History

Blastomycosis was first suspected in 1894 by Gilchrist who described a proliferative skin disease in people that had microscopic yeast-like bodies in biopsy sections. In 1914, Stober demonstrated that the organism would grow on paper, cardboard, cotton, sawdust, fruit, and vegetables. He investigated several cases in Chicago and found that 50% of patients lived in buildings in downtown Chicago with wet or decayed wood floors that were built directly over or in contact with soil. Because of these early descriptions, the clinical presentation was referred to as Gilchrist’s disease and Chicago Disease. Some 70 years later, the mold form of the organism was identified. Before amphotericin, therapy was of limited success and consisted of iodide, sulfonamides, or radiation.

Organism

Numerous fungi inhabit our environment but only a few are pathogenic. Those fungi that are known pathogens such as Blastomyces are able to transform from mold to yeast and from yeast to mold. Dimorphism is a term that is applied to fungi that can have two entirely different morphologic and cultural features.

Blastomyces dermatitidis is a dimorphic fungus that exists as a mold or yeast depending on growth conditions. Mold forms tend to grow at temperatures below mammalian body temperatures and reproduce sexually. Yeast forms grow best at body temperatures and reproduce by budding asexually. In nature, the organism exists as a mold, probably as a nidus in the environment, and under appropriate conditions (temp, humidity, unknown) mycelia grow which generate conidia or spores. Conidia can survive several weeks in soil. Release of conidia from mycelia is far more efficient (99% release) under moist conditions. High moisture conditions (fog, rainfall, humidity, spring weather) are weather changes frequently associated with release of conidia. Once inhaled, the conidia develop into the yeast form (asexual) in a few hours. Temperature appears to be the main factor that controls the growth of the organism between

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the mold and yeast phase but there is growing debate that this may not be the sole factor. The organism does not depend on the infection of animals or people to maintain its life cycle.

Isolation and growth of the yeast form from infected people or animals is fairly routine. Attempts to isolate the mold from suspected premises have been extremely difficult. In the literature, there are approximately 2400 attempts to isolate the organism. Of these, only 21 were successful; 19 required animal inoculation and only 2 were successful by in vitro cultures. Specific studies on requirements for growth in natural settings indicate that the organism has minimal requirements and that a variety of wood by-products and animal waste substrates can support growth. The mold form may not have or require a specific molecular niche, but may be a survivor that is able to tolerate major changes in temperature, pH, nutrients, and hydration. In order to survive other competitors and environmental extremes, the mold may temporarily bloom and generate spores. Thus, providing a mechanism for point source infections.

**Human Outbreaks in Wisconsin**

1979 Seven people in a group of eight developed blastomycosis during a canoeing trip on the Namekegon River near Hayward. The point source was thought to be a campsite along the river. Attempts to isolate the organism 30 days after exposure were negative.

1984 Forty-eight of 95 participants in a visit to an environmental camp had clinical blastomycosis. Visitors to the camp had direct access to an abandon beaver lodge and dam. Features of the environment included acidic soil, decaying wood and vegetation, water environment, recent rainfall, accumulation of animal waste, and a connection with beaver habitat. Samples of the lodge and dam were positive by mouse inoculation. However, several attempts to repeat the isolation were unsuccessful. No clinical cases were identified in tour groups that visited the beaver lodge or dam two weeks before the outbreak or in the weeks that followed.

1985 Seven of 12 people developed clinical disease after fishing during a two day period along a bank of the Tomorrow River in Portage County. The bank was shaded, high in organic content, and was adjacent to a farm (animal waste source) and also had groundhog burrows nearby. The bank was steep and the soil was mechanically disrupted. Fungus was isolated from the soil by mouse inoculation.

1986 Six boys and one adult developed blastomycosis by playing in an underground timber fort along the Crystal River in Waupaca County. The environment was moist with decaying wood. An animal burrow was located nearby and was disturbed.

1988 Three people and one dog had confirmed clinical disease from a point source infection on a lakeshore in Vilas County. Infection was also suspected in one adult and four dogs. Dust carried by prevailing winds from an excavation site at a resort across the lake was thought to be the source of spores. Additional people were tested and 16 asymptomatic infections were detected. This data indicated that many were exposed and infected, but only a few developed clinical disease.

1990 Ten people developed clinical disease during a seven month period. All patients lived along a stretch of the Oconto River in Oconto County. In addition to residential proximity, patients participated in hunting, excavating around their homes, or digging near water. Results of retrospective studies of these outbreaks indicate that a large number of subclinical infections were found suggesting that many are exposed, but fewer individuals actual develop clinical disease. Human outbreaks at Crystal, Eagle, and Tomorrow Rivers occurred following rain. Shaded moist locations and recent excavation or soil movement are contributing factors. Incubation following exposure ranged from 21-106 days with a median of 43 days following inhalation of spores. There is no evidence to suggest transmission between animals and people. Cases in dogs and their owners have occurred, but are believed to be the result of a common point source of inhaled spores.
Incidence in the general population is 0.5 cases/100,000 population. Residents of seven counties in Northern and Central Wisconsin have a higher incidence. In Vilas County the incidence has been 40 cases/100,000 population.

Occupational risk factors are multifaceted and include a variety of occupations. In the medical field, pathologists, veterinarians, veterinary technicians, and microbiologists are at risk because of direct inoculation of the yeast or inhalation of the spores from culture plates. In the general population, outdoor occupation or participation in outdoor activities allows for greater risk of exposure. In one study, 48% of 372 cases came for the combined occupations of farmer, agriculture, laborer, mine worker, heavy equipment operator, and other outdoor occupation.

Ecology

Unlike many of the other systemic fungi, the natural habitat of the *Blastomyces* has long been a mystery. Information on the biology and location of the organism in the environment is based 20% on fact and 80% on speculation. The natural habitats of the other major fungal pathogens have been delineated as follows: *Histoplasma* - bat droppings, chicken coops, or blackbird roosts; *Cryptococcus* - pigeon droppings, *Coccidioides* - desert soil around animal burrows.

The ecological distribution of *Blastomyces* is not understood completely, but data collected in the last 20 years indicate that the mold is associated with sandy soils that have high organic content, abundant moisture, acid pH, shade from direct sun, and enriched with animal waste. However, soil is not necessary for the mold to grow.

Archer (1985) summarized 200 canine cases in WI and found that 68% lived within 500 feet of a body of water. Four waterways were noted in the study as high incidence areas: Pigeon River, Eagle River, Plover River, and Namekegon River. Human outbreaks have occurred along Namakegon, Eagle, Crystal, Tomorrow, and Bigfork rivers. In Vilas County, 82% of the people with blastomycosis lived or visited within 500 meters of water; 95% of the canine cases lived within 400 meters of water.

Factors that limit growth in the environment include: drying, lysis, mycostatic and mycolytic factors, and competing soil fungi. In soil incubated in 100% humidity, viable conidia increased by 100%. In a desiccated environment, viable conidia decreased by 95% in 5–10 days. Conidia are resistant to temperature extremes of freezing and 40°C.
Disease Pathogenesis

Up to 1950, two types of disease presentation, cutaneous and systemic, were the result of two routes of infection, direct contact and inhalation, respectively. Current thinking is that nearly all infections occur by inhalation with a rare infection caused by a penetrating wound.

Transmission: Blastomycosis is not a contagious disease with rare exception. Once conidia are inhaled, the disease may evolve along several different pathways: 1) severe pulmonary disease, 2) inapparent pulmonary infection that resolves spontaneously, 3) disseminating to multiple organs resulting in systemic disease, 4) disseminating to a single organ where it may remain occult for several years, 5) cutaneous disease that may involve bone. Other modes of transmission include: accidental inoculation through the skin, dog bites, sexual transmission, and intrauterine. Needle sticks, contamination of wounds with exudate, inhalation of conidia from culture plates are modes of transmission that veterinarians should be aware of. Transmission by contaminated bronchoscopes was also reported.

Incubation period: Route of infection influences the incubation period. Following inhalation of spores, disease is usually apparent in 2 - 14 weeks. Skin lesions appear in 1-5 weeks following a penetrating wound.

Blastomyces Antigens and Cellular Resistance:

Antigens: 1) Blastomycin- culture filtrate of mycelial form that was used for the outdated skin test, 2) B-ASWS- extract of outer cell wall that causes granuloma formation, 3) A antigen- extract of yeast broth, 4) BAD-1 (WI-1)- extract of cell wall surface protein. BAD-1 is an adhesion protein that is important for the pathogenicity of Blastomyces because it promotes the adherence of the yeast to lung tissue and cells and it influences host immunity by blocking TNF-alpha production by phagocytes which further promotes progression of infection. Specifically, the reduction in TNF-alpha causes a skewed inflammatory cell response dominated by neutrophils instead of T cells, which allows for the yeast to flourish. BAD-1 is the antigenic target of the humoral and cellular immune responses. Antibody generated against BAD-1 does not improve clinical outcome and may actually enhance the severity of infection.

Cellular Resistance: Generation of Blastomyces specific antibodies, although detectable, plays no discernible role in host defense mechanisms. Therefore, infected or exposed dogs and people must rely on cellular resistance for defense and protection. PMNs will phagocytose and kill conidia very rapidly by oxidative burst and generation of hydrogen peroxide and superoxide. After initial exposure, 80-90% are killed in 2 hours. With added time, only 50% were killed. The yeast form of Blastomyces is far more difficult for PMNs to phagocytose and kill. Yeast forms are 50x more resistant to peroxide killing than the conidia. In one study, co-culture of yeasts and PMNs produced a 60% enhancement of yeast replication suggesting that PMNs may actually enhance the infectivity and severity of infection. Macrophages surround and inhibit the replication of yeast forms initially during the first 24 hours but with time (24-72 hrs) the organisms are able to replicate freely. In similar culture conditions, neutrophils were not able to kill yeast forms and their presence was associated with enhanced (60%) replication. Growth of yeasts in vivo and in vitro was enhanced by the presence of live or dead murine neutrophils. Therefore, host neutrophils and macrophages may actually exacerbate rather than inhibit disease.

Canine Blastomycosis

Occurrence is influenced by location in North America. The Mississippi drainage system that extends from the Gulf of Mexico to the upper Midwest and Central Canada delineates the distribution of human and canine cases of
Blastomycosis. In WI, disease peaks in summer, fall, and early winter. The incidence in Vilas County is 1420:100,000 (1420 cases per 100,000 dogs). In the Deep South, disease occurs throughout the year because winters are more moderate. In an endemic area, the organism does not appear to be uniformly distributed but is localized to neighborhoods or certain bodies of water. Dogs are by far the most susceptible domestic animal and are 10x more likely to be infected than people. There is experimental evidence that many dogs contract subclinical, self-limiting infections and spontaneously recover.

Large breeds, especially sporting types, four years old or younger, in the weight range of 22-45 kg are most likely to contract the disease. Some studies indicate that males are more susceptible (60-70%). A study in Guinea pigs indicated that females were more resistant to disease.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Survival</th>
</tr>
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<tbody>
<tr>
<td>Intact Females</td>
<td>52%</td>
</tr>
<tr>
<td>Intact Males</td>
<td>7%</td>
</tr>
<tr>
<td>Spayed Females</td>
<td>80%</td>
</tr>
<tr>
<td>Castrated Males</td>
<td>40%</td>
</tr>
<tr>
<td>Spayed Females + Testosterone</td>
<td>47%</td>
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Clinical Signs: Following inhalation and establishment of pulmonary infection, the organism spreads to other tissues. Sites that are most frequently involved include skin, eyes, bones, lymph nodes, and external nares. Clinical signs include fever, anorexia, weight loss, cough, cutaneous and subcutaneous skin lesions, lymph node enlargement, lameness, and ocular disease. Cutaneous lesions often involve the planum nasale, face, and nail bed. Less frequent signs include prostatitis, testicular enlargement, mammary abscess, seizures, regurgitation, nasal discharge, and oral lesions. Radiographic evidence of pulmonary disease is evident in 85% of cases and consists of nodular interstitial pattern and bronchial lymph node enlargement. The former is difficult to differentiate from other fungal diseases or metastatic neoplasms. Regional or generalized lymph node enlargement occurs in 65% of cases. Ocular disease is noted in 40% of cases. In one case summary, 30% of cases had bone lesions that usually appeared as osteolytic lesions in distal limb bones.

Occurrence of Clinical Signs
- Fever 40%
- Lung lesions 85%
- Eye disease 40%
- Skin 30-40%
- Bone 30%

Ocular Disease with Blastomycosis: Ocular involvement occurs in 30-40% of the cases. Manifestations include conjunctival hyperemia, corneal edema, iridocyclitis, chorioretinitis, retinal separation, glaucoma, and blindness. Severity and extent of involvement determines the clinical outcome. The infection begins as a pyogranulomatous choroiditis/chorioretinitis. Once the disease progresses to the anterior segment, the prognosis for vision is diminished. The development of endophthalmitis and secondary glaucoma is associated with a very poor prognosis. Treatment consists of itraconazole or a combination of amphotericin and ketoconazole. Fluconazole may achieve superior penetration of the blood-retinal and blood-aqueous barriers. Affected globes may serve as a nidus for recurrence of disease; removal may be indicated after initial treatment.

Laboratory Features: The CBC results reveal lymphopenia and monocytosis in 35% and 47% of dogs, respectively. Anemia of inflammation is evident 35% of cases. Hypercalcemia, hyperglobulinemia, and mild hypoalbuminemia may be noted in the serum chemistries.

Diagnostic Specimens: The diagnosis is usually made by cytologic detection of organisms in FNA/impression smears of lymph nodes, transtracheal wash (50% success in dogs with lung involvement), lung aspirates, skin lesions, posterior chamber of the eye, prostate or bone lesions. Organisms can be seen in urine. One case report has demonstrated the yeast form in feces from a clinical case. Since GI involvement is rare the organisms were probably coughed up from the respiratory tract and swallowed. This may serve as a mechanism for environmental contamination in the domicile of an infected dog. For culture, tissue specimens should be placed in saline or distilled water and refrigerated but not frozen. Hospital staff can be infected by accidental needle sticks or by opening blood agar plates or fungal plates and aerosolizing spores.

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Serology: The value of serologic tests for diagnosis of blastomycosis is a major question in clinical practice. The tests available currently detect antibodies to prepared antigens either by RIA or AGID. Dogs with severe clinical disease are often seronegative because of anergy or antigen excess with consumption of antibody. Conflicting results have been published on the performance of these tests in infected and control dogs. In one publication, the agar gel immunodiffusion test had a sensitivity and specificity of 90%. The test can be negative in early infections. Klein compared the detection of antibody against WI-1 antigen by RIA with the A-antigen by AGID. In dogs with clinical disease, 92% were positive by WI-1 RIA and 42% were positive by AGID. In treated dogs, WI-1 titers were high initially, declined during treatment, but persisted for months. AGID titers decreased quickly. Control dogs had no detectable antibodies with either test. Thus, sensitivity for RIA and AGID was 92% and 41% respectively and specificity was 100% for both tests.

A urine Blastomyces urine antigen test is available and detect Blastomyces antigen in a voided urine specimen. This test has been very useful in detecting latent infections or infections in dogs with vague symptoms. This test is available at Marshfield Labs by submitting a urine sample for the Miravista Blastomyces urine antigen test.

Serum from dogs and people inhibits the growth of *Blastomyces* in culture. For most pathogens, inhibition of growth has been related to iron binding by iron-binding proteins (transferrin, lactoferrin). Transferrin binding of iron inhibits the growth of *Candida*, *Histoplasma*, and *Cryptococcus*. Growth of *Blastomyces* is independent of iron-binding proteins and these molecules are not effective in the defense against infection or clinical disease. Further studies reveal that albumin is a serum factor that inhibits the growth of *Blastomyces*. The activity does not require direct contact between albumin and yeast, but does require the site 1 drug-binding domain (rHSA I-II) on the albumin molecule. If a drug or compound occupies this site, does this diminish the inhibitory effect?

Treatment of Canine Blastomycosis: Several drugs have been used with varying success to treat blastomycosis either alone or in combination. Amphotericin B is a polyene that has been the drug of choice until the advent of the azole drug group. All of the azole antifungal drugs that include ketoconazole, itraconazole, and fluconazole interfere with the synthesis of the fungal cell membranes. A clinical trial in dogs compared itraconazole with amphotericin B. The cure rates for amphotericin B and itraconazole at dosages of 5mg/kg and 10 mg/kg (60 day course) were similar at 57%, 54%, and 54%. Recurrence and mortality rates were also similar among all treatments at 20% and 25% respectively. Seventy five percent of the dogs that died did so during the first week of treatment. In vitro determinations of minimum inhibitory concentrations (MIC) and minimum lethal concentrations (MLC) of itraconazole, ketoconazole, and fluconazole reveal that MIC and MLC drug levels in serum can be consistently achieved for ketoconazole and itraconazole, but not for fluconazole. However, a recent paper suggests that a higher dose of fluconazole (2x) will achieve similar clinical responses to the other drugs. Itraconazole was most active against *Blastomyces* in vitro.

Amphotericin B is the drug of choice for genitourinary, CNS or optic nerve involvement, but causes significant nephrotoxicity. This drug has been used for 40 years and can only be given intravenously. The drug produces large molecular pores in the lipid membrane of the fungus, which causes leakage of vital contents leading to death of the fungus. Incorporation of the drug into liposomes reduces the toxic effects and targets the drug towards the fungus. Amphotericin produces a more rapid clinical response than with the azole drugs because of its mode of action and route of administration.

Itraconazole is effective against many fungi and dermatophytes and is the drug of choice for non-meningeal, non-life threatening blastomycosis in people. The drug does penetrate the eye and has been used to treat ocular involvement, provided there was no evidence of optic neuritis. Itraconazole is not excrated in the urine and should not be used for animals with renal or urinary blastomycosis. This drug has a broader spectrum, fewer side effects, and is more expensive than the other antifungal drugs. Itraconazole requires an acid environment for absorption. Therefore, antacids and H2 blockers are contraindicated. Anorexia and increases in hepatic leakage enzymes are noted in dogs on high doses of itraconazole (10mg/kg). High doses can also cause focal ulcerative dermatitis. During the initial treatment, many patients will become anorexic and depressed, associated with rapid killing of the Yeats. These adverse reactions diminish at lower dosages. Giving a nonsteroidal anti-inflammatory such as metacam with antifungal treatment can diminish the severity of anorexia and depression.

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Ketoconazole requires an acid gastric environment for absorption. Therefore, antacids and H2 blockers interfere with the efficacy of this drug. Ocular, CNS, or genitourinary penetration is minimal. Major side effects are related to the gastrointestinal tract and the liver. Vomiting, anorexia, increases in hepatic leakage enzymes, and liver damage are most frequent. This drug is only available in an oral preparation.

Fluconazole is less toxic than ketoconazole and penetrates the CNS, eye, and prostate. This drug has fewer side effects than ketoconazole and has been used successfully in treating blastomycosis. Side effects are usually mild and limited to vomiting, anorexia, and occasional diarrhea.

**Drug Dosages**

**Itraconazole:** Initial dose is 5 mg/kg q12h for five days followed by 5 mg/kg per day. Treatment should be continued for 2-4 months and for at least one month beyond the detection of clinical signs.

**Amphotericin B:** 0.5 mg/kg IV three times a week until a cumulative dose of 8-10 mg/kg is achieved. The drug is very irritating and can cause phlebitis. Hydration status is important and fluids should be administered concurrently. BUN should be monitored. If values approach or exceed 50 mg/dL, therapy should be interrupted.

**Amphotericin Lipid Complex:** Is far less toxic than traditional amphotericin, but is very expensive. The dose is 1 mg/kg IV three times a week until a cumulative dose of 12 mg/kg is achieved.

**Amphotericin B and Itraconazole or Ketoconazole Combination:** 0.5 mg/kg IV three times a week until a cumulative dose of 4-6 mg/kg is achieved then begin by giving itraconazole at the dose given above or by giving ketoconazole at a dose of 10-15 mg/kg q12h. BUN should be monitored during amphotericin treatment. Azole is continued for 2-4 months and for at least one month beyond the detection of clinical signs.

**Prognosis and Relapse:** Three factors have been identified that influence prognosis and include: 1) severity of radiographic evidence of lung disease, 2) presence of leukocytosis with a left shift, and 3) the influence of gender in that females may have a better recovery rate than males. With severe lung or CNS disease, the prognosis is guarded. Radiographic infiltrates in the lungs will become more severe during the first week of treatment due to rapid death of organisms. The mortality rate is highest during the first week of treatment, especially for dogs with advanced lung disease. Short-term glucocorticoid therapy may be necessary if respiratory distress is significant during initial treatment. Relapse occurs in 20% of the dogs usually within the first six months and is most likely the result of reactivation of a latent infection rather than reinfection from the environment. Relapses are more frequent in dogs with severe lung involvement. Fungal resistance to itraconazole has not been described.

![Slide of Blastomycosis](image1)

![Slide of Histoplasmosis](image2)