IN THE BLEACHERS

HE HAS TO WEAR IT FOR ANOTHER WEEK. MEANWHILE, HIS SCORING AND REBOUNDING HAVE SUFFERED...

BY STEVE MOORE

MORE

ALONG WITH HIS PRIDE.

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Well, that idiot's done it again... left for work with only one eye.

1984

How birds see the world.
Fungi of Ophthalmic Importance

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Mycology

- Fungi are eukaryotic organisms

**Yeast**
- Unicellular
  - Elliptical to spherical
  - Generally 3-5 µm in diameter
  - Reproduce by budding

**Molds**
- Multi-cellular
  - Branching tubular hyphae
  - 2-10 µm in diameter
Dimorphic

- Fungi that can grow as either yeasts or molds

Polymorphic

- Fungi that exhibit multiple forms simultaneously
- Environmental temperature, nutrient factors and kinetic factors determine the type of growth observed.
Reproductive Cycle of Fungi

- Sexual, asexual or both
- Simple mitosis of somatic nuclei and budding
- Apical extension of the cell wall
- Septa
- Fungal cell walls contain ergosterol
Fungi and the eye

- Not permanent floral residents
- Transient colonizers of the external eye

Predominant fungal isolates from normal equine eyes

- Aspergillus
- Fusarium
- Alternaria
- Cladosporium
- Penicillium
- Absidia
Ocular defenses

- An intact corneal epithelium provides resistance to fungal penetration and infection.

- Unfavorable environment for the growth of any opportunistic fungi due to:
  - Normal ocular surface flora
  - Normal lacrimal flow
  - Mechanical movements of eyelids and nictitans
Normal body temperature prevents many fungi from becoming pathogenic.

The lower temperature of the cornea may partially explain the predilection for keratomycosis.
Immunity

- Primarily cell mediated
- Local immunosuppression by corticosteroids may predispose an animal to a fungal infection
- Systemic or topical antibacterial agents
  - alter normal flora
  - decrease natural microbial barriers
  - encourage colonization and growth of fungi
Vascularization

- Certain fungal isolates produce metabolites that inhibit vascular tubule formation \textit{in vitro}, thus altering the host vascular response to the fungi.

Keratomycosis

- Most commonly diagnosed in the horse but has been reported in the dog, cat and bird.
Equine Keratomycosis

Common environmental fungi
- Aspergillus
- Penicillium
- Fusarium

Other organisms
- Candida infections
Diagnosis of fungal disease

- Cytology
- Culture
- Histopathology
Confocal microscopy
Treatment

- Sensitivities to standard drugs vary by region.
- Voriconazole - 1% topical solution - broad spectrum of activity and can penetrate into the anterior chamber.
- Silver sulfadiazine can be applied every 4 to 5 hours.
- No fungi were routinely sensitive to fluconazole.
Canine fungal keratitis

- Predisposing factors
- Use cytology with culture
- Many types of fungal organisms have been isolated.
Systemic Mycoses

Blastomyces
Cryptococcus
Coccidioides
Histoplasma
Aspergillus
Most of these fungi are dimorphic.

All cause uveitis, chorioretinitis, endophthalmitis and optic neuritis.

The host inflammatory response is generally suppurative acutely and pyogranulomatous chronically.

Host tissues can be damaged directly by inflammatory processes or by degradative enzymes produced by the fungi.
Pathogenesis

- Dimorphic switching
- Residing in phagocytes
- Remodeling cell walls
- Adherence
Dimorphic fungi adaptations

- All primary fungal pathogens.
- All have features that facilitate growth in tissue.
- Phase transition or dimorphic switching is essential for the pathogenesis for all thermally dimorphic fungi.

FEMS Microbiol Rev. 2015
Signalling pathways for dimorphic switch

- These signalling pathways also co-regulate processes important for adaptation to this environment, such as adaptation to oxidative stress.
- Phase specific genes have been identified, but little is known about controlling expression.
- The predominant stimulus for switch is temperature.
- Conversion to yeast is accelerated following phagocytosis of *B. dermatitidis* spores by alveolar macrophages.
Some fungi reside within phagocytic cells of the host as this shields organisms from the rest of the immune system.

Other dimorphic fungi use the yeast cell form to avoid phagocytosis and the cytotoxic environment of the phagolysosomal system; instead, they are adapted to tolerating the adaptive immune responses.
Surviving within the macrophage phagolysosome

- **Iron**
  - Host cells restrict iron through sequestration by high-affinity iron-binding proteins.
  - Host T-cells produce (IFNγ) which downregulates surface transferrin receptors and NO production.
  - Fungi utilize high-affinity iron uptake systems such as reductive iron assimilation and non-reductive, siderophore-based iron assimilation.

- **Calcium plays an important role in *H. capsulatum* infection.**
  - Large amounts of calcium are essential for *H. capsulatum* hyphal growth suggesting that yeast cells are adapted to surviving in low calcium environments such as the macrophage phagolysosome.
  - Bad1 is a yeast-specific calcium-binding protein which is essential for pathogenicity in *B. dermatitidis*. 
Other survival strategies

- Rapid remodeling of the cell wall to prevent recognition by phagocytic cell PRRs.
- Proteins required for oxidative stress resistance are components of the extracellular proteome of *H. capsulatum* yeast cells.
- Heat shock proteins have developed to allow for a response to changes in environmental temperature.
- BAD-1 protein allows binding to host lung tissue, the extracellular matrix, and cellular receptors via glycosaminoglycans.
Blastomycosis

- *Blastomyces dermatitidis*
- Dimorphic fungus
- Occurs primarily in dogs and humans
- Primarily in North America, also occurs in India and Africa
- Tolerance for ammonia
- Cases in close proximity to water
Trends

- Increase in prevalence over 2 decades
- Increasing trend in diagnoses from February to November
- No correlation between temperatures and rainfall
Human cases in endemic states

- Increased percentage of population over age 65
- Decreased maximum temperature
- Increased mercury and decreased copper soil content.
Transmission

- Inhalation
- Nares
- Infection establishes in the lungs initially.
- Then hematogenous or lymphatic spread.

J Immunol, 2015, 194: 1796–1805
Morphology

- 5 to 20 µm in diameter
- A thick, refractile, double-contoured cell wall
- Broad-based budding
- PMNs and granulomas
Pathogenesis

- Generally considered an extracellular pathogen
- Nearly 80% of spores were inside cells at 24 h postinfection
- Most spores were located inside of alveolar macrophages
- Real-time imaging showed rapid uptake of spores, conversion to yeast, and intracellular multiplication during in vitro coculture.
- Depletion of alveolar macrophages actually curtailed infection.

J Immunol, 2015, 194: 1796–1805
Blastomycosis in Dogs

- Young large breed dogs
Blastomycosis

- Ocular disease occurs in 30% to 43% of dogs.
- Conjunctival nodules
- Anterior uveitis
- Chorioretinitis
- Retinal detachment
- Panophthalmitis
- Secondary glaucoma
- Optic neuritis
- Orbital cellulitis
Blastomycosis
In Cats

- Less common than in dogs
- Common clinical signs
  - respiratory
  - CNS
  - lymph nodes
  - skin
  - GI tract
  - urinary systems
- Ocular signs
  - anterior uveitis
  - rubeosis iridis
  - keratic precipitates
  - retinal detachment
  - chorioretinitis with granulomas
Blastomycosis in Other Species

- a dolphin, bats, horses, a polar bear, black bear, rat, non-domestic felides, alpaca and kinkajou.
- Ocular lesions have not been reported.
- Transmission to humans from bites, scratches, necropsies
Diagnosis

- Aspirates
  - draining tracts
  - enlarged lymph nodes
  - vitreous
- MVista™ Blastomyces antigen EIA (urine)
- AGID commonly results in false-negatives.
- Easily cultured
Histopathology

- Most commonly: choroiditis and retinal detachment
- Pyogranulomatous inflammation
- Lens rupture is seen in 50% of enucleated eyes.
- Choroidal inflammation is more severe in the nontapetal choroid
- Organisms are observed primarily in the choroid
Treatment

- Itraconazole
  - Commercial vs generic vs compounded
- Fluconazole
- Amphotericin B
- +/- Prednisone
Prognosis

- Varies by location and extent of inflammation within the eye and response to therapy.
Cryptococcosis

- *C neoformans* and *gattii*

- A saprophytic, round, yeast-like fungus, 3.5-7 µm in diameter

- Forms a 1-30 µm thick heteropolysaccharide capsule

- Always produces a capsule in tissues
Form 1 or 2 buds, blastoconidia, that are connected to the parent cell by a narrow isthmus.

Buds may break off when small.

 Unlike other dimorphic fungi, the yeast form is always found under normal laboratory conditions and in infected tissues.
C neoformans

- Worldwide distribution esp temperate regions
- Infects a variety of domestic and wild animals and humans.
- Many serotypes
- Usually compromised immune systems
C gattii

- Isolated from the bark and leaf litter of eucalyptus trees and trees in Pacific Northwest.
- Incidence is increasing in Pacific Northwest and California.
- Usually non-immunocompromised people.
- Incubation has been > 8 years.
Pathogenesis

- The prevalence in cats is ≥ that of dogs
- Inhalation of airborne organisms
- Unencapsulated in the environment
- Arrival in upper respiratory tract causes nasal granulomas.
- 7-14% of animals have asymptomatic colonization of the nasal passages.
- Production of a thick capsule and abundant release of glycoprotein into the circulation are hallmarks of virulence.
Cryptococcosis

- Capsule interferes with
  - antigen presentation
  - subsequent immune response
  - elimination of the organisms
- Hematogenous dissemination
- Vertical transmission
Cryptococcosis neoformans

- Establishment and spread of infection in the host are highly dependent on immunity.
- Incidence in AIDS patients is decreasing dramatically.
- Immunosuppression does not appear to play a role in veterinary patients.
Immune response

- The complement system and phagocytic effector cells are the major players in the non-specific host immune response.
- Phagocytosis is triggered by direct recognition of the yeast or by receptor-mediated recognition via complement or antibodies.
- Macrophages’ importance is becoming obvious.
- Melanin-like production
Disease in Dogs
- Young, adult dogs
- CNS signs are typically multifocal
- Cutaneous disease
- 25% have fever
Disease in Dogs

Ocular abnormalities

- Granulomatous chorioretinitis
- Retinal hemorrhage
- Papilledema
- Retinal detachment
- Optic neuritis
- Anterior uveitis
- Retrobulbar abscess with lysis

Cryptococcosis
Disease in Cats

- Upper respiratory signs
- Hard subcutaneous nodules over the bridge of the nose
- Lymphadenomegaly
- Ulcerative or proliferative lesions in the oral cavity
- Papules or nodules
- Neurologic signs depend on location
Ocular Abnormalities

- exudative retinal detachment
- granulomatous chorioretinitis
- panophthalmitis
- anterior uveitis
- optic neuritis
- adnexal disease
Disease in Other Species

- Mallocan Cockatoo
  - bilateral retinal detachments
  - exophthalmia secondary to disease in the sinuses

- Ferret
  - chorioretinitis
  - carriers
Diagnosis

- Clinical signs
- Identification or culture
- Serology
  - The latex cryptococcal agglutination test for antigen
  - CSF serology may be more sensitive for CNS cryptococcosis
Histopathology

- Gelatinous mass with little inflammation
- Severe granulomatous reaction
- Ocular lesions predominantly in the optic nerve, retina and choroid
- Dissemination found more often in dogs than cats
- In cats, granulomas typically found in affected tissues
Histopathology

- poorly stained pleomorphic yeasts
- wide capsular halo
- “soap bubble” appearance on H&E
Treatment

- Azole antifungals
- Amphotericin B
- Strain resistance
- Judicious use of steroids
Coccidioidomycosis

- *Coccidioides immitis* and *Coccidioides posadasii*
- Only found in the mycelial phase in specific locals
- Lower Sonoran Life Zone
  - sandy, alkaline soil, high environmental temperatures, low annual rain fall, and low elevation
Pathogenesis

- Inhalation
- Incubation period is 1-3 weeks
- Dissemination involves a reproductive cycle of spherules to endospores to new spherules.
- Disseminated disease affects bones, eyes, heart, pericardium, testicles, brain, spinal cord and visceral organs
- Cell mediated immune response
Disease in Dogs

Systemic signs

- cough
- fever
- weight loss
- bone involvement 65%
- localized peripheral lymphadenomegaly
- draining skin lesions
- CNS involvement
- cardiac
Ocular signs

- Conjunctivitis
- Keratitis
- Glaucoma / Iris bombé
- Uveitis
- Hyphema
- Cataracts
- Retinal detachment
- Granulomatous panuveitis
- Chorioretinitis
- Orbital cellulitis
- Most cases are unilateral
Disease in dogs

- Young, adult, large breed
- Outdoors
- Roaming
- Desert access
Disease in Cats

Skin lesions are most common

Systemic signs
- fever
- inappetance
- bone lesions
- respiratory disease
- weight loss

Ocular signs
- conjunctival masses
- fluid filled periorbital swellings
- anterior uveitis
- chorioretinitis
- retinal detachment

Coccidioidomycosis
Coccidioidomycosis

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Disease in Other Species

Ring tailed lemur
Llama
Rhinoceros
Chimpanzee
Koala
Diagnosis

Clinicopathologically

- Hyperglobulinemia
- Hypoalbuminemia

Radiography

- Osteomyelitis
- Diffuse interstitial pattern in the lungs
Diagnosis

Cytologically

- unstained preparations show 10-80 µm round, double-walled structures with endospores.

- IgM both appears and disappears early
- IgG persists longer.
- The CF titer is indicative of severity.
Histopathology

- More suppurative, destructive, and more prone to progress to panophthalmitis than other mycoses
- Anterior uveal involvement may be more common with *C. immitis*.
Histoplasmosis

- *Histoplasma capsulatum* is widely distributed in soil.
- Most of N. and S. America
- A mycelial stage is present in the soil.
- Fungi convert to a budding yeast phase in lungs.
- The organisms in the yeast phase are 2-4 µm.
Pathogenesis

- Grow as mold preferably on bird and bat guano at ambient temps
- Probably acquired by inhalation of microconidia
- Incubation ~ 12-16 days
- Reproduce by budding
Pathogenesis

- Organisms are phagocytized by mononuclear cells and undergo intracellular replication.

- Lymphatic and hematogenous dissemination then occur.
Disease in Dogs

- GI tract or liver.
- Pyrexia, malaise and coughing occur.
Ocular signs in dogs

- Relatively rare
- Blepharitis
- Conjunctivitis
- Pyogranulomatous chorioretinitis
- Anterior uveitis
- Optic neuritis

Experimentally, ocular lesions are seen in 66% of cases.
Disease in Cats

- Similarly susceptible hosts as dogs
- Young cats (<4 years of age)
- Most cats have disseminated disease
  - bone lesions
  - skin lesions
Ocular signs

- conjunctivitis
- granulomatous blepharitis
- granulomatous chorioretinitis
- retinal detachment
- optic neuritis
Treatment and diagnosis

- Itraconazole
  - Median duration of 5 months
  - Overall survival at time of discharge for cats was 55%.
- Fluconazole has similar mortality and recrudescence rates.

- Antigenuria was detected in 17/18 cats with a histopathologic or cytopathologic diagnosis.
Disease in Horses

- *Histoplasmosis farciminosum*
- Africa, the Middle and Far East, and India
- Ocular signs
  - Serous then purulent discharge with blepharitis
  - Ulcerating conjunctival nodules
  - Swelling of the eyelids with palpebral granulomas
  - The horses may not be able to open their eyes
- Pneumonia, abortion and disseminated disease
- Reportable disease
Disease in other species

- Snow leopards in Mexico
- Wild European hedgehog in Germany
- Bengal tiger in Wisconsin
- Eclectus parrot
- Rabbit

Brandao JVDI 2014
Diagnosis

- Histology (using GMS)
- Culture
- Urine antigen
- Serology using AGID is very specific but only about 80% sensitive
- Splenic changes in cats
Histopathology

- varies from other mycoses
- diffuse granulomatous and lymphocytic choroiditis with little suppuration and destruction
- usually numerous organisms
- visible as small spherical bodies within the cytoplasm of macrophages
Aspergillus spp.

- Ubiquitous in the environment
- *A. fumigatus*
  - 3-5 µm in diameter
  - Repeated dichotomous branching
  - Uniform directional orientation
  - Hyphae branch at approximately a 45° angle from the main hyphae
- Septae

- Aspergillosis has been reported in many species.
- In most species, except for the dog, *Aspergillus* is usually confined to the pulmonary system.
Transmission

- Inhalation of microconidia
- Nasal cavity is the most frequently reported area of infection in the dog
- German Shepherds
- Most human ophthalmic infections are related to immunosuppression and spread from adjacent sinus infections.
A *felis*

- Novel heterothallic species in Aspergillus section Fumigati
- Human with chronic invasive pulmonary aspergillosis
- Cats with invasive fungal rhinosinusitis
- Dog with disseminated invasive aspergillosis.
- Refractory to aggressive antifungal therapeutic regimens.

*Aspergillus spp.*
Disease in dogs

- Paraparesis, paraplegia, limb weakness
- Pyrexia
- Diskospondylitis
- Watery diarrhea
- Discharging sinus tracts
Ocular signs

- Swollen irides
- Vitreal cells
- Chorioretinitis
- Retinal detachments
- Orbital aspergillosis 2° to invasion from the sinus
- Ocular signs often occur several mos before generalized illness
**Disease in cats**

- *A. fumigatus*, *Neosartorya* spp., *A udagawae* and *A viridinutans*
- Sino-orbital aspergillosus
  - history of sneezing and nasal discharge
  - exophthalmia or presence of a mass or ulcer in the pterygopalatine fossa.
- Lesions are characterized by necrosis, well-vascularized granulomatous inflammation and PAS + fungal hyphae.
- Variable resistance to antifungals
- Very poor prognosis even with medical and surgical therapy.
Disease in other species

- **Alpaca**
  - Blind, retinal detachments and hemorrhages
  - optic neuritis
  - necropsy revealed pulmonary and renal lesions

- **Birds**
  - Air sac disease
  - One week after an acute episode in a turkey flock, cloudiness of the eye with severe conjunctivitis was associated with paralysis in broiler breeders.
  - Blepharitis, keratitis and keratoconjunctivitis (turbid discharge, cloudy cornea, and cheesy yellow exudates within the conjunctival sac) in numerous species.

Aspergillus spp.
Pathology

Gross findings on necropsy

- white nodular lesions in the myocardium, skeletal muscles, diaphragm, kidneys, liver, and spleen

- Ocular findings may include foci of lymphocytes, macrophages, fibrin and aggregates of septate hyphae adjacent to the lens capsule. In addition, the hyphae may penetrate the lens capsule.

- Pyogranulomatous

- Organisms can be seen in the vitreous, retina and choroid.
Pathology

- The organisms are PAS positive, linear with 5-15 segments, occasional focal bulges, club formation and branching.
Diagnosis

- +/- humoral antibodies
- associated with debilitation or immunologic suppression
- identification and culture
- vitreocentesis
- galactomannan
Disseminated *Rhizopus*

- *Rhizopus* is an opportunistic fungus.
- Calf with disseminated *Rhizopus* infection
- Bilateral ocular lesions, including endophthalmitis and intumescent immature cataracts
- Histologic ocular abnormalities
  - vasculitis
  - suppurative keratitis
  - anterior uveitis
  - chorioretinitis
  - intumescent immature cataracts
- Hyphae were noted in all ocular tissues.
**Candida albicans**

- Dimorphic fungus
- May cause either localized or generalized disease
- Local proliferation in wounds or mucosal surfaces is the first step of infection
- Usually in immunosuppressed or debilitated animals
Protothecosis

- Achlorophyllous algae
- *P. zopfii* and *P. wickerhamii* are pathogenic
- *P. zopfii* is most commonly isolated from dogs
- Cells are spherical to oval
- Range from 1-13 µm in diameter
- Hyaline cell wall is ~ 0.5 µm thick
- The cytoplasm is granular and basophilic
- The nucleus is small and centrally located
Protothecosis

- Reproduction: endosporulation with cleavage of the parent cell resulting in the release of 2-20 or more endospores.
- Empty hyaline shells may be visible among intact cells within lesions.
Protothecosis

- environmental contaminant
- minimally pathogenic
- does not spread between hosts
- Europe, Asia, Oceania, and N. America
Pathogenesis

- Immunosuppression, especially if cell-mediated immunity, favors establishment of infection.
- Another mechanism is the inability of the host’s PMNs to specifically destroy *Prototheca* after phagocytosis.
- Biofilms may assist in pathogenesis.
- In humans, infection is usually cutaneous, subcutaneous, or bursal.
- The colon is the probable site of primary infection and entry for disseminated disease in dogs.
Fluorescent microscopy image of *P. wickerhamii* ("biofilm-negative") on the bottom of the well after 3-day culture (scale bar = 50 µm).

Three-dimensional reconstructed images of biofilms obtained with confocal laser scanning microscope (b) fluorescent microscopy images of biofilm at various magnifications (scale bar = 50 µm);

Disease in Dogs

- hemorrhagic diarrhea or colitis is primary sign
- blindness may be initial complaint
- predisposition for the Collie breed and females
- lymphatic, nervous, renal and pulmonary systems are commonly involved
- very poor prognosis
Clinical signs

- Weight loss
- Debility
- Renal failure
- Intermittent bloody diarrhea
- Chronic ulcerative skin lesions
- CNS
  - severe depression
  - deafness
  - ataxia
  - circling
  - paresis

Protothecosis
Clinical signs

- anterior uveitis
- retinal detachment
- chorioretinitis
- blindness
- 2º glaucoma
Diagnosis

- Wright’s stain can be done on specimens obtained by vitreocentesis, CSF tap, or colonic scrapings.
- GMS or PAS
- Extracellular, round to oval
- Thin, unstained walls
- May contain endospores
- Readily grown in culture
Histopathology

- lesions identical to those of blastomycosis
- the algae are free or within macrophages
- spherical to oval from 2-20 µm
- a refractile cell wall that stains with PAS or GMS
- multiple daughter cells form enclosed within a single cell wall
"Of course I'll need to run some tests; but offhand I'd say it's some sort of fungus infection."

"I'm afraid you have a yeast infection."