We describe a fatal case of imported coccidioidomycosis in India in a 22-year-old male who worked in Tucson, Arizona, approximately four years prior to his illness. The diagnosis was based on the presence of characteristic spherules with endospores in biopsy tissue of lymph nodes, bone and pus from a chronic discharging sinus in the left gluteal region and isolation of \textit{Coccidioides immitis} in culture. \textit{C. immitis} is one of the most infectious and virulent fungal pathogens and poses a serious occupational hazard for laboratory personnel, especially in areas where the disease is not endemic. To reduce the role of laboratory-acquired infection, all procedures that involve manipulation of cultures of \textit{C. immitis} should, whenever possible, be conducted in a biological safety cabinet.

**Keywords** biosafety, \textit{C. immitis}, endemic mycosis, imported coccidioidomycosis, India

Coccidioidomycosis is a rare disease in India. There is only one previous case report describing a case of disseminated disease. This occurred in a 24-year-old man of Indian origin living in Arizona who developed persistent fever and cough while on vacation in India [1]. The diagnosis was based on the presence of characteristic spherules with endospores in biopsied lymph nodes and caseating granulomas in the lungs. The diagnosis was further confirmed by strongly positive serologic tests of serum and cerebrospinal fluid (CSF). We describe a second Indian case of disseminated coccidioidomycosis in a 22-year-old male with a history of working in Tucson, Arizona four years prior to his illness.

**Case report**

A 22-year-old male was admitted to the Apollo Hospitals, Chennai, South India, on 7 November 2000.

He presented with a 6-month history of recurrent fever, a chronic discharging sinus in the left gluteal region (May 2000), and deterioration in consciousness level. He had previously been diagnosed elsewhere as having pyogenic meningitis and tuberculous meningitis and had been treated with multiple antibiotics. At the time of admission, the patient was on irregular antituberculous therapy with rifampin, isoniazid, ethambutol and pyrazinamide.

Initial investigations including analysis of the CSF revealed a raised protein concentration (116 mg dl$^{-1}$), lowered glucose concentration (glucose 5 mg dl$^{-1}$) and a high leucocytosis (differential count of 50 cells showed 12 polymorphonuclear leucocytes and 38 lymphocytes). Microscopic examination of Gram-stained CSF smears showed moderate numbers of pus cells but no fungal cells or acid-fast bacilli. Blood cultures carried out on three occasions showed no growth. He was serologically negative for HIV infection. Magnetic resonance imaging (MRI) performed at an outside hospital showed brain-stem infarction [2]. A computed tomography (CT) scan, performed on 9 November 2000, showed destructive lytic lesions involving $L_5$, $S_1$ and $S_2$ on the left side, left iliac...
bone and prevertebral soft tissue components, and a sinus tract draining into left gluteal region.

Biopsy tissue sections from the left gluteal region and inguinal lymph node were stained with hematoxylin and eosin, and Gomori’s methenamine silver stain procedures. Microscopic examination of the tissue slides showed a granulomatous and suppurrative tissue response surrounding numerous spherical, oval, spherules 20–30 μm in diameter, many containing endospores. Direct examination of pus from the gluteal abscess showed many spherules containing endospores (Fig. 1). The pus was cultured on Sabouraud’s dextrose agar containing chloramphenicol with or without cycloheximide at 25 °C. White to off-white, downy colonies became evident after 72–96 h of incubation. Microscopic examination of the growth revealed hyaline, septate branching hyphae in 2–72–96 h of incubation. Microscopic examination of the tissue slides showed a granulomatous and suppurrative tissue response surrounding numerous spherical, oval, spherules 20–30 μm in diameter. Many lateral branches formed rectangular, or barrel-shaped arthroconidia that measured 2.5–4.0 by 3–6 μm. The older arthroconidia showed alternate empty cells typical of Coccidioides immitis. The isolate was submitted to the Mycotic Diseases Branch, CDC, where its identity was confirmed by a chemiluminescent DNA probe (Accuprobe, Gen Probe Inc., San Diego, CA, USA). It has been deposited in the Mycotic Diseases Branch culture collection as strain B-6037.

Treatment with intravenous amphotericin B (50 mg day⁻¹) was initiated on 11 November 2000. On 21 November, the dosage was reduced to 25 mg day⁻¹ because of high urea and creatinine levels. In addition, the patient received oral itraconazole (400 mg day⁻¹) and intravenous fluconazole (2.5 mg 4 times daily) for a total of 4 days. He was treated with intrathecal amphotericin B (0.1 mg kg⁻¹) on alternate days. The second dose was increased to 0.2 mg kg⁻¹. The patient received only two doses before he expired. In spite of antifungal therapy, the patient suffered respiratory failure with deterioration of consciousness and died on 28 November 2000.

Conclusions

C. immitis is endemic in the Western hemisphere, occurring as a soil-inhabiting mould in the Southwestern United States, Mexico, Nicaragua, Guatemala and Honduras in Central America; as well as Brazil, Bolivia, Colombia, Venezuela, Paraguay, and Argentina in South America [3]. Increased travel has led to more cases of coccidioidomycosis being diagnosed among foreign visitors to endemic areas in the USA. Such cases have been reported among travelers from Europe, Canada, and some Asian countries, including India [1]. A few cases of the disease have been reported in persons who have never visited an endemic area, but who had been exposed to products from endemic areas [4–6]. In our case, the patient had a history of working in Tucson, Arizona, an area known to be highly endemic for coccidioidomycosis [7]. It is possible that the osteolytic lesions of the vertebrae led to his meningitis.

There have been five previous case reports of presumed coccidioidomycosis from India [1,8–11]. However, in none of those cases was C. immitis isolated in culture. In one case [1], the diagnosis of coccidioidomycosis was confirmed by the complement fixation test. In the four earlier case reports [8–11], the presumptive diagnosis was based on histologic examination of the tissues. However, it appears that sporangia of R. seeberi were seen in tissue and were misidentified as spherules of C. immitis [8–11]. This was despite the fact that C. immitis is not endemic in India, and that in the two human cases [8,9], the individuals concerned had not visited any endemic areas in North, Central or South America. Similarly, in two cases in animals [10,11], these were neither imported from the endemic areas nor were they exposed to any contaminated products from the endemic areas. Rhinosporidium seeberi is the etiologic agent of rhinosporidiosis, a common proctistan disease of humans and animals in India. Mature sporangia of R. seeberi are much larger than mature spherules of C. immitis. In addition, the sporangiospores of R. seeberi are uniformly stained with special fungal stains and usually contain 10 or more globular eosinophilic bodies. At the periphery of the sporangium, flattened sporogenous cells are often present. Mature endosporae of C. immitis, however, are round regardless of their location within the spherules [12].

The present case emphasizes the importance of using microbiological and/or serological methods as well as

Fig. 1 Direct examination of KOH mount of pus from a chronic sinus in left gluteal region showing an open spherule and released endospores. Original magnification ×750.
histological tests to establish a diagnosis of an endemic mycosis such as coccidioidomycosis in non-endemic areas. In the case of coccidioidomycosis, the diagnosis should be based not only on the presence of spherules in the affected tissue but also on isolation of \textit{C. immitis} in culture. Where available, serologic tests including complement fixation and immunodiffusion are extremely useful for the diagnosis of localized and disseminated forms of coccidioidomycosis. In the absence of a positive culture, a histologic diagnosis should be confirmed by using a \textit{C. immitis}-specific fluorescent–antibody conjugate.

Increased international travel has led to more cases of coccidioidomycosis being diagnosed in countries remote from the endemic areas, by laboratorians unfamiliar with the risk involved in handling live cultures of \textit{C. immitis} without proper containment facilities. \textit{C. immitis} is one of the most virulent and infectious fungal pathogens and one which poses an occupational hazard to laboratorians as well as to other personnel in the close vicinity. The risk is a serious one, owing to the large numbers of small arthroconidia most isolates produce in culture. The aerosol dose to which these workers may be exposed while examining plates, or making slide preparations or subcultures is likely to be much greater than would be encountered in nature. Thus it is not surprising that many laboratory-acquired cases of coccidioidomycosis have been symptomatic, or that severe or lethal disseminated infections have occurred. Nor is the risk confined to persons handling cultures: office and maintenance staff, as well as visitors, have also been affected [13]. To reduce the risk of laboratory-acquired infection, all procedures that involve manipulation of cultures of \textit{C. immitis} should, whenever possible, be conducted in a biological safety cabinet [14]. The cultures should be examined at frequent intervals and discarded as soon as possible after identification is completed.

References