfort.

Systemic disease

Hematologic and/or serum biochemical analysis (SBA) was performed at presentation in 6 horses with CD and 18 horses with CBK. For CD, tests included complete blood count (CBC) and SBA ($n = 4$) or renal panel ($n = 2$). For horses with CBK, tests included CBC and SBA ($n = 6$), CBC and renal panel ($n = 1$), SBA only ($n = 1$), CBC, SBA, and leptospirosis titers ($n = 1$), renal panel only ($n = 5$), creatinine only ($n = 2$), coagulation panel ($n = 1$), or baseline ACTH ($n = 1$). Of the 20 horses evaluated with a SBA or renal panel, none had altered calcium and/or phosphorus concentrations or azotemia. The coagulation panel was performed prior to enucleation for unstated reasons. Seven of 69 horses (10%) in the current study were diagnosed with concurrent systemic disease. Concurrent systemic disease tended to be diagnosed more frequently in horses with CBK ($n = 6$) than those with CD ($n = 1$; $P = 0.051$; OR 0.11; CI 0.01–1.01). Of the 6 horses diagnosed with CBK and concurrent systemic disease, 4 were diagnosed with PPID; horses with CD were significantly less likely to be diagnosed with PPID than were horses with CBK ($P = 0.03$; OR 0.12, CI 0–0.83). Three horses with CBK were diagnosed with PPID using unknown methodology by referring veterinarians prior to presentation. One horse with CBK was diagnosed on the day of presentation using a baseline ACTH concentration. Two of the 4 horses diagnosed with PPID had clinical signs of the disease characterized by hypertrichosis and muscle atrophy; the other 2 that were receiving treatment for PPID did not have evidence of clinical signs recorded. No other horses diagnosed with CD or CBK had baseline ACTH blood work performed at any UCD-VMTH visit.

Table 3. Chelation with ethylenediaminetetraacetic acid (EDTA) was performed significantly less frequently in equine patients with corneal degeneration (CD) vs. calcific band keratopathy (CBK). Treatments administered to horses following diagnosis with CD or CBK. The EDTA chelation, EDTA topical gel, keratectomy, and enucleation percentages are in relation to the number of eyes diagnosed with CD (n = 42) or CBK (n = 45), whereas euthanasia is in relation to the number of horses diagnosed with CD (n = 33) or CBK (n = 36). Frequency of treatments between groups was analyzed using logistic regression.

	Number of eyes (%)				
Treatment	EDTA chelation	EDTA topical gel	Keratectomy	Enucleation	Euthanasia
CD	3 (7.1%)*	2 (4.8%)	0 (0%)	3 (7.1%)†	0/33 (0%)
CBK	14 (31.1%)*	4 (8.9%)	1 (2.2%)	11 (24.4%)†	2/36 (5.6%)

*EDTA = ethylenediaminetetraacetic acid.
Significantly differed between the two groups (P = 0.012; OR 0.17; CI 0.04–0.70).
†Trend toward significance between the two groups (P = 0.074; OR 0.27; CI 0.06–1.14).

Systemic diseases other than PPID were diagnosed prior to presentation and included one mare with CBK and prior idiopathic cellulitis/lymphangitis and ulcerative mucosal lesions and another mare with CBK along with suspected right dorsal colitis secondary to chronic NSAID administration for treatment of ERU. The only horse with CD and systemic disease was suspected to have right dorsal colitis, diagnosed 5 days after presentation. The prevalence of systemic disease other than PPID did not significantly differ between horses with CBK and those with CD (P = 0.610).

Follow-up

No significant difference in the number of follow-up visits (P = 0.630) or length of follow-up time (P = 0.875) was detected between horses diagnosed with CD vs. those diagnosed with CBK. Nineteen eyes (15 horses) with CD did not have any follow-up. Twenty-three eyes (18 horses) had follow-up, and the median (range) number of recheck appointments was 1 (1-14) over a median (range) of 0.53 (0.07–79) months from initial presentation. Two eyes diagnosed with CD with follow-up were treated for mineral deposition using 4% EDTA chelation (n = 1) or topically applied 1% EDTA gel (n = 1). Reduction in corneal mineralization was documented in the eye treated with a single EDTA chelation; however, the resultant iatrogenic ulcer required 1.5 months to heal and new lesions formed within 6 months. The eye treated topically with 1% EDTA gel for approximately 6 months showed no change in number, extent, or density of lesions at a recheck examination 6 months following initial presentation; no further follow-up examinations were performed.

Sixteen eyes (12 horses) with CBK did not return for follow-up examinations. Twenty-nine eyes (24 horses) with CBK had follow-up, and the median (range) number of recheck examinations was 1 (1-15) conducted over 0.47 (0.13–144) months. Nine of these 29 eyes underwent follow-up examinations after treatment for CBK. Improvement was documented in 8 of the 9 treated eyes (88.9%), with decreased or resolved corneal mineral deposition noted in eyes treated by EDTA chelation (n = 5), superficial keratectomy (n = 1), topical application of EDTA gel (unstated concentration) 4 times daily for 5 months (n = 1), and EDTA chelation followed by topical administration of 1% EDTA ointment for 1 month (n = 1). Complete resolution occurred in 3 of 8 eyes, all treated with a single EDTA chelation. Temporary resolution following 4% EDTA chelation occurred in 1 of 8 eyes; mineral deposits recurred to a minimal degree approximately 5 months later but did not require additional treatment. Reduced mineralization was noted in 1 of 8 eyes following chelation with 4% EDTA; however, a presumptive infectious stromal abscess developed following the procedure that resolved over 1 month (Fig. 4). Reduced mineral deposition was noted in 1 of 8 eyes treated by EDTA chelation and topical EDTA ointment, but was enucleated 54 months later due to refractory uveitis and secondary glaucoma. The only treated eye with follow-up in which improvement was not documented underwent chelation with 4% EDTA and was enucleated 7 months later due to refractory discomfort.

DISCUSSION

This retrospective study of CD and CBK in horses demonstrates a few notable differences and many similarities between the two conditions. The major differences noted were that systemic disease, specifically clinical PPID, was more common in horses diagnosed with CBK than those with CD, and horses with CBK were more likely to undergo EDTA chelation than were horses diagnosed with CD. By contrast, the equine populations diagnosed with CD or CBK did not significantly differ in terms of breed or gender. Taken together, these findings raise the possibility that CBK may be a more severe manifestation of CD rather than a distinct disease entity; however, prospective studies are required to confirm this hypothesis. Regardless, it is notable that Appaloosas were 18-fold over-represented in the study vs. hospital population and represented 33% of all horses that were presented for CD or CBK in the present study. Further, all Appaloosas with CD or CBK had also been diagnosed with ERU. As Appaloosas are predisposed to ERU, and as >50% of the horses in the study were concurrently diag-

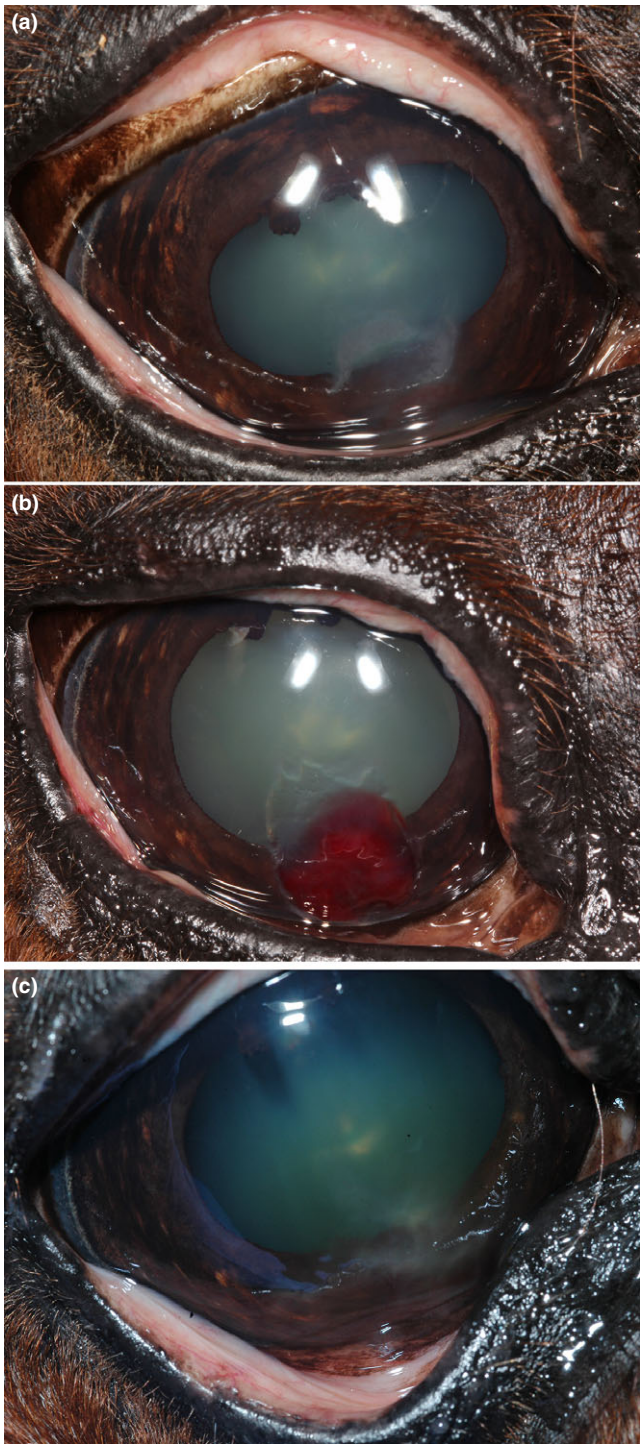

Figure 4. Improvement in mineral deposition in the right eye of a 25-year-old American saddlebred gelding diagnosed with calcific band keratopathy (CBK) and treated with EDTA chelation. (a) External photograph of the right eye with mineral deposition in the visual axis consistent with a diagnosis of CBK at presentation. (b) External photograph of the right eye immediately following chemical chelation with 4% EDTA. Note the marked intrastromal corneal hemorrhage. (c) External photograph of the right eye 18 days post-EDTA chelation showing reduced mineral deposition. Note the stromal infiltrate that was clinically suspicious for an infected stromal abscess and treated with topical antimicrobials for 1 month.

nosed with ERU, this association is not surprising but has not been documented in previous studies of CBK.[16]

The present study demonstrates that horses diagnosed with CD and CBK are significantly older in comparison with the equine hospital population, and horses diagnosed with CD tended to be younger than those diagnosed with CBK. These observations suggest that compromised corneal health as a result of age and/or chronicity related to the horse's primary intraocular disease may play a role in the pathogenesis of both conditions, particularly CBK. Similarly, CBK has been found to primarily affect older dogs with concurrent ophthalmic and systemic conditions,[5] and in humans, most cases of CBK are slowly progressive over years.[17] Due to the retrospective nature of the present study, it was impossible to assess how quickly mineral deposition developed because some of the horses were seen at a single time point after the disease process had begun.

The association between PPID and CBK may also be an age-related phenomenon. A common endocrine disorder, PPID is caused by a pituitary adenoma or hyperplasia and has a mean age of onset of 20 years.[18] Older age and PPID have been associated with decreased corneal sensitivity in the horse, which may lead to impaired corneal wound healing,[19] and subsequent corneal mineral deposition. It is also possible that PPID contributes directly to the disease process in a similar manner to which hyperadrenocorticism does in dogs. Canine hyperadrenocorticism leads to rearrangement of collagen fibers with secondary calcium deposition.[3,5,10] Due to the retrospective nature of this study, it was often difficult to establish at what point PPID was diagnosed relative to the keratopathy, and difficult to ascertain how the diagnosis of PPID was determined if it was made prior to referral. In general, a presumptive (and often correct) diagnosis is based on clinical signs of hypertrichosis, redistribution of body fat with muscle atrophy, polyuria/polydipsia, and sometimes laminitis/recurrent hoof abscesses, while a more definitive diagnosis is typically made by assessing plasma ACTH concentration.[18] Two of the 4 horses in this study that were diagnosed with CBK and with untreated PPID had long hair coats and muscle atrophy, whereas the other 2 that were receiving treatment for PPID did not have evidence of clinical signs. The lack of association between PPID and CD may also be due to the lower median age of the CD relative to the CBK population, with resultant absence of clinical signs consistent with PPID that would normally prompt a diagnosis or further testing. Regardless, this is the first study to identify an association between CBK and PPID in horses, and, as a result, equine patients with PPID should be closely monitored for corneal disease, while CBK may prompt further investigation for PPID, especially in horses showing other typical signs. A future study assessing ACTH concentrations in horses with CBK is warranted. Additional systemic diseases that alter serum calcium and phosphorous concentrations, such

as metastatic calcification in the dog and chronic renal failure, hyperparathyroidism, and sarcoidosis in humans have been associated with CBK.[5,20,21] However, of the 20 horses with CD or CBK and in which an SBA or renal panel was performed, none had altered calcium and/or phosphorus concentrations or azotemia. Herpetic keratitis can precede CBK in both humans and cats.[6,22] While an association between nonulcerative keratitis and equine herpesvirus has been investigated,[23] an analysis of the relationship between CD or CBK and infection with equine herpesviruses has not been performed but may be warranted.

Topical corticosteroids were commonly administered to horses diagnosed with CD or CBK in the present study. Although this observation is expected given that topical corticosteroids are commonly employed in the treatment of ERU, the proportion of eyes receiving topical corticosteroids (63%) was less than the percentage of eyes diagnosed with ERU (76%) in the present study. The tendency for corticosteroid use to be associated with CD or CBK development shown in the present study supports previous studies and theories regarding the pathogenesis of mineral deposition in horses and other species.[1,7] The relationship between topical corticosteroid use, ERU, and CD or CBK, however, remains unestablished. Studies currently support an association between corticosteroids and mineral deposition but not necessarily causation in horses.[7,9] Mineral deposition may be primarily due to an underlying intraocular inflammatory process (with corticosteroid application being a confounding factor) or may be directly due to corticosteroid therapy. One hypothesis for the pathogenesis underlying CBK involves increased pH at the corneal epithelium which allows calcium salts to precipitate, in combination with increased water evaporation at the interpalpebral fissure relative to other regions of the cornea.[1,7] The pH at more exposed regions (i.e., interpalpebral fissure) is higher due to increased carbon dioxide release at the site.[11] Topical corticosteroids may further increase the pH of the cornea and thus encourage calcium precipitation.[7] In addition, topical corticosteroids are often compounded with phosphate, which, when added to the naturally high phosphate concentration in tear film, may result in phosphate concentrations that exceed the solubility product.[8,9] Topical corticosteroids, topical pilocarpine, and injection of tissue plasminogen activator for intraocular disease have also been associated with CBK in humans[5,24]; however, the most common association is in children with juvenile idiopathic arthritis and uveitis.[4] CBK is also associated with uveitis in dogs [3,10] and has been induced by corneal cryo- or laser therapy or by inducing hypervitaminosis D in rabbits.[7] To explore whether ERU or topical application of corticosteroids is more important in development of CD or CBK, it may be interesting to measure tear phosphate concentrations in horses with or without ERU of which some are treated with topical corticosteroids and some are not.

Histologically, CD and CBK with mineral deposition appear similar with extracellular, amorphous, basophilic granular deposits visible at the lamina propria of the corneal epithelium and the superficial stroma; CD may also appear to have clefts within the lamina propria reflecting lipid deposits.[1] It should be noted that CD and CBK are clinical terms and histologically would be referred to as either mineral or lipid keratopathy depending on the composition of the deposits. In this study, cytology did not confirm a diagnosis of CBK or CD in any cases tested; however, it was useful for identifying or making less likely other diagnoses such as infectious or neoplastic keratitis. By contrast, histology confirmed mineral deposition in 50% of eyes in which it was performed, including 70% of eyes clinically diagnosed with CBK in the present study. Indeed, all eyes in which mineral deposition was histologically confirmed had CBK, perhaps because mineral in the interpalpebral fissure is more likely to be in the plane of histologic section. Importantly, lipid or mineral was not identified in any eyes diagnosed with CD in the present study. There are a number of potential reasons for this. The regional and often paraxial distribution of CD (as observed in the clinical descriptions in this study population) may reduce the likelihood of mineral/lipid being identified in histologic sections unless specifically requested. Also, CD includes both mineral and lipid deposition, and lipid deposits are often challenging to confirm with typical fixation and staining techniques because the lipid itself is lost during processing, leaving a cleft in its place. Finally, 2 eyes clinically diagnosed with CD and examined histologically were fixed in Bouin's fixative, which may have decalcified samples and led to false-negative interpretation of mineral deposition. Mineral deposition is typically confirmed with Von Kossa's stain; however, fixation in Bouin's with subsequent decalcification renders this stain less useful.[25] Ocular specimens submitted for histologic confirmation of mineral deposition should be fixed in formalin to prevent inadvertent decalcification.

In the present study, chemical chelation with 4% EDTA was used significantly more frequently to treat CBK than CD. Increased prevalence of treating CBK with EDTA chelation is likely due to distribution of the mineral and/or lipid within the visual axis, although other factors may have included density of the opacity, clinical assessment that the corneal deposits were more likely mineral than lipid, and ocular discomfort due to ulceration. While not statistically significant, more horses diagnosed with CBK (57.8%) were concurrently diagnosed with ulcerative keratitis compared to those diagnosed with CD (40.5%). Chelation with 4% EDTA is also a common treatment option for CBK in humans, with reported symptomatic relief reported in 98% of patients and lesion recurrence in 17.8% of patients an average of 17.7 years later.[12,17] In horses, the procedure appears to be successful with improvement documented in 75% of patients follow-

ing treatment in the present study. Furthermore, this procedure was carried out with standing sedation in the majority of eyes ($n = 14$) in this study, with associated benefits of lower cost, fewer and less severe anesthetic risks, and a procedural time of <1 h. If mineral deposition is severe enough, a second chelation may be required, and this occurred in 6% of horses in the present study in which at least 4 months of follow-up was available. In the human literature, the most common complication of chemical chelation was increased healing time for the iatrogenic ulcer; specifically, a mean healing time of 8 days was noted in comparison with 2–3 days for uncomplicated ulcers in otherwise healthy eyes.[12] It is postulated that increased time to corneal healing is due to the underlying intraocular condition leading to delayed reepithelialization.[12] In this study, complications following EDTA chelation included 1 eye with CD (and concurrent ERU and glaucoma) with an unexpectedly long time to reepithelialization (1.5 months) following chelation and mild recurrence of the presumed mineral plaque at 6 months. A second eye with CBK treated by chelation developed a presumed infected stromal abscess following the procedure, which resolved within 1 month.

Historically, the treatment most commonly described for removal of corneal mineral deposits in veterinary patients is SK.[7] Rebhun and colleagues described use of SK for 19 of 21 horses affected with CBK.[7] Complications included corneal scarring ($n = 17$), prolonged healing at the surgery site (number of horses not stated), or severe bacterial or fungal infection ($n = 5$). Despite these complications, 89% of horses that underwent the procedure eventually achieved corneal healing and resolution of clinical signs.[7] In humans, EDTA chelation and other more recent therapies have superseded the use of SK, but it is still popular in developing countries.[11] While corneal fibrosis appears to be a common disadvantage in horses,[7] other disadvantages associated with SK in humans include a resultant roughed corneal surface following treatment and difficulty controlling the depth and area of the cornea that is treated.[11] Of the cases presented here, only 3 horses were treated with keratectomy, 1 for CD and 2 for CBK. The patient with CD underwent keratectomy for treatment of corneolimbal SCC. Improvement after SK was reported in 1 of the 2 horses with CBK, and no complications directly associated with the surgery were reported for any of the patients treated in this manner.

Additional alternative therapies for CD and CBK in veterinary patients include topical application of EDTA and diamond burr debridement. Topically applied EDTA drops, gels, or ointment have been used in animals and humans as preparatory therapy prior to ≥4% EDTA chelation, or in patients with mineral deposition deemed insufficiently severe to warrant chelation or SK.[3,26] In one case of CBK reported here, 1% EDTA gel was topically applied prior to and after EDTA chelation with clinical improvement subjectively noted. Concentrations of EDTA applied topically range widely with 10% solutions reported in human patients vs. 0.4–1.38% solutions in veterinary patients.[3,11] Diamond burr debridement has recently been described for treatment of nonhealing corneal ulcers in horses; however, it was not used in any of the horses in the present study.[13] Other treatment options reported in humans for mineral deposition within the cornea include lamellar keratoplasty, Nd:YAG laser, and phototherapeutic keratoplasty (PTK).[12,27] Laser and PTK are useful in cases where deposits are deeper within the corneal layers and therefore less amenable to treatment with SK or EDTA chelation.[27,28] In addition, amniotic membrane transplantation in conjunction with EDTA chelation and PTK has been described in humans following mineral removal from the anterior stroma.[27] Benefits of amniotic membrane include more rapid corneal epithelialization, action as a structural bandage, absorption of inflammatory cytokines, and improved ocular comfort.[27–29] In horses, amnion has been reported as treatment for melting ulcers or bullous keratopathy and after keratectomy for corneal neoplasms or immune-mediated keratitis, with mild corneal fibrosis and maintenance of vision in the majority of cases.[30]

Limitations of the present study were largely due to its retrospective nature. Many horses were lost to follow-up, making it challenging to assess long-term treatment outcomes. For example, the authors could find records with sufficient follow-up to document response to treatment in only 9 of 19 eyes that were treated for CBK and 2 of 5 eyes treated for CD. Prospective studies are required to determine the short- and long-term effects of different treatment protocols. A multicenter study to evaluate treatment-based outcomes for mineral deposition within the equine cornea, taking into account ophthalmologist preference for treatment choice and variability in follow-up, would be ideal.

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REFERENCES

1. Clode AB. Diseases and surgery of the cornea. In: *Equine Ophthalmology*, 2nd edn (ed. Gilger BC). Elsevier Saunders, St. Louis, MO, 2011; 225–226.
2. Rebhun WC. Corneal dystrophies and degenerations in horses. *Compendium on Continuing Education for the Practicing Veterinarian* 1992; **14**: 945–950.
3. Gelatt KN, Gilger BC, Kern TJ editors. *Veterinary Ophthalmology*, 5th edn. Wiley-Blackwell, Ames, IA, 2013.
4. BenEzra D, Cohen E, Behar-Cohen F. Uveitis and juvenile idiopathic arthritis: a cohort study. *Clinical Ophthalmology* 2007; **1**: 513–518.
5. Sansom J, Blunden T. Calcareous degeneration of the canine cornea. *Veterinary Ophthalmology* 2010; **13**: 238–243.

6. Nasisse MP, Guy JS, Davidson MG *et al.* Experimental ocular herpesvirus infection in the cat. Sites of virus replication, clinical features and effects of corticosteroid administration. *Investigative Ophthalmology Vision. Science* 1989; **30**: 1758–1768.
7. Rebhun WCM, Murphy CJ, Hacker DV. Calcific band keratopathy in horses. *The Compendium on Continuing Education for the Practicing Veterinarian* 1993; **15**: 1402–1410.
8. Pucket JD, Boileau MJ, Sula MJ. Calcific band keratopathy in an alpaca. *Veterinary Ophthalmology* 2013; **17**: 286–289.
9. Brooks DE. Calcium degeneration and ocular surface failure in the horse. *Equine Veterinary Education* 2012; **24**: 8–11.
10. Laus JL, dos Santos C, Talieri IC *et al.* Combined corneal lipid and calcium degeneration in a dog with hyperadrenocorticism: a case report. *Veterinary Ophthalmology* 2002; **5**: 61–64.
11. Jhanji V, Rapuano CJ, Vajpayee RB. Corneal calcific band keratopathy. *Current Opinion in Ophthalmology* 2011; **22**: 283–289.
12. Najjar DM, Cohen EJ, Rapuano CJ *et al.* EDTA chelation for calcific band keratopathy: results and long-term follow-up. *American Journal of Ophthalmology* 2004; **137**: 1056–1064.
13. Lassaline-Utter M, Cutler TJ, Michau TM *et al.* Treatment of nonhealing corneal ulcers in 60 horses with diamond burr debridement (2010-2013). *Veterinary Ophthalmology* 2014; (Suppl. 1): 76–81.
14. Bernauer W, Thiel MA, Kurrer M *et al.* Corneal calcification following intensified treatment with sodium hyaluronate artificial tears. *The British Journal of Ophthalmology* 2006; **90**: 285–288.
15. Preacher KJ. 2001. Calculation for the chi-square-test: an interactive calculation tool for chi square-tests of goodness of fit and independence [Computer software]. [cited 2015 Jul 13]. Available from http://quantpsy.org
16. Fritz KL, Kaese HJ, Valberg SJ *et al.* Genetic risk factors for insidious equine recurrent uveitis in Appaloosa horses. *Animal Genetics* 2014; **45**: 392–399.
17. Moisseiev E, Gal A, Addadi L *et al.* Acute calcific band keratopathy: case report and literature review. *Journal of Cataract and Refractive Surgery* 2013; **39**: 292–294.
18. McFarlane D. Equine pituitary pars intermedia dysfunction. *The Veterinary Clinics of North America Equine Practice* 2011; **27**: 93–113.
19. Miller C, Utter ML, Beech J. Evaluation of the effects of age and pituitary pars intermedia dysfunction on corneal sensitivity in horses. *American Journal of Veterinary Research* 2013; **74**: 1030–1035.
20. Johnston RL, Stanford MR, Verma S *et al.* Resolution of calcific band keratopathy after lowering elevated serum calcium in a patient with sarcoidosis. *The British Journal of Ophthalmology* 1995; **79**: 1050.
21. Porter R, Crombie AL. Corneal calcification as a presenting and diagnostic sign in hyperparathyroidism. *The British Journal of Ophthalmology* 1973; **57**: 665–668.
22. Albietz JM, Lenton LM. Late reactivation of herpes zoster keratitis results in band keratopathy. *Optometry and Vision Science: Official Publication of the American Academy of Optometry* 2014; **91**: 149–155.
23. Hollingsworth SR, Pusterla N, Kass PH *et al.* Detection of equine herpesvirus in horses with idiopathic keratoconjunctivitis and comparison of three sampling techniques. *Veterinary Ophthalmology* 2015; **18**: 416–421.
24. Pavicic-Astalos J, Lacmanovic-Loncar V, Petric-Vickovic I *et al.* Eye drops preservative as the cause of corneal band keratopathy in long-term pilocarpine hydrochloride treatment. *Acta Clinica Croatica* 2012; **51**: 107–111.
25. Bussolati G. A fixation-decalcification procedure for bone biopsies. *Histopathology* 1978; **2**: 329–334.
26. Yilmaz STM, Maden A. Adjunctive preoperative ethylenediaminetetraacetic acid drops for surgical chelation for calcific band keratopathy. *Asian Journal of Ophthalmology* 2006; **8**: 143–146.
27. Kwon YS, Song YS, Kim JC. New treatment for band keratopathy: superficial lamellar keratectomy, EDTA chelation and amniotic membrane transplantation. *Journal of Korean Medical Science* 2004; **19**: 611–615.
28. Im SK, Lee KH, Yoon KC. Combined ethylenediaminetetraacetic acid chelation, phototherapeutic keratectomy and amniotic membrane transplantation for treatment of band keratopathy. *Korean Journal of Ophthalmology* 2010; **24**: 73–77.
29. Anderson DF, Prabhasawat P, Alfonso E *et al.* Amniotic membrane transplantation after the primary surgical management of band keratopathy. *Cornea* 2001; **20**: 354–361.
30. Plummer CE, Ollivier F, Kallberg M *et al.* The use of amniotic membrane transplantation for ocular surface reconstruction: a review and series of 58 equine clinical cases (2002-2008). *Veterinary Ophthalmology* 2009; **12**(Suppl. 1): 17–24.