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Algaemia Due to *Prototheca wickerhamii* in a Patient with Myasthenia Gravis

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***Prototheca wickerhamii* is a rare cause of systemic infection in humans. While some cases occur in previously healthy individuals, others are associated with a variety of preexisting diseases. Here we present, for the first time, a case of *P. wickerhamii* algaemia in a patient with myasthenia gravis. The patient was successfully treated with amphotericin B.**

Prototheca is an achlorophyllous alga and is a rare cause of human infection. A review of the literature published in 1992 revealed 60 documented cases of human protothecosis (9). Since then, eight additional cases have been described (1, 10–13, 15, 25). Human protothecosis occurs worldwide and affects both immunocompromised and immunocompetent patients. About 90% of the infections are localized to the skin and subcutaneous tissues. To our knowledge, only seven cases of systemic protothecosis have been reported (2, 3, 8, 10–12, 15), with algaemia documented for only four patients (3, 8, 11, 12). Here we present a fifth case of protothecal algaemia; however, this is the first described association of systemic protothecosis with myasthenia gravis.

A 75-year-old white male was admitted to Zale Lipshy University Hospital, Dallas, Tex., in January 1997 with acute exacerbation of his myasthenia gravis (diagnosed two years previously). His medications included prednisone and cyclosporine. Plasmapheresis was initiated, but on the third day after admission the patient became ventilator dependent. Two weeks after admission, he developed a fever of 38.2°C, with possible pneumonia. He was also noted to have a papulonodular rash on his right hand.

Blood cultures had been drawn 1 day before the development of fever, inoculated into aerobic and anaerobic blood culture bottles (BacT/Alert; Organon Teknika Corp., Durham, N.C.), and incubated at 35°C. Growth was detected in the aerobic bottle after 3 days of incubation, although no organisms were initially seen on a Gram stain. Retrospective review of the Gram stain revealed rare atypical yeast-like organisms. The culture in the aerobic bottle was subcultured to Trypticase soy agar with 5% sheep blood, chocolate agar with hemoglobin and IsoVitaleX, and enriched MacConkey agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.) and incubated at 35°C with 7% CO_2. After 48 h, pinpoint white, creamy colonies were seen on blood and chocolate agars. A Gram stain of the colonies showed yeast-like cells of variable size. The germ tube test (16) was negative, and the organism failed to metabolize urea after 72 h of incubation (urea slant agar; Becton Dickinson Microbiology Systems). The colonies were subcultured onto Sabouraud-dextrose agar (SAB; Becton Dickinson Microbiology Systems) and incubated in ambient air at 30°C. After 24 h, colonies from the SAB were reinoculated onto cornmeal agar with Polysorbate 80 (Becton Dickinson Microbiology Systems) and into an API 20 C system (bioMerieux Vitek, Inc., Hazelwood, Mo.).

Microscopic examination of growth on cornmeal agar revealed findings typical for *P. wickerhamii*: small spherules, in aggregates, ranging from 7 to 15 μm in diameter, with multiple internal cleavages (Fig. 1). *Prototheca* species are nonfermenters, and the different species can be distinguished by their sugar-assimilating properties. Both *P. wickerhamii* and *P. zopfii* metabolize glucose and galactose, but only *P. wickerhamii* utilizes trehalose (24). Seventy-two hours of incubation in the API 20 C strip yielded the following analytical profile index (code 6 000 040): *P. wickerhamii*, 1/2; *Torulopsis glabrata*, 1/7; *P. zopfii*, 1/10019. Susceptibility testing gave the following results (MIC, in micrograms per milliliter): amphotericin B, 0.25; 5-fluorocytosine, >64; fluconazole, >64; itraconazole, 2 (Fungus Testing Laboratory, University of Texas Health Science Center at San Antonio, San Antonio, Tex.). The culture of a central venous catheter tip, removed soon after isolation of the organism, was negative.

Four days after the first positive blood culture, *P. wickerhamii* was isolated again in the aerobic bottles from two additional blood culture sets. This time, Gram stains of the blood bottles revealed many yeast-like organisms of variable sizes (Fig. 2). One day after collection of blood for these blood

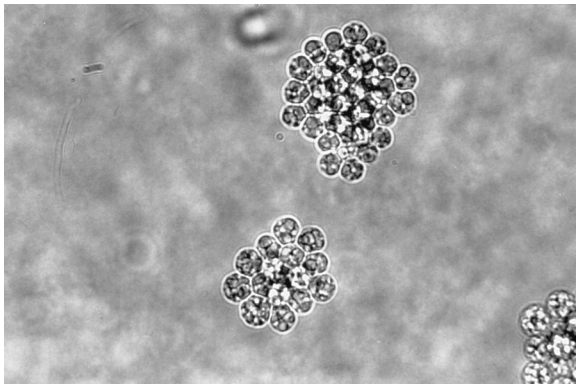

FIG. 1. Microscopic colony morphology on cornmeal agar. Three aggregates of spherules are shown. Each spherule measures between 7 and 15 μm and has multiple cleavages. Magnification, ×400.

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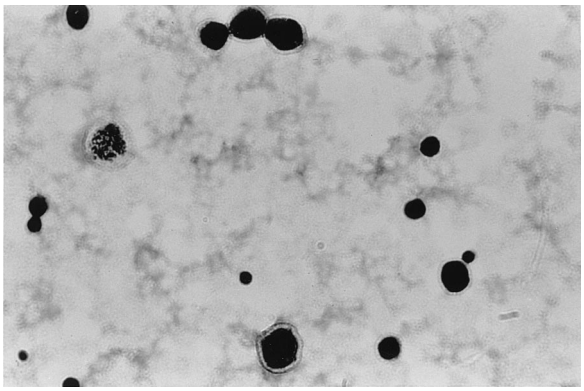

FIG. 2. Gram stain of blood culture. Yeast-like organisms of various sizes are shown. The Gram stain does not reveal the internal cleavages. Abutting *P. wickerhamii* organisms of different sizes are visible (lower right) and could easily be confused with budding yeast. Magnification, ×1,000.

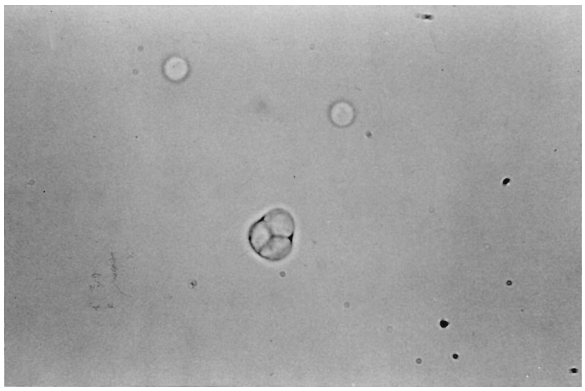

FIG. 4. Wet mount of growth from blood agar plate. Two small unicellular *P. wickerhamii* organisms are visible in the upper portion of the field. The larger, cleaved structure is a sporangium, containing sporangiospores. Magnification, ×1,000.

cultures, an aspirate was drawn from one of the papulonodular eruptions on the patient's right hand. A Gram stain showed many leukocytes and few yeasts (Fig. 3). The aspirate was plated onto blood and chocolate agars and, 2 days later, grew pinpoint colonies from which a wet-mount preparation revealed typical *Prototheca* cells (Fig. 4). The colonies were subcultured to duplicate SAB plates and incubated for 24 h at 30 and 35°C. Colonies were also subcultured on cornmeal agar and incubated at 30°C. Growth on SAB was obtained at both temperatures, which distinguished *P. wickerhamii* and *P. zopfii* from *P. stagnora*, which grows only at 30°C (15). Microscopic colony morphology on cornmeal agar was again consistent with *P. wickerhamii.*

Therapy with amphotericin B was started on the day the hand aspiration was performed. In addition, the patient was weaned off immunosuppressive therapy over a 3-week period. The patient responded well, as assessed by reduction of fever and improvement of rash, and by subsequent negative blood cultures. After the patient had received 635 mg of amphotericin B, a lipid complex preparation of amphotericin B (5 mg/kg of body weight/day) was substituted because of drug-induced renal toxicity. Treatment was given over 21 days. Following clearance of *Prototheca*, the patient remained ventilator dependent for an extended period of time. He had several recurrences of fevers, but these were attributed, respectively, to herpes zoster eruptions of the left shoulder, colonization of an intravenous catheter by coagulase-negative staphylococci, and probable pneumonia. The patient was appropriately treated for each of these episodes. Infection with *P. wickerhamii*, however, never recurred. Eventually, the patient's condition stabilized and he was weaned off the ventilator. Immunosuppressive therapy was not restarted. He was discharged 14 weeks after admission, demonstrating notable improvement in his underlying disease.

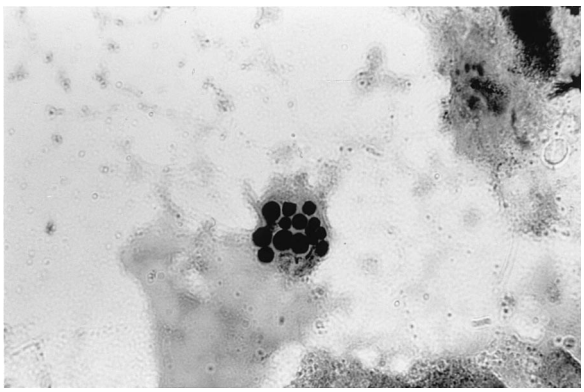

FIG. 3. Gram stain of aspirate from right-hand lesion. Yeast-like organisms within the cell are *P. wickerhamii*. Internal structures are not demonstrated by Gram staining. Magnification, ×1,000.

The genus *Prototheca* belongs to the family *Chlorellaceae*. It is a unicellular organism that reproduces asexually by internal septation and cleavage, to form sporangia (6 to 26 μm in diameter) containing sporangiospores (14, 20). *Prototheca* is a ubiquitous organism that lives saprophytically on decaying organic matter. It has been recovered from fresh and marine water, fish tanks, swimming pools, waste stabilization ponds, vegetables, and meat products such as beef, pork, crabs, and clams (3, 19, 21, 22). The first case of human protothecal infection (*P. zopfii*) was described by Davis et al. in 1964. The patient was a rice farmer in Sierra Leone with a localized chronic skin lesion (4, 5). The first case of multiorgan systemic protothecal infection was described by Cox et al. in 1974. The patient was a 29-year-old man who had involvement of the liver, peritoneum, lymph nodes, skin, and blood (3). A similar case of visceral protothecosis, in a 39-year-old man, was later described; the infection mimicked sclerosing cholangitis (2). A third case of visceral protothecosis, in a 13-year-old Japanese boy, was described in 1992. In this patient, the liver, spleen, small intestine, lymph nodes, and central nervous system were involved (15). The other cases of systemic protothecosis consisted of an AIDS patient with protothecal meningitis (10), a Hickman catheter-related algaemia in a 7-year-old child on chemotherapy for Hodgkin's disease (8), and two cases of non-catheter-related protothecal sepsis in patients with a lung transplant and leukemia, respectively (11, 12). In all but one, the species involved was *P. wickerhamii*. From the lung transplant patient, *P. zopfii* was isolated (12).

Systemic algaemia, with recovery of *Prototheca* species from the blood, occurred in only four of these cases. Our case represents a fifth report of protothecal algaemia and is the first in which systemic protothecosis has been associated with myasthenia gravis. There is one previous report of a protothecal infection in a patient with myasthenia gravis, but this was localized to the skin (7). Our patient was on immunosuppressive therapy and was noted to have a papulonodular eruption on his right hand, from which *P. wickerhamii* was subsequently

isolated. According to his wife, the patient had a persistent rash on his right hand for approximately 6 to 8 weeks prior to admission. It is possible that this initial rash was a localized mild infection due to *P. wickerhamii*, which then disseminated as a systemic algaemia, when the patient suffered an acute exacerbation of his myasthenia gravis. The role of the prior plasmapheresis in the progression of his protothecal disease is unknown. Alternatively, the cutaneous eruption could have resulted from hematogenous seeding of a preceding algaemia. *Prototheca* species have previously been isolated from stools (2, 23), urine (12), and sputum (18, 23). For our patient, urine and sputum cultures were negative for *Prototheca* species; a stool specimen was not cultured for *Prototheca*. Cultures of four additional intravenous catheter tips were negative. The source of infection, in this patient, remains undetermined.

It is important to make the correct laboratory diagnosis of protothecosis. *Prototheca* species may be confused with yeasts such as *Candida* and *Cryptococcus* species on Gram stains. The API 20 C strip used in our study gave a similar likelihood of the tested organism being either *P. wickerhamii* (1/2) or *T. glabrata* (1/7). Since infections with the latter are far more common than those with *Prototheca* species, the unwary technologist might dismiss *P. wickerhamii* in the interpretation of the API 20 C strip. Thus, cellular and colony morphological examination is essential. The typical morphology of *Prototheca* species can readily be demonstrated by stereoscopic colony examination of growth on cornmeal agar; a wet mount is also helpful. Susceptibility testing helps in selecting therapeutic options, usually amphotericin B, although in vitro testing may not always reflect in vivo response to treatment (2, 3, 6, 10, 17, 21). Our patient responded to intravenously administered amphotericin B, with subsequent clearing of the organism from the blood.

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