The fungal plaque form of equine keratomycosis

Kathleen M. Maxwell, Caryn E. Plummer and Dennis E. Brooks*

*Correspondence: brooksd@ufl.edu

College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA.

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

Introduction

Horses commonly develop keratomycosis although the specific

reasons are not clear. Fungi are normal inhabitants of the equine environment and corneal/conjunctival microflora, and exist in

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

Figure 1. Case #1 displays a brown-yellow plaque raised above the corneal epithelium that is a classic presentation of a fungal plaque. Corneal vascularization is approaching the edge of the plaque dorsally and reached it ventrally. The edges of the plaque are loosely attached. A dematiaceous mold was cultured from this plaque.

Figure 2. Histology of a plaque from surgical pathology from case #21. The epithelium is absent at the top of the figure. Massive numbers of fungal hyphae (black) infiltrate the anterior and posterior corneal stroma to form the plaque. There are few neutrophils and minimal cellular and karyorrhectic fungal debris. (GMS; bar=50 microns).

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 re-growth of epithelial cells [15,16]. Standing keratectomies to

remove loose pieces at the edge of a plaque may be performed with a topical anesthetic and motor nerve block. Keratectomies to remove the entire plaque and place a graft over the defect can be done under general anesthesia and are warranted when there is extensive stromal tissue destruction and loss of stroma. Surgical procedures done under general anesthesia to protect the weakened cornea with tissue loss from keratomycosis include conjunctival grafts, collagen biomaterial grafts, and amniotic membrane transplants, and corneal transplants [1,15].

Retrospective studies of horses treated for the various forms of keratomycosis except the plaque form of keratomycosis show positive visual outcomes in 43-75% of horses [5-7,10,17]. The purpose of this study is to characterize the clinical presentation of fungal plaques in horses, and to discuss the diagnosis, medical and surgical treatment, and visual outcomes of cases of the fungal plaque form of keratomycosis.

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 cytologic 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 xylazine¹ or detomidine². A motor nerve block was then performed using 1% lidocaine³ and tetracaine⁴ 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 [18]. 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, 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- <i>Aspergillus</i> sp.	Staphylococcus, gram+ bacteria, dematiaceous mold	SK	Keratotomy- keratitis, focally extensive, severe, with intra-lesional fungal hyphae; appearance consistent with <i>Aspergillus</i> 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	<i>Scopulariopsis</i> sp., <i>Fusarium</i> sp.	SK multiple times	Keratotomy- 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/Biosisist/ AMT	Keratotomy- keratitis, neutrophilic, with neovascularization, chronic, diffuse, moderate	Visual, mild fibrosis
4	2 y, E, T	B-Y plaque, 30% of cornea	Too much edema to assess	Presentation- mild Final- same	Bacterial and fungal sepsis	<i>Staphylococcus xylosum</i> , <i>Streptococcus</i> spp., gram+ bacteria	SK multiple times	Keratotomy- large number of fungal hyphae, suppurative 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	<i>Pseudomonas</i> , <i>Fusarium</i> sp	SK	Enucleation- keratitis, ulcerative, necrosuppurative, focal, mild to moderate, with intralesional hyphae; anterior uveitis, suppurative, 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, <i>Dactylaria constricta</i>	None	--	Visual

Continuation of Table 1

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	<i>Aspergillus</i> , <i>Staphylococcus epidermis</i>	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, G, Qx	Y plaque, 40% of cornea	Mild flare	Presentation - moderate Final - infiltrative	Fungal keratitis	Non-hemolytic <i>Staphylococcus</i> , unknown fungus	SK	Keratotomy - large number of fungal hyphae, suppurative inflammation	Visual
10	16 y, G, WP	W plaque w/B rim, 80% of cornea	Severe (2+ flare)	Presentation - mild Final - infiltrative	Fungal sepsis w/neutrophilic inflammation	<i>Pseudomonas aeruginosa</i>	SK	Keratotomy - large number of fungal hyphae, suppurative inflammation, subacute	Visual, mod fibrosis
11	6 y, F, T	B plaque, 10x5mm	1+ flare	Presentation - mild Final - infiltrative	Fungal keratitis	Mold	SK	Keratotomy - suppurative, severe, acute to subacute, diffuse, with numerous intralesional fungal hyphae	Visual
12	17 y, F, Q	B-Y plaque, 15% of cornea	Mild	Presentation - mild Final - severe	Fungal keratitis	<i>Staphylococcus epidermis</i>	SK	Keratotomy - 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	<i>Aspergillus</i>	SK	Keratotomy - 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	<i>Staphylococcus aeruginosa</i> , <i>Nocardia</i> sp	GK-GA, CF	Keratotomy - 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	Keratotomy - large number of fungal hyphae; neutrophilic, with neovascularization, chronic, diffuse, moderate	Visual, mild fibrosis

Continuation of Table 1

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 Prior to enucleation moderate at site of AMT	Fungal sepsis	<i>Ralstonia picketti</i>	K-GA, AMT- 2 layers	Keratotomy- large number of fungal hyphae	Iris prolapse Enucleated
17	17 y, F, Q	W plaque, 20% of cornea	3+ flare	Presentation- mild Prior to enucleation moderate	Bacterial and fungal sepsis	<i>Stenotrophomonas maltophilia</i>	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 synechiae	Iris prolapse, Enucleated
18	10 y, G, T	B-Y plaque, 40% of cornea	None	Presentation- mild Final- moderate	Fungal keratitis	None	SK	Keratotomy- large number of fungal hyphae with many neutrophils	Visual, major fibrosis
19	10 y, F, Q	B plaque, 20mmx5mm	Could not assess	Presentation- moderate Final- infiltrative	Fungal keratitis	α -hemolytic streptococcus, mold	SK	Keratotomy- 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 Final- infiltrative	Neutrophilic keratitis w/ fungal sepsis	<i>Aspergillus</i>	SK	Keratotomy- large number of fungal hyphae with many neutrophils	Visual, mild fibrosis
21	6 y, F, C	W, 60% of cornea	None noted	Presentation- none Prior to enucleation mild, progressing slowly	Fungal keratitis	<i>Aspergillus</i>	K-GA, Biosist, AMT	Keratotomy- 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 synechia, 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 Final- infiltrative	Fungal keratitis	<i>Fusarium</i> sp., <i>Stenotrophomonas maltophilia</i>	DALK, K-GA, voriconazole, Biosist, AMT	Keratotomy- large number of fungal hyphae, suppurative	Visual, major fibrosis

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: Neovasc: Neovascularization;
Enuc: Enucleation; Surgical Interventions: SK: Standing keratectomy;
K-GA: Keratectomy under General Anesthesia;
GK: Grid Keratotomy; AMT: Amniotic membrane transfer;
CF: Conjunctival Flap; DALK: Deep anterior lamellar keratoplasty;
Breed: T: Thoroughbred; Q: Quarter Horse; S: Saddlebred;
W: Warmblood; P: Crossbred pony; C: Crossbred horse;
Ap: Appaloosa

for *Fusarium solani* and *Cladorrhinum bulbiliosum*. 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 voriconazole⁷ (n=11); the antibiotics cefazolin² (n=12), tobramycin⁸ (n=7), neopolygram⁹ (n=11), ciprofloxacin¹⁰ (n=8), and neomycin-polymyxin-bacitracin¹¹ (n=3); the anti-proteinases EDTA² (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, neopolydex¹⁷, 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 (Biosist²¹) (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

¹RompunTM, 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.

²Dormosedan®, Pfizer Animal Health, Exton, PA, USA.

³Lidocaine, Sparhawk Pharmacies Inc, Lenexa, KS, USA.

⁴Tetracaine®, OCuSOFT inc, Richmond, TX, USA.

⁵Natacyn®, Alcon Labs Inc, Fort Worth, TX, USA.

⁶Compounded.

⁷Voriconazole solution, Roerig, New York, NY, USA.

⁸Tobramycin Ophthalmic Solution, Akorn Inc, Lake Forest, IL, USA.

⁹Neomycin & Polymyxin B Sulfates & Gramicidin Ophthalmic solution, Bausch and Lomb Inc, Tampa, FL, USA.

¹⁰Ciprofloxacin Ophthalmic Solution, Hi-Tech Pharmacal Co Inc, Amityville, NY, USA.

¹¹Vetropolycin® Dechra Vet Products, Overland Park, KS, USA.

¹²Steri-Units®, Alcon Labs Inc, Fort Worth, TX, USA.

¹³Tropicamide Ophthalmic Solution, Akorn Inc, Lake Forest, IL, USA.

¹⁴Phenylephrine Hydrochloride Ophthalmic Solution, Bausch and Lomb Inc, Tampa, FL, USA.

¹⁵MURO 128.5%, Bausch and Lomb, Tampa, FL, USA.

¹⁶Flurbiprofen Sodium Ophthalmic Solution, Bausch and

Lomb Inc, Tampa, FL, USA.

¹⁷Neomycin & Polymyxin B Sulfates & Dexamethasone Ophthalmic Susp, Bausch and Lomb Inc, Tampa, FL, USA.

¹⁸Flunixinamine®, Zoetis, Florham Park, NJ, USA.

¹⁹GastroGard, Merial, Duluth, GA, USA.

²⁰TMP-SMZ, Mutual Pharmaceutical Co, Philadelphia, PA, USA.

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.

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 [1]. 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, suppurative keratitis that is associated with fungi [2,3]. 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 [1-3].

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 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 and have been reported to cause dark colored corneal plaques in humans [20]. The presence of a brown colored fungal hyphae in the scraping material strongly indicates the presence of a dematiaceous fungus [20]. The term "hyalohyphomycosis" is used to group together infections caused by hyaline non-dematiaceous *hyalohyphomycete* fungal pathogens, other than *Aspergillus*, such as *Fusarium* and *Scopulariopsis* that can also cause tissue coloration and were found in horses 2, 6 and 22 [21]. *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 [22].

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 [23]. 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.

Case #14 had a grid keratotomy performed by the referring veterinarian prior to presentation at the University of Florida. 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 equine keratomycosis with plaque formation, most eyes (91%) required some form of surgical intervention. It was our clinical impression that the plaque removal increased the comfort of the eye and sped vascularization to improve patient comfort and cause resolution of the fungal plaque. The majority of surgical interventions were standing keratectomies performed to remove necrotic plaque tissue and fungal debris in order to hasten the healing process. In a few cases, portions of the plaque sloughed off of the cornea without any surgical intervention. This spontaneous extrusion of the plaque was associated with stromal vascularization and corneal epithelial movement centripetally under the plaque.

Thirty-two percent (n=7) of horses that were cultured had bacterial infections in addition to the fungal infection. 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 negative results with a clinical appearance of a fungal plaque are likely due to the surface of the plaque containing only dead fungi. When negative PCR results were found in conjunction with positive

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 hastened 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	✓	✓	✓

Acknowledgement

We would like to thank Dr. Michael Dark, Dr. Lisa Farina, and Dr. Julia Conway from the surgical pathology group at University of Florida Veterinary Medical Center for examining the cytology specimens and biopsies.

Publication history

EIC: Olivier Sparagano, Coventry University, UK.
Received: 24-Feb-2015 Final Revised: 04-May-2015
Accepted: 07-May-2015 Published: 15-May-2015

References

- Brooks DE and Matthews AG. **Equine ophthalmology**. In Gelatt KN (4th Ed), **Veterinary Ophthalmology**. 2007; 1165-1274. | [Book](#)
- de Linde Henriksen M, Andersen PH, Thomsen PD, Plummer CE, Mangan B, Heegaard S, Toft N and Brooks DE. **Equine deep stromal abscesses (51 cases - 2004-2009)--Part 1: the clinical aspects with attention to the duration of the corneal disease, treatment history, clinical appearance, and microbiology results**. *Vet Ophthalmol*. 2014; **17 Suppl** 1:6-13. | [Article](#) | [PubMed](#)
- de Linde Henriksen M, Andersen PH, Mietelka K, Farina L, Thomsen PD, Plummer CE, Mangan BG, Heegaard S, Coleman JK, Toft N and Brooks DE. **Equine deep stromal abscesses (51 cases - 2004-2009)--Part 2: the histopathology and immunohistochemical aspect with attention to the histopathologic diagnosis, vascular response, and infectious agents**. *Vet Ophthalmol*. 2014; **17 Suppl** 1:14-22. | [Article](#) | [PubMed](#)
- Brooks DE, Andrew SE, Denis H, Strubbe DT, Biros DJ, Cutler TJ, Samuelson DA and Gelatt KN. **Rose bengal positive epithelial microerosions as a manifestation of equine keratomycosis**. *Vet Ophthalmol*. 2000; **3**:83-86. | [Article](#) | [PubMed](#)
- Galán A, Martín-Suárez EM, Gallardo JM and Molleda JM. **Clinical findings and progression of 10 cases of equine ulcerative keratomycosis**. *Equine Vet. Educ*. 2009; **21**:236-42. | [Article](#)
- Voelter-Ratson K, Pot SA, Florin M and Spiess BM. **Equine keratomycosis in Switzerland: a retrospective evaluation of 35 horses (January 2000-August 2011)**. *Equine Vet J*. 2013; **45**:608-12. | [Article](#) | [PubMed](#)
- Wada S, Hobo S, Ode H, Niwa H and Moriyama H. **Equine keratomycosis in Japan**. *Vet Ophthalmol*. 2013; **16**:1-9. | [Article](#) | [PubMed](#)
- Andrew SE, Brooks DE, Smith PJ, Gelatt KN, Chmielewski NT and Whittaker CJ. **Equine ulcerative keratomycosis: visual outcome and ocular survival in 39 cases (1987-1996)**. *Equine Vet J*. 1998; **30**:109-16. | [Article](#) | [PubMed](#)
- Gaarder JE, Rebhun WC, Ball MA, Patten V, Shin S and Erb H. **Clinical appearances, healing patterns, risk factors, and outcomes of horses with fungal keratitis: 53 cases (1978-1996)**. *J Am Vet Med Assoc*. 1998; **213**:105-12. | [Article](#) | [PubMed](#)
- Reed Z, Thomasy SM, Good KL, Maggs DJ, Magdesian KG, Pusterla N and Hollingsworth SR. **Equine keratomycoses in California from 1987 to 2010 (47 cases)**. *Equine Vet J*. 2013; **45**:361-6. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
- Hendrix DV, Brooks DE, Smith PJ, Gelatt KN, Miller TR, Whittaker C, Pellicane C and Chmielewski N. **Corneal stromal abscesses in the horse: a review of 24 cases**. *Equine Vet J*. 1995; **27**:440-7. | [Article](#) | [PubMed](#)
- Mitchell JS and Attleberger MH. **Fusarium keratomycosis in the horse**. *Vet Med Small Anim Clin*. 1973; **68**:1257-60. | [PubMed](#)
- Cutler TJ. **Corneal epithelial disease**. *Vet Clin North Am Equine Pract*. 2004; **20**:319-43. | [Article](#) | [PubMed](#)
- Zhu WS, Wojdyla K, Donlon K, Thomas PA and Eberle HI. **Extracellular proteases of *Aspergillus flavus*. Fungal keratitis, proteases, and pathogenesis**. *Diagn Microbiol Infect Dis*. 1990; **13**:491-7. | [Article](#) | [PubMed](#)
- Galera P and Brooks D. **Optimal management of equine keratomycosis**. *Vet. Med: Research and Reports*. 2012; **3**:7-17. | [Article](#)
- Welch PM, Gabal M, Betts DM, Whelan NC and Studer ME. **In vitro analysis of antiangiogenic activity of fungi isolated from clinical cases of equine keratomycosis**. *Vet Ophthalmol*. 2000; **3**:145-151. | [Article](#) | [PubMed](#)
- Grahn B, Wolfer J and Keller C. **Equine keratomycosis: Clinical and laboratory findings in 23 cases**. *Progress in Vet. and Compar. Ophthalmol*. 1993; **3**:2-7.
- Turenne CY, Sanche SE, Hoban DJ, Karlowsky JA and Kabani AM. **Rapid identification of fungi by using the ITS2 genetic region and an automated fluorescent capillary electrophoresis system**. *J Clin Microbiol*. 1999; **37**:1846-51. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
- Ollivier FJ. **Medical and surgical management of melting corneal ulcers exhibiting hyperproteinase activity in the horse**. *Clin. Tech. in Equine Prac*. 2005; **4**:50-71. | [Article](#)
- Gajjar DU, Pal AK, Ghodadra BK and Vasavada AR. **Microscopic evaluation, molecular identification, antifungal susceptibility, and clinical outcomes in fusarium, Aspergillus and, dematiaceous keratitis**. *Biomed Res Int*. 2013; **2013**:605308. | [Article](#) | [PubMed Abstract](#) | [PubMed Full Text](#)
- Burgess LW and Liddell CM. **Laboratory manual for Fusarium research. 2nd Edition**. *Fusarium Research Laboratory, Department of Plant Pathology and Agricultural Entomology*. The University of Sydney. | [Book](#)
- Klich MA. **Identification of common *Aspergillus* species**. Utrecht, The Netherlands, Centraalbureau voor Schimmelcultures. 2002. | [Book](#)
- Thomas PA. **Fungal infections of the cornea**. *Eye (Lond)*. 2003; **17**:852-62. | [Article](#) | [PubMed](#)

Citation:

Maxwell KM, Plummer CE and Brooks DE. **The fungal plaque form of equine keratomycosis**. *Vet Med Anim Sci*. 2015; **3**:2. <http://dx.doi.org/10.7243/2054-3425-3-2>