Small Animal Fungal Disease

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Fungal Classification

- Organism that grows as a unicellular yeast or a multicellular mould
- Shared characteristics with eukaryotes include membrane bound organelles (nucleus), lack of chloroplasts, and use of organic compounds as energy. They also contain a cell wall which is structurally different due to presence of chitin (also found in exoskeleton of arthropods), chitosan, glucan (also found in plants), and mannan. Fungi do not use cellulose in their walls.
- Reproduction is via sexual and asexual means. A fungus in the sexual state is termed a teleomorph and in the asexual state is termed an anamorph.

Fungal Terminology

- Hyphae are cylindrical, thread-like structures that grow at the tips and can either branch or fork. They can also fuse, which is referred to as hyphal fusion or anastomosis.
- Micelium are an interconnected network of hyphae that develops as hyphae grow. They can be septate or coenocytic.
  - Septate micelium are divided into compartments separated by cross walls formed at right angles. Each septa has pores and each compartment has a single nucleus.
  - Coenocytic mycelium are not compartmentalized.
- Conidia are responsible for asexual reproduction and are nonmotile spores generated by mitosis and can come in many shapes and sizes.

Normal Skin Flora and Microbiome

- Opportunistic pathogens infect individuals with compromised immune systems.[4]
- True pathogens can cause disease in normal individuals.[4]
- Clinically healthy cats and dogs can carry *Alternaria, Aspergillus, Cladosporium, Penicillium, Rhizopus,* and *Trichoderma.*[2]
  - Represent repeated transient contamination by airborne fungi or soil fungi.[6]
  - Cats with FIV or FeLV have a greater fungal diversity on their skin.[6]

Normal Cutaneous and Immune Defenses Against Fungal Diseases

- Two major physiological barriers to fungal growth[4]:
  - Temperature
    - The optimal growth range for fungi is considerably lower than body temp.
  - Oxidation-reduction potential
- Fungi must also penetrate into hair shafts or stratum corneum, before being desquamated.[6]
- Fungistatic fatty acids and sphingosine produced by keratinocytes.[6]

- Major reactions to fungal infections include (1) acute suppurative inflammation and microabcess formation, (2) chronic pyogranulomatous or granulomatous inflammation, and (3) necrosis from blood vessel infarction and subsequent tissue death.[6]

Dermatophyte

Geographic Distribution of Fungal Organisms

- Dermatophytosis occurs worldwide, is the most important superficial mycosis, and is common in humans and animals, especially cats.[5]
- *Microsporum canis* has been identified from about 2% of clinically healthy pet cats.[1]
- In healthy stray and shelter cats in the US, *M. canis* prevalence is higher in subtropic climates (17.5%) than temperate climates (4%).[2]
- *Microsporum gypseum* is most common in humid, tropic, and subtropic areas. It is more common in the summer and autumn.[2]

Characteristics of Fungal Organisms

- More contagious than other fungal infections.[5]
- Dermatophytes are isolated as asexual fungal organisms (anamorphs).[7]
- Species[5]:
  - Zoophilic dermatophytes
    - *Microsporum canis, M. equinum,* and *T. equinum* are adapted to living on animals, rarely found in the soil, and will occasionally infect humans.[4,5]
    - Sylvatic dermatophytes
      - *T. mentagrophytes, M. persicolor* are zoophilic dermatophytes adapted to living on rodents or hedgehogs.[4,5]
  - Anthropophilic dermatophyte
    - *Epidermophyton, T. tonsurans, T. rubrum* - adapted to humans, rarely infects animals as a reverse zoonosis.[4,5]
  - Geophilic dermatophyte
    - *Microsporum gypseum* occurs in the soil as saprophytes, but under favorable conditions, can infect humans and animals if skin or immune system is compromised.[5]
- Arthrospores - typical infective portion. Form by segmentation and fragmentation of fungal hyphae.[5]

Pathophysiology of Dermatologic Disease

- Predispositions
  - Young animals in in overcrowded, dirty, or damp areas. Inadequate nutrition, immunosuppression.[5]

  - Breeds: Yorkshire terriers, Pekingese, Persian and Himalayans to *M. Canis.* Parson Russell Terrier to *T. mentagrophytes.*[4,5]
  - Large catteries have up to 35% of cat culture positive.[4]
  - Excessive bathing/grooming can remove fungistatic sebum and serum.[2,4]
- Host
  - *M. canis* - source is usually an infected cat[4,7]
  - *T. mentagrophytes* - source is usually rodents or their immediate environment.[4,7]
- Vector
  - Fleas from infected animals can potentially transmit dermatophytes.[2]
- Mode of Transmission
  - Acquired by contact with infected animals, infective material (shed scales, hair), or fomites (combs, clippers).[5]
    - Hair fragments containing infectious arthrospores can remain infectious for more than 18 months if protected from UV light.[4]
  - *M. gypseum* is acquired from digging or rooting in contaminated soil.[4]
  - Three stages of infection occur[2,5]:
    - Arthrospores adhere strongly to keratin.
    - Conidial germination within 6 hours of contact.
    - Invasion of cornified tissues (stratum corneum, hair shafts, claws) by producing proteolytic enzymes (e.g. keratinase, elastase, and collagenase)
    - Mechanical injury and increased humidity facilitate penetration
  - Infection of the hair shaft does not progress below zone where cornification occurs (keratogenous zone, Adamson's fringe)[4,5]
  - Infection continues as long as downward growth of fungus is in equilibrium with keratin production. If keratin production stops, fungus is sloughed and hair is cleared of infection.[4]
    - When hair enters telogen, keratin production stops and fungal growth ceases.[4]
    - Hairs are weakened, become brittle and easily broken.[4]
  - Incubation period until lesions develop is usually 1-3 weeks.[2]
- Immune Response
  - The normal dry condition of the skin surface and fungistatic properties of serum and sebum are natural host defense mechanisms.[2]
  - Cell-mediated immune response is the principal means of resolving dermatophytosis and developing resistance to reinfection.[2,5]
    - Humans who recover from acute dermatophyte infections develop develop positive delayed intradermal test reactions and are usually immune to further infection.[2]
    - Humans who develop chronic, unrelenting dermatophytosis persisting for months to years typically develop strong immediate intradermal test reactions and weak delayed reactions.[2]

- Positive delayed intradermal reactions in cats are often weaker in cats with active infections and those who were treated with antifungals, suggesting the entire course of disease must be run to develop immunity.[2]

- Fungal products and cytokines released from damaged keratinocytes lead to epidermal hyperplasia (hyperkeratosis, parakeratosis and acanthosis) and dermal inflammation.[5]
- Superficial perivascular dermatitis and white blood cell exocytosis into cornified layers leads to intracorneal microabscesses.[5]
  - White blood cell exocytosis into follicular walls and lumens leads to mural and luminal folliculitis, and eventually furunculosis.[5]
    - Secondary bacterial infection increases the severity of folliculitis and furunculosis.[5]
- Dermatophyte antigens in the host immune response include cell wall carbohydrates, cell wall proteins and secreted keratinases. Glycopeptide is the immunologically active portion of the cell wall. The carbohydrate portion is involved in immediate-type hypersensitivity and the peptide portion is involved in delayed-type hypersensitivity.[2]

Diagnosis of Dermatophytosis

- Skin lesions can be variable.
  - Asymptomatic - some cats carry the organisms with no clinical disease.[5]
  - Kerion - eruptive nodular mass that may ulcerate and develop draining tracts. These are usually solitary nodules on the face and forelimbs of dirty digging dogs.[4,5]
  - Pseudomycetoma - deep granulomatous nodular dermal and subcutaneous mass containing distorted fungal hyphae seen almost exclusively in Persian cats, may have tissue grains.[4,5]
  - Onychomycosis - discolored, malformed, friable, broken, or sloughed claws.[5]
  - Gross lesions are primarily due to follicular disease - annular or irregularly shaped, scaly to crusty alopecia that can coalesce.[2,5]
    - *Ringworm* - central portion of lesion contains dead fungi, but viable fungi at periphery of annular lesion cause a red ring.[5]
    - Hair loss is due to breakage of shafts and loss of shafts from inflamed follicles. In adult cats, patchy mild alopecia or broken hairs with little skin change can be the only sign.[4,5]
    - Hyperpigmentation is rare in cats and most often associated with ringworm.[2]
    - Follicular papules, pustules are common. Draining tracts can be see with deep furunculosis.[5]
    - Pruritus can be absent to moderate. Focal pruritic lesions that resemble eosinophilic plaques may occur. Unilateral or bilateral pinnal pruritus can be seen with *M. canis*.[2,4]
    - Recurring chin folliculitis resembling feline acne or “stud tail” dermatitis may be seen.[4]
  - *M. persicolor* causes prominent scaling but minimal alopecia because it infects surface keratin but does not invade hair.[4]
- Systemic clinical signs
- Wood’s Lamp
  - The use of ultraviolet light, 320-400nm wavelength is a fast screening tool for the presence of *M. canis.* The fluorescence is the result of a water soluble chemical metabolite, pteridine,

produced by actively growing fungi within the cortex or medulla of hair, and not on scale or claw material.[2,7]

- Debris, scale, link and topicals can produce false fluorescence.[2]
- Wood's lamp can be helpful for monitoring endemic infection in a cattery or shelter.[2]

• Be patient. Let the lamp warm up and spend a few minutes with the room lights out for best success. Battery operated, small lamps may not be as effective as larger, plug-in lamps.[2]
• Hairs may still fluoresce long after the hair shafts are culture negative.[7]

- Trichogram
  • Hairs can be suspended using 10-20% KOH overnight at room temp or for 10 minutes with heat. You can also use mineral oil to suspend hairs and Wood's lamp to find glowing hairs.[2]
  • Dermatophytes form hyphae and arthroconidia (ectothrix spores) on hair and scale. Macroconidia never formed in tissue. If ectothrix spores seen, start treatment while awaiting culture. Infected hairs are thicker, frayed and indistinct with a filamentous appearance.[2]
- Dermoscopy
  • Illuminated magnification of the skin. Dermatophytes will cause "comma hairs" to be visible. These are slightly curved or broken hairs with a homogenous thickness.[7]
- Culture techniques and identification, PCR testing
  • Culture provides definitive diagnosis, but is not perfectly sensitive or specific.[2]
    - Dermatophyte test medium (DTM) contains nutrient medium, bacterial and saprophytic fungal growth inhibitors (gentamicin, tetracycline, and cycloheximide), and phenol red as a pH indicator.[2]
    - Cultures incubated above room temperature have increased sporulation. Light exposure does not adversely affect sporulation, so incubation does not have to be in the dark.[2]
    - Colonies appear 5-7 days after inoculation and almost always within 14 days. Cultures from treated animals should be evaluated for 21 days.[2]
    - Daily evaluation will show color change of medium to red with concurrent growth of white to buff colored, powdery to cottony mycelium. Other nonpathogens can cause color change immediately (*Scopulariopsis)* or after colonies have grown, so all colonies should be viewed microscopically. Black, brown, gray and green colonies are not dermatophytes. True infections result in numerous colonies. Early or mild infection, recovery, innocent carrier cats living with infected cats or poor sampling may all cause few colonies.[2]
    - Use cellophane tape to sample the colony, then evaluate with lactophenol cotton blue or new methylene blue. If spores are not visible, repeat in 4-7 days, some colonies produce spores as they mature.[2]
    - *M. canis* colonies have a white top surface, yellow-orange reverse pigment underside, are flat with a depressed center, and have a cotton or wool consistency.[2]
      • Microscopic appearance of macroconidia is spindle or canoe-shaped, each containing ≥6 cells with thick walls with outer spines on surface and terminal knob. Young colonies may have <6 cells. Microconidia may have a single cell.[2]

- *M. gypseum* colonies have a cinnamon brown top surface, yellow to tan underside, are flat, and have a face powder consistency.[2]
  - Microscopic appearance of macroconidia is rowboat shaped, each containing <6 cells with thin walls. Microconidia may have a single cell.[2]
- *Trichophyton* spp. colonies have white to cream top surface and tan to brown to red underside.[2]
  - Microscopic appearance of macroconidia is cigar shaped and rare. Microconidia are common and often spinal hyphae.[2]

- Polymerase chain reaction
  - A positive PCR can be the result of active infection, fomite carriage or nonviable fungal organisms from a successfully treated infection.[7]

- Sample selection and acquisition
  - Lesions can be plucked or brushed to obtain hair and scale. A new, inexpensive human toothbrush should be vigorously combed through areas of suspected infection for 2-3 minutes. Brush and attached hairs should be pressed lightly and repeatedly (20-30 times) onto the surface of the culture medium, "planting" hairs in agar.[2]
    - You can teach clients to obtain cultures to avoid bringing infective pets into the clinic.[2]
    - Make sure you brush the face and the inside of the bell of the ear in kittens, which are often the first areas infected and commonly undersampled.[2]
- Report interpretation
  - Microscopic patterns include perifolliculitis, luminal folliculitis, or furunculosis and epidermal hyperplasia with intracorneal microabscesses. Orthokeratotic and parakeratotic hyperkeratosis with variable acanthosis may be seen.[4,5]
    - Dermatophytic pseudomycetoma contains masses of fungal elements surrounded by granulomatous or pyogranulomatous inflammation in deep dermis or subcutis.[4]
    - Septate hyphae or spores seen in hair shafts and in stratum corneum of epidermis or follicles. Arthrospores are formed on the outside of the hairs (ectothrix) or within the hairs (endothrix).[4,5]
    - Histopathology is not as sensitive as culture for diagnosis and is most useful in nodular forms.[4]
    - H&E can identify fungal organisms in most cases, but PAS and GMS may be necessary.[4]
      - GMS stains fungal structures brownish-black against a pale green background.[2]
      - PAS stains fungal structures dark red against a contrasting background.[2]

Management of Dermatophytosis

- Many infections will spontaneously resolve in 3 months, but specific therapy is often recommended for affected animals to decrease shedding of infective scales and hairs, particularly because of zoonotic potential.[5]
  - Chronic dermatophytosis can occur in dogs with *Trichophyton* spp. and *M. persicolor* lasting up to 5 years without any immunodeficiency, suggesting production of an inhibitory substance that prevents an effective immune response needed to eliminate the organism.[4]

- Mycological cure is defined by two negative fungal cultures taken at two weeks apart.[7] Monitoring of colony forming units on fungal plates can be used to assess response.[7]
- Topical Therapies
  - Reduces contamination on the haircoat and environmental contamination, resulting in faster mycologic cure than systemic therapy alone.[2]
    - This is important for young animals who are going through critical socialization periods to allow them to safely interact with other animals and people as soon as possible.[2]
  - Clipping haircoat removes fragile hairs that could break and release spores into the environment, as well as improves topical therapy.[2]
  - Topical treatment alone may predispose animal to chronic subclinical infections.[2]
  - Twice weekly application of lime sulfur, enilconazole or miconazole/chlorhexidine (not miconazole solely) shampoo are recommended. Accelerated hydrogen peroxide, climbazole, and terbinafine shampoos may be effective but need more studies.[7]
- Systemic Therapies
  - Reduces number of weeks to complete cure. Systemic treatment targets site of infection.[2,7]
  - Itraconazole (noncompounded) and terbinafine are the most effective and safest treatments.[7]
  - Griseofulvin is effective, but has increased risk of adverse effects.[7]
  - Ketoconazole and fluconazole are less effective and ketoconazole has more risk of AEs.[7]
  - Lufenuron has no efficacy and antifungal vaccines do not protect against exposure.[7]
- Environmental control
  - Reduces chances the infection will spread to other animals/humans in house, as well as reducing time to mycologic cure.[2]
  - Antifungal disinfectants include sodium hypochlorite, enilconazole, accelerated hydrogen peroxide, potassium peroxymonosulfate, and OTC disinfectants with anti-*T. mentagrophytes* action. These should be used following removal of organic debris from surfaces.[7]
  - Laundry can be washed in any temperature without sodium hypochlorite. The most important thing is not to overload the machine, as agitation is what removes dermatophytes. Two washings on the longest cycle are recommended.[7]
  - Carpets should be vacuumed, treated with a disinfectant, then washed with a beater brush shampooer. Wood floors can be cleaned with Swiffer sheets for electrostatic properties and then washed with wood oil soap.[7]

Zoonoses and Reportable Fungal Diseases

- *Microsporum canis and Trichophyton mentagrophytes* are primarily animal pathogens, but can infect humans.[5]
- There has been a shift from *M. audouinii, Epidermophyton floccosum,* and *T. rubrum* to *M. canis* as the most common dermatophyte causing infection.[2]
- At risk populations include children, transplant and cancer patients, immunocompromised or older adults, and veterinary professionals.[2]

Resistance, Virulence Factors, and Immune Response

- Virulence factors include keratinolytic enzymes, endoproteases, and exoproteases which hydrolyze keratin, allowing invasion of the hair shaft. Keratinase, elastase and collagenase have been isolated from dermatophytes.[2,4]
- Normal skin is relatively inhospitable to fungal growth because of low moisture conditions, antifungal substances in the surface film and normal resident flora. Sebum contains fungistatic fatty acids.[4]
  - Continuous shedding of the stratum corneum removes infecting organisms with sloughed keratin.[4]

Malassezia

Geographic Distribution of Malassezia Organisms

- Malassezia pachydermatis commonly colonizes healthy dogs and cats, with less common colonization by *Malassezia furfur, Malassezia sympodialis, Malassezia globosa, Malassezia slooffiae* (feline claw fold), and *Malassezia nana* (feline ear canal).[1,6]
- M. pachydermatis commonly colonizes the chin, lips, interdigital skin, external ear canal, anal sacs, and less commonly axillae and groin of dogs.[1,5]
- *Malassezia* dermatitis is more common in topical climates and during warm, humid months in more temperate latitudes.[3]

Characteristics of Malassezia Organisms

- *Malassezia pachydermatis* - historically defined as lipophilic, but non-lipid-dependent yeast because it is the only *Malassezia* that can grow on Sabouraud's dextrose agar.[3,5] Recently, genome sequencing has confirmed that *M. pachydermatis* lacks a fatty acid synthase gene like other *Malassezia* spp., but is able to utilize lipid fractions within the peptone component of Sabouraud's dextrose agar. *M. pachydermatis* cannot grow on a lipid-free media, so should technically be defined as "lipid dependent."[3]
  - Commensal organism in dogs and cats that lives in the stratum corneum and becomes a pathogen when predisposing factors alter host cutaneous microenvironment, epidermal barrier, or immune system.[5]
  - Thought to have a symbiotic relationship with staphylococci.[5]
- All other *Malassezia* species are lipid dependent. Currently 18 described species.[3,4]
- *Malassezia* are oval to footprint or peanut-shaped 2x4 µm yeast in stratum corneum or crusts.[4]
- Reproduce by budding on a broad base and from the same site at one pole (monopolar blastic development). Yeast have a thick cell wall, ~0.12µm.[1]

Pathophysiology of Dermatologic Disease

- Predispositions
  - Dogs:

  - Underlying conditions: allergies, keratinization defects, endocrinopathies and presence of skin folds.[6]
  - Breeds: basset hounds, West Highland white terriers, cocker spaniels, toy and miniature poodles, dachshunds, boxers, Cavalier King Charles spaniels, Shih Tzus, Australian and silky terriers, and German shepherd dogs.[1] Bull terriers with lethal acrodermatitis have large numbers of *Malassezia*.[6]
  - High serum titers of IgG and IgE antibody and have greater skin test reactions, suggesting hypersensitivity may be involved in the pathogenesis of clinical disease.[3,4]
- Cats:
  - Underlying conditions: allergies, metabolic disease, immunosuppressive viruses (FeLV, FIV) and paraneoplastic diseases (pancreatic paraneoplastic alopecia, exfoliative dermatitis with thymoma).[2,3,6]
  - Breeds: Devon rex and sphynx cats predisposed to seborrheic *Malassezia* dermatitis.[1,2,6]
- Increased heat and humidity.[5]

- Immune Response
  - *Malassezia* can stimulate innate, antibody and cell mediated immune responses, as well as hypersensitivity reactions.[3]
  - Keratinocytes recognize *Malassezia* antigens via Toll-like receptors, which leads to up regulation of IL-10 and TGF-β and down regulation of IL-1α.[3]
  - Cell mediated responses can lead to delayed type hypersensitivity in some atopic patients.[3]

Diagnosis of Malassezia Dermatitis

- Skin lesions
  - Skin lesions in dogs commonly occur in warm, moist anatomic sites, including interdigital skin, ventral cervical skin, lips, ear canals, axillae, groin, and other intertriginous skin.[2] Lesions include mild to severe pruritus, erythema with greasy exudate, malodor, traumatic alopecia, lichenification and hyperpigmentation.[2] Paronychia presents as pedal pruritus, reddish-brown discoloration of the claw and/or ungual exudate.[2]
  - *Malassezia* infections are less common in cats and most often present as ceruminous otitis externa, chronic chin acne, facial dermatitis with large dark brown to black scales and follicular casts, and refractory paronychia. Skin lesions may include exfoliative erythroderma, greasy exudation, and pruritus. Devon rex cats with seborrheic dermatitis can have greasy, tightly adherent, brown ungual exudate, as well as dark, greasy exudate on interdigital skin, axillae and groin.[1,2,4]
- Cytology
  - Surface scale and *Malassezia* sp. can be lost during tissue processing, so cytology is more reliable than biopsy.[5]
  - Cytology is obtained through impression smears or tape-strip to identify footprint/penguin/peanut/snowman shaped yeast using modified Wright Giema stain (“Diff-Quik”). Dogs with hypersensitivity to *Malassezia* may have low numbers on cytology, but a significant improvement in clinical signs in response to therapeutic trial.[1,2]
- Culture techniques and identification, PCR testing

- Rarely needed.[5]
- *Malassezia pachydermatis* is the only *Malassezia* that can be grown on Sabaroud's dextrose agar.[2] All other *Malassezia* species must have additional lipid supplementation for in vitro cultivation.[2] On lipid-enriched media, such as modified Dixon's agar, *Malassezia* colonies are cream to yellowish, smooth or lightly wrinkled, glistening or dull, with margin being either entire or lobate.[1]
- Most wild-type *Malassezia* remains susceptible to common azoles. Recent sporadic reports of therapeutic failure with azoles.[3]

- Report interpretation
  - Biopsy - hyperkeratosis, focal parakeratosis, variable spongiotic pustular dermatitis, lymphocytic exocytosis, acanthosis, perivascular and interstitial mixed cellular (lymphocytes, plasma cells, histiocytes, eosinophils) dermatitis.[4,5]
    - Organisms are best visualized with PAS or GMS stain.[4]
  - Because *Malassezia* can be identified in normal skin and seen in association with other disease processes, their role in causing or contributing to skin disease must be interpreted in regards to clinical findings and may ultimately come down to response to treatment.[5]

Management of Fungal Dermatologic Diseases

- Topical Therapies
  - Shampoos, gels and lotions are appropriate because *Malassezia* lives within the stratum corneum. Shampoo allows for mechanical removal of scale and exudate.[1]
  - Strong evidence of 2% miconazole and 2% chlorhexidine shampoo used twice weekly. Moderate evidence for 3% chlorhexidine shampoo.[1]
- Systemic Therapies
  - Often more expensive than topicals, but may be necessary when topicals are challenging or ineffective. Combined topical/systemic may be optimal for generalized/severe lesions.[1]
  - Moderate evidence in canines for use of ketoconazole (5-10mg/kg q12-24) or itraconazole (5mg/kg q24 or 2 consecutive days/week). Compounded formulations have limited bioavailability. Fluconazole and terbinafine require additional studies.[1]
  - Itraconazole is recommended for cats.[1]
- Allergy-Specific Immunotherapy
  - Improvement has been noted in studies evaluating ASIT, but further studies are required.[1]

Zoonoses and Reportable Fungal Diseases

- Overall risk for zoonosis is low, however good hand hygiene by individuals in contact with pet dogs and cats should be emphasized, particularly for those who are immunocompromised or work with immunocompromised humans.[1]
- *M. pachydermatis* has been implicated in some zoonoses, including a neonatal ICU when a nurse transmitted it from her pet dogs to infants receiving lipid emulsions. Rigorous hand hygiene should be used whenever there are immunocompromised humans.[3]

Resistance, Virulence Factors, and Immune Response

- PAMPS - mannan, zymosan.[2]
  - PRRs - Langerin, a C-type lectin (protein that binds carbohydrates in a calcium -dependent manner), is expressed by Langerhans cells and recognizes mannose and beta-glucans.[1]
- Virulence factors include hydrolases (lipase, phospholipase, aspartyl proteases, and acid sphingomyelinases) and the production of biofilm.[4]
- Resistance:
  - Mis-sense mutation in the *ERG11* gene that encodes lanosterol 14-alpha-demethylase, a target side for azoles.[3]
  - Mutation in the drug efflux pumps has been reported.[3]

Candidiasis

Geographic Distribution of Fungal Organisms

- *Candida* sp. are normal inhabitants of the mucocutaneous skin and GI tract, genital tract.[4,5]
- Rare infections in domestic animals, such as dogs, cat, pigs, horses, goats and a llama.[4,5]

Characteristics of Fungal Organisms

- *Candida* spp. Reproduce by asexual budding. Usually have a budding, ovate appearance, but may also appear filamentous, particularly after adherence to mucosal and catheter sites during biofilm formation. *C. albicans* is the most common species isolated from people and animals.[3]

Pathophysiology of Dermatologic Disease

- Predisposing factors
  - Compromised host resistance (diabetes mellitus, hyperadrenocorticism, neoplasia, viral infections, cytotoxic chemotherapy, or prolonged glucocorticoid treatment), factors that alter superficial keratin barrier (maceration, chronic trauma, or burns), upset normal flora (prolonged broad spectrum antibiotic therapy).[4,5]
  - Bull terriers with lethal acrodermatitis commonly have infections on foot pads, nail beds, noses, and perianal regions.[3]
- Immune Response
  - Neutrophils and T-cell mediated immunity are major defense mechanisms against *Candida*

Diagnosis of Dermatologic Disease

- Skin lesions
  - Exudative and pustular to ulcerative inflammation of the lips (cheilitis), oral mucosa (stomatitis), and external ear canal (otitis externa).[5]

  - Sharply delineated ulcers with erythematous borders and a malodorous surface with moist gray-white exudate can occur. Chronic lesions are thickened, alopecia, and hyperkeratotic with prominent folds.[4]
- Systemic clinical signs
  - UTIs can occur. GI overgrowth can be seen with chronic antibiotics.[3]
- Cytology
  - Skin cytology can reveal budding organisms. Urine sediment can show budding or filamentous organisms.[3]
- Culture techniques and identification, PCR testing
  - Readily grow on blood agar within 48 hours.[3]
  - Sabouraud's dextrose agar grows white to cream colored colonies with 5-7µm diameter rounded yeast cells, pseudohyphae, and 3-5µm wide septate hyphae. Positive cultures should be interpreted in light of clinical signs.[3]
- Report interpretation
  - Biopsy - spongiotic neutrophilic pustular inflammation, parakeratosis, and ulceration with exudation. *Candida* present in superficial exudates.[5]
  - Yeasts, pseudohyphae, and hyphae are best visualized with PAS or GMS stains
    - Yeast are most numerous on the surface of the lesions, whereas hyphae and pseudohyphae extend into the epidermis.[4]

Management of Fungal Dermatologic Diseases

- Topical antifungals combined with oral azaols are most commonly used. Infected areas should be kept dry and clean.[3]

Zoonoses and Reportable Fungal Diseases

- Zoonotic transmission between people and animals has not been reported.[3]

Resistance, Virulence Factors, and Immune Response

- Virulence factors - yeast can adhere to and colonize mucosa via ligand-receptor and/or hydrophobic interactions. Ligands include those in the afflutinin-like (AL) family and hyphal wall protein (Hwp) family. Mannose and mannoproteins can act as adhesins. Receptors on mucosal epithelial cells include E-cadherin, fibrinogen, fibronectin, thrombin, collagen, laminin, and vitronectin-binding proteins. Mucosa invaded using invasin proteins. Hydrolytic aspartyl proteinases injure the mucosa.[5]

Subcutaneous Mycoses

- Fungal infections involving skin and subcutaneous tissues as a result of saprophytic fungi gaining entry by traumatic implantation. Infection tends to be indolent and usually remains localized to site of entry with slow spread.[4]

- Eumycotic mycetoma, dermatophyte pseudomycetoma, subcutaneous phaeohyphomycosis, subcutaneous hyalohyphomycosis, sporotrichosis, subcutaneous entomophthoromycosis (zygomycosis), and oomycosis (pythiosis and lagenidiosis).

Geographic Distribution of Fungal Organisms

- Eumycotic mycetoma reported worldwide, but more frequent near the Tropic of Cancer.[4]
- Phaeohyphomycosis organisms are worldwide in distribution and widespread in soil, wood and vegetation. Occasionally can be cultured from the skin of healthy people and animals.[4]
- Sporothrix schenckii is more common in tropical, subtropical and temperate zones. It is endemic in Central and South America and Africa.[4]
  - In Rio de Janeiro and Southern Brazil, there has been an ongoing epidermic since 1998.[4]
- Oomycosis infections are more common in tropical or subtropical climates, including the Gulf Coast of the US, Thailand, Australia, India, Indonesia, and Costa Rica. Most infections occur in the late summer and fall.[4,5]
- Zygomycosis infections are more common in tropical or subtropical climates.[5]

Characteristics of Fungal Organisms

- Eumycotic mycetoma
  - Mycelial morphology in tissue surrounded by eosinophilic hyaline material (Splendore-Hoeppli reaction: antigen-antibody complexes) resulting in macroscopic granule or grain.[4,5]
  - Most commonly due to saprophytes, such as *Curvularia geniculata*, *Madurella*, *Acremonium*, and *Pseudallescheria.*[4,5]
- Phaeohyphomycosis (chromomycosis)
  - Caused by species of pigmented fungi (dematiaceous) with dark-walled, septate hyphae, including *Alternaria, Drechslera, Exophiala, Phialophora, Bipolaris* spp., *Cladosporium* spp., *Curvularia* spp., and *Wangiella* spp.[4,5]
  - Typically plant pathogens, soil saprophytes or normal flora.[5]
  - Occurs in cattle, horses, cats and rarely dogs.[5]
- Hyalohyphomycosis (paecilomycosis, adiaspiromycosis)
  - Similar to phaeohyphomycosis, except fungal hyphae are non pigmented (nondematiaceous), including *Pseudallescheria* sp., *Acremonium* sp., *Fusarium* sp., *Paecilomyces* sp., and *Geotrichum* sp. Organisms have septet branching or non-branching hyphal (mycelial) tissue morphology.[4,5]
- Sporotrichosis
  - Caused by *Sporothrix schenckii* complex, including *Sporothrix brasiliensis, S. schenckii, Sporothrix globosa*, and *Sporothrix luriei.*[5]
  - *S. schenckii* is a saprophytic dimorphic fungus found in moist organic debris that grows as hyphae at environmental temperatures and yeast form in tissue.[4,5]
- Oomycosis (Pythiosis and Lagenidiosis)
  - Caused by aquatic, dimorphic water molds *Pythium insidiosum* or *Lagenidium* sp.[5]

- Pythiosis is also known as leeches, bunkers, swamp cancer, bursattee. *P. insidiosum* is not a true fungus because its cell walls do not contain chitin and instead have cellulose and β-glucan. The cytoplasmic membrane lacks ergosterol, the sexual process is oogamy, and the organism develops biflagellate zoospores (infective stage) in wet environments.[4]

- Zygomycosis (entomophthoromycosis and mucormycosis)
  - Entomophthoromycosis caused by saprophytic fungi *Basidiobolus* sp. & *Conidiobolus* sp.[4,5]
  - Mucormycosis is caused by fungi from the order Mucorales, including *Rhizopus, Mucor, Lichtheimia, Saksenaea* and others. Very uncommon in small animals.[4,5]
- Dimorphic fungi grow as molds at room temperature and yeast at human body temperature
  - Include some species of *Sporothrix, Histoplasma, Blastomyces,* and *Coccidioides*[5]

Pathophysiology of Dermatologic Disease

- Predisposing factors
  - German Shepherds may be predisposed to phaeohyphomycosis, although this is seen much more commonly in cats, and hyalohyphomycosis.[4]
- Mode of Transmission
  - Traumatic inplantation of fungi that can remain localized or spread to lymph vessels.[5]
  - Phaeohyphomycosis is through to result from wound contamination or traumatic inplantation of wood slivers, thorns and sticks.[4]
  - Sporotrichosis is caused by puncture wounds from thorns, wood splinters or contaminated claws and is considered an occupational hazard for people who work with soil or plants.[4]
  - Oomycosis occurs in conjunction with exposure to free-standing water and contamination of minor skin wounds. Zoospores contact skin, mucosa, and encyst on the tissue. Encysted zoospores secrete a sticky amorphous glycoprotein that mediates adhesion. Germ tube (hypha) development is stimulated by host body temperature and invades blood vessels. Proteases weaken host tissue.[4,5]
  - Zygomycosis can occur through inhalation or traumatic inplantation by wounds or insects.[5]

Diagnosis of Dermatologic Disease

- Skin lesions
  - Mycetomas are characterized by triad of tumefaction (swelling), draining tracts, and grains. Grains are composed of aggregates of fungal organisms varying from 0.1mm to several millimeters in size and may be white, yellow, pink-red, brown or black.[4]
    - *Curvularia* spp. and *Madurella* spp. are associated with black-grained mycetomas and *Scedosporium/Pseudallescheria complex* is usually associated with white grains.[4]
  - Most subcutaneous fungal infections cause lesions similar to deep bacterial granulomatous infections with one or more ulcerative nodules with or without draining sinuses.[5]
  - Phaeohyphomycosis produces pigmented nodules that may be mistaken for melanoma, typically on the face (nose and pinnae) and paws of cats.[4]

- Hyalohyphomycosis typically infects claw beds, face, head, eyes and joints and lesions range from well-circumscribed ulcers covered by exudate to non ulcerated nodular masses with variable alopecia and desquamation.[4]
- Sporotrichosis is subdivided into 3 clinical forms[4]:
  - Primary cutaneous form consists of raised alopecia, ulcerated, crusted nodules or plaques at the point of entry in an animal with high immunity preventing further spread. Course may be chronic and grooming behavior of cats may cause autoinnoculation into surrounding tissue.
  - Cutaneous-lymphatic form involves skin, subcutaneous tissue and lymphatics, which become thick and corded, eventually developing secondary nodules that may ulcerate and drain. This is the most common form in horses and humans.
  - Extracutaneous/disseminated form - see systemic clinical signs.
- Pythiosis affects skin of limbs and trunk of horses, cattle, dogs and cats. Lagenidiosis has only been reported in dogs.[5]
  - Extensive tissue destruction by inflammation and necrosis can occur.[5]
- *Basidiobolus* has only been reported in the horse. *Conidiobolus* has been reported in horses, llamas, sheep, and dogs.[5]

- Systemic clinical signs
  - Sporotrichosis rarely causes visceral signs in the extracutaneous/disseminated form.
    - May involve a single extracutaneous tissue, such as osteoarticlar sporotrichosis, or multiple organs. This form occurs most commonly in cats and does not require immunosuppression to occur. Pyrexia, depression and anorexia are often present.[4,5]
  - Oomycosis can often cause severe systemic disease.
    - Pythiosis in dogs can cause a rapidly progressive, debilitating and often fatal disease most often in young, large-breed dogs. Gastric pythiosis can also occur in large-breed dogs. Eosinophilia is often seen on hematology.[4,5]
    - Lagenidiosis can cause lesions in organs other than skin and lymph nodes.[5]
- Cytology
  - Sporotrichosis may show ovoid to elongate (cigar-shaped) bodies in FNA or impression smear of exudative lesions in cats because of sufficient numbers of yeasts.[4,5]
- Culture techniques and identification, PCR testing
  - Definitive identification of organism in eumycotic mycetoma and phaeohyphomycosis requires culture.[4]
  - Tissue culture, immunohistochemistry or fluorescent antibody staining may be necessary for definitive diagnosis of sporotrichosis. On Sabouraud's agar at room temp, fungus grows as white mold that turns brown to black as pigmented conidia grow and the saprophytic phase has septate hyphae with oval or tear-shaped conidia (2-3µm by 3-6µm) in clusters on conidiophores and along hyphae. At 35-37ºC on blood agar, budding pleomorphic yeasts seen as cigar bodies that can also be round and up to 10µm long.[4]
  - Oomycosis does not respond to anti fungal therapy. They must be differentiated from fungi.
    - Pythiosis can be diagnosed using serologic testing (ELISA), culture or PCR.

        - Specific handling and culture techniques required. ELISA can be used to monitor response to therapy in affected patients. Decrease in antibody levels should be seen 2-3 months after successful treatment, whereas patients with unsuccessful treatment have static antibody levels.[5]
    - Lagenidiosis must be cultured from fresh tissue followed by ribosomal RNA gene sequencing.[5]
  - Zygomycosis requires culture to differentiate between Oomycetes, because zygomycosis can respond to anti fungal treatment.[5]
- Submission
  - Dimorphic fungi can be dangerous to culture in routine microbiology lab settings because mycelial phase in infective to humans. Lab personnel should be notified if *Sporothrix, Histoplasma*, *Blastomyces,* and *Coccidioides* are suspected.[5]
- Report intepretation
  - Eumycotic mycetoma histopathology - nodular masses of granulomatous inflammation with fibrosis and exudate containing embedded granules of masses of septet, branching fungal hyphae measuring 0.2-6 µm in diameter, bordered by hyaline eosinophilic Splendore-Hoeppli material arranged radially or smoothly contoured.[4,5]
  - Phaeohyphomycosis histopathology - foci of granulomatous, pyogranulomatous, or lymphocyte-rich granulomatous dermatitis and panniculitis containing pigmented fungal organisms in small aggregates throughout the lesion (compared to mycetomas, which form discrete, organized granules). Hyphae are septate, 2-6µm wide, and branched or unbranched. Fungal elements are usually intracellular within epithelioid macrophages and multinucleate giant cells. Melanin stains, such as Fontana-Masson, can be used to confirm presence of melanin in hyphae. Fungal stains can demonstrate the organisms but mask the natural color.[4,5]
  - Sporotrichosis histopathology - nodular to diffuse pyogranulomatous or granulomatous dermatitis and panniculitis. Yeast surrounded by stellate radial corona of brightly eosinophilic material (asteroid body/Splendore-Hoeppli reaction) can be seen. Sparsely distributed ovoid 2-6µm diameter to elongate (cigar-shaped) bodies 2x3 to 3x10µm are difficult to find. Organisms are more numerous in cats and rare in dogs and horses. Yeast have a refractile cell wall and processing may cause the cytoplasm to shrink, giving the appearance of a capsule, which can sometimes cause misidentification as *Cryptococcus neoformans* or *Histoplasma capsulatum.*[4,5]
  - Oomycosis histopathology - hyphae or hyphal-like structures are in areas of eosinophilic to pyogranulomatous dermal or subcutaneous inflammation. May not be readily seen on H&E stain. *Pythium* stains poorly with PAS because of the lack of chitin. Gomori's methenamine silver may be needed to see thick-walled, sparsely septate hyphae, 2-7 µm in diameter, with occasional branching.[4,5]
  - Zygomycosis histopathology is similar to oomycosis. Multifocal-to-diffuse eosinophilic and granulomatous dermatitis and panniculitis with multifocal necrosis with eosinophilic coagulated material. Fungi are in necrotic foci and appear as clear linear or circular hyphal "ghosts" surrounded by thick (2.5-25 µm) eosinophilic sleeve (Splendore-Hoeppli), compared to oomycosis which has thin sleeves. Best visualized with GMS, as they stain poorly with PAS.[4,5]

Management of Subcutaneous Fungal Diseases

- Eumycotic mycetoma
  - Cutaneous eumycotic mycetoma are not life-threatening. Surgical excision can be difficult and amputation could be required. Antifungal chemotherapy should be based on susceptibility and continue 2-3 months past clinical cure. Local hypothermia can be used.[2]
- Phaeohyphomycosis
  - Surgical excision of small lesions with wide margins may be curative, but recurrence is common. Ketoconazole, itraconazole (10mg/kg/day for >3months), amphotericin B and flucytosine used with varying results. If acquired while immunosuppressed, immunosuppressive medications should be discontinued.[2]
- Hyalohyphomycosis
  - Disseminated or CNS disease have grave prognosis. Systemic antifungals often result in short-term clinical improvement and may prolong survival, but recurrence of clinical signs is common and there are few reports of survival with disseminated disease. Localized disease should be removed surgically.[2]
- Sporotrichosis
  - Cats can be treated with itraconazole 1.25-1.5mg/kg q24 >2 months, but are difficult to treat
  - Dogs and horses are treated with potassium iodide, but toxic iodize can occur, resulting in ocular and nasal discharge, dry scaly coat, vomiting, depression and collapse. If this occurs, stop treatment for 1 week, then restart at a lower dose.[2]
  - Ketoconazole, terbinafine, local hypothermia, amphotericin B and surgical resection are options. Treatment should continue for 1 month after clinical cure.[2]
- Oomycosis (pythiosis and lagenidiosis)
  - Poor prognosis. Wide surgical excision or amputation is the best option for cure, but local postoperative recurrence is common and can occur at resection site or local lymph nodes.[2]
  - Antifungal chemotherapy for >2-3 months with itraconazole or terbinafine. *P. insidiosum* does not contain ergosterol, which is the target for majority of anti fungal drugs, so medical management is often unrewarding. Caspofungin targets β-glucan and can be considered.[2]
- Zygomycosis
  - Not much information available. Surgical excision followed with anti fungal chemotherapy based on susceptibility is recommended for a minimum of 2-3 months.[2]

Zoonoses and Reportable Fungal Diseases

- Sporotrichosis is considered a zoonotic disease due to reported transmission from cats to humans. Infected cats pose a significant public health danger.[4]
- Oomycosis is not considered zoonotic because the infective stage, zoospore, does not form in tissue, only in standing water.[4]

Resistance, Virulence Factors, and Immune Response

- Melanin may act as a virulence factor in the development of phaeohyphomycosis.[4]

Systemic Mycoses

- Infections of the internal organs with cutaneous involvement resulting from hematogenous dissemination. Skin lesions from direct inoculation are rare. Animals with skin lesions caused by these organisms should be assumed to have systemic infection until proven otherwise.[4]
- *Blastomyces dermatitidis, Coccidioides immitis, Cytopcoccus neoformans,* and *Histoplasma capsulatum.*

Blastomyces dermatitidis

Geographic Distribution of Fungal Organisms

- Common in moist, acidic soil rich in organic material, like beaver dams.[2]
- Primarily found in North America, most prevalent in Mississippi, Missouri, and Ohio River Valleys, mid-Atlantic states, and Canadian provinces of Quebec, Manitoba, and Ontario.
  - Has been found in Africa, India, Europe and Central America.[2]

Characteristics of Fungal Organisms

- Dimorphic saprophytic soil fungus produces conidia (~2-10µm diameter) which are carried by the air when soil is disrupted.[2,5]

Pathophysiology of Dermatologic Disease

- Predisposing factors
  - Immunocompromised animals are more severely affected, but infections can occur in animals with apparently normal immune function.[5]
  - Sporting dogs and hounds may be at greater risk.[2]
- Mode of Transmission
  - Inhalation of conidia, which are deposited on mucus layer of airway mucosa. Conidia bind to alveolar macrophages via surface adhesins, such as BAD1 (*Blastomyces* adhesion factor 1), which can cause phagocytosis and killing of conidia by mucosal macrophages and neutrophils, spread of conidia via leukocyte trafficking through mucosae, and acute inflammation with tissue damage facilitated by pro inflammatory cytokines released from macrophages. Incubation is 3-15 weeks.[5]
  - Conidia are rapidly killed by macrophages, so they rapidly transition to yeast form (12-15µm diameter) to resist phagocytosis by shedding surface adhesins and/or producing masking capsules. If they are phagocytosed, yeast and yeast-derived debris are released from dead macrophages and act as chemokines/cytokines to recruit additional macrophages and neutrophils, which results in significant pyogranulomatous inflammation.[5]
- Immune Response
  - Effective immune response requires a T-lymphocyte, cell mediated response directed to BAD-1.[2]

Diagnosis of Dermatologic Disease

- Skin lesions
  - Solidary or multiple nodules with ulceration and draining sinuses.[5]
- Systemic clinical signs
  - Anorexia, weight loss, cough, dyspnea, ocular disease, lameness. Signs can be present for a few days or up to a year.[2]
  - 85% of dogs have lung lesions.[2]
- Cytology
  - Fungal organisms can be seen in about 50% of cases on cytology from draining tracts.[2]
- Culture techniques and identification, PCR testing
  - Culture should only be done in laboratories with proper facilities to protect lab workers from pulmonary blastomycosis.[2]
  - 25-30ºC on Sabouraud's agar for 3 weeks. Hyphae produce condidiophoes with spherical or oval smooth-walled conidia. Colonies are white and cottony and may become light tan.[2]
  - At 37ºC thick-walled yeast, 5-20µm in diameter develop. Colonies are dirty white to brown color and develop in 3-4 days.[2]
  - Fungal serology has 41-90% sensitivity and 90-100% specificity.[2]
  - Urine ELISA highly specific for infection and useful in monitoring response to treatment.[2]
- Report interpretation
  - Nodular areas of granulomatous or pyogranulomatous inflammation in the dermis and subcutis with thick-walled yeast cells with single buds attached by broad base. PAS, Gridley, and GMS stains are helpful in identifying microorganisms.[2]

Management of Fungal Dermatologic Diseases

Systemic Therapies

- Multimodal therapy recommended. Good prognosis, but relapse seen in 20-25% of cases.[2]
- Amphotericin B is nephrotoxic and must be given IV, so itraconazole is more commonly used and is equally safe and effective. AMB can be life saving in dogs who cannot absorb itraconazole or who did not respond. Fluconazole can be considered for dogs with urinary tract disease because it is excreted in the urine.[2]
- Dogs with severe lung disease can have acute inflammatory response within first week of treatment. About 50% of dogs with severe lung disease may die during this time. Low dose glucocorticoids can help reduce the inflammatory response.[2]

Zoonoses and Reportable Fungal Diseases

- Dogs have a 10x greater risk of infection compared to humans, so they are an important epidemiological marker.[2]

Resistance, Virulence Factors, and Immune Response

- Virulence factors of the yeast phase include glucans on cell wall, and other virulence factors like melanin that provide resistance to phagocytosis and killing. Yeast change surface polysaccharides and hide in phagosomes to evade the immune system.[5]

- BAD1 mediates adherence to CR3 and CD14 on cell membranes. BAD1 also depresses the production of tumor necrosis factor-alpha, which is important for phagocyte killing of fungal organisms and recovery from disease.[2,5]

***Coccidioides immitis* (Coccidioidomycosis)**

Geographic Distribution of Fungal Organisms

- Common in Southwest US, especially Arizona. Areas of high temperature and low rainfall.[2] After a period of rainfall, fungi replicate in the upper soil surface, sporulate, and release large numbers of arthroconidia that are disseminated by the wind.[2]
- Referred to as *Valley Fever* due to epidemic that occurred in San Joaquin Valley of California.[2]

Characteristics of Fungal Organisms

- Dimorphic life cycle. The fungus is present in the soil. Suitable growing conditions will lead to production of arthroconidia (~3-6µm diameter) that are aerosolized during soil disruption (construction, farming, earthquakes, dust storms).[5]

Pathophysiology of Dermatologic Disease

- Predisposing factors
  - Immunocompromised animals are more severely affected, but infections can occur in animals with apparently normal immune function.[5]
  - Young, male dogs, boxers and dobermans more affected.[5]
- Mode of Transmission
  - Highly infectious, but not typically contagious. Very few, <10, arthroconidia needed to produce disease.[2]
  - Inhalation of arthroconidia, which are deposited and trapped on mucus layer of airway mucosae. Arthroconidia rapidly transition to spherules to protect from phagocytosis. Spherules express additional virulence factors that cause acute inflammation resulting in mucosal injury and colonization.[5]
    - Spherules grow to 20-60µm diameter (up to 100µm) and can form intraspherular endospores (1-5µm diameter) through endosporulation. Spherules become too big to be phagocytized. When spherules are damaged, endospores are released and phagocytosed by macrophages and grow into second-generation spherules protected within macrophages, eventually releasing 200-300 endospores when infected cells are lysed. Proinflammatory cytokines recruit macrophages which aid in colonization and leukocyte trafficking of endospores to lymph nodes, skin, bone, muscle, adrenals, and CNS.[5]
  - Rare primary skin infections can occur from direct infection to damaged skin.[5]

Diagnosis of Dermatologic Disease

- Skin lesions

- Solidary or multiple nodules with ulceration and draining sinuses. Most often over areas of infected bone.[5]
- Skin lesions are the most frequent type of infection in cats.[2]

- Systemic clinical signs
  - Cough, dyspnea, fever, anorexia, weight loss, ocular disease.[2]
  - Most common form is asymptomatic or mild, undiagnosed, lower respiratory tract infection.
  - Cardiac, CNS, ocular, boney and arthritic lesions can occur.[2]
- Cytology
  - Definitive diagnosis through cytology or histologic visualization of organism.[2]
    - If demonstration of organism isn't possible due to cost or invasive procedures, history, clinical findings and serologic testing can provide diagnosis.[2]
  - Suppurative, pyogranulomatous or granulomatous inflammation with sparse fungal elements. Spherules are 10-100µm diameter with thick (>2µm) wall and are best visualized with Pap stain (capsular wall is refractile and purple-black, cytoplasm yellow, and endospores red-brown) or PAS stain. Wright's stain can also be used. Not all spherules contain endospores, but when present, they are small, round to oval, densely aggregated with eccentric nuclei and can be extracellular or phagocytized. The presence of endospores and extreme variation in size of sphere differentiates between *B. dermatitidis*.[2]
- Culture techniques and identification, PCR testing
  - No attempts should be made to culture and identify this organism within the veterinary practice. Must be cultured in a bio containment lab.[2]
  - Blood agar at 37ºC and Sabouraud's agar at 25ºC promote mycelial growth within 1 week. Barrel-shaped arthroconidia seen in lactophenol cotton blue wet mount. Colonies are white and cottony with tan, brown, pink or yellow pigment.[2]
  - Canine IgM detectable within 2-5 weeks of infection. IgG detectable 8-12 weeks after infection and IgG titers are not consistent with severity of clinical signs.[2]
- Report interpretation
  - Nodular areas of granulomatous or pyogranulomatous inflammation in the dermis and subcutis, sometimes with fibrosis. Occasional ensosporulating or nonendosporulating spherules can be seen. PAS and GMS enhance organism identification.[5]

Management of Fungal Dermatologic Diseases

- Mononuclear phagocytes remove the organism and if the animal recovers, lifelong immunity is conferred. Overall recovery rate is about 60% Azole antifungals should be used for a minimum of 1 year and at least 3-6 months after clinical signs resolve.[2]
- Fluconazole has best penetration, fewest side effects and recommended for CNS disease.[2]
- Treatment should be discontinued based on resolution of clinical disease, radiographic appearance of bone and lung lesions, and serologic titers.[2]
- Prognosis for localized respiratory coccidioidomycosis is good. Treatment is recommended to decrease dissemination, as animals typically die or are euthanized without treatment. CNS disease has guarded to poor prognosis.[2]

Zoonoses and Reportable Fungal Diseases

- Not typically contagious, but rare infections have developed in veterinary personnel handling infected animals/tissue.[2]
- Culturing organism is extremely dangerous.[2]

Resistance, Virulence Factors, and Immune Response

- Virulence factors include production of spherule outer wall glycoprotein that modules immune response and compromises cell-mediated immunity, depletion of spherule outer wall glycoprotein on the surface of endospores (preventing phagocytosis), and production of host tissue arginase I and coccidioidal urease (causing tissue damage).[5]
  - Exposed laminin and collagen serve as receptors for fungal ligands, enhancing adhesion.[5]

Crytopcoccus neoformans and C. gatti

Geographic Distribution of Fungal Organisms

- Present in soil-derived aerosols from moist and humid environments and from bird droppings and nests.[5]
- *C. Neoformans* has a worldwide distribution. *C. gatti* associated with tropical and subtropical climates.[5]

Characteristics of Fungal Organisms

- Dimorphic life cycle with mycelial (basidiospore) phase extracellularly and yeast phase intracellularly.[5]
- 5 serotypes identified (A, B, C, D, AD). *C. neoformans* are serotypes A and D. *C. gatti* are serotypes B and C.[5]
- Most common systemic mycosis in cats, but also affects humans, dogs, ferrets, horses, goats, sheep, cattle, and koalas.[5]

Pathophysiology of Dermatologic Disease

- Predisposing factors
  - Immunocompromised animals are more severely affected, but infections can occur in animals with apparently normal immune function.[5]
  - Rare in dogs. Dobermans, GSD, and cocker spaniels may be predisposed.[2,5]
  - Siamese and Abyssinians may be predisposed.[2,5]
- Mode of Transmission
  - Inhalation of blastoconidia, basidiospores, or poorly encapsulated yeast cells (~1.8-3µm diameter). Basidiospores quickly germinate to yeast in mucosae or within phagosomes to avoid being killed by neutrophils and macrophages. Yeast-derived glucosylceramide synthase is essential for survival of yeast in mucosae.[5]
  - Once phagocytosed, a polysaccharide capsule is formed, which dilutes lysosomal hydrolases and provides physical separation between yeast and membrane of phagosome. Macrophages become grossly distended with the capsule (>30µm diameter). The capsule is

composed of glucuronoxylomannan and galactoxylomannan (polysaccharides), which also suppress the immune system.[5]

Diagnosis of Dermatologic Disease

- Skin lesions
  - Solidary or multiple nodules with ulceration and draining sinuses.[5]
- Systemic clinical signs
  - CNS signs such as CN abnormalities. Ocular disease and osteomyelitis also seen.[2]
  - Felines develop intranasal granulomatous disease (80% of cases).[2]
  - Fever, lethargy, and weight loss also noted.[2]
  - Dogs frequently develop severe disseminated disease.[2]
- Cytology
  - Pyogranulomatous inflammation with pleomorphic (round to elliptical) 2-20µm yeast like organism with narrow based budding with mucinosis capsule forming refractile halo.[2]
- Culture techniques and identification, PCR testing
  - Not a public health hazard to culture. Sabouraud's dextrose agar at 25-30ºC and 37ºC forms white, creamy colonies that yellow with age, usually within 2-3 days, sometimes 10 days. Canavanine-glycine-bromothymol blue agar (CGB) differentiates *C. gatti* from *C. neoformans*, as *C. gatti* grows and turns agar blue.[2]
  - Susceptibility testing is recommended.[2]
  - CSF analysis can show increased protein levels and mixed cellular pleocytosis, mostly neutrophils and large mononuclear cells. Cryptococcal yeasts may or may not be visible. India ink is used for observation of presence or absence of capsules of fungal cells against a dark background.[2]
  - Latex agglutination detects polysaccharide capsule Ag using latex particles coated with anticryptococcal Ab and can be used to monitor therapy.[2]
  - Serology can be obtained from serum or CSF.[2]
- Report interpretation
  - Nodular areas of granulomatous or pyogranulomatous inflammation in the dermis and subcutis. Cystic degeneration or vacuolation of the dermis/SQ is most common.[5]
    - *C. neoformans* can cause a granulomatous response, but inflammation is usually less severe than other fungi.[5]
    - Cryptococcal organisms have a mucinous capsule that does not stain with H&E, which can give a multicystic appearance when inflammation is mild. Mucicarmine-positive staining capsule, appears rose-red, organism appears pink against a blue background. PAS, methenamine silver, or Fontana-Masson stain can identify organism, but capsule still does not stain well with these methods.[5]

Management of Fungal Dermatologic Diseases

- Surgical excision recommended if possible, followed by anti fungal chemotherapeutics long term.

- Amphotericin B is most effective and can eradicate CNS infections, despite poor CNS penetration. It is pro inflammatory and can stimulate host immunity.[2]
  - Optimal therapy is combination AMB and flucytosine for meningoencephalitis.[2]
  - Flucytosine can promote rapid resistance if used as a sole agent.[2]
    - Dogs receiving flucytosine can develop severe cutaneous drug reaction with 10-14 days.
  - Fluconazole is ideal due to CNS penetration and urinary excretion, but resistance has been seen in strains of *C. gattii* from North America.[2]
  - Itraconazole is more effective than ketoconazole and comparable to slightly inferior to fluconazole for infections that are not in the CNS, eye or urinary tract.[2]
  - Ketoconazole is least expensive, but high doses needed.[2]
  - Voriconazole and Posaconazole are expensive, but effective.[2]
- Antigen titer should be monitored every 2 months and anti fungal therapy continued until the antigen titer is zero. Favorable prognosis with early detection, worse with CNS signs.[2]

Zoonoses and Reportable Fungal Diseases

- Not considered a zoonotic disease in terms of pet owners acquiring infection from pets. Pets may act as a sentinel species for exposure of humans to bird excrement.[2]
- Cryptococcosis is the most important opportunistic fungal infection of humans with HIV.[2]

Resistance, Virulence Factors, and Immune Response

- Yeast produce phospholipase that injure alveolar type II epithelial cells and hinder production and function of surfactant, enhancing adhesion to pneumocystis and improving changes of being phagocytosed by alveolar macrophages.[5]
  - Polysaccharide capsule of yeast has antiphagocytic properties and may be immunosuppressive. The negative charge of the capsule from glucuronoxylomannan (GXM) inhibits phagocytosis and killing by neutrophils and macrophages and causes complement depletion, antibody unresponsiveness and dysregulation of cytokine secretion by monocytes/macrophages. The capsule also inhibits chemotaxis of leukocytes from bloodstream, which may account for lack of inflammation in cysts.[5]
  - Melanin facilitates yeast survival during CNS infection by acting as an antioxidant and eliminating reactive oxidant species that could kill yeast.[5]

Histoplasma capsulatum (Histoplasmosis)

Geographic Distribution of Fungal Organisms

- Worldwide distribution, but more common in Central US.[2]

Characteristics of Fungal Organisms

- Dimorphic life cycle; mycelial (microconidia) phase occurs in the extracellular environments (25ºC/77ºF) and yeast phase occurs intracellularly within monocyte-macrophage system (37ºC/98.6ºF).[5]
- Organisms are present in soil-derived aerosols from moist and humid environments.[5]

Pathophysiology of Dermatologic Disease

- Predisposing factors
  - Immunocompromised animals are more severely affected, but infections can occur in animals with apparently normal immune function.[5]
  - Pointers, weimaraners, Brittany Spaniels, and working dogs are predisposed.[5]
  - Second most commonly reported systemic fungal disease in cats.[5]
- Mode of Transmission
  - Inhalation of microconidia into bronchi and bronchioles.[5]
- Immune Response
  - Neutrophils and alveolar macrophages phagocytize microconidia. Microconidia rapidly transition to yeast form, as microconidia can be killed by macrophages. Transitioning from microconidia to yeast is a requirement for fungal pathogenicity.[5]
  - Phagolysosomes attempt to kill yeast, but yeast prevent lysis by synthesizing proteins that inhibit acidification and activity of lysosomal proteases. Yeast becomes protected while hidden in phagosomes of viable macrophages and spreads to lymphoid tissue through leukocyte trafficking and affects more macrophages. When macrophages die (6-16 days), yeast released and, along with yeast-derived surface polysaccharide antigens, recruit more macrophages. This process is repeated and leads to increase in inflammatory exudate.[5]

Diagnosis of Dermatologic Disease

- Skin lesions
  - Solidary or multiple nodules with ulceration and draining sinuses.[5]
- Systemic clinical signs
  - Thickened small intestinal walls, hepatomegaly, splenomegaly, mesenteric lymphadenopathy due to accumulation of granulomatous inflammatory cells in perivascular spaces. This can lead to generalized organ enlargement with increased pallor or formation of 1+ solid white-yellow nodules within tissue. Bone marrow and eyes may be affected.[5]
  - Inappetence, weight loss, fever, respiratory signs, GI signs (large bowel diarrhea with tenesmus, mucous and frank blood).[5]
- Cytology
  - Organisms are numerous in infected tissues. Usually seen within mononuclear cells on Wright or Giemsa's stains as small, 2-4µm round body with basophilic center and lighter halo caused by shrinkage of the yeast during staining.[2]
- Culture techniques and identification, PCR testing
  - Highly pathogenic, risky to culture. Colonies are reddish and wrinkled before appearance of cottony brownish to white mycelium with 7-10 days on routine media at room temp.[2]

- Report interpretation
  - Nodular areas of granulomatous or pyogranulomatous inflammation in the dermis and subcutis. Yeast have a basophilic center with lighter halo because of shrinkage during staining. PAS, GMS, or Gridley's fungal stain should be used to enhance detection of the organisms.[2,5]

Management of Fungal Dermatologic Diseases

- This is a life-threatening infection caused by rapid proliferation in many tissues. Chronic airway obstruction from hilar lymphadenopathy may occur.[2]
- Itraconazole +/- glucocorticoids to reduce inflammatory airway disease. Steroids do not cause worsening or dissemination of disease. Treatment should continue for 4-6 months minimum and at least 2 months after clinical resolution.[2]
- Amphotericin B can be used alone or in combination with itraconazole for severe pulmonary or GI disease.[2]
- Fluconazole has better penetration into CNS and eye and may be considered, but is not recommended over itraconazole or amphotericin B. Ketoconazole not recommended.[2]
- Voriconazole is active against *Histoplasma* and penetrates CSF. Posaconazole has been shown to be highly effective.[2]

Resistance, Virulence Factors, and Immune Response

- Heat shock proteins 70 and 83, M-phase cell cycle enzymes, and tubular proteins are activated when conidia are incubated. Walls of yeast contain more chitin and less mannose and amino acids than mycelial-phase cells. When phagocytosed, yeast raise the intracellular pH slightly by releasing urease, ammonia, and bicarbonate, facilitating the absorption of intracellular iron. Yeast also produce IL-4, which can interfere with cellular immune response. Melanin is also an important virulence factor.[2]

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