A RARE CASE OF LABORATORY-CONFIRMED COCCIDIOIDOMYCOSIS IN BULGARIA, OCCURRING AFTER A 20-YEAR INTERVAL

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ABSTRACT

Coccidioidomycosis is a systemic fungal infection with no local distribution in Europe , associated with travelling to endemic regions of the world. The disease is defined as local but of global importance due to the increasing number of travellers to endemic areas.

We present a rare case of coccidioidomycosis in a 52-year-old woman living in the state of Arizona, USA. The patient had no disease symptoms, but computed axial tomography performed at the annual follow-up showed a small dense mass in the right lung. Positron emission tomography was also performed for suspicion of neoplasia, without conclusive evidence of malignancy. On her annual holiday in Bulgaria, the woman decided to consult a microbiologist at the National Centre of Infectious and Parasitic Diseases in Sofia, based on her information about the disease and the regions at risk of infection. The patient was referred to a consultation with a pulmonologist. As recommended by the pulmonologist, the lung nodule was surgically removed and subsequent histological and microbiological studies (Sabouraud agar medium culture) confirmed the diagnosis of coccidioidomycosis.

This is only the second case of this systemic mycosis registered in Bulgaria, showing that the diagnosis is difficult due to the lack of specific symptoms. A multidisciplinary approach is essential for the rapid diagnosis and timely treatment, which in turn is a prerequisite for a favorable outcome of the disease.

Keywords: coccidioidomycosis; endemic mycoses; case report

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INTRODUCTION

Coccidioidomycosis is caused by fungi of the genus *Coccidioides*. The latter contains two species, *Coccidioides immitis* and *Coccidioides posadasii*. also known as 'endemic mycoses' Together with *Histoplasma* and *Blastomyces*, *Coccidioides* only occur in certain geographical regions [1]. Coccidioides are 'dimorphic fungi', meaning they can grow in the human body like yeast, but in the environment (soil) they grow like moulds and form spores called arthroconidia. When the integrity of the soil is disturbed (agriculture, construction, wind, etc.), the spores become airborne and can be inhaled into human lungs. This can cause coccidioidomycosis, known as "valley fever". This form is often mild and asymptomatic. One to 3 weeks after the exposure, flu-like symptoms may occur, such as fever, weight loss, cough, fatigue, headache, chest pain, and myalgia [2, 3].

Coccidioidomycosis is not transmitted from person to person [4]. The asymptomatic forms can be detected during routine chest imaging. Pulmonary nodules are seen that can persist for years and be metabolically active, making them difficult to differentiate from malignant nodules [1].

The severity of the disease varies depending on various factors (immune deficiency, transplantation, comorbidities, pregnancy, diabetes, age, etc.). Infection can progress from community-acquired pneumonia to disseminated form spreading throughout the body (skin, bones, liver, brain, heart, meninges). One of the most aggressive forms is the cutaneous manifestation with ulcers, skin lesions and abscesses (erythema nodosum and erythema multiforme). Mediastinal fibrosis is rare and is associated with hemoptysis. Most people recover from pneumonia without complications. A small percentage may develop lung cavities that resolve spontaneously or are surgically removed. Prolonged antifungal therapy is

needed because of the risk of relapse [1, 3, 5].

Coccidioides inhabit soils with a limited geographical distribution. In the USA, the endemic regions are California, Arizona, New Mexico, Utah and Texas. The endemic region for coccidioidomycosis extends south into the deserts of Mexico and parts of Central and South America [1]. The disease is not endemic in Bulgaria, and isolated imported cases acquired after travelling to endemic areas have been reported [5].

By presenting a clinical case of an extremely rare infection in Bulgaria, we would like to point out that the appearance of imported cases is not excluded, and knowledge of diagnostic algorithms and treatment can be essential for a favorable outcome of the disease.

Case presentation

We describe a clinical case from 2024 of a 52-year-old woman who has lived for 10 years in the state of Arizona, North America, known for its mostly dry climate. She worked in the field of advertising, has been an active smoker for 30 years, and did not report any allergies, alcohol consumption, elevated blood glucose level, or concomitant diseases. In addition, she was living in a house with a flower garden, where she often cultivated flowers.

A prophylactic imaging study conducted in late 2023 (computed tomography scan of the lung) revealed the presence of a pulmonary nodule (8x11 mm) situated in the superior lobe of the right lung. The oval lesion had a small excentric cavitation. There were no other focal or infiltrative changes, lymphadenomegaly, or evidence of pleural effusion. Follow-up of the nodule in three to six months was recommended.

A follow-up CT scan six-months later revealed that the lesion had slightly increased in size (12x12 mm) while maintaining the same characteristics. The nodule was characterized by clearly demarcated borders, with no evidence of pleural or pericardial effusion, and without compression or dislocation of trachea and main bronchi (Fig. 1).

To elucidate the etiology of the lung formation, a positron emission tomography (PET-CT) scan was performed, which revealed no evidence of enlarged and metabolically active cervical, axillary, mediastinal and inguinal lymph nodes. No evidence of pleural or pericardial effusion was observed, and no patholog-

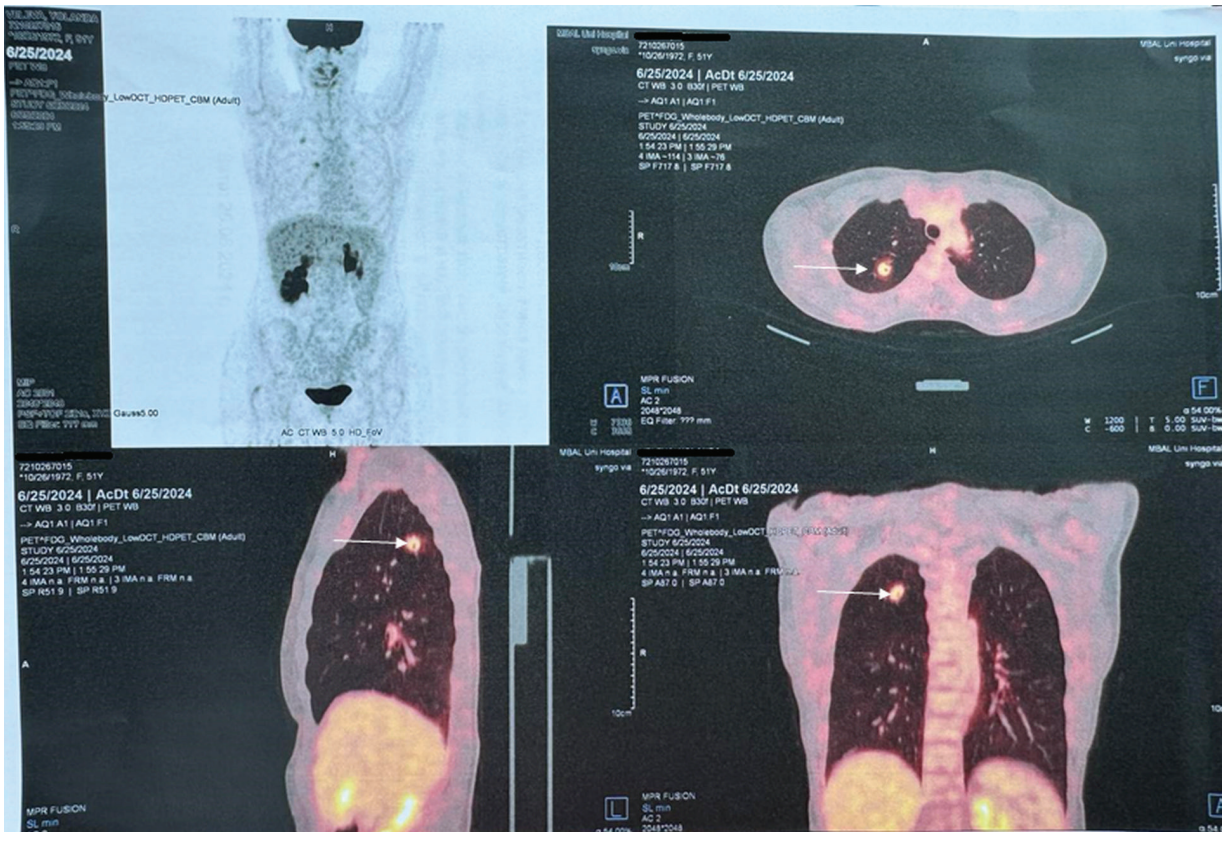

Fig. 1 Axial CT image showing an oval lesion with small eccentric cavitation in different sections (white arrow).

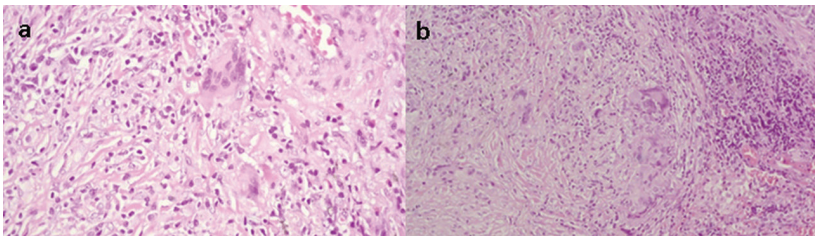

Fig. 2 (a, b) Histological slides of a surgically removed lung nodule showing a caseous granuloma with a concentration of inflammatory cells.

ical foci were identified in the abdomen and pelvis minor. In conclusion, the described lesion could not be definitively related to a primary neoplastic process, and there was no evidence of lymphogenous or haematogenous dissemination. The patient did not present any subjective complaints at the time of the imaging studies.

In the summer of 2024, the patient came to Bulgaria during her annual leave. Based on her personal information, she visited the National Reference Laboratory "Mycoses" at the National Centre of Infectious and Parasitic Diseases (NCIPD), Sofia, regarding a possible "valley fever" infection. As an initial screening serological immunodiffusion tests were conducted for detection of antibodies to the endemic fungi *Coccidioides*, *Histoplasma* and *Blastomyces* using the following procedure:

- Phenol agar is spilled in a few petri plates, and immediately refrigerated at 4°C.
- Smooth-edged wells are formed, and the following are dropped:

• Control serum (commercial kit IMMY, USA)
• Antigen (commercial kit IMMY, USA)
• Patient's serum

Precipitation lines are sought following incubation in a wet camera at 25°C for a few days.

The results were negative.

To further refine diagnosis, we recommended a microbiological and histological examination of bronchoalveolar lavage or of biopsy material from the lesion. According to the recommendation of a pulmonologist, the pulmonary nodule was excised through thoracoscopy , and the biopsy material was delivered to the Mycoses Reference Laboratory. A histological examination of the biopsy material (lung parenchyma fragment) revealed the presence of a caseous granuloma comprised of giant multinucleated cells of the Langhans type, lymphocytes, and epithelioid cells (Fig. 2).

The morphological picture was comparable with that of specific granulomatous inflammation, and a differential diagnosis for tuberculosis was conducted. The results of the tuberculosis test were negative. At the same time, the culture of the biopsy material on Sabouraud agar medium at 30°C demonstrated on the fifth day the expansion of an unpigmented fungus with substrate and aerial mycelia, which became clearly visible after another two to three days. At macroscopic observation, the fungus was found to be similar to Coccidioides (Fig. 3).

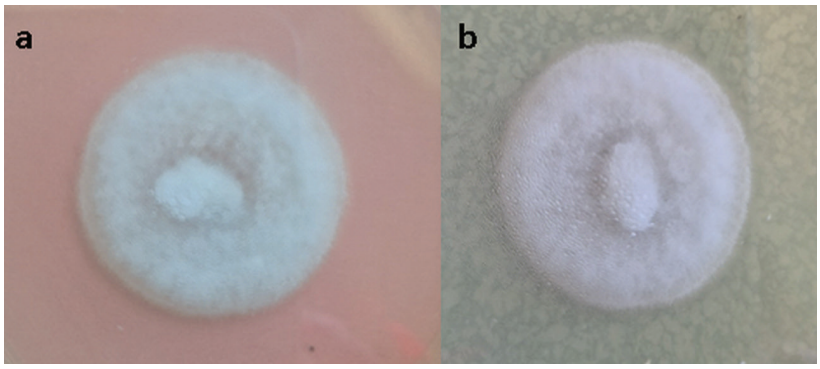

Fig. 3 (a, b) Growth of unpigmented fungus with substrate and aerial mycelium on Sabouraud's medium

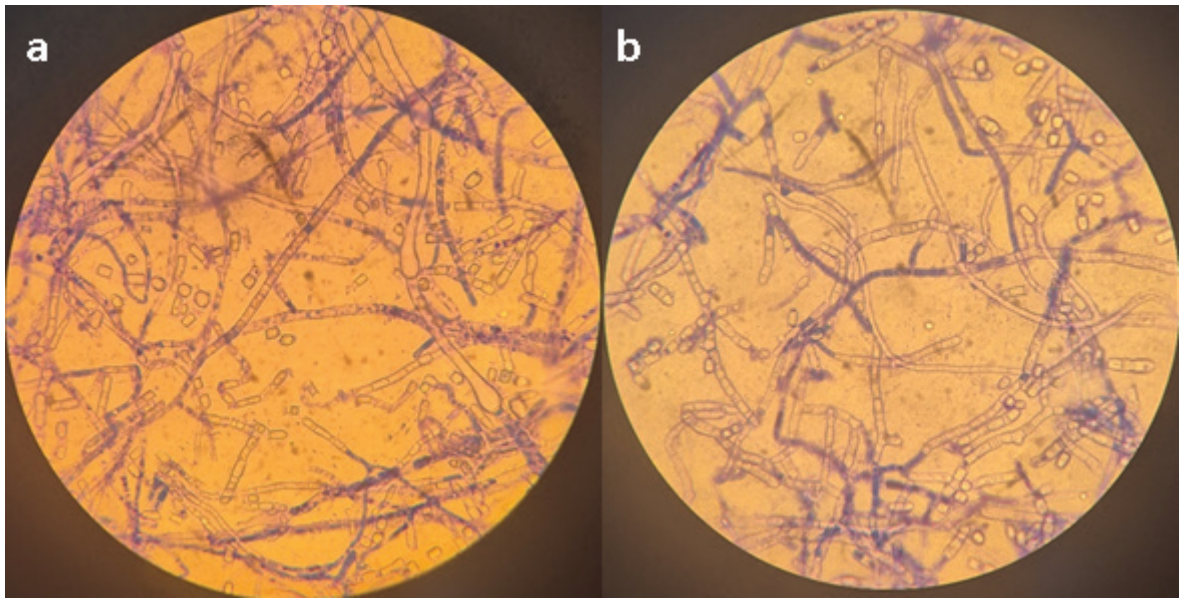

Fig. 4 (a, b) Hyphae of Coccidioides with the presence of arthroconidia

Microscopic evaluation confirmed the identification by visualising the characteristic hyphae and the presence of arthroconidia (Fig. 4).

A microscopic examination was conducted following occupational safety requirements, given the infectious nature of the fungus spores. The patient was prescribed antimycotic therapy with fluconazole for six months, given the elevated risk of recurrence (7), and follow-up has not yet been completed.

DISCUSSION

Coccidioidomycosis is a disease of limited prevalence in certain geographic regions but is of global importance given the potential exposure of increasing numbers of travellers and residents in endemic areas [1]. It is estimated that 60% of coccidioidomycoses are asymptomatic, while the remaining cases present with clinically manifested respiratory disease. In some cases, this can lead to disseminated life-threatening conditions, particularly among individuals with impaired immune systems [6].

The case presented is the second diagnosed in Bulgaria. The first was described in 2004 in a woman who underwent a surgical intervention for nodular lesions in the lung. Histological slides of the lesions showed morphological changes typical of infection. The aetiological diagnosis was based on the detection of specific antibodies against *Coccidioides immitis* by immunodiffusion with fungal antigen. The epidemiological history showed that the disease developed about one month after a stay in the state of Arizona, USA. The patient recovered after 9 months of therapy [7]. Although the travel history is important for diagnosis of endemic mycoses, especially in non-endemic countries, it is not always easy to obtain [8]. The diagnosis of coccidioidomycosis is based on a combination of epidemiological information, clinical findings, physical examination and laboratory/radiological data. All patients should have a carefully recorded history and physical examination, including assessment for possibly disseminated disease [1]. Various microbiological diagnostic methods exist. In the present case, we used a culture method and microscopic detection of the pathogen. Immune-based diagnostic method was used in the other case described in the country 20 years ago . In both cases, the epidemiological history and the histological examination of specimens obtained after surgical interventions provided the clue for a targeted microbiological study. Similar data were obtained in a study of endemic mycoses in Italy, where most cases were diagnosed by histology and/or culture. In recent years, PCR diagnostics has become essential, while serological and antigenic tests are of limited diagnostic value [9].

In Europe, the infection is generally rarely seen, mainly in people returning from areas where

coccidioidomycosis is known to be endemic. The last published case was a 26-year-old Dutch man who returned from a trip to California, North America, in 2017 [10]. In 2016, one case of pulmonary coccidioidomycosis was reported in Ireland in a patient also residing in California [11]. To date, ten cases have been described in Italy, six of which after travelling to the United States and South America and four – without a travelling history. Only one case has been reported in an immunocompromised person with AIDS [9]. All published cases from Europe, as well as the Bulgarian one, were pulmonary coccidioidomycosis and all had a favourable outcome after treatment. Most of them, including the present one, were treated with fluconazole in generally accepted doses and regimens.

Given the increasing number of international travelers and the relatively large proportion of individuals with compromised immunity due to causes other than HIV infection (organ transplantation, cancer, systemic corticosteroid use, etc.), clinicians should be particularly cautious in approaching individuals with pulmonary symptoms and evidence of residence in a region endemic for coccidioidomycosis. In our experience, though from a single case, culture diagnosis has a sufficiently high sensitivity and specificity when appropriate clinical material is available. It is important to observe strictly workplace safety rules because of the high risk for the laboratory personnel performing the diagnostic tests. The timely application of contemporary therapy minimizes the possibility of infection dissemination, complications and, consequently, poor prognosis.

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