Bacterial and Fungal Keratitis in the Equine Patient
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Objectives:

- A thorough ocular examination is key to appropriate diagnosis and prompt treatment
- Understanding the etiology of bacterial and fungal keratitis
- How to improve therapeutic outcomes

Introduction: Ulcerative keratitis is a common condition in horses due to their large, prominent eyes and can be potentially sight limiting if not diagnosed early and treated properly. Superficial, non-infected ulcers commonly heal rapidly; whereas stromal degradation associated with infected ulcers can rapidly progress to perforation of the cornea. This is characterized by increased proteolytic activity in tear film and stroma, which is responsible for the melting and necrosis of the cornea. Since melting ulcers in horses can progress rapidly, a swift diagnosis and suitable treatment are essential to salvaging the eye. A thorough ocular examination and medical history are crucial and can aid in determining the severity of the corneal disease, as well as choosing the appropriate method of treatment whether medical, surgical or a combination.

Pathophysiology: Injury to the cornea activates multiple systems that produce a series of complex and synchronized cellular and biochemical processes that can result in either corneal destruction or healing. Ocular surface failure most often appears as corneal ulceration in the horse and occurs after trauma, inflammation, or infection. Ulcers can range in depth from simple, superficial, to full-thickness and in some cases the iris will prolapse. Excessive proteinase activity of the tear film and stroma intensify the problem. The major sequelae of corneal ulceration include infection, iridocyclitis, and disregulation of tear film protease activity. These areas are thus targets for medical therapy.

Formation of plasmin by plasminogen activator is an early step in response to ulceration. The damaged cornea causes the production of plasmin, and subsequently the production of matrix metalloproteinases (MMP), a group of zinc-dependent proteolytic enzymes that function in the remodeling of extracellular matrix within tissues. There are two main types of MMPs important in remodeling and degradation of corneal stromal collagen: MMP-2 and MMP-9. These MMPs are produced by corneal keratocytes and act as surveillance in the normal cornea. They become locally active when collagen molecules are damaged to aid in the removal of cells. In diseased states, leucocytes release massive quantities of degradative proteases, peroxide radicals, and peroxides, which damage the corneal stroma. This causes a breakdown of stromal collagen and stromal extracellular matrix resulting in “melting” of the cornea. Response to injury causes the release of prostaglandins, leucotrienes, and cytokines from epithelial cells and sub epithelial keratocytes. Neutrophils eliminate bacteria, but are too small to phagocytize fungi. Antibiotics can rapidly sterilize corneal ulcers; however, after neutrophils have been recruited, they remain to cause severe corneal stromal damage and are often the resulting cause of the keratomalacia.
When bacterial and fungal organisms resist the natural corneal defense mechanisms like eyelashes, washing effect of the tear film, blinking, ocular mucin, and antimicrobial substances, colonization of the corneal epithelium and stroma occur. Young horses, especially, are at an increased risk for gram-negative bacterial infections caused by *Pseudomonas* sp., as well as gram-positive bacterial infections including, *Staphylococcus* sp., *Listeria* sp., and *Streptococcus* sp. Bacteria such as *Pseudomonas* sp. readily adhere to injured or diseased epithelium, but do not adhere well to intact epithelium. Once these organisms reach the stroma, they spread by migrating through the stromal collagen lamellae. After establishment, release and activation of large amounts of inflammatory cytokines follows, thus causing extensive infiltration of white blood cells from limbal blood vessels and the PTF. The destruction of the stroma is caused by the release of proteolytic enzymes by both the microbial organisms and the neutrophils. The white blood cells begin a destructive process that involves necrosis of stromal keratocytes and phagocytosis of these degenerating stromal cells. As the white blood cells migrate peripherally in the anterior stroma, the overlying epithelium is destroyed which enlarges the defect and enhances the migration of the white blood cells into the corneal stroma. Soon after, the collagen fibrils are lost and stromal destruction increases with the peripheral migration.

By contrast, fungal organisms particularly, *Fusarium* and *Aspergillus* spp., have the ability to adhere to healthy equine corneal epithelial cells when the normal PTF layer is compromised. These fungi penetrate and tunnel into the stroma toward the Descemet’s membrane. This causes the addition of MMPs. Recent research suggests that fungal organisms, produce metabolites that inhibit tubule formation in vitro and these metabolites may play a significant role in the altering of the host response to fungal infections in the cornea. The leukocytic response to fungal hyphae present in the corneal stroma is primarily mediated by neutrophilic inflammation. Neutrophils are ineffective at digesting large fungal hyphae. Macrophages are found in small numbers and have the ability to effectively remove fungal hyphae. However, sufficient quantities of macrophages are unable to migrate to the corneal stroma. Leukocyte infiltration of the cornea is thus highly undesirable if present in excessive numbers. Total corneal ulceration ultimately requires the degradation of the collagen framework of the corneal stroma. Most evidence indicates that true corneal collagenolysis results from the proteases produced by the PMNs primarily and only minimally by fungal organisms. Even if the fungal organisms can be halted with anti-fungals or the immune response, the inflammatory response still ensues for some time. Thus, one of the hallmarks of treating keratomycosis is to quiet the inflammatory response.

Ulcerative keratitis has been linked with initially high levels of tear film proteolytic activity. Normalizing this activity in the tear film is a principal objective in the treatment of melting corneal ulcers. After stopping the destructive process, the ocular surface can undergo a course of repair. This includes vascularization, glycosaminoglycans synthesis, collagen resynthesis, and reepithelialization. The rate and efficiency of that healing depends on a number of factors; location and depth of the wound, presence of microbial or foreign body contaminants, and topical application of drugs. Epithelial cells migrate at a rate of 0.6-1.2 mm/day in non-infected horse corneas. Corneal vascularization occurs at a rate of 1 mm/day. Tear film proteases are elevated two to four times normal levels in ulcerated eyes and reduction must occur before healing can be completed.
Clinical presentation: Clinical signs for corneal lesions include blepharospasm, conjunctival and episcleral hyperemia, epiphora, corneal edema, and neovascularization, photophobia, miosis, and protraction of the nictitating membrane. Ocular pain is a hallmark finding in keratomycosis and will range in severity depending on location and depth of the corneal defect, as well as the presence or absence of reflex uveitis. Serous to mucoid ocular discharge, focal or diffuse corneal edema, corneal vascularization, and keratomalacia can be grossly appreciated in many cases. At first, anterior uveitis and corneal vascularization may not be pronounced, but as the defect progress, these characteristics can be appreciated. Corneal trauma causes a concurrent iridocyclitis, or inflammation of the iris and ciliary body. Penetration deep into the Descemet’s membrane is a potential outcome secondary to equine corneal ulceration. If the ulcer has previously received prolonged antibiotic or corticosteroid therapy with little to no improvement, fungal involvement should be suspected.

Superficial keratomycosis presenting as microerosions is characterized by multifocal whitish subepithelial opacities, staining positively with rose bengal dye, indicative of epithelial devitalization, with or without accompanying ulceration. Both superficial ulceration and stromal abscessation will yield a more painful eye, deeper vascularization, and anterior uveitis is more likely to be present. One of the more characteristic features of fungal keratitis is focal, creamy yellow, somewhat “fluffy” corneal opacities at the advancing edge of the lesion – so-called satellite lesions. A similar presentation would yield a whitish-yellow, necrotic plaque of corneal stroma within an area of corneal ulceration -- “cake-frosting” appearance. Of note is that ulcerative keratitis in the foal often differs from the adult horse. Neonates may not show characteristic signs of ocular pain. For this reason, ulcers are often missed.

Diagnosis: Any painful eye as evidenced by blepharospasm, epiphora or photosensitivity should be examined and stained. A thorough ocular examination performed with proper restraint, sedation, and blocking will facilitate the veterinarian in arriving at an appropriate assessment of the severity of the lesion. Even if the surface of the cornea does not appear to be abraded, fluorescein staining should always be performed as minor, superficial ulcers can be missed although could promptly respond to appropriate therapy if diagnosed early. Examination should include assessment of the anterior chamber, iris, lens and posterior chamber for evidence of underlying or complicating pathology such as chronic uveitic changes, foreign body, tumor or laceration.

Definitive diagnosis of ulcerative keratomycosis depends on a positive fungal culture, but, which this is ideal, final results lag behind clinical decision making and treatment necessity. Thus, it is prudent to evaluate corneal scrapings cytologically for evidence of fungal hyphae, mold or yeast to aid in a presumptive and timely tentative diagnosis. Additionally, histopathologic examination of a keratectomy sample or Polymerase Chain Reaction (PCR) of corneal scrapings and biopsies may be helpful diagnostic modalities.

Treatment: While medical therapy is the mainstay of ulcer treatment, the addition of surgical procedures may be used adjunctively to speed healing and improve outcomes. In consideration of medical therapy, the author uses the “Five A’s of corneal ulcer treatment” approach. This is a multi-pronged approach including: Antibiotics, Antifungals, Atropine, Anti-inflammatory (NSAIDs) and Antiproteinases. Treatment will initially be frequent (q 2-4h) with multiple
medications, horses are often painful, and quickly become resentful of treatment. Thus, a subpalpebral lavage (SPL) system should be used when warranted. This allows topical application and increased absorption of medications. The goal of any therapeutic plan is to sterilize the ulcer, reduce tear film protease activity, and decrease uveitis. First and foremost, the progression of fungal and bacterial agents must be halted with topical antibiotics and antifungals specifically tailored to the pathologic agent present.

**Antibiotics:** Triple antibiotic or tobramycin are good first line drugs with broad spectrum of activity that can be used as initial treatment or until cytology results can aid in a more tailored approach. Gentamicin, ciprofloxacin, or tobramycin ophthalmic solutions can be used to treat gram-negative bacterial ulcers. Gentamicin was previously effective against *Pseudomonas* spp. but currently 50% of organisms are resistant and it is irritating to healthy corneas, thus amikacin or ciprofloxacin are recommended in cases of *Pseudomonas* infection. Gram-positive organisms, particularly beta-hemolytic *Streptococcus* sp. should be treated with cefazolin. However, this drug is very irritating so should be conservatively. Chloramphenicol is broad spectrum and non-irritating to corneas and can be a reasonable choice. It is recommended that you use the most benign, broad-spectrum antibiotic and change only if you are not getting improvement.

**Antifungals:** Fungal ulcers have been successfully treated with miconazole, natamycin, fluconazole, voriconazole, clotrimazole, and itraconazole with susceptibility of fungal agents varying depending upon geographic distribution. These antifungal agents need to be used for several weeks; systemic antifungals can be used, but their efficacy has not been proven. Antifungal therapy is difficult because fungicidal levels are not easy to obtain and often result in fungistatic activity only. It is ideal to determine the species of fungi infecting the cornea, and base the antifungal drug selection from that discovery, however culture results often lag weeks behind the case. Therefore, familiarity with the key organisms and efficacy of antifungals in the region of one’s practice can help guide therapy. Topical antifungal therapy can be initiated as natamycin and miconazole in combination (Brooks, UFl), or voriconazole alone (Boggs, MSU), applied three to four times per day, over the first couple of days. Sudden death of stromal fungi can occur and initiate acute iridocyclitis if higher doses are used. It is not uncommon to experience an increase in ocular inflammation with initiation of therapy as significant fungal death occurs. The author recommends tapering up and down with the antifungals at onset and discontinuation of treatment as well as using full dose NSAIDs at initiation of treatment.

**Atropine:** Atropine should be used to address anterior uveitis as this is a significant source of pain for horses, can lead to ongoing ocular inflammation and can ultimately result in blindness. It stimulates pupillary dilation, which protects the visual axis from occlusion by preventing formation of synechiae. Atropine also stabilizes the blood aqueous barrier to reduce aqueous flare and induces relaxation of the ciliary muscles, which aids in preventing ciliary spasms, a source of ocular discomfort. If adhesions between the iris and the lens (synechiae) have already occurred, tissue plasminogen activator can aid in breakdown. One should assume that every horse with a corneal ulcer has uveitis to some degree and thus topical atropine should be used to effect (mydriasis) as well as systemic NSAIDs. Remember, horses are exquisitely sensitive to the anticholinergic effects of atropine, so should be monitored closely for signs of colic. In severe uveitis, one may initially need to use atropine 4 or more times per day, but as soon as mydriasis is observed the frequency should be decreased. If you note sluggish PLR in the contralateral eye, this indicates the atropine has become systemic and the
dose should be significantly decreased. While one should use caution with atropine it is an important component of treating anterior uveitis so should be considered for all cases of corneal ulceration unless very mild.

**Antiinflammatories:** Phenylbutazone orally or flunixin meglumine (banamine) orally or intravenously can be used to reduce uveal exudation and relieve ocular discomfort, however flunixin meglumine is considered by most ophthalmologists to be more efficacious for ocular inflammation and pain.

**Antiproteinases:** Serum has been advocated in ulcerative keratomycosis, to inhibit collagenase activity. The alpha-2-macroglobulin portion inhibits corneal collagenases. Serum is effective against both types of proteases (NE and MMP). Other adjunctive modalities that have been shown to inhibit MMPs are: 0.17% EDTA, topical 0.1% doxycycline, 5% N-acetylcysteine (NAC), and tetanus antitoxin. Because these compounds use different mechanisms to inhibit various families of proteases in the tear film of horses, a combination of these protease inhibitors may be beneficial for treatment of corneal ulcers in horses. Furthermore, EDTA is effective in breaking down the “biofilm” that surrounds fungi, thus increasing efficacy of antifungals. The author uses autogenous serum and EDTA but does not mix them. The combined treatment reduces MMPs by ~80% after 4-7 days. An easy way to prepare 0.17% EDTA is to take a purple top tube, fill it with sterile water and shake. Serum can be harvested directly from the patient. Draw 8-10 red top tubes, spin down the serum and use for up to 7 days (stored at room temperature or refrigerated), then replace.

**Surgical treatments:** Combined medical and surgical therapy may be warranted in particularly deep ulcers, non-responsive ulcers, or melting corneal ulcers. To treat superficial corneal ulcers, a grid keratotomy, or direct surgical debridement of dead and necrotic tissues may be useful. This procedure can aid in minimizing scarring and increase the rate of healing. This is also useful in decreasing the stimulus for iridocyclitis. Grid keratotomy should never be performed in cases of suspected fungal keratitis as inadvertent penetration of fungal organisms into the deeper cornea can occur. Deep, melting, and large corneal ulcers can be treated with conjunctival flaps or corneal grafts. Conjunctival autografts contain limbal stem cells, blood vessels, and lymphatics. Corneal scarring is a common sequela of this procedure. Another way to improve the surface of the cornea, but decrease scarring at the same time, is by using an amniotic membrane graft. This type of graft can be used to facilitate migration and differentiation of epithelial cells. The cellular adhesion of those same cells can be reinforced, as well as decrease ocular surface inflammation. Amnion has anti-inflammatory and antiangiogenic properties and thus can reduce the severity of corneal melting and corneal neovascularization to improve the likelihood of repair of both the epithelial and stromal defects. Amnionic membrane grafts modulate corneal fibroblasts and decrease scarring to improve corneal transparency. Amnion is harvested from mares, washed, stored frozen with antibiotics then thawed and sutured in place when necessary.

If corneal graft rejection occurs, full-thickness microsurgical corneal transplantation can be performed. Penetrating keratoplasty removes layers of the corneal epithelium, stroma, and Descemet’s membrane. This is a common practice in human medicine and has recently become popular in instances with severe inflammatory non-ulcerative and ulcerative keratopathies, and keratomalacia in horses.
**Prognosis:** Prognosis depends on the type of ulcer present as well as the ability to prevent or decrease protease activity caused by microbes as well as within the tear film. If bacterial keratitis is treated with appropriate antibiotics and with suitable surgical methods as adjunct, a positive visual outcome can be expected. In all cases of fungal keratitis, prognosis is guarded. Because most anti-fungal agents are fungistatic instead of fungicidal, recurrence is possible, if appropriate drugs are not administered that can penetrate the cornea and reach therapeutic levels. The depth of the lesion also plays a role in prognosis. Perforation and endophthalmitis occurs in some cases and in those instances, enucleation is necessary.

**Summary:** The high incidence of bacterial and keratitis in horses is attributed to several predisposing factors including: large prominent laterally positioned eyes, naturally aggressive physical activity, and frequent environmental exposure to pathogens. Fungal keratitis should be suspected if a corneal ulcer has received prolonged antibiotic or corticosteroid therapy, or both, with either slight or no improvement; if there is a history of corneal injury with vegetative material; or if an ulcer fails to vascularize. Globe rupture, minimal to massive corneal scarring, phthisis bulbi, and blindness are all possible sequelae to kertaomycosis, however favorable outcomes are possible with appropriate diagnosis and prompt, long-term therapy.

**References:** Available upon request.