California Association for Medical Laboratory Technology

Distance Learning Program

COCCIDIOIDOMYCOSIS (VALLEY FEVER):
A REEMERGING MYCOSIS

Course # DL-993

by
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Approved for 2.0 CE
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Level of Difficulty: Intermediate

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COCCIDIOIDOMYCOSIS (VALLEY FEVER): A REEMERGING MYCOSIS

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2.0 CE
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OBJECTIVES
After completing this course, the participant will be able to:
1. Discuss discovery and identification of *Coccidioides* as a fungal pathogen.
2. Outline principal characteristics and life cycle of *Coccidioides*
3. Describe classification of *Coccidioides* as a select agent.
4. Outline epidemiology of Valley Fever (coccidioidomycosis).
5. Discuss clinical forms of coccidioidomycosis.
6. Summarize risk factors that contribute to disseminated coccidioidomycosis.
7. List laboratory methods used for diagnosis of coccidioidomycosis.
8. Explain the nature of immune response to *Coccidioides* infection.
10. Describe current state of vaccine development.
11. List experimental models of coccidioidomycosis.

HISTORICAL BACKGROUND
The first reported case of coccidioidomycosis occurred in Argentina. In 1892 Alejandro Posadas, a medical intern in Buenos Aires, attended a patient who had severe ulcerative skin lesions and recurrent fever. The patient eventually died after suffering seven years of progressive skin lesions. Skin biopsy specimens showed organisms that resembled protozoa, specifically coccidia that parasitize humans.

A year after this case was reported a patient was hospitalized in San Francisco with skin lesions resembling those of Posadas’ patient. The San Francisco patient was a manual laborer who lived and worked in the San Joaquin Valley. Clinical specimens from this patient showed organisms that resembled protozoa. The patient did not recover from his illness; he died several years later. Specimens from the patient were studied by Gilchrist, who was a pathologist at Johns Hopkins Medical School, as well as by Rixford, a San Francisco surgeon. In 1896 Gilchrist concluded that the organisms in the patient’s samples were protozoa and resembled *Coccidia*.

The newly isolated pathogen was named *Coccidioides* (resembling *Coccidia immitis* (not mild)) by Gilchrist and Rixford.

Four years later William Ophuls and Herbert Moffitt cultured clinical material from a patient whose symptoms resembled the case described by Rixford and Gilchrist. Ophuls and Moffitt inoculated samples from this patient into male guinea pigs, producing orchitis (inflammation of the testes). Spherules could be observed microscopically in pus removed from the guinea pigs. When the spherule preparation was reexamined on the following day, mycelia had developed. Ophuls and Moffitt demonstrated that *C. immitis* was not a protozoan. It was a fungus that existed in mycelial form when cultured and as spherical protozoa-like bodies within tissues.
In 1929 a laboratory accident provided valuable information on transmission of coccidioidomycosis. Harold Chope, a medical student at Stanford, opened an old culture of *C. immitis* and inhaled the fungal spores. Chope developed severe pneumonia from which he recovered after several months of illness. Subsequently, when Harold Chope left Stanford, one of his classmates, Charles Smith, became involved in the coccidioidomycosis project. Later Charles Smith continued his research at the University of California Berkeley where he and his research group made invaluable contributions to the study of coccidioidomycosis. Smith’s work included development of skin test reagents, diagnostic serologic methods, epidemiological studies, investigation of clinical forms of disease, and studies of disease transmission (1).

**PRINCIPAL CHARACTERISTICS OF *COCCIDIOIDES***

**TAXONOMY**

Fungi are classified on the basis of their sexual reproduction. Although a sexual state had not been observed for *Coccidioides*, nucleic acid sequence data and biochemical analysis place *Coccidioides* in the group of fungi that belong to the order Onygenales. This order is in the phylum Ascomycota which contains many medically important fungi. Members of the phylum Ascomycota have a sexual reproductive phase characterized by formation of ascospores.

Two species have been identified in the genus *Coccidioides*:
- *C. immitis*
- *C. posadasii*

These species may be differentiated by genomic sequence analysis but cannot be distinguished phenotypically. Laboratory animal studies fail to show any difference in the virulence of these two species or in their growth and development. *In vitro*, *C. posadasii* had been reported to show a faster growth rate than *C. immitis*. *C. posadasii* is known as the “non-Californian” species. Results of molecular epidemiological studies suggest that *C. posadasii* originated from a single North American population in Texas and rapidly multiplied in parts of Texas, Arizona, Central Mexico, and in certain areas of Venezuela, Brazil, and Argentina. *C. immitis* is the “Californian” species and appears to be localized in central and southern California.

**NATURAL HABITAT**

*Coccidioides* is a soil fungus. It requires hot summers and mild winters and propagates in the semiarid regions of the southwestern United States, Mexico, and Central and South America. *Coccidioides* habitat is in areas that have plant life, soil composition, and climate typical of the Lower Sonoran Life Zone. *Coccidioides* has been demonstrated in soil in the following geographical areas considered to be endemic regions for coccidioidomycosis:
- Southern and central part of California, the San Joaquin Valley in particular
- Southwestern Texas (especially El Paso)
- Arizona, with highest fungal concentration in the Tucson and Phoenix areas
- Southern Utah and one recently discovered site in northeastern Utah
- Parts of southern Nevada
- South-Central Washington State
- Southern New Mexico
- Northwestern Mexico, especially the states of Sonora and Chihuahua
- Certain regions in Central and South America
The distribution of *Coccidioides* in soil is highly focal; fungal growth is concentrated in selected areas. *Coccidioides* prefers alkaline soils with increased salinity, although fungal mycelia are able to tolerate a wide pH range (pH 2 to pH 12). Fungal spores are often found in abundance in areas of high organic content, such as in the vicinity of rodent burrows and around Indian ruins and burial grounds. Very fine sand and silt is abundant in all of the *Coccidioides*-bearing soils. Sandy soil composition appears to be the most common shared feature of all sites where *Coccidioides* is found.

*Coccidioides* propagates in the upper layers of moist soils, forming septate hyphae (multicellular filaments) that develop into a mycelium (a mass of branching hyphae). When soil dries, arthrospores (arthroconidia) form by a process that involves disarticulation of the septate hyphae. Arthrospores may become airborne due to wind or various soil disturbances and may travel great distances from their place of origin. The spores are highly resistant to adverse environmental conditions and are able to remain viable for long periods of time. It is estimated that some arthrospores have existed in a viable state for thousands of years (2).

**MORPHOLOGY AND LIFE CYCLE**

*Coccidioides* is a dimorphic fungus. It grows in the soil as a filamentous fungus, forming mycelia. *Coccidioides* converts to a unicellular spherule phase when the fungus invades an animal host or when cultured in a special medium and incubated at high temperature in the presence of elevated carbon dioxide concentration. Thus, the temperature of incubation does not appear to be the only variable that controls *Coccidioides* dimorphism and spherule formation. Mycelial growth of *Coccidioides* is known as the saprophytic phase. Septate hyphae and reproductive spores called arthroconia or arthrospores are produced during this growth phase in a self-perpetuating cycle. The process of spore formation involves a series of coordinated events: progressive septation of the hyphae, condensation of the cytoplasm in certain hyphal compartments, autolysis of adjacent hyphal cells, and synthesis of a new wall layer.

Arthroconidia are typically barrel-shaped and separated from each other by empty disjunctor cells (autolysed hyphal cells). Each spore has at least two nuclei. The spores measure 2.5 to 4 micrometers in width and 3 to 6 micrometers in length. They are easily dispersed into the air and may spread throughout the environment. When arthrospores are inhaled, their minute size allows them to reach the alveoli of the lungs and to initiate the infectious process. Within host tissues the conversion of arthrospores to spherules begins by the rounding up and swelling of the spores, followed by nuclear divisions and segmentation. This process begins the parasitic phase of the fungal growth cycle.

The central portion of a growing spherule is occupied by a vacuole. The area surrounding the vacuole develops single-celled compartments which differentiate into endospores. As the spherule matures, the vacuole ruptures and endospores fill the entire spherule. A mature spherule measures 30 to 100 micrometers and may contain 200 to 1000 endospores. These endospores are approximately 2 to 5 micrometers in size. Rupture of the mature spherule releases the endospores which continue the parasitic cycle by growing into second generation spherules. When endospores are released from host tissues under suitable environmental conditions such as temperature, these spores may initiate the fungal saprophytic growth cycle: an endospore will form a tubular outgrowth that develops into hyphae.

**CLASSIFICATION AS SELECT AGENT**

Both species of *Coccidioides* are classified as select agents of bioterrorism by the federal government. The Select Agent Rule was enacted to establish a system of safeguards to be followed when specific agents are acquired, transported, transferred, or maintained. Laboratories
isolating such agents must follow strict rules. The laboratories must either be registered with the government to work with such agents or, if not registered, must follow regulations for reporting and destroying select agent isolates. Federal regulations for handling Select Agents include:

- Documentation and inventory control of select isolates
- Background checks of involved employees
- Restricted access to work areas
- Monthly reports to the Centers for Disease Control and Prevention (CDC)

Unregistered laboratories must destroy all isolates and report to the CDC each isolate’s disposition within 7 days of identification.

**GENETICS**

Genetic studies have been done with several strains of *C. immitis* from California and with *C. posadasii* strains from Texas, Arizona, Mexico, and Argentina. These studies indicated that *Coccidioides* strains fall into two groups. Additional genetic studies led to the current classification of *C. posadasii* and *C. immitis* as separate species within the genus *Coccidioides*.

In spite of genetic differences, infection with one of the *Coccidioides* species protects against infection with either of the two species. Frequent genetic recombination occurs among individual members of each species. Isolates from outbreaks of coccidioidomycosis represent a diversity of genotypes. The genetic heterogeneity of *Coccidioides* explains, in part, the difference in the virulence of *Coccidioides* strains. Experimental studies in mice demonstrate that the mean time to death of 50% of mice in a study group may vary from 11.5 days to over 45 days depending on the fungal strain.

**VIRULENCE FACTORS**

*Coccidioides* is a pathogen capable of establishing disease in immunocompetent as well as in immunocompromised individuals. The ability of *Coccidioides* to survive and multiply within a hostile host environment is a function of certain characteristics of the fungus. These characteristics are virulence traits that contribute toward the success of *Coccidioides* as a pathogen and include the spherule outer wall glycoprotein, production of arginase, and production of urease.

**Spherule outer wall glycoprotein (SOWgp)**

**Immunogenic characteristics of SOWgp**

As the growth of *Coccidioides* spherule progresses, a membranous layer composed of lipids, polysaccharides, and proteins is deposited at the cell surface. This material has a high level of immunoreactivity in assays of humoral and cellular immunity. The immunodominant component in the spherule outer wall material is a single glycoprotein known as SOWgp. This glycoprotein stimulates antibody-mediated and cellular immune responses in patients infected with *Coccidioides*. Although an immune response to the pathogen is critical in recovery from infection, extensive clinical studies with coccidioidomycosis patients have shown that cellular immunity rather than antibody production is crucial for the patient’s recovery. Patients recovering from coccidioidomycosis without antifungal treatment develop delayed-type hypersensitivity with only low levels of antibody against *Coccidioides*. Patients who do not recover spontaneously tend to have high levels of specific antibody to *Coccidioides*.

**SOWgp as a modulator of immune response**

Immune modulation is an effective strategy to lessen host defenses. Experimental evidence suggests that SOWgp may modulate the patient’s immune response in favor of a subset of T cells called T helper 2 (Th2) lymphocytes. Two functionally distinct subsets of T cells have been identified: T helper 1 (Th1) and T helper 2 (Th2) lymphocytes. Different sets of host factors...
(cytokines) are produced by the two T cell subsets. Th1 cells produce interleukin-2, tumor necrosis factor, interleukin-12, and gamma-interferon. Th2 cells secrete interleukins-4, 5, 6, and 10. The Th1 cytokine profile correlates with resistance to *Coccidioides* infection while the Th2 cytokine profile correlates with susceptibility. Interleukin-6 plays a key role in acute inflammation and in the differentiation of T lymphocytes into cells that secrete interleukin-17. Persistent activation of the interleukin-17 pathway has been shown to result in significant degrees of host tissue damage. The shift of the immune response in the direction of the Th2 pathway mediated by SOWgp offers a distinct advantage to *Coccidioides* by affecting the host cellular immune defenses (3).

**Production of enzyme arginase**

The synthesis of enzyme arginase 1 in macrophages and in dendritic cells is increased upon exposure to certain Th-2 cytokines. Arginase 1 competes with the inducible enzyme nitric oxide synthase for the common substrate, L-arginine. This results in reduced synthesis of nitric oxide by phagocytic cells, as well as an increased production of host ornithine and urea. The availability of ornithine at the site of fungal infection may promote fungal growth and proliferation. The presence of urea provides a substrate for the fungal enzyme urease which releases ammonia from urea, resulting in a more alkaline environment. To summarize, the enzyme arginase decreases efficiency of phagocytic cells, indirectly promotes fungal growth, and aids the activity of the fungal enzyme urease.

**Production of enzyme urease**

Urease is produced within the fungal spherules and is released from the ruptured spherules at the time of endosporulation. Urease activity is a major source of ammonia. It creates a highly alkaline environment and promotes an inflammatory response with accompanying tissue damage. In addition, ammonia interferes with the host’s immune reactivity by inhibiting the expression of major histocompatibility complex class II antigens on the surface of macrophages.

**COCCIDIOIDES INFECTION AND CLINICAL DISEASE**

**EPIDEMIOLOGY**

*Coccidioidomycosis in areas endemic for Coccidioides*

*Coccidioidomycosis* is considered a re-emerging disease because of a dramatic increase in the number of cases during the past decade in endemic areas. A large increase in incidence has been reported in Arizona and in California, particularly in Kern and Tulare counties and in the southern part of San Joaquin Valley. Between 1997 and 2006 the number of coccidioidomycosis cases in Arizona had quadrupled, while during the same period California cases increased 3 to 4-fold. It has been estimated that 150,000 primary coccidioidomycosis cases occur each year in the United States in endemic areas. Since most primary infections with *Coccidioides* are mild and not reported, it is difficult to determine the precise number of these infections. In 2011 coccidioidomycosis incidence in states with endemic regions (Arizona, California, Nevada, New Mexico, and Utah) was 42.6 cases per 100,000 persons.

Approximately 10% to 50% of individuals in endemic areas test positive with the coccidioidin skin test, indicating prior exposure to the fungus. The incidence of reported disease in Arizona was 91 cases per 100,000 persons in 2006, while in California the incidence was 8.4 cases per 100,000 persons rising to 18.8 cases per 100,000 persons in 2017. Among California counties, Kern county had the highest rate of coccidioidomycosis cases.

Some of the reasons for an increase in coccidioidomycosis cases in endemic areas are:

- Population growth in endemic areas
- Migration of a pool of susceptible individuals to California and Arizona
- Influx of new residents who are over 65 years of age
- New construction of homes and businesses, involving soil disturbance
- Climate change, such as an unusually rainy spring that follows a prolonged drought
- Increased awareness of coccidioidal infections among health care providers
- Improved diagnostic methods
- Better compliance with reporting cases

**Coccidioidomycosis in non-endemic areas**

More extensive travel to and from endemic areas has resulted in cases of coccidioidomycosis occurring in geographic locations where the disease is not generally found. For example, between 1992 and 1997, New York State had a total of 161 cases of coccidioidomycosis, all of which occurred in persons who had traveled to endemic areas.

Dust storms are capable of carrying arthrospores great distances from the site of origin. In 1977 in California an especially severe dust storm carried dust from San Joaquin Valley to the San Francisco Bay area. This resulted in hundreds of cases of non-endemic coccidioidomycosis.

Soil movement, as it occurs in earthquakes and in landslides, dislodges the arthrospores in the soil. An increase in the number of cases of coccidioidomycosis frequently follows these natural disasters. In January 1994 an earthquake centered in Northridge, California resulted in 170 cases of acute coccidioidomycosis due to dust from landslides in Ventura County, which is geographically many miles from Northridge.

**Risk factors for acquiring coccidioidomycosis**

a) Infections are more likely to occur during certain seasons. In Arizona, the highest rate of infection is seen in the summer during June and July. A second peak in infections occurs in the fall from October to November. In California, the risk of infection is highest between the months of June through November.

b) Opportunity for exposure to fungal spores: anyone who lives, visits, or travels through the areas where the fungus grows may contract coccidioidomycosis. Persons in occupations such as construction, excavation, agricultural work, archaeological digging and other occupations that disturb the soil are at an increased risk of infection. Military trainees participating in training exercises in endemic areas are also at risk of acquiring infection (4).

**Transmission of infection**

a) Inhalation of arthrospores is the most common means of acquiring *Coccidioides* infection.

b) Percutaneous inoculation of arthrospores may happen among laboratory workers. This can result in primary cutaneous coccidioidomycosis characterized by a painful lesion at the site of inoculation and an involvement of regional lymph nodes. Most cases tend to remain localized.

**Coccidioidomycosis in institutions**

Person to person transmission of coccidioidomycosis had not been demonstrated. The large numbers of coccidioidomycosis cases that have been noted in correctional institutions situated within or near endemic areas are due to availability of susceptible individuals in the correctional facility.

**Coccidioidomycosis in other animal species**

Coccidioidomycosis can affect many species of animals. These include dogs, cats, cattle and other livestock, horses, llamas, apes and monkeys, kangaroos, wallabies, tigers, bears, badgers, otters, marine animals such as sea otters and dolphins, skunks, cougars, coyotes,
rodents, bats, and snakes. Infection of marine animals indicates that spores are blown long
distances over the water where these animals inhale them and become sick.

**PRIMARY INFECTION**

Most cases of primary coccidioidomycosis are acquired by inhalation of arthrospores and are very mild or asymptomatic. As many as 60% of cases may be inapparent, with an additional 30% of cases exhibiting mild or moderate symptoms. Many cases resolve spontaneously. The fatality rate is estimated to be less than 1%.

**Primary coccidioidomycosis**

Most commonly reported symptoms of primary coccidioidomycosis are:

- fatigue
- cough
- joint pain
- chest pain
- fever
- headache
- rash (toxic erythema). This is a non-specific rash associated with fever and is present on the trunk and on the extremities. Rash develops in 10% to 30% of patients within the first few days of illness and disappears shortly thereafter.

**Pulmonary coccidioidomycosis**

Approximately 5% of patients with primary coccidioidomycosis develop persistent pulmonary coccidioidomycosis. Chronic progressive pneumonia may be accompanied by formation of pulmonary cavities and nodules. Many of these resolve spontaneously. A few patients develop chronic pulmonary involvement with symptoms of weight loss, cough, fever, and chest pain that may persist for years.

**Valley fever complex**

The Valley fever complex syndrome develops in approximately 5% of all cases of primary coccidioidomycosis. This syndrome is characterized by arthritis in the joints, a mild conjunctivitis, tender subcutaneous reddish nodules (erythema nodosum), fever, feeling of malaise, itching of the skin, and multiple skin lesions (erythema multiforme). These symptoms occur in female patients more frequently than in male patients and are thought to be associated with a hypersensitive reaction to the infectious agent. Simultaneously, the patient shows a strong delayed-type hypersensitivity reaction to skin testing with coccidioidin. The symptoms of erythema nodosum and erythema multiforme may resolve within 2 to 6 weeks.

**Risk factors for symptomatic coccidioidomycosis**

Treatment of patients with corticosteroids, immunosuppressive medications, and inhibitors of tumor necrosis factor appear to be risk factors for symptomatic coccidioidomycosis.

**Disseminated coccidioidomycosis**

It is estimated that approximately 1% to 4% of patients with primary coccidioidomycosis develop disseminated disease. The infectious agent may spread via blood stream from the original site of infection to various organs. Dissemination, when it occurs, happens early in the disease process and may occur in the absence of clinical evidence of previous pulmonary infection. Skin and subcutaneous tissues are the most common sites of disseminated coccidioidomycosis. Other body sites affected include bone, meninges, lymph nodes, spleen, liver, kidneys, pleura, or any part of the body with the exception of the gastrointestinal tract.
which has not been observed as a site for disseminated coccidioidomycosis. If multiple tissues or meningitis are involved, the mortality rate may exceed 50%.

**Risk factors for disseminated coccidioidomycosis**

There is conclusive evidence of genetically determined susceptibility to disseminated coccidioidomycosis. Epidemiological studies have shown that Filipinos were 176 times more likely to develop disseminated disease than Caucasians. African Americans and Mexican American were, respectively, 14 and 3 times more likely than Caucasians to develop disseminated disease.

- Genetic susceptibility: Filipinos, African Americans, Mexicans, American Indians are more susceptible to dissemination.
- Gender: males are reported to have a 3.5 to 5-fold higher occurrence of dissemination compared to females.
- Age: children 5 and under and adults over 50 years of age have greater susceptibility to dissemination.
- Pregnancy: an increased susceptibility to dissemination occurs in pregnancy, particularly during the third trimester, when hormonal levels are high and cell-mediated immunity may be depressed.
- Immunosuppression: depressed cellular immunity contributes to dissemination. Persons with HIV, immunosuppressive conditions, malignancies, collagen vascular disease, organ transplant recipients, and anyone undergoing immunosuppressive therapy including adrenal corticosteroid therapy is under increased risk of disseminated coccidioidomycosis.
- Miscellaneous factors associated with dissemination: diabetes, cigarette smoking.

**HOST DEFENSES**

Host defenses in coccidioidomycosis include the inflammatory response and a specific immune response to fungal antigens. The development of skin testing reagents by Smith, Levine, and co-workers laid the foundation for understanding the immunology of coccidioidomycosis. Smith introduced coccidioidin, a filtrate of mycelial cultures of *Coccidioides*. The coccidioidin skin test detects delayed hypersensitivity to fungal antigens. The delayed hypersensitivity reaction is mediated by T lymphocytes and indicates activation of cellular immunity.

The development of a culture medium for laboratory propagation of the spherule-endospore phase facilitated the introduction of spherulin by Levine. Spherulin is a soluble aqueous lysate of spherule cultures and is an alternate skin-testing reagent that detects delayed hypersensitivity to fungal antigens.

**KEY FEATURES OF IMMUNE RESPONSE TO COCCIDIOIDES INFECTION**

- Persons with asymptomatic coccidioidal infections or with mild disease show delayed hypersensitivity to coccidioidin and a very low level or an absence of circulating antibody to *Coccidioides*.
- Persons with severe, chronic, or progressive coccidioidomycosis show polyclonal B-cell activation as evidenced by an increase in serum levels of immunoglobulins G, A, and E. Such patients are hyporesponsive or nonresponsive to coccidioidal skin testing.
- Recovery from active disease may be associated with the appearance of T-cell reactivity to coccidioidal antigens and a decrease in circulating antibody titers.
- Cellular immunity is crucial for recovery from coccidioidal infection.
Cellular immunity and delayed hypersensitivity are long lived and prevent re-infection. Recurrent infections have not been observed.

Reactivation of a prior infection occurs only in cases of profound immunosuppression.

Initial response to infection

Inflammation is the initial response of the infected host. It occurs in two stages: early and late. In the early stage polymorphonuclear neutrophils (PMNs), monocytes, macrophages, and natural killer cells are attracted to the site of infection. Phagocytosis is restricted to arthroconidia, to the initial stages of differentiation of arthrospores into spherules, and to endospores. The mature spherules are too large in size for effective phagocytosis. Generally, phagocytosis is only moderately effective; fewer than 20% of fungal spores are destroyed by phagocytic cells. In some instances fungal spores are capable of surviving within macrophages. The late inflammatory response takes place within infected tissues. This takes the form of a granulomatous reaction characterized by an influx of lymphocytes, plasma cells, macrophages, and multinucleated giant cells formed through fusion of macrophages. Formation of granulomas is a chronic inflammatory reaction designed to wall off the infection. The center of a coccidioidal granuloma consists of necrotic area surrounded by cellular infiltrate.

Immune response to fungal antigens

Antigen presenting cells, such as dendritic cells, are activated and interact with B and T lymphocytes in secondary lymphatic organs. Dendritic cells are able to regulate T and B cell responses by producing cytokines that polarize T lymphocyte responses and regulate the activity of effector cells, such as neutrophils, macrophages, natural killer cells, and T and B cells. The cytokines produced by various cell types are also important regulators of the immune response. For example, activation of the interleukin-12 pathway is critical in induction of Th-1 cellular immune response.

TREATMENT

Most coccidioidal infections are inapparent or very mild. Many primary pulmonary infections are self-limited and resolve spontaneously without anti-fungal treatment. Drug treatment is generally reserved for patients with more severe clinical manifestations, such as excessive weight loss, extensive lung involvement, patients with symptoms that persist for over two months, and persons at high risk for severe disease.

Two classes of drugs are used for treatment of coccidioidal infections: triazoles and amphotericin B. The latter was the first antifungal drug to show activity against *Coccidioides*. The drawbacks of amphotericin B are that it must be administered intravenously and that its use may be accompanied by serious side effects. The newer formulation of lipid-associated amphotericin B has decreased the likelihood and severity of renal toxicity shown by amphotericin B deoxycholate.

Currently azoles are the main drugs used in treating coccidioidomycosis. These drugs can be taken orally and have few side effects, with a few exceptions. Fluconazole is the azole of choice for treatment of uncomplicated pulmonary and disseminated coccidioidomycosis. Itraconazole is used to a lesser extent. Two newer drugs, voriconazole and posaconazole, are used to treat infections that do not respond to treatment with fluconazole or itraconazole. The duration of treatment varies from several months to life-long therapy. Azoles are also used for prophylaxis against infection in high-risk patient population.

Guidelines for treating patients with more advanced coccidioidal disease have been developed by the Infectious Disease Society of America. Patients with acute pneumonia are...
treated with an azole for 3 to 6 months, 200-400 mg daily. Those with chronic progressive pneumonia are treated with azoles for 12 months or longer. If the patient is not responding to treatment with azoles, amphotericin is administered. The general dose for amphotericin B is 0.5-1.5 mg/kg of body weight daily or every other day intravenously. Lipid-associated amphotericin B is given daily in a dose of 2.0-5.0 mg/kg of body weight. Disseminated coccidioidomycosis is treated for 12 months or longer with azoles and amphotericin if response to primary treatment with azoles is not satisfactory.

A new antibiotic, nikkomycin Z, is in the process of testing and development at the Valley Fever Center, University of Arizona.

Certain patients such as those with meningeal disease, HIV, transplant recipients, and pregnant women present special treatment issues.

Patients with coccidioidomycosis that disseminated to the meninges are given oral azoles at high dose level or are treated with amphotericin B administered into the spinal canal. The treatment may be life-long to prevent disease reactivation.

Patients with HIV are severely immunosuppressed and are known to be susceptible to coccidioidal infection. The infection may be a primary event or a reactivation of prior infection. It is difficult to diagnose these infections because the patient has a diminished immune response and immunological diagnostic tests are often negative. In such cases it is necessary to demonstrate coccidioidal spherules in biopsy specimens or a positive fungal culture. HIV patients with coccidioidomycosis are treated with azoles. Unfortunately, an interaction between anti-retroviral drugs used in treatment of AIDS and azoles has been reported.

Patients who receive organ transplants are given immunosuppressive drugs. Such patients are highly susceptible to coccidioidomycosis. Historically, a 7% to 9% incidence of coccidioidomycosis in organ recipients has been reported. The fatality rate may be as high as 72%. The infection may be primary or reactivation of an existing condition. Transmission of coccidioidomycosis through the donor organ may also occur. The patients can be treated with azoles, but the level of immunosuppressive medications the patients receive must be carefully monitored. Azoles interfere with metabolism of certain immunosuppressive drugs, resulting in possible renal toxicity.

Coccidioidomycosis in pregnant women, although rare, may carry the risk of dissemination, particularly if the infection is acquired during the third trimester. The risk of dissemination is especially high if the patient is in one of the risk groups for disseminated disease. Amphotericin B deoxycholate or lipid-associated amphotericin constitute preferred treatment for pregnant women with coccidioidomycosis. These medications have no known fetal risk. The azoles, unfortunately, may cause fetal malformations.

**DIAGNOSIS OF COCCIDIOIDOMYCOSIS**

Laboratory tests are very important in the diagnosis of coccidioidomycosis. Primary pulmonary coccidioidomycosis is usually a mild disease with flu-like symptoms. Pneumonia caused by *Coccidioides* is clinically difficult to distinguish from other forms of acute pneumonia, such as community-acquired pneumonia. Clinical symptoms alone are generally insufficient for diagnosis of pulmonary coccidioidomycosis without laboratory tests.

Historically, skin testing with coccidioidin antigens was used to identify infected persons. These antigens are no longer in use. A reformulated spherule-derived coccidioidin antigen, Spherusol, is now available for skin tests. It is designed for testing patients aged 18 to 64. Spherusol is used for detection and monitoring of cell-mediated immunity to Coccidioides in
persons with history of coccidioidomycosis. It takes 2 to 4 weeks after infection before skin reactivity appears. A positive skin test indicates immunity to Coccidioides and resistance to reinfection.

A number of serologic methods are useful in diagnosis of coccidioidomycosis and are the most frequently used diagnostic tests. Molecular methods for direct identification of fungal DNA are being developed. These procedures offer a high level of specificity and sensitivity. Molecular techniques for detection and identification of *C. immitis* can be divided into nucleic acid hybridization methods in which the probes can be used to confirm the identification of the culture or to identify the fungi in tissue sections, and nucleic acid amplification methods which include all PCR-based techniques (polymerase chain reaction techniques). The hybridization methods use chemiluminescent probes such as the Accuprobe and Diversi-Lab systems commercially available from Gen-Probe, (San Diego, CA) and Bacterial Barcodes, Inc., (Athens, GA), respectively. PCR techniques are mostly used in research laboratories and offer the greatest potential specificity and sensitivity for detection and identification of *Coccidioides*. These techniques are not generally used by hospital laboratories. The most definitive and generally accepted methods of confirming diagnosis of coccidioidomycosis is by demonstration of typical fungal spherules in tissues and by culture and isolation of the infecting organism.

**MICROSCOPIC EXAMINATION OF CLINICAL SPECIMENS**

Demonstration of endospore-containing spherules in clinical samples is diagnostic of coccidioidomycosis. Wet mounts in potassium hydroxide (KOH) may be examined, or the calcofluor white fluorescent stain may be used. The KOH wet mounts are easy to prepare but rather difficult to interpret, requiring considerable experience. The calcofluor fluorescent stain is highly sensitive but may nonspecifically bind plant or fatty acid material.

Tissue biopsy specimens may be stained with hematoxylin-eosin stain, periodic acid Schiff stain, or the Grocott methenamine silver stain, which is very sensitive but may stain other tissue elements. Hematoxylin-eosin stain is the least sensitive of the three stains. The presence of endospores within spherules is considered diagnostic for coccidioidomycosis. The presence of a few spherules without internal structures is considered presumptive evidence for the disease. Occasional mycelia and arthroconidia-like structures may be present in certain clinical specimens.

**CULTURE AND ISOLATION OF COCCIDIOIDES**

*Coccidioides* species are not fastidious and can grow on most selective and nonselective fungal media, such as brain-heart infusion agar, potato dextrose or potato flakes agar, and Sabouraud dextrose agar, with and without cycloheximide. *Coccidioides* species also grow well on many bacteriological media: sheep blood agar, chocolate agar, and media designed for isolation of *Bordetella* and of *Legionella*. Growth of colonies is apparent within 2 to 16 days after inoculation of culture media, with an average time of 4 to 5 days. Colonies on Sabouraud’s agar incubated at 25°C are grayish-white with tan to brown color on the reverse side of the colony. Initially, colonies appear membranous but become white and fluffy as growth progresses. Colony morphology and growth rate show variation. Media containing cycloheximide and other antimicrobial agents as well as nonselective media should be used for clinical specimens with potentially mixed flora. All handling of *Coccidioides* species cultures should be performed under a laminar-flow biological safety cabinet because of ease of dispersal of arthroconidia and their high infectivity. Microscopic examination of colonies of *Coccidioides* species shows branched septate hyphae with alternating barrel-shaped arthroconidia separated by clear zones called disjunctors. These are suggestive of *Coccidioides*

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species but are not diagnostic. The identity of suspected colonies in the past was confirmed by the exoantigen immunodiffusion test, propagation of the spherule phase in Converse medium, or by mouse inoculation tests. The last two procedures promote conversion of the fungal mycelial phase of growth to the spherule phase. These older methods are no longer used in the clinical laboratory. The current method of choice for identification of the fungal isolate at the genus level is the genetic probe, ACCUPROBE, GenProbe, San Diego, CA, which recognizes but does not differentiate between the two species of Coccidioides. A heat-inactivated previously identified isolate is used as control. A non-invasive Coccidioides strain is also available for control functions. Identification of the isolate as Coccidioides species is diagnostic for coccidioidomycosis since there is no state of colonization. Differentiation of the two Coccidioides species relies on molecular techniques, although C. posadasii shows a faster growth rate in vitro than C. immitis. Molecular techniques that separate the two species, such as PCR and molecular sequencing, are available in reference laboratories.

Recovery rate of Coccidioides species from cultured clinical samples varies from 8.3% for samples from the respiratory tract to 0.4% for blood samples. When specimens from patients with known coccidioidomycosis were cultured, 20% of sputum specimens and 53% of bronchoscopy samples were positive.

ANTIBIOTIC SUSCEPTIBILITY TESTS

The Clinical and Laboratory Standards Institute defined standards in 2002 for in vitro susceptibility testing of Coccidioides isolates. A number of testing methods are available, including disk diffusion tests and microdilution assays, as well as newer methods such as the quantitative gradient strip, flow cytometry, and fluorimetry using fluorescent probes. Potential antibiotic resistance of isolates can be determined by minimum inhibitory concentration and minimum effective concentration testing. In vitro susceptibility testing of fungal isolates is not routinely done in clinical laboratories. Coccidioides species are generally susceptible in vitro to the following drugs: amphotericin B, fluconazole, itraconazole, voriconazole, and posaconazole.

SEROLOGIC TESTS

Circulating antibody to Coccidioides does not provide protection against coccidioidomycosis. Presence of antibody and its titer are indicative of the pathogen’s level of activity. Persistently high antibody levels may point to continued infection or to dissemination. Determination of circulating antibody titers is useful in diagnosis as well as prognosis of disease.

Circulating antibody becomes detectable within one to three weeks after onset of infection and belongs to the immunoglobulin M (IgM) class. After the second week of infection antibody of the IgG class can be detected. A negative serologic test does not rule out infection, since the sensitivity of serologic tests is not as high as was originally thought. Immunocompromised patients may have very low antibody titers or negative serologic tests.

A number of serologic methods are available for determination of antibody response to coccidioidomycosis. These are enzyme immunoassays (EIAs), immunodiffusion tests, and complement fixation tests.

The EIA assay is more sensitive than the other two types of tests in detecting the presence of antibody early in infection. The immunodiffusion and complement fixation tests are more sensitive later in the course of disease. The immunodiffusion assay and EIA are able to measure IgM antibody class as well as the IgG antibody. The complement fixation test detects IgG antibody and is the only standard quantitative assay for measuring antibody titers. Although the immunodiffusion test is not considered to be a quantitative test, it can be modified to determine antibody titers.
The complement fixation test was introduced early in the history of coccidioidomycosis as an important diagnostic and prognostic tool. The assay measures complement-fixing antibody of the IgG class and is useful in evaluation of antifungal therapy and in assessing progression of disease. Although not as sensitive as EIA and immunodiffusion assays, the complement fixation test is important in diagnosis of more severe cases of coccidioidomycosis. It is the primary serologic test for diagnosis of coccidioidal meningitis. Currently there are several complement fixation assay protocols and a variety of reagents available for complement fixation tests.

ANTIGEN DETECTION TESTS

Coccidioidal antigen may be found in the serum and other body fluids of 47% of infected persons with antibody to *Coccidioides*. A number of tests have been developed to detect the fungal antigen. For example, Mira Vista Diagnostics introduced a *Coccidioides* antigen EIA test that detects coccidioidal galactomannan. The Exoantigen test (immunodiffusion) had been used for identification of cultures prior to introduction of genetic probes. To perform this test the unknown organism is grown on a slant for six days or longer. The culture is extracted overnight in a merthiolate solution and then concentrated 25-50 times. The concentrate is reacted against a known anti-*Coccidioides* serum in an immunodiffusion assay. The test is incubated for 24 hours and precipitin lines are compared to positive and negative controls.

MOLECULAR TESTS:

Polymerase chain reaction test (PCR) has been approved by the Food and Drug Administration for detection of *Coccidioides* directly from lower respiratory tract specimens. In 2005 patients with coccidioidomycosis were shown to have cross-reactivity to *Histoplasma* antigens. Approximately 58% of patients with systemic coccidioidomycosis give a positive reaction in a *Histoplasma* antigen test. A *Histoplasma* urine test is currently being evaluated for a possible application to the diagnosis of coccidioidomycosis.

VACCINE DEVELOPMENT

The search for a vaccine that offers protection against coccidioidomycosis goes back at least 50 years. Early vaccine studies employed attenuated viable cells or whole killed cells. The mycelial-phase cultures of the fungus were used. Once a medium for cultivation of spherules was developed, vaccine studies with the spherule-phase of fungal growth became possible. Since reversion to virulence occurs with attenuated strains, most studies utilized killed fungal cells. Charles Smith’s coccidioidomycosis research group with Levine, Pappagianis, Kong, and others had been involved in vaccine development in the early 1960s. In 1965 Levine, Kong, and Smith published a study on the use of a formalin-killed spherule vaccine. This preparation was effective in protecting mice and monkeys from challenge with viable *Coccidioides*. The favorable results in animal tests led to clinical trials in order to assess the vaccine’s toxicity and its potential use in humans. The protective capacity of the spherule vaccine was evaluated in a double-blind study between 1980 and 1985. A total of 2,867 healthy persons participated in the study. The vaccine recipients were given 3 injections of the formalin-killed spherule vaccine. The subjects in the test and control groups were observed for new cases of coccidioidomycosis for a period of 5 years. After the 5-year follow-up period there was no significant difference in the incidence or severity of coccidioidomycosis in the two groups of subjects. The lack of effectiveness of the formalin-killed spherule vaccine may have been due, in part, to the low vaccine dose used in clinical trials as well as to the very low incidence of disease during the test period; only 21 cases of coccidioidomycosis occurred in the two study groups.
The search for an effective vaccine continued in a number of different laboratories. Various cell-derived antigens and purified subcellular components had been tested for their ability to offer protection against coccidioidomycosis. The cell-derived antigens included extracts of spherule cell walls, extracts of mycelial cell walls, spherule outer wall fraction, urease, heat shock protein 60, and heat-stable exoantigen.

More recently, the emphasis in vaccine development has shifted to the use of recombinant protein subunits, synthetic peptides, protein-polysaccharide conjugates, and plasmid DNA. A number of recombinant coccidioidal protein antigens, as well as two attenuated mutant strains, had been identified by investigators with Valley Fever Vaccine Project to offer protection in murine models. Other possible vaccine candidates are fungal cell wall proteins expressed by *Escherichia coli*, dendritic cell-based vaccines, heat-killed and viable *Saccharomyces cerevisiae*, genetically engineered live, attenuated strain of *C. posadasii*, and many others. The genetically engineered strain of *C. posadasii* was developed by disruption of two chitinase genes that activate chitinolytic enzymes during the early stage of endospore development. The altered fungal strain was not infectious and could not produce endospores because chitin is a major component of fungal cell walls. Subcutaneous immunization of mice with viable spores of the attenuated strain resulted in localization of spores to the site of inoculation, differentiation of spores into spherules without endospore formation, and activation of dendritic cells. Injected mice developed granulomas with diminished inflammation and an immune response that involved both Th-1 and Th-2 lymphocytes.

Although many vaccine preparations show promise, there is a need to identify additional vaccine candidates for use in monovalent and multivalent component vaccines. There is also a need to develop additional adjuvants, since aluminum-based mineral salts (alum) are the only adjuvants currently available for human use.

ANIMAL MODELS

The understanding of pathogenesis of coccidioidomycosis, host resistance to infection, nature of the immune response, vaccine development, as well as development of more effective antifungal therapies has relied heavily on the use of animal models. Mice are used as the primary laboratory animal species for models of this disease because of availability of genetically defined strains, ease of handling, and lower cost. Studies in murine models have provided evidence for the crucial role of cellular immunity in host defense against coccidioidal infection. Various aspects of host resistance, pathogenesis, and antifungal therapy have also been investigated in murine models. In addition, murine models of primary pulmonary disease, intraperitoneal disease with dissemination, intravenous infection that simulates systemic disease, and intracranial infection as a model of meningeal disease have been developed. A rabbit model of meningeal disease has also been shown to simulate human meningitis.

A variety of other animal species including dogs, primates, and guinea pigs have been used to study host response to coccidioidomycosis and vaccine efficiency.

SUMMARY

Coccidioidomycosis was first reported in Argentina at the end of the 19th century. The distinguishing symptoms of the disease were fever and ulcerative skin lesions. A few years later similar cases were reported in California. While the nature of the pathogen remained controversial, its morphology in host tissues resembled protozoa-like organisms. The isolate was classified as a protozoan and named *Coccidioides immitis*. The ability of *Coccidioides* to form
mycelia at room temperature caused re-classification of the organism as a fungus with two morphological phases. The pathogen was dimorphic: it had a mycelial phase at room temperature and a unicellular spherule phase in host tissue.

*Coccidioides immitis* is classified as a member of the phylum Ascomycota, which contains many medically important fungi. Two *Coccidioides* species are now recognized: *C. immitis* and *C. posadasii*. Although the two species differ genetically, they cannot be distinguished morphologically and are similar in their virulence. Immunity to one species protects the host against infection with either of the two species. Genetic analysis is used to distinguish *C. immitis* from *C. posadasii*. *C. immitis* is found in California, while the natural habitat for *C. posadasii* is in areas outside California.

*Coccidioides* is a soil fungus. It is found in sandy, alkaline soil that has a high organic content. The fungus requires hot summers and mild winters and propagates in semi-arid regions of the southwestern United States, Mexico, and Central and South America. *Coccidioides* grows in the upper layers of moist soils forming septate hyphae. These develop into a mycelium and form spores called arthroconidia or arthrospores. The spores are highly resistant to adverse environmental conditions, are easily spread by wind or soil disturbances, and are highly infective. When inhaled by humans or animals, the spores initiate the infectious process. The parasitic phase of fungus development is initiated within host tissues. Arthrospores are converted into spherules by a stepwise process. The spherules undergo a process of differentiation and form endospores. Each spherule, when mature, may contain from 200 to 1,000 endospores. When the mature spherule ruptures, the endospores are released into host tissues. Each endospore has the capacity to develop into a second generation spherule. Because of its high infectivity, *Coccidioides* has been classified as a select agent of bioterrorism and is subject to federal regulations.

The ability of *Coccidioides* to invade tissues and cause disease is aided by fungal virulence factors. These factors include the spherule outer wall glycoprotein and enzymes arginase and urease. The cell wall glycoprotein modulates host immunity in the direction of the Th2 pathway. This results in a decreased cellular immune response. Arginase affects activity of phagocytic cells and urease promotes an alkaline environment that facilitates inflammation and tissue damage.

*Coccidioidomycosis* is endemic in certain areas of California and Arizona. The incidence of disease in these areas has increased considerably over the past decade. A number of factors are responsible for this increase, such as population growth, rise in construction, influx of susceptible residents, and improved diagnostic methods. The incidence of coccidioidomycosis in non-endemic areas has also increased due to rise in travel between endemic and non-endemic areas and natural disasters, such as earthquakes and dust storms.

Infection with *Coccidioides* is generally mild or asymptomatic with one third of infected persons showing flu-like symptoms. A small percentage of these persons develop pulmonary coccidioidomycosis while others show symptoms of Valley Fever. These symptoms include arthritis in the joints, subcutaneous nodules, fever, and multiple skin lesions. Approximately 1% to 4% of patients infected with *Coccidioides* develop disseminated disease, which may affect many body organs. If several organs are involved or meningitis develops, the mortality rate may be 50% or higher. A number of factors affect the risk of dissemination of disease: genetic susceptibility, gender, age, pregnancy, and immunosuppression.

The immune response to *Coccidioides* infection involves development of circulating antibody and cellular immunity. The cellular immune response is a major factor in recovery from
infection and in preventing a recurrent coccidioidal infection. Circulating antibody does not offer protection and rising antibody titers are associated with progressive disease.

Two classes of drugs are used for treatment of coccidioidomycosis: triazoles and amphotericin B. Triazoles can be taken orally and are the main drugs currently in use. If the patient does not respond to azoles, amphotericin is used. Coccidioidomycosis in pregnant women is treated with amphotericin to avoid fetal damage by azoles.

Laboratory diagnosis of coccidioidomycosis is based on direct examination and culture of clinical specimens and serologic tests. Demonstration of endospore-containing spherules in clinical samples is considered diagnostic of coccidioidomycosis. Similarly, cultivation of Coccidioides species from clinical material confirms diagnosis of coccidioidomycosis. Procedures are available for in vitro antibiotic susceptibility tests. Serologic tests used in diagnosis of coccidioidomycosis include enzyme immunoassays, immunodiffusion tests, and complement fixation tests. The complement fixation test is a quantitative assay and is the primary serologic test for diagnosis of coccidioidal meningitis.

The search for a vaccine that offers protection against coccidioidal infection is continuing. Many vaccine candidates have been tested in animal models. Possible vaccine candidates have included attenuated and killed fungal cells, cell-derived antigens, purified sub-cellular components, recombinant protein subunits, synthetic peptides, attenuated mutant strains, genetically engineered fungal strains, and many others. Animal models have been essential for testing vaccine effectiveness. Murine models had been used most frequently in vaccine studies as well as for testing anti-fungal drugs and for developing models of clinical disease.

REFERENCES
REVIEW QUESTIONS
Course #DL-993
Choose the one best answer:

1. *Coccidioides* was originally classified as
   a. a bacterium
   b. a fungus
   c. a protozoan
   d. an alga

2. Coccidioidomycosis is endemic in
   a. New York
   b. San Joaquin Valley
   c. San Francisco
   d. Oregon

3. *C. immitis* infections
   a. are always asymptomatic
   b. invariably progress to meningitis
   c. may result in disseminated disease
   d. are always more severe than *C. posadasii* infections

4. The natural habitat of *Coccidioides* is
   a. soil
   b. water
   c. animal fur
   d. human intestinal tract

5. *C. immitis* and *C. posadasii* can be differentiated by
   a. colony pigmentation
   b. serologic tests
   c. genetic analysis
   d. animal inoculation

6. *Coccidioides* species grow best in
   a. swamps
   b. sandy soil with high organic content
   c. clay soil
   d. any acidic soil

7. *Coccidioides* species
   a. form sexual spores called basidiospores
   b. do not produce spores
   c. form arthroconidia
   d. form yeast cells
8. *Coccidioides* forms a mycelium  
   a. in the soil  
   b. only in host tissue  
   c. only at body temperature  
   d. only in a liquid culture medium

9. Arthrospores are  
   a. sexual reproductive spores  
   b. formed within hyphae by a process of segmentation  
   c. formed only within host tissues  
   d. formed only in a culture medium

10. Coccidioidomycosis is spread  
    a. by person-to-person contact  
    b. by ingestion of contaminated food  
    c. by drinking contaminated water  
    d. by inhaling arthrospores

11. *Coccidioides* has the following characteristics:  
    a. dimorphism  
    b. yeast-like growth  
    c. inability to grow *in vitro*  
    d. inability to infect warm-blooded animals

12. *Coccidioides* species are  
    a. non-pathogenic for humans  
    b. plant pathogens  
    c. select agents of bioterrorism  
    d. aquatic fungi

13. Spherules are formed  
    a. during the parasitic phase of fungal development  
    b. during the saprophytic phase of fungal development  
    c. during fungal dormancy  
    d. only *in vitro*

14. Which of the following is a *Coccidioides* virulence factor?  
    a. fungal ribosomes  
    b. fungal hyphae  
    c. chitin in fungal cell walls  
    d. spherule outer wall glycoprotein
15. New cases of coccidioidomycosis
   a. have decreased during the past decade
   b. have increased during the past decade
   c. no longer occur
   d. are impossible to identify because all are asymptomatic

16. The enzyme urease
   a. is found only in bacteria
   b. produces high acidity within fungal cells
   c. is one of Coccidioides virulence factors
   d. is not produced by fungi

17. A major factor in recovery from coccidioidomycosis is
   a. circulating antibody
   b. cellular immunity
   c. diet rich in animal protein
   d. phagocytic cells

18. Drugs used for treatment of coccidioidomycosis include:
   a. penicillin
   b. tetracycline
   c. streptomycin
   d. triazoles

19. Amphotericin B
   a. is never used to treat coccidioidomycosis
   b. is used for treatment of pregnant women with coccidioidomycosis
   c. can only be taken orally
   d. is known to present a fetal risk

20. Diagnosis of coccidioidomycosis can be confirmed by observing the following fungal structures in clinical material:
   a. hyphae
   b. arthrospores
   c. endospores within spherules
   d. empty spherules