The fungal plaque form of equine keratomycosis

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Abstract

Objective: To describe clinical findings and visual outcomes of the fungal plaque form of equine keratomycosis.

Design: Retrospective medical records study.

Animals studied: Medical records of horses with the fungal plaque form of keratomycosis that presented to the University of Florida Veterinary Medical Center from 2001 to 2013 were reviewed.

Procedures: Data collected for all horses with corneal fungal plaques at the University of Florida Veterinary Medical Center from 2001 to 2013 included photographs at various points during treatment, and the signalment and clinical description of ocular lesions, medical and surgical treatments, diagnostic test results, and therapeutic outcomes from medical records. Diagnosis of the plaque form of keratomycosis was based on the clinical appearance in addition to cytology and/or histologic results.

Results: Twenty-two horses with keratomycosis with fungal plaques were identified. Corneal scraping found septate hyphae in all 22 eyes. Thirteen eyes (59.1%) had positive fungal culture results from the plaque. Dematiaceous fungi (n=3), Fusarium and Scopulariopsis (n=3), Aspergillus sp (n=4) and unidentified fungi or molds (n=4) were cultured from fungal plaques. Thirty-two percent (n=7) of the eyes cultured had bacterial infections in addition to the fungal infection. Fungi alone were cultured in six eyes (27.3%). Bacteria only were cultured in 6 eyes (27.3%). Culture did not reveal positive bacterial or fungal presence in three (13.7%) plaques. Medical therapy was utilized in all twenty two horses. Ninety-one percent (n=20 horses) of the horses were treated with at least one topical antifungal and at least one topical antibiotic, while one horse was treated with only topical antifungals and one horse was treated with only topical antibiotics. Nine of the horses received one topical antifungal medication and twelve received a combination of two topical antifungal medications with natamycin and either miconazole or voriconazole being the most common combination. Topical antiproteases and mydriatic/cyloplegics, and systemic nonsteroidal anti-inflammatory drugs were used in all horses. Ninety-one percent (n=20 horses) received surgical intervention of some form with 68% (n=15 horses) receiving at least one standing keratectomy and 23% (n=5 horses) receiving a superficial keratectomy under general anesthesia. Mean time to resolution was 6.5±4.1 weeks. Following medical and surgical therapy, 73% (n=16) of the eyes were visual and 27% (n=6 horses) were enucleated. The mean time to resolution of horses in which vision was preserved was 7.5±3.6 weeks.

Conclusions: The visual outcome of therapy of 22 horses with the dark fungal plaque form of keratomycosis was positive in 73%. Successful treatment of the fungal plaque form of keratomycosis was aided by plaque removal, usually hastened by performing one or more keratectomies, and concurrent successful management of corneal ulceration and accompanying uveitis.

Keywords: Cornea, fungus, horse, keratomycosis, plaque
symbiosis with ocular surface bacteria of the horse [1]. Some of these fungal species may be innately pathogenic, while others can become pathogenic following corneal injury and/or alterations in the microenvironment of the ocular surface. A lack of integrity and stability of the precorneal tear film, and corneal epithelial cell injury predispose and encourage fungal adhesion, invasion and infection of the horse cornea. It may be that unique metabolic and immunologic characteristics of the horse tear film, the normal low resting temperature of the horse cornea, the large corneal surface area, and/or interspecies alterations in corneal immunoprotection make the horse cornea more susceptible than that of other animal species to keratomycosis [2,3]. It also seems likely that the role and activity of horse neutrophils versus that of horse macrophages in infiltrating and influence the healing process of the diseased horse cornea may predispose to fungal infection [1-3].

Keratomycosis has a variety of clinical presentations in the horse that may represent a continuum of lesions. They include tear film disruption [4], epithelial microerosions [4], ulcerations of variable stromal depths [1,5-9], corneal dissolution and melting [5,9], corneal perforations and subsequent iris prolapse [10], stromal abscess [1,7,10,11], and stromal plaque formation [1,5,6,10]. It seems clear that tear film instability and disruption of corneal epithelial integrity are necessary for most forms of keratomycosis to occur in the horse [1-10].

The term “fungal plaque” in the horse was first used in a 1973 case report that described a one centimeter diameter, off-white, opaque stromal plaque containing hyphae that was covered with epithelium and did not retain fluorescein dye [12]. Since then, fungal plaques as a form of stromal keratomycosis have been reported in multiple retrospective reports on equine keratomycosis [1,5,6,9,10]. Fungal plaques are composed of a dense proliferation of fungal elements in or on the anterior aspect of the corneal stroma admixed with host inflammatory cells and necrotic corneal tissue (Figures 1 and 2). The plaque color is possibly related to the fungal species associated with plaque formation. The fungal plaques in horses are typically dark to light brown, yellow, or tan, may cover a very large portion of the cornea, and often thicken with time. Leukocytic invasion posterior to the plaque is generally quite dramatic [13]. Corneal stromal vascularization can surround the plaque [1,13].

When treating keratomycosis of any form in horses, the goal of therapy is to have a positive visual outcome by sterilizing the ocular surface by killing the fungi, removing any degenerate corneal stromal tissue, minimizing stromal damage from proteases produced from inflammatory cells attracted to the corneal infection, controlling uveitis that occurs as a result of the reflex response when the cornea is injured, and providing support to the weakened cornea [13]. When a fungal infection is present in the horse cornea, there is an increase in the amount and activity of proteases in the cornea and the tear film elaborated by the organisms, keratocytes [14] and leukocytes [1] that cause serious and progressive damage to the cornea. This protease activity must be suppressed in order to maintain the integrity and transparency of the cornea. Typical treatment regimens for keratomycosis include one or more topical antifungals, antibiotics if there is a bacterial component to the infection or to prevent secondary bacterial infection, one or more topical antiproteases, and a mydriatic/cycloplegic agent. Keratectomies and surgical debridement of the fungal plaque may be particularly helpful in order to remove fungal material that inhibit vascularization and regrowth of epithelial cells [15,16]. Standing keratectomies to
Methods

Medical records of horses that had keratomycosis with fungal plaques at the University of Florida Veterinary Medical Center from 2001 to 2013 were reviewed. Data collected from the medical records included signalment and clinical description of ocular lesions, types of medical and types of surgical treatments, diagnostic test results, and therapeutic outcomes.

Clinical examinations utilized slit lamp biomicroscopy for examination of the anterior segment, fluorescein staining, corneal scraping for cytology, aerobic and fungal culture of the corneal plaque site, and direct ophthalmoscopy of the fundus when possible. All horses were photographed at various points during treatment.

Diagnosis was based on the clinical appearance of a plaque during examination and treatment of an ulcerative or non-ulcerative keratopathy in a horse. Cytologic, histologic and culture results, and response to antifungal therapy were used to confirm the keratomycosis.

Plaque specimens were obtained with debridement, scraping and superficial keratectomy, and the cytopathic and/or histologic specimens examined by light microscopy. Scrapings for cytology were obtained aseptically from the base and edges of each ulcer using a disposable blade. Superficial keratectomies, debridement and, plaque scrapings were performed standing following sedation with xylazine\(^1\) or detomidine\(^2\). A motor nerve block was then performed using 1% lidocaine\(^3\) and tetracaine\(^4\) was applied to provide topical anesthesia to the cornea. Loose pieces of the plaque were carefully removed with small scissors and placed in formalin. Plaques associated with corneal melting, corneal groove formation, and that were tightly adhered to the stroma were removed with keratectomy under general anesthesia.

PCR was performed at the University of Florida using universal fungal primers for the conserved 5.8S and 28S ribosomal DNA regions. The PCR products were then analyzed for length of the ITS2 region of the amplicon using the ABI PRISM 310 Genetic Analyzer. The analyzer tests for 12 Candida species, 9 non-Candida yeasts, and 21 opportunistic and dematiaceous fungi including 4 Aspergillus species. Horses were selected for PCR based on the availability of the technology at the time of presentation.

Results

The clinical findings, cytology, histology results and culture results, type of surgical intervention if utilized, and visual outcomes for 22 horses presenting to the University of Florida Veterinary Medical Center with the fungal plaque form of keratomycosis are found in Table 1. Fungal plaques in the 22 horses were diagnosed based on clinical appearance of the corneal plaque, and cytology, biopsy and culture results confirming keratomycosis.

The mean age of the 22 horses at the time of diagnosis was 11±7 years (range 2-24 years). Eleven horses were mares, ten horses were geldings, and one was a stallion. Thoroughbreds (n=7), Quarter Horses (n=6), and Warmbloods (n=4) were the most common breeds affected. There was one each of the following breeds: Appaloosa, Saddlebred, Welsh Pony, crossbreed pony, and crossbreed horse.

Corneal scraping found hyphae in all 22 eyes. Cytology reports from corneal scrapings of horses with fungal plaques were submitted to Clinical Pathology Service and typically described fragments of septate fungal hyphae with bulbous terminal swellings and large branching structures. Sheets of corneal epithelial cells, moderate to large numbers of mature neutrophils, and necrotic cellular debris were noted in addition to the fungal elements. There was a noticeable paucity of macrophages. Melanin granules were present in one report. Biopsies from keratectomies under general anesthesia (n=4 horses) or after enucleation (n=6 horses) described the plaque margins as eosinophilic and homogenous, and infiltrated by massive numbers of fungi underlain by larger numbers of degenerate neutrophils and necrotic cellular debris. The stroma of the plaques is infiltrated by septate fungal hyphae with parallel dual walls admixed with degenerate neutrophils, and cellular and karyorrhectic debris. The hyphae were 3-8 microns in diameter. The plaques also contain eosinophilic fibrillar disorganized connective tissue with multiple clusters of small caliber vessels (neovascularization) that are frequently surrounded by occasional to moderate numbers of leukocytes.

Thirteen eyes (59.1%) had positive fungal culture results from the plaque. Dematiaceous fungi were cultured in horse cases 1, 2, and 7. Fusarium and/or Scopulariopsis were found in horses 2, 6 and 22, Aspergillus sp were cultured in horses 8, 13, 18, 20 and 21. Horses had unidentified fungi or molds in horses 5, 9, 11, and 19. Fungi alone were cultured in cases 2, 5, 11, 13, 20 and 21. Bacteria alone were cultured in cases 4, 10, 12, 14, 16 and 17. Thirty-two percent (n=7; cases 1, 6-9, 19 and 22) of eyes that were cultured had bacterial infections in addition to the fungal infection. No infection was detected by culture or histology in cases 3, 15 and 18. Four horses (cases 1-4) had fungal PCR performed. Two had positive results, one each...
Table 1. Clinical findings, cytology, histology and culture results, surgical interventions, and visual outcomes for 22 horses presenting to the University of Florida Veterinary Medical Center with the fungal plaque form of keratomycosis.

| Case # | Age, sex, breed | Clinical Appearance | Degree of Uveitis at Presentation | Degree of Vascularization | Cytology | Culture | Surgical Intervention | Histology Results | Outcome |
|--------|-----------------|---------------------|----------------------------------|---------------------------|----------|---------|----------------------|-------------------|---------|
| 1      | 20 y, F, T      | B-Y plaque, 40% of cornea (Figure 1) | 1+ flare | Presentation-moderate, reaching lesion Final- moderate | Neutrophilic keratitis with fungal infection- *Aspergillus* sp. | Staphylococcus, gram+ bacteria, dematiaceous mold | SK | Keratectomy- keratitis, focally extensive, severe, with intra-lesional fungal hyphae; appearance consistent with *Aspergillus* spp. | Visual, mild fibrosis |
| 2      | 8 y, F, W       | W-Y plaque, 3x3mm | 1+ flare | Presentation-moderate, 2 mm from lesion Final- infiltrating neovasc | Fungal keratitis | *Scopulariopsis* sp., *Fusarium* sp. | SK multiple times | Keratectomy- large number of fungal hyphae | Visual, mild fibrosis |
| 3      | 12 y, F, Q      | W plaque, 0.5x0.5 mm | 1+ flare | Presentation-mild Final- infiltrating neovasc | Neutrophilic keratitis, single encapsulated structure of suspected fungal origin | No growth | K-GA/Biosist/ AMT | Keratectomy- keratitis, neutrophilic, with neovascularization, chronic, diffuse, moderate | Visual, mild fibrosis |
| 4      | 2 y, F, T       | B-Y plaque, 30% of cornea | Too much edema to assess | Presentation-mild Final- same | Bacterial and fungal sepsis | *Staphylococcus xylosus*, *Streptococcus* spp., gram+ bacteria | SK multiple times | Keratectomy- large number of fungal hyphae, suppurrative inflammation | Visual |
| 5      | 23 y, G, S      | W-Y plaque, B rim, 15x5 mm (Figure 3) | 2+ flare | Presentation-none Prior to enuc- 5-6 mm subepith 360 deg neovasc, progressing slowly | Fungal keratitis | Unknown fungus | None- Recommended- K and CF, declined for financial reasons | Enucleated- Melting ulcer and groove; financial |
| 6      | 24 y, G, T      | W plaque, 1x0.7mm | 2+ flare | Presentation-mild Prior to enuc-mild | Fungal keratitis | *Pseudomonas*, *Fusarium* sp | SK | Enucleation- keratitis, ulcerative, necrosuppurative, focal, mild to moderate, with intra-lesional hyphae; anterior uveitis, suppurrative, multifocal, mild to moderate, anterior chamber, iris, ciliary body, and filtration angle | Iris prolapse, Enucleated |
| 7      | 4 y, G, Q       | W-Y plaque, 6x6mm | 2+ flare | Presentation-mild Final- mild | Fungal keratitis | α-hemolytic strep, *Dactylaria constricta* | None | -- | Visual |
| Case # | Age, sex, breed | Clinical Appearance | Degree of Uveitis at Presentation | Degree of Vascularization | Cytology | Culture | Surgical Intervention | Histology Results | Outcome |
|--------|-----------------|---------------------|-----------------------------------|--------------------------|----------|---------|-----------------------|------------------|----------|
| 8      | 9 y, G, W       | W-Y plaque, 80% of cornea **(Figure 4)** | Unknown                         | Presentation-prolapsed iris | Fungal keratitis | Aspergillus, Staphylococcus epidermis | SK                  | Enucleation- keratitis, suppurative, multifocal, subacute, severe with intralesional fungal hyphae; corneal perforation; hyphema, acute, marked, anterior chamber; iris prolapse and anterior synechia, anterior chamber, | Iris prolapse, Enucleated |
| 9      | 9 y, Qx         | Y plaque, 40% of cornea | Mild flare                      | Presentation-moderate Final- infiltrative | Fungal keratitis | Non-hemolytic *Staphylococcus*, unknown fungus | SK                  | Keratectomy- large number of fungal hyphae, suppurative inflammation | Visual |
| 10     | 16 y, WP        | W plaque w/B rim, 80% of cornea | Severe (2+ flare)               | Presentation-mild Final- infiltrative | Fungal sepsis w/neutrophilic inflammation | Pseudomonas aeruginosa | SK                  | Keratectomy- large number of fungal hyphae, suppurative inflammation, subacute | Visual, mod fibrosis |
| 11     | 6 y, E, T       | B plaque, 10x5mm     | 1+ flare                        | Presentation-mild Final- infiltrative | Fungal keratitis | Mold | SK                  | Keratectomy- suppurative, severe, acute to subacute, diffuse, with numerous intralesional fungal hyphae | Visual |
| 12     | 17 y, E, Q      | B-Y plaque, 15% of cornea | Mild                            | Presentation-mild Final- severe | Fungal keratitis | *Staphylococcus* epidermis | SK                  | Keratectomy- large number of fungal hyphae, neutrophilic, with neovascularization, chronic, diffuse, moderate | Visual, mild fibrosis |
| 13     | 10, G, T        | Y plaque, 10% of cornea | Mild                            | Presentation-mild Final- severe | Fungal keratitis | Aspergillus | SK                  | Keratectomy- large number of fungal hyphae, neutrophilic, with neovascularization, chronic, diffuse, moderate | Visual |
| 14     | 4 y, G, W       | Y plaque, 15% of cornea | None                            | Presentation-mild Final- severe | Fungal keratitis | *Staphylococcus* aeruginosa, *Nocardia* sp | GK-GA, CF | Keratectomy- large number of fungal hyphae | Visual |
| 15     | 21 y, S, T      | Y-W plaque, 5% of cornea | Mild                            | Presentation-mild Final- severe | Fungal keratitis | None | SK                  | Keratectomy- large number of fungal hyphae; neutrophilic, with neovascularization, chronic, diffuse, moderate | Visual, mild fibrosis |
| Case # | Age, sex, breed | Clinical Appearance | Degree of Uveitis at Presentation | Degree of Vascularization | Cytology | Culture | Surgical Intervention | Histology Results | Outcome |
|--------|-----------------|---------------------|----------------------------------|---------------------------|----------|---------|----------------------|-------------------|---------|
| 16     | 7 y, G, W        | W plaque, 10x20 mm  | 2+ flare                         | Presentation - none       | Fungal sepsis | Ralstonia picketti | K-GA, AMT - 2 layers | Keratectomy - large number of fungal hyphae | Iris prolapse, Enucleated |
| 17     | 17 y, F, Q       | W plaque, 20% of cornea | 3+ flare                         | Presentation - mild       | Bacterial and fungal sepsis | Stenotrophomonas maltophilia | SK | Enucleation - corneal ulcer, locally extensive, severe, chronic with intralesional fungal hyphae and with: panophthalmitis suppurative and histiocytic, multifocal, mild to severe, chronic; iris prolapse and anterior synchiae | Iris prolapse, Enucleated |
| 18     | 10 y, G, T       | B-Y plaque, 40% of cornea | None                             | Presentation - mild       | Fungal keratitis | None | SK | Keratectomy - large number of fungal hyphae with many neutrophils | Visual, major fibrosis |
| 19     | 10 y, F, Q       | B plaque, 20mmx5mm   | Could not assess                 | Presentation - moderate   | Fungal keratitis | a-hemolytic streptococcus, mold | SK | Keratectomy - large number of fungal hyphae with many neutrophils | Visual, mild fibrosis |
| 20     | 6 y, F, P        | W w/B rim, 15mmx13mm | 1+ flare                         | Presentation - mild       | Neutrophilic keratitis w/ fungal sepsis | Aspergillus | SK | Keratectomy - large number of fungal hyphae with many neutrophils | Visual, mild fibrosis |
| 21     | 6 y, F, C        | W, 60% of cornea     | None noted                        | Presentation - none       | Fungal keratitis | Aspergillus | K-GA, Biosist, AMT | Keratectomy - keratitis, suppurative, severe, acute to subacute, diffuse, with numerous intralesional fungal hyphae, cornea. Enucleation- ulcerative keratitis, with disruption of Descemet's membrane and iris prolapse, posterior synchiae, scleritis, uveitis, suppurative, acute, multifocal, moderate with intracorneal fungi. | Iris prolapse, Enucleated |
| 22     | 5 y, F, Ap       | W, 10% of cornea     | 2+ flare                         | Presentation - moderate   | Fungal keratitis | Fusarium sp., Stenotrophomonas maltophilia | DALK, K-GA, voriconazole, Biosist, AMT | Keratectomy - large number of fungal hyphae, suppurative | Visual, major fibrosis |

Continuation of Table 1
Table 1 Abbreviations:
Sex: F: Mare; G: Gelding; S: Stallion; 
Plaque Colors: W: White; Y: Yellow; B: Brown; R: Red; Bl: Black; 
Degree of Vascularization: N: None; Nv: Neovascularization; 
Enufic: Enucleation; Interventions: Ap: Appaloosa; 
Breed: T: Thoroughbred; Q: Quarter Horse; S: Saddlebred; 
CF: Conjunctival Flap; DALK: Deep anterior lamellar keratoplasty; 
GK: Grid Keratotomy; AMT: Amniotic membrane transfer; 
K-GA: Keratectomy under General Anesthesia; 
Enuc: Enucleation; Surgical Interventions: SK: Standing keratectomy; 
Degree of Vascularization: NeovASC: Neovascularization; 
Sex: F: Mare; G: Gelding; S: Stallion; 
Table 1 Abbreviations: 
Maxwell et al. 2015, http://www.hoajonline.com/journals/pdf/2054-3425-3-2.pdf

for Fusarium solani and Cladorrhinum bulbillosum. Two had negative results with the finding of fungal infection confirmed by culture and cytology in one horse (case 1), and the other (case 3) had a negative fungal culture result, but cytology showed an encapsulated structure of fungal origin.

In the majority of cases, corneal ulcers were apparent surrounding or under the plaque based on clinical appearance and uptake of fluorescein dye, although the plaques themselves did not retain fluorescein. Fungal plaques may cover 50% of the cornea and were estimated to be as thick as 1-2 mm from caliper measurements. The plaques loosen with time and so most of the plaques could be removed, albeit in pieces, over a several week period. Surgical pathology biopsy results typically showed histologic evidence of the stroma infiltrated by massive numbers of fungal hyphae admixed with moderate to large numbers of neutrophils, multiple small areas of hemorrhage, and low numbers of macrophages and lymphocytes (Figure 2 is a representative section).

Ninety-one percent (n=20 horses) of the horses were treated with at least one topical antifungal and at least one topical antibiotic, while one horse was treated with only topical antifungals and one horse was treated with only topical antibiotics. Nine of the horses received only one topical antifungal medication and twelve received a combination of two topical antifungal medications with six horses receiving natamycin and miconazole, and six horses receiving natamycin and voriconazole. Topical medications utilized included the antifungal medications natamycin5 (n=14), miconazole6 (n=8), and voriconazole2 (n=11); the antibiotics cefazolin7 (n=12), tobramycin8 (n=7), neopolygram9 (n=11), ciprofloxacin10 (n=8), and neomycin-polymyxin-bacitracin11 (n=3); the anti-proteinases EDTA12 (n=13), serum (n=22), and amniotic membrane homogenate (n=1); the mydriatic/cycloplegics atropine12 (n=21), tropicamide13 (n=1), and phenylephrine14 (n=1); and other topical ophthalmic drugs Muro-12815 (n=7) and flurbiprofen16 (n=1). In two patients, an antibacterial-steroid combination, neoploydex17, was used by the referring veterinarian prior to referral to our teaching hospital, which may have contributed to or exacerbated the development of the fungal plaques by interfering with cellular immunity [19]. Parenteral medications included flunixin meglumine18 (n=21), omeprazole19 (n=12), and sulfamethoxazole and trimethoprim20 (n=2).

Surgical interventions were performed to obtain diagnostic samples and when significant plaque formation was present that did not display evidence of spontaneous sloughing. These included standing keratectomies (n=15) and keratectomies under general anesthesia (n=5). When medical therapies were not yielding significant improvement or the integrity of the cornea was severely compromised by corneal melting, progression of a corneal stromal groove, or iris prolapse occurrence, amniotic membrane transplantation (n=4), collagen biomaterial grafting (Biosist15) (n=2) in combination with amniotic membrane transplantation, and conjunctival flap (n=1) surgeries were performed.

Medications were discontinued when the plaque had been sloughed spontaneously or was removed surgically, aqueous flare was absent and the pupil dilated, the cornea was fluorescein negative, and the eye was comfortable. At that point, seventy-three percent (n=16) horses were considered visual by a positive menace response and dazzle reflex. Visual globes did have mild to moderate corneal opacification from subepithelial and stromal corneal fibrosis. Six horses (27%) were enucleated due to corneal perforation, although one of those was enucleated due to owner financial constraints.

The mean time to resolution of all horses, including enucleation cases, was 6.5±4.1 weeks (range 1-12 weeks). With globe retention, the mean time to resolution of all horses was 7.5±3.6 weeks (range 4-12 weeks). Sloughing of the plaque (Figures 1, 3 and 4) occurred associated with neutrophilic and vascular infiltration beneath the plaque, formation of a stromal groove, and was typically also aided by keratectomies (Figures 1, 4-8). In cases with a positive visual outcome, plaque sloughing was followed by stromal and epithelial healing with vascularization and minimal to moderate corneal opacification (Figures 7 and 8).

Medication
1Rompun™, Bayer Healthcare LLC, Shawnee Mission, KS, USA.

Figure 3. Case #5 shows a white-yellow plaque with a brown rim, and thinning of the stroma to form a stromal groove and furrow dorsal and temporal to the plaque margins. Neovascularization was notably not yet present near the plaque in this case.
Figure 4. A white fungal plaque in case #7 is loosely attached to the cornea. A gutter and cellular infiltrate are present under the plaque. Blood vessels are moving toward the plaque site. Hypopyon is also present.

Figure 5. Right eye of case #20 three days after presentation to the University of Florida Veterinary Medical Center for a corneal ulcer, a fungal plaque has developed. *Aspergillus* was cultured from the plaque edge. The ventral margin was loosely attached and had been removed with standing keratectomy. There is no neutrophilic infiltration beneath the plaque and little corneal vascularization at this time.

Figure 6. Same horse as Figure 5. Case #20; 18 days after presentation to the University of Florida Veterinary Medical Center. A standing keratectomy was performed the day prior to this photo to remove part of the plaque, and neutrophilic infiltration can now be seen. Blood vessels have reached the edge of the plaque. Standing keratectomies to remove loose pieces of the plaque were repeated over several days.

Figure 7. Same horse as Figures 5 and 6, case #20; 26 days after presentation to the University of Florida Veterinary Medical Center, the plaque has sloughed, the epithelium is present, and the stroma shows evidence of fibrovascular tissue.

2 Dormosedan®, Pfizer Animal Health, Exton, PA, USA.
3 Lidocaine, Sparhawk Pharmacies Inc, Lenexa, KS, USA.
4 Tetracaine®, OCuSOFT inc, Richmond, TX, USA.
5 Natacyn®, Alcon Labs Inc, Fort Worth, TX, USA.
6 Compounded.
7 Voriconazole solution, Roerig, New York, NY, USA.
8 Tobramycin Ophthalmic Solution, Akorn Inc, Lake Forest, IL, USA.
9 Neomycin & Polymyxin B Sulfates & Gramicidin Ophthalmic solution, Bausch and Lomb Inc, Tampa, FL, USA.
10 Ciprofloxacin Ophthalmic Solution, Hi-Tech Pharmacal Co Inc, Amityville, NY, USA.
11 Vetropolyclin® Dechra Vet Products, Overland Park, KS, USA.
12 Steri-Units®, Alcon Labs Inc, Fort Worth, TX, USA.
13 Tropicamide Ophthalmic Solution, Akorn Inc, Lake Forest, IL, USA.
14 Phenylephrine Hydrochloride Ophthalmic Solution, Bausch and Lomb Inc, Tampa, FL, USA.
15 MURO 128.5%, Bausch and Lomb, Tampa, FL, USA.
16 Flurbiprofen Sodium Ophthalmic Solution, Bausch and
This paper reports on the visual outcomes of horses with fungal plaques. The term “phaeohyphomycosis” is used to characterize infections caused by dematiaceous or pigmented filamentous fungi which contain melanin or chromic metabolic by-products in their cell walls and produce dark colonies in culture and in vivo. Dematiaceous fungi were found in horse cases 1, 2, and 7 which had been reported to cause dark colored corneal plaques in humans. The presence of a brown colored fungal hyphae in the scraping material strongly indicates the presence of a dematiaceous fungus. The term “hyalohyphomycosis” is used to group together infections caused by hyaline non-dematiaceous fungi which can also cause tissue coloration and were found in horses 2, 6, and 22. Aspergillus species such as Aspergillus niger can also form colored lesions in vitro and in vivo, and were noted in our cases 8, 13, 20, and 21.

In human corneas, it has been noted that the genus of fungi involved may change the course of the progression with Fusarium solani causing markedly more stromal destruction than Aspergillus sp. However, in the horses examined here, Aspergillus sp. were cultured in four cases, and 50% (n=2) of those cases ended with enucleation of the globe, while Fusarium sp. were cultured in three cases, and one of those ended in enucleation of the globe.

Figure 8. Same horse as Figures 5-7, case #20; six years after presentation to the University of Florida Veterinary Medical Center the eye is comfortable and the cornea has a slight haze due to subepithelial fibrosis.

We speculate, based on nine of our cases, that the plaque color is related to the fungal species associated with the plaque formation although other factors may be involved. The majority of these cases were treated with at least one topical antifungal and at least one topical antibiotic. Although culture results were helpful for targeting certain fungi and bacteria with treatment, some patients with obvious fungal plaques had negative culture results. These results were likely due to the surface of the plaque containing only dead fungi. When negative PCR results were found in conjunction with positive plaques had negative culture results.

Discussion
This paper reports on the visual outcomes of horses with fungal plaques as a form of keratomycosis and shows a positive visual outcome in 16 of 22 (73%) horses. This percentage is similar to the percentages noted for all forms of keratomycosis, indicating that, when properly treated, horses with fungal plaques have a strong possibility for a positive visual outcome.

Plaque formation, rather than progression to a different form of keratomycosis, may be unique and dependent on the pathologic capability of certain fungal species. We hypothesize that following corneal ulceration, the fungi initially proliferate on the surface of the anterior corneal stroma to form a mat of hyphae. The fact that the fungi initially remain superficial may be due to an inherent lack of protease activity of the causative fungal species such that they do not move deeper into the stroma. Neutrophils are then attracted to the stroma to infiltrate beneath the fungal plaque to result in collagen degradation and later vascularization. In fungal deep stromal abscesses, the fungi produce more proteases to allow movement posteriorly within the stroma where they then attract neutrophils at the level of Descemet's membrane. The cellular infiltrate in fungal plaques is a neutrophilic, supplicative keratitis that is associated with fungi. Macrophages and monocytes are rarely seen histologically in our fungal plaque cases. This may be important to plaque formation, as there is a strong suggestion that the chemoattraction of macrophages instead of neutrophils would reduce the pathologic capabilities of fungi in horse keratopathies.

In equine keratomycosis with plaque formation, most eyes (91%) required some form of surgical intervention. The grid keratotomy may have precipitated keratomycosis and plaque formation in that case by allowing commensal fungal organisms to penetrate into the anterior stroma.

In human corneas, it has been noted that the genus of fungi involved may change the course of the progression with Fusarium solani causing markedly more stromal destruction than Aspergillus sp. However, in the horses examined here, Aspergillus sp. were cultured in four cases, and 50% (n=2) of those cases ended with enucleation of the globe, while Fusarium sp. were cultured in three cases, and one of those ended in enucleation of the globe.
cytology and/or culture results, it is likely that the sample used for PCR was taken from a sterile area of the plaque that contained only cellular debris from the cornea.

Conclusion

The visual outcome of therapy of 22 horses with the fungal plaque form of keratomycosis was positive in 16 (73%) with the average length of therapy 7.5±3.6 weeks. Successful treatment of the dark thick fungal plaque form of keratomycosis was aided by plaque removal, usually by performing one or more keratectomies, and concurrent successful management of corneal ulceration and accompanying uveitis.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

| Authors' contributions       | KMM | CEP | DEB |
|------------------------------|-----|-----|-----|
| Research concept and design  | ✓   | ✓   | ✓   |
| Collection and/or assembly of data | ✓ | ✓ | ✓ |
| Data analysis and interpretation | ✓ | ✓ | ✓ |
| Writing the article          | ✓   | ✓   | ✓   |
| Critical revision of the article | ✓ | ✓ | ✓ |
| Final approval of article    | ✓   | ✓   | ✓   |

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