Section VIII-B: Fungal Agents

Blastomyces dermatitidis

Blastomyces dermatitidis is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia) that are the infectious particles; these convert to large budding yeasts under the appropriate culture conditions *in vitro* at 37°C and in the parasitic phase *in vivo* in warm-blooded animals. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Three groups are at greatest risk of laboratory-acquired infection: microbiologists, veterinarians and pathologists.[1] Laboratory-associated local infections have been reported following accidental parenteral inoculation with infected tissues or cultures containing yeast forms of *B. dermatitidis*.[2-8] Pulmonary infections have occurred following the presumed inhalation of conidia from mold-form cultures; two persons developed pneumonia and one had an osteolytic lesion from which *B. dermatitidis* was cultured.[9,10] Presumably, pulmonary infections are associated only with sporulating mold forms.

Natural Modes of Infection

The fungus has been reported from multiple geographically separated countries, but is best known as a fungus endemic to North America and in association with plant material in the environment. Infections are not communicable, but require common exposure from a point source. Although presumed to dwell within the soil of endemic areas, *B. dermatitidis* is extremely difficult to isolate from soil. Outbreaks associated with the exposure of people to decaying wood have been reported.[11]

Laboratory Safety and Containment Recommendations

Yeast forms may be present in the tissues of infected animals and in clinical specimens. Parenteral (subcutaneous) inoculation of these materials may cause local skin infection and granulomas. Mold form cultures of *B. dermatitidis* containing infectious conidia, and processing of soil or other environmental samples, may pose a hazard of aerosol exposure.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with clinical materials, animal tissues, yeast-form cultures, and infected animals. BSL-3 practices, containment equipment, and facilities are required for handling sporulating mold-form cultures already identified as *B. dermatitidis* and soil or other environmental samples known or likely to contain infectious conidia.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Coccidioides immitis and Coccidioides posadasii

Coccidioides spp. is endemic to lower sonoran deserts of the western hemisphere including northern Mexico, southern Arizona, central and southern California, and west Texas. The original species (*C. immitis*) has been divided into *C. immitis* and *C. posadasii*.[12] These species are dimorphic fungal pathogens existing in nature and in laboratory cultures at room temperature as filamentous molds with asexual spores (single-cell arthroconidia three to five microns in size) that are the infectious particles that convert to spherules under the appropriate culture conditions *in vitro* at 37°C and *in vivo* in warm-blooded animals.

Occupational Infections

Laboratory-associated coccidioidomycosis is a documented hazard of working with sporulating cultures of *Coccidioides* spp.[13-15] Occupational exposure has also been associated in endemic regions with archeology[16] and high dust exposure.[17] Attack rates for laboratory and occupational exposure are higher than for ambient exposure when large numbers of spores are inhaled. Smith reported that 28 of 31 (90%) laboratory-associated infections in his institution resulted in clinical disease, whereas more than half of infections acquired in nature were asymptomatic.[18] Risk of respiratory infection from exposure to infected tissue or aerosols of infected secretions is very low. Accidental percutaneous inoculation has typically resulted in local granuloma formation.[19]

Natural Modes of Infection

Single spores can produce ambient infections by the respiratory route. Peak exposures occur during arid seasons. *Coccidioides* spp. grow in infected tissue as larger multicellular spherules, up to 70 microns in diameter and pose little or no risk of infection from direct exposure.

The majority of ambient infections is subclinical and results in life-long protection from subsequent exposures. The incubation period is one to three weeks and manifests as a community-acquired pneumonia with immunologically mediated fatigue, skin rashes, and joint pain. One of the synonyms for coccidioidomycosis is desert rheumatism. A small proportion of infections is complicated by hematogenous dissemination from the lungs to other organs, most frequently skin, the skeleton, and the meninges. Disseminated infection is

much more likely in persons with cellular immunodeficiencies (AIDS, organ transplant recipient, lymphoma).

Laboratory Safety and Containment Recommendations

Because of their size, the arthroconidia are conducive to ready dispersal in air and retention in the deep pulmonary spaces. The much larger size of the spherule considerably reduces the effectiveness of this form of the fungus as an airborne pathogen.

Spherules of the fungus may be present in clinical specimens and animal tissues, and infectious arthroconidia in mold cultures and soil or other samples from natural sites. Inhalation of arthroconidia from environmental samples or cultures of the mold form is a serious laboratory hazard. Personnel should be aware that infected animal or human clinical specimens or tissues stored or shipped in such a manner as to promote germination of arthroconidia pose a theoretical laboratory hazard.

BSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, and processing animal tissues. ABSL-2 practices, containment equipment, and facilities are recommended for experimental animal studies when the route of challenge is parenteral.

BSL-3 practices, containment equipment, and facilities are recommended for propagating and manipulating sporulating cultures already identified as *Coccidioides* spp. and for processing soil or other environmental materials known to contain infectious arthroconidia. Experimental animal studies should be done at BSL-3 when challenge is via the intranasal or pulmonary route.

Special Issues

Select Agent Some *Coccidioides* spp. are select agents requiring registration with CDC and/or USDA for possession, use, storage and/or transfer. See Appendix F for additional information.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Cryptococcus Neoformans

Cryptococcus neoformans is a monomorphic fungal pathogen existing in nature, in laboratory cultures at room temperature and *in vivo* as a budding yeast. The sexual stage is grouped with the Basidiomycetes and is characterized by sparse

hyphal formation with basidiospores. Both basidiospores and asexual yeasts are infectious.

Occupational Infections

Accidental inoculation of a heavy inoculum of *C. neoformans* into the hands of laboratory workers has occurred during injection or necropsy of laboratory animals.[20,21] Either a local granuloma or no lesion was reported, suggesting low pathogenicity by this route. Respiratory infections as a consequence of laboratory exposure have not been recorded.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with pigeon feces. Infections are not transmissible from person-to-person, but require common exposure via the respiratory route to a point source.

Laboratory Safety and Containment Recommendations

Accidental parenteral inoculation of cultures or other infectious materials represents a potential hazard to laboratory personnel, particularly to those who may be immunocompromised. Bites by experimentally infected mice and manipulations of infectious environmental materials (e.g., pigeon feces) may also represent a potential hazard to laboratory personnel. *C. neoformans* has been isolated from bedding of cages housing mice with pulmonary infection indicating the potential for contamination of cages and animal facilities by infected animals.[22] Reports of cutaneous cryptococcal infection following minor skin injuries suggests that localized infection may complicate skin injuries incurred in laboratories that handle *C. neoformans*.[23]

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for activities with known or potentially infectious clinical, environmental, or culture materials and with experimentally infected animals. This agent and any samples that may contain this agent should also be handled in a Class II BSC.

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts under the appropriate culture conditions *in vitro* at 37°C

and in the parasitic phase *in vivo*. The sexual stage is an Ascomycete with infectious ascospores.

Occupational Infections

Laboratory-associated histoplasmosis is a documented hazard in facilities conducting diagnostic or investigative work.[24-27] Pulmonary infections have resulted from handling mold form cultures.[28,29] Local infection has resulted from skin puncture during autopsy of an infected human,[30] from accidental needle inoculation of a viable culture,[31] and from spray from a needle into the eye.[32] Collecting and processing soil samples from endemic areas has caused pulmonary infections in laboratory workers.[33] Conidia are resistant to drying and may remain viable for long periods of time. The small size of the infective conidia (less than 5 microns) is conducive to airborne dispersal and intrapulmonary retention. Work with experimental animals suggests that hyphal fragments are capable of serving as viable inocula.[24]

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with starling and bat feces. It has been isolated from soil, often in river valleys, between latitudes 45°N and 45°S. Histoplasmosis is naturally acquired by the inhalation of infectious particles, usually microconidia.[24] Infections are not transmissible from person-to-person, but require common exposure to a point source.

Laboratory Safety and Containment Recommendations

The infective stage of this dimorphic fungus (conidia) is present in sporulating mold form cultures and in soil from endemic areas. The yeast form in tissues or fluids from infected animals may produce local infection following parenteral inoculation or splash onto mucous membranes.

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling and processing clinical specimens, identifying isolates, animal tissues and mold cultures, identifying cultures in routine diagnostic laboratories, and for inoculating experimental animals, regardless of route. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

BSL-3 practices, containment equipment, and facilities are recommended for propagating sporulating cultures of *H. capsulatum* in the mold form, as well as processing soil or other environmental materials known or likely to contain infectious conidia.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Sporothrix schenckii

Sporothrix schenckii is a dimorphic fungal pathogen existing in nature and in laboratory cultures at room temperature as a filamentous mold with asexual spores (conidia); these are the infectious particles that convert to small budding yeasts in the parasitic phase *in vivo*. The sexual stage is unknown.

Occupational Infections

Most cases of sporotrichosis are reported sporadically following accidental inoculation with contaminated material. Large outbreaks have been documented in persons occupationally or recreationally exposed to soil or plant material containing the fungus. However, *S. schenckii* has caused a substantial number of local skin or eye infections in laboratory personnel.[34] Most occupational cases have been associated with accidents and have involved splashing culture material into the eye,[35,36] scratching,[37] or injecting[38] infected material into the skin or being bitten by an experimentally infected animal.[39,40] Skin infections in the absence of trauma have resulted also from handling cultures[41-43] or necropsy of animals[44] without any apparent trauma.

Natural Modes of Infection

The fungus is distributed worldwide in the environment and is associated with sphagnum moss and gardening, often involving sphagnum moss and traumatic implantation. Infections are not transmissible from person-to-person, but require common exposure to a point source. Rare respiratory and zoonotic infections occur. It is thought that naturally occurring lung disease results from inhalation.

Laboratory Safety and Containment Recommendations

Although localized skin and eye infections have occurred in an occupational setting, no pulmonary infections have been reported as a result from laboratory exposure. It should be noted that serious disseminated infections have been reported in immunocompromised persons.[45]

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for laboratory handling of suspected clinical specimens, soil and vegetation, and experimental animal activities with *S. schenckii.* Gloves should

be worn during manipulation of *S. schenckii* and when handling experimentally infected animals. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Dermatophytes (Epidermophyton, Microsporum, and Trichophyton)

The dermatophytes are biologically related species of the genera, *Epidermophyton, Microsporum,* and *Trichophyton* that exist as monomorphic pathogens in nature, in laboratory cultures at room temperature and *in vivo* as filamentous molds. The sexual stages, when known, are Ascomycetes with infectious ascospores. These fungi are distributed worldwide, with particular species being endemic in particular regions. The species are grouped by natural environment habitat as being primarily associated with humans (anthrophilic), other animals (zoophilic), or soil (geophilic).

Occupational Infections

Although skin, hair, and nail infections by these molds are among the most prevalent of human infections, the processing of clinical material has not been associated with laboratory infections. Infections have been acquired through contacts with naturally or experimentally infected laboratory animals (mice, rabbits, guinea pigs, etc.) and, occasionally, with handling cultures.[26,29,45,46]

Systemic dermatophytosis is a rare condition. Superficial chronic infections occur frequently among immunocompromised individuals as well as elderly and diabetic persons. Susceptible individuals should use extra caution.[47-50]

Natural Modes of Infection

Infections can be transmissible from person-to-person, or acquired from common exposure to a point source. The dermatophytes cause infection (dermatophytosis) by invading the keratinized tissues of living animals and are among the most common infectious agents of humans. This fungal group encompasses members of three genera: *Epidermophyton, Microsporum,* and *Trichophyton*. The severity of infection depends on the infective species or strain, the anatomic site and other host factors. One of the most severe dermatophytoses is favus, a disfiguring disease of the scalp caused by *Trychophyton schoenleinii*.

Laboratory Safety and Containment Recommendations

Dermatophytes pose a moderate potential hazard to individuals with normal immune status. In the clinical laboratory setting, the inappropriate handling of cultures is the most common source of infection for laboratory personnel. The most common laboratory procedure for detection of the infective dermatophyte is the direct microscopic examination of contaminated skin, hair, and nails, followed by its isolation and identification on appropriated culture media. Direct contact with contaminated skin, hair, and nails of humans could be another source of infection.[48,49] In research laboratories, dermatophytosis can be acquired by contact with contaminated soil (source of infection: geophilic species) or animal hosts (source of infection: zoophilic species).

BSL-2 and ABSL-2 practices, containment equipment, and facilities are recommended for handling cultures and soil samples. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

Miscellaneous Molds

Several molds have caused serious infection in immunocompetent hosts following presumed inhalation or accidental subcutaneous inoculation from environmental sources. These agents include the dimorphic mold, *Penicillium marneffei*, and the dematiaceous (brown-pigmented) molds, *Bipolaris* species, *Cladophialophora bantiana*, *Exophiala (Wangiella) dermatitidis*, *Exserohilum* species, *Fonsecaea pedrosoi, Ochroconis gallopava (Dactylaria gallopava), Ramichloridium mackenziei (Ramichloridium obovoideum), Rhinocladiella atrovirens*, and *Scedosporium prolificans*.[51]

Occupational Infections

Even though no laboratory-acquired infections appear to have been reported with most of these agents, the gravity of naturally-acquired illness is sufficient to merit special precautions in the laboratory. *Penicillium marneffei* has caused a localized infection in a laboratory worker.[52] It also caused a case of laboratory-acquired disseminated infection following presumed inhalation when an undiagnosed HIV-positive individual visited a laboratory where students were handling cultures on the open bench.[53]

Natural Modes of Infection

The natural mode of infection varies by specific species; most are poorly characterized.

Laboratory Safety and Containment Recommendations

Inhalation of conidia from sporulating mold cultures or accidental injection into the skin during infection of experimental animals are potential risks to laboratory personnel.

BSL-2 practices, containment equipment, and facilities are recommended for propagating and manipulating cultures known to contain these agents. Any culture identifying dimorphic fungi should be handled in a Class II BSC.

Special Issues

Transfer of Agent Importation of this agent may require CDC and/or USDA importation permits. Domestic transport of this agent may require a permit from USDA/APHIS/VS. A DoC permit may be required for the export of this agent to another country. See Appendix C for additional information.

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