

## Primary *Aspergillus* and *Fusarium* keratitis in a Holstein cow

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### Abstract

A 5-year-old female Holstein cow was examined for ocular discharge, periorbital swelling, an area of full-thickness corneal cellular infiltrate, fibrin, hypopyon, diffuse corneal edema, and miosis. The patient was diagnosed with a corneal stromal abscess and secondary anterior uveitis. Histopathology, mycotic culture, and polymerase chain reaction positive for *Aspergillus* and *Fusarium* DNA confirmed the presence of fungal infection. Response to therapy was adequate, and follow-up with the patient 1 year after diagnosis revealed a focal area of corneal fibrosis.

**Key Words:** abscess, *Aspergillus*, bovine, cornea, *Fusarium*

### INTRODUCTION

Keratomycosis is extremely uncommon in cattle. There are currently no reports in the literature describing primary bovine keratomycosis. In contrast, however, keratomycosis is common in horses, and reports of equine ocular fungal infection are prevalent in the literature. Several factors likely influence the relative greater occurrence of keratomycosis in horses compared to cattle. Predisposing corneal trauma, environmental and intrinsic host factors, and the frequency of topical antibiotic and corticosteroid therapeutic intervention in horses are significant and likely contribute to the greater prevalence of equine ocular fungal infection. The intent of this case report is to elaborate the clinical presentation, diagnosis, therapeutic intervention, and response to treatment of a Holstein cow with primary fungal keratitis and secondary uveitis.

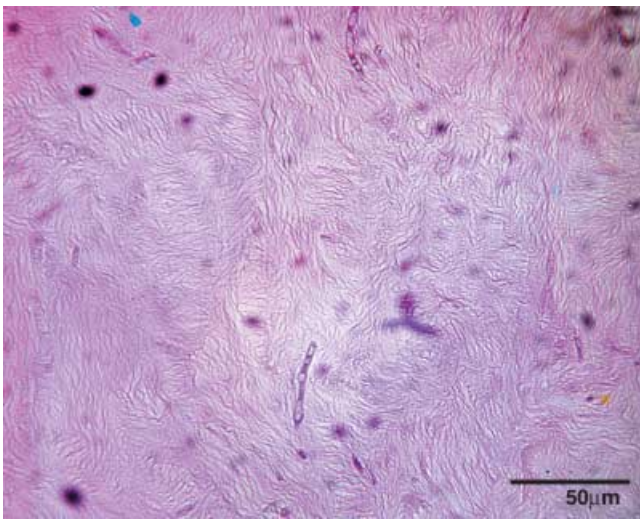
### CASE REPORT

A 5-year-old female Holstein cow presented to The Ohio State University College of Veterinary Medicine Teaching Hospital for recurrent ketosis as well as evaluation of periorbital swelling and ocular discharge involving the left eye (OS). Pertinent history included hospitalization at the teaching hospital 3 weeks prior to presentation for an internally displaced abomasum with secondary ketosis and hypocalcemia. An omentopexy was performed and the patient was discharged 1 week later. On general physical examination at the time of presentation, mild dehydration, soft tissue swelling at the omentopexy incision site, and moderate to high ketonuria were appreciated.

Examination OS revealed blepharospasm, blepharedema, photophobia, enophthalmia, copious green ocular discharge, and a 3–4 mm paraxial abscess. Direct and consensual pupillary light reflexes were absent OS because of a severely miotic pupil, but were normal in the right eye (OD). Fluorescein stain OS was negative. An auriculopalpebral nerve block with carbocaine (Abbot Laboratories, North Chicago, IL, USA) was performed to facilitate examination. Biomicroscopic evaluation of the left eye revealed moderate (2+) diffuse corneal edema, a 3–4 mm full-thickness, yellow cellular infiltrate located in the ventral region of the cornea, and the presence of fibrin and hypopyon within the anterior chamber. Deep stromal vessels invaded the cornea circumferentially and extended to the ventral margin of the cellular infiltrate. A focal area of clear cornea was evident dorsally (Fig. 1). The lens and posterior segment could not be visualized. Biomicroscopic examination OD was unremarkable. Cytology of the corneal lesion OS revealed the presence of septate, branching hyphae, epithelial cells, and numerous neutrophils. The presence of bacteria was not observed on cytology. Corneal swabs were obtained and submitted for fungal and bacterial culture. Vannas scissors were used to debride the diseased cornea and to obtain samples for histopathology and fungal polymerase chain reaction (PCR). A diagnosis of fungal corneal stromal abscess with secondary anterior uveitis was made. Topical therapy OS was initiated with natamycin 5% solution (Alcon Laboratories, Fort Worth, TX, USA) every 6 h, Neo-Poly Bac ophthalmic ointment (Butler Co., Columbus, OH, USA) every 6 h, and atropine sulfate 1% ointment (Butler Co.) once daily. Flunixin meglumine (Schering-Plough, Union, NJ, USA) 1 mg/kg was given intramuscularly every 12 h.



**Figure 1.** Focal area of yellow cellular infiltrate surrounded by diffuse corneal edema and vascularization of the cornea.



**Figure 2.** Necrotic corneal stroma containing scattered septate, dichotomously branching fungal hyphae. H&E 40X.

Histopathology revealed a necrotic corneal stroma containing numerous, scattered, septate, dichotomously branching fungal hyphae (Fig. 2). Bacterial culture was negative, but *Aspergillus* sp. grew on fungal culture, and PCR was positive for *Fusarium* sp. and *Aspergillus* sp. DNA.

Within 1 week after topical therapy was initiated, the stromal abscess had begun to resolve, and atropine administration was discontinued. Because of a decline in the patient's metabolic status 9 days following admission, the frequency of systemic flunixin meglumine therapy was decreased to once daily and administration of 7100 mg Oxytetracycline (Vedco, St. Joseph, MO, USA) once daily was initiated. By 2 weeks after commencement of ocular topical therapy, the stromal abscess was completely vascularized and the patient's comfort level had significantly improved. Treatment with natamycin was discontinued, and administration of miconazole 1% solution (compounded at the teaching hospital pharmacy) every 6 h was initiated. The patient was discharged



**Figure 3.** One year after medical therapy, a focal area of corneal fibrosis remains.

17 days following admission with instructions to instill miconazole 1% solution every 6 h, Neo-Poly Bac ophthalmic ointment every 6 h, and atropine sulfate 1% ointment once daily as needed for pain for 14 days following discharge. Follow-up with the patient 1 year after diagnosis revealed only a small, focal area of corneal fibrosis OS (Fig. 3).

## DISCUSSION

Fungal keratitis is a leading cause of human ocular morbidity worldwide.<sup>1</sup> Among domestic animals, fungal keratitis is most common in the horse.<sup>2,3</sup> Bovine keratomycosis is extremely uncommon. The microbial flora of the normal bovine eye is predominantly gram-positive bacteria. The three most common bovine ocular bacterial isolates are *Staphylococcus epidermidis*, *Streptococcus faecalis*, and *Bacillus* sp.<sup>2,4</sup> Gram-negative nonhemolytic *Moraxella bovis* and *Branhamella catarrhalis*, as well as *Mycoplasma bovovulvi* also predominate in healthy and diseased cattle eyes.<sup>4,5</sup> Few descriptions of bovine ocular fungal flora exist in the literature. One study of the conjunctival fungal flora of several domestic animal species, including horses and cattle, incorporated 25 cows and 43 horses without clinical evidence or recent history of keratitis or other ocular disease.<sup>3</sup> Fungi were isolated from conjunctival swabs of 100% of the bovine and 95% of the equine subjects. Of a total of 95 bovine fungal isolates, 75 of which were identifiable, *Penicillium* sp. and *Cladosporium* sp. were most frequently isolated, accounting for 11 (12%) and 16 (16%) of the isolates, respectively. Only three (3%) of the bovine fungal isolates were identified as *Aspergillus* sp. Of a total of 88 equine conjunctival fungal isolates, 81 of which were identifiable, 23 of the isolates (56%) were identified as *Aspergillus* sp. *Penicillium* sp. were the next most frequently isolated fungi among the horses, accounting for 19 (22%) of the total isolates.

Diagnosis of keratomycosis has been conventionally established by recognition of disease symptoms, isolation and culture of organisms, or by morphologic or biochemical identification.<sup>6</sup> Novel methods of diagnosing fungal infection

include immunologic detection of fungal toxins and PCR. One recent study reported in the literature concluded that antisera against low molecular weight *Aspergillus fumigatus* toxin may be a potentially useful diagnostic indicator of fungal infection.<sup>7</sup> PCR technology has recently evolved from use predominately in the realm of research to a viable clinical diagnostic modality.<sup>6,8-10</sup> PCR technology is currently being utilized to identify ocular fungal infection at The Ohio State University College of Veterinary Medicine Teaching Hospital. PCR fungal diagnosis requires the preselection of a specific target DNA region for which DNA extracted from tissue samples is examined. Universal fungal primers and specific primers for *Fusarium* sp. and *Aspergillus* sp. are currently in use at the teaching hospital.

The clinical appearance of fungal keratitis is less predictable than the appearance of bacterial keratitis.<sup>11</sup> A deep infection with no visible epithelial defect may result during the course of keratomycosis from either fungal migration through the cornea or epithelialization over an infected stroma. Equine fungal keratitis is typically characterized by epithelial ulceration with considerable stromal edema, deep corneal vascularization, pain, serous to purulent discharge, and a white to gray stromal cellular infiltrate. Clinical signs associated with improvement of equine keratomycosis vary depending upon the chronicity of infection and the rate of corneal melting when treatment was initiated.<sup>11</sup> A reduction in corneal edema, cellular infiltrate, and pain are the most reliable clinical signs of improvement. Corneal clearing is usually accompanied by deep stromal vascularization. The areas of opacification become denser as they diminish in size as a result of fibrosis.

Two mechanisms have been proposed for the establishment of ocular fungal disease. Fungal colonization can occur following disruption of the anatomic integrity of the eye, or alternatively, colonization may occur via hematogenous extension of fungal organisms to the eye. A break in the integrity of the corneal epithelium is the usual prerequisite for the establishment of keratomycosis in horses.<sup>2</sup> Common pathophysiologic mechanisms for the development of equine keratomycosis include direct inoculation via penetration of the cornea or treatment of corneal ulceration with topical corticosteroids that inhibit cell-mediated immune response allowing fungal overgrowth.<sup>11</sup> In a study of 191 human patients with fungal keratitis, predisposing risk factors were identified in 79% of the study population.<sup>12</sup> Infection was associated with corneal trauma in 42% of the patients, contact lens wear in 25% of the patients, and topical corticosteroid administration in 21% of the patients. In humans, oculomycoses can also occur during systemic immune depression.<sup>13</sup> However, in animals, systemic mycoses often occur in endemic areas in otherwise healthy individuals.<sup>2</sup>

Systemic fungal infection is not uncommon in cattle. Fungal abortion and pulmonary and mammary aspergillosis have been reported, and the portal of entry of *Aspergillus* implicated in systemic infection is usually the gastrointestinal tract.<sup>14-16</sup> However, there is little evidence in the literature to support hematogenous spread of fungal organisms as a primary cause

of ocular fungal infection. There is one report in the literature of a calf with disseminated *Rhizopus* infection and ocular involvement.<sup>17</sup> However, there are no reports in the literature describing ocular manifestations of systemic fungal disease involving either *Aspergillus* or *Fusarium*. Systemic debilitation of the Holstein cow presented in this report may have contributed to the inception of keratomycosis, which is exceedingly rare in bovine species.

Both medical and surgical options exist for the treatment of keratomycosis. Topical antifungal agents commonly used to treat equine ocular fungal infection include miconazole, natamycin, itraconazole, ketoconazole, econazole, and sulfadiazine cream.<sup>11,18-21</sup> There are several reports in the literature of studies initiated to determine the comparative efficacy of these therapeutic agents. One study reported that fungi causing equine keratomycosis were equally susceptible to natamycin and miconazole, followed by itraconazole, then ketoconazole.<sup>19</sup> Another investigation found topical natamycin to be the therapy of choice for filamentous fungal keratitis in humans, followed by itraconazole, especially for *Aspergillus* or *Curvularia* sp.<sup>20</sup> A third study involving human patients found that the concurrent use of 5% natamycin and 2% econazole offered no additional benefits over monotherapy with 5% natamycin for the management of fungal keratitis.<sup>21</sup> Systemic antifungal agents are also efficacious in the treatment of equine keratomycosis.<sup>22</sup> A significant obstacle to the successful medical management of keratomycosis in veterinary patients remains the sustained delivery of the therapeutic agent to the site of infection.

Objectives of surgical treatment of keratomycosis include accurate diagnosis by means of biopsy of the infected tissue, removal of the diseased tissue, and the structural reinforcement of injured tissue.<sup>11</sup> Surgical management typically involves a superficial, deep, or penetrating keratectomy to debride infected corneal stroma, followed by a grafting procedure using conjunctiva, as with a rotational pedicle graft, or cornea in a lamellar or penetrating keratoplasty.<sup>23</sup> Regardless of whether treatment is medical, surgical, or both, the efficacy of treatment can be evaluated by the presence of cytological changes such as poorly staining walls and indistinct hyphae, both of which indicate mycotic cell death.<sup>11</sup>

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