

## BRIEF REPORT

## Fatal Myositis Due to the Microsporidian *Brachiola algerae*, a Mosquito Pathogen

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**M**ICROSPORIDIA ARE OBLIGATE INTRACELLULAR EUKARYOTES THAT have emerged as a cause of chronic diarrheal syndromes in patients with human immunodeficiency virus (HIV) infection.<sup>1</sup> Most cases have been caused by *Enterocytozoon bieneusi*. In addition, there have been clinical reports of skeletal-muscle infection with pleistophora species, *Trachipleistophora hominis*, and *Brachiola vesicularum*.<sup>1</sup> We identified the mosquito pathogen *B. algerae* (formerly *Nosema algerae*) as the cause of fatal myositis in a patient who was receiving infliximab for rheumatoid arthritis.<sup>2</sup> To our knowledge, *B. algerae* has not previously been isolated from deep tissue in a person.

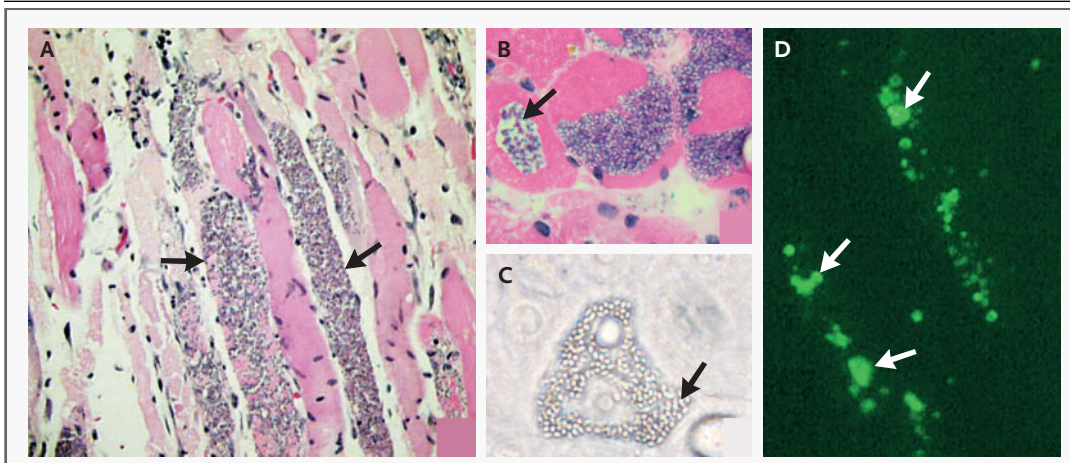
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### CASE REPORT

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In November 2002, a 57-year-old woman with rheumatoid arthritis and diabetes presented with a six-week history of increasing fatigue, generalized muscle and joint pain, profound weakness, and fever. Her symptoms initially limited her daily activities and finally confined her to bed. In the preceding year, she had been taking 15 mg of methotrexate per week and 20 mg of leflunomide per day. She had also been receiving 3 to 10 mg of prednisone daily for several decades. In the six months before admission, she began taking infliximab (a total of four doses of 3 mg per kilogram of body weight at intervals of three to four weeks and one dose of 5 mg per kilogram within the month before admission). The patient resided in a small town in northeastern Pennsylvania and had no recent travel history. She had had no contact with animals. A muscle-biopsy specimen from the left anterior thigh contained microorganisms that were consistent with microsporidia (Fig. 1).

The patient was admitted to a tertiary care facility, corticosteroids and infliximab were discontinued, and treatment was begun with 400 mg of albendazole in 40 ml of canola oil twice daily through a nasogastric tube, along with clindamycin, metronidazole, and atovaquone. Magnetic resonance imaging of the brain with the use of gadolinium demonstrated chronic ischemia of the small vessels with no leptomeningeal enhancement. The creatine kinase level was 4103 U per liter on admission and continued to rise, peaking at 6337 U per liter. A test for HIV was negative. A biopsy specimen of the right quadriceps muscle obtained four days after admission also revealed organisms consistent with microsporidia. The morphologic appearance suggested brachiola species, and the identity was confirmed by the polymerase chain reaction (PCR) with the use of primers specific for *B. algerae* (Nalg6F and Nalg178R).<sup>4</sup> No amplification was seen with the use of primers specific for *T. hominis* — TRACH1R (5'CACC-AGGTTGATTCTGCCTG3') and TRACH1F (5'TTATGATCCTGCTGCTCC3'). At this



**Figure 1. Photomicrographs of *Brachiola algerae* in Muscle-Biopsy Specimens and Tissue Culture.**

In Panel A, a low-power photomicrograph of the initial muscle-biopsy specimen obtained after the patient presented with myositis and muscle pain shows multiple organisms in the muscle fibers (arrows) with associated cell lysis but little or no inflammation (hematoxylin and eosin,  $\times 100$ ). In Panel B, a high-power photomicrograph of the second muscle-biopsy specimen demonstrates cytolysis (arrow) of the muscle fibers surrounding spores ( $\times 400$ ). In Panel C, a phase-contrast photomicrograph of *B. algerae* isolated from muscle shows spores (arrow) and proliferative forms throughout the cytoplasm of the host cell ( $\times 400$ ). In Panel D, a high-power photomicrograph shows clear immunofluorescence staining (arrows) of the initial muscle-biopsy specimen with antiserum against *B. algerae* (human corneal isolate<sup>3</sup>) ( $\times 400$ ).

point, treatment with itraconazole (400 mg twice daily) was begun and clindamycin and atovaquone were discontinued.

Narcotic analgesics were necessary to control the patient's worsening muscle pain. During the next two weeks, the patient became increasingly debilitated, and she required mechanical ventilation after respiratory insufficiency developed. A chest film revealed bilateral pulmonary infiltrates; *Pneumocystis jirovecii* was detected on direct fluorescence antibody testing of a smear from the tracheal aspirate; an examination for microsporidia was negative. Trimethoprim-sulfamethoxazole was added to the regimen, and the chest film cleared over the next week. After 16 days of therapy, microsporidia were seen in a third biopsy specimen, from the right quadriceps muscle. The frequency of albendazole was increased to three times a day but was curtailed because of increasing liver-enzyme levels. Four weeks after admission, the patient died from a massive cerebrovascular infarction, evident on computed tomography of the brain. A postmortem muscle biopsy revealed necrosis and persistent organisms.

#### METHODS

Muscle tissue was fixed in 10 percent neutral buffered formalin, embedded in paraffin, sectioned, and

stained with hematoxylin and eosin, periodic acid-Schiff, and Wright-Giemsa according to standard techniques.<sup>5</sup> Slides were also incubated with a 1:400 dilution of rabbit antiserum against *B. algerae* (human corneal isolate<sup>3</sup>), rabbit antiserum against *B. algerae* (Undeen mosquito isolate<sup>6</sup>), rabbit antiserum against *Vittaforma corneae*, or rabbit antiserum against *Encephalitozoon cuniculi* at 37°C for 30 minutes; washed three times with phosphate-buffered saline; and incubated at 37°C for 30 minutes in a 1:100 dilution of fluorescein-labeled goat antirabbit IgG. The specimens were then washed three times with phosphate-buffered saline, mounted with glycerol, and examined and photographed with an Olympus BX60 microscope equipped with epifluorescence optics.

For transmission electron microscopy, muscle-biopsy specimens and cultures were fixed in 0.1 M cacodylate-buffered 2.5 percent glutaraldehyde and then processed according to standard methods.<sup>7</sup> Thin sections were stained with uranyl acetate and lead citrate. The samples were examined with the use of a Tecnai 12 transmission electron microscope at the Rutgers University electron-microscopy facility.

A piece of fresh muscle-biopsy tissue measuring 8 by 8 mm was mechanically triturated and inoculated onto RK13 (rabbit kidney) and L6E9 (rat

myoblast) cells. RK13 cells were maintained in minimal essential medium (GIBCO-BRL) containing 7 percent fetal-calf serum and a 1 percent solution of penicillin, streptomycin, and amphotericin B.<sup>5,8</sup> L6E9 cells were maintained in Dulbecco's minimal essential medium (GIBCO-BRL) containing 10 percent fetal-calf serum and a 1 percent solution of penicillin and streptomycin.<sup>8</sup> Cells were incubated at 37°C and 30°C.

Conserved primer pairs (ss18f and ss1492r and ss530f and ls530r) for the large and the small subunit of the ribosomal RNA (rRNA) gene were used to amplify the microsporidian rRNA gene from muscle-biopsy material according to previously described methods.<sup>4</sup> The PCR products were cloned into a TA cloning vector (PCR 2.1, Invitrogen) and sequenced on an ABI Prism DNA Sequencer (model 377, PerkinElmer), and data sets were assembled with the use of SeqMan sequence-analysis software (DNASar). The resulting rRNA sequence was deposited in GenBank (accession number AY230191).

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## RESULTS

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### LIGHT MICROSCOPY

Sections of skeletal muscle from the first biopsy, of the left quadriceps muscle, demonstrated distended myofibers filled with basophilic microorganisms on staining with hematoxylin and eosin (Fig. 1A). The small spores measured 4 by 2  $\mu\text{m}$ . Polar granules were apparent on staining with periodic acid–Schiff. Several areas of the biopsy specimen contained ruptured myofibers with extracellular organisms but no associated inflammatory-cell response. A second biopsy specimen obtained eight days later, after the dose of immunosuppressive agents had been tapered and antimicrobial therapy had been initiated (Fig. 1B), had a similar microbial load; however, necrotic myofibers and a neutrophilic inflammatory-cell response were now apparent. A third biopsy specimen obtained 19 days after the first specimen demonstrated a necrotizing myopathy with regeneration of myofibers; however, the inflammatory response was no longer evident. Tissue sections reacted strongly with antiserum against *B. algerae* (Fig. 1D) but not antiserum against either *V. corneae* or *E. cuniculi*.

### ELECTRON MICROSCOPY

Within infected muscle cells, both developmental and spore stages of microsporidium were present (Fig. 2A and 2B). The spores contained a polar fil-

ament with approximately nine coils (seen in cross section in Fig. 2B) surrounding the paired, abutting nuclei (diplokaryon) and cytoplasmic organelles. These structures were encased within the thick-walled spore coat. The plasmalemma of the developing parasite was coated with electron-dense secretions and had vesiculotubular appendages. All stages were diplokaryotic and were in direct contact with the host-cell cytoplasm. These features are characteristic of the genus *brachiola* of the phylum Microsporidia.<sup>1</sup>

### PCR AND rRNA-SEQUENCE DATA

On PCR, the sequence of rRNA obtained from muscle-biopsy specimens was identical to the sequence of rRNA from spores obtained from this organism in tissue culture. BLAST analysis demonstrated that this sequence had 99.7 percent identity with the GenBank rRNA sequence of *B. algerae* (accession number AF069063) obtained from *Anopheles stephensi*.

### CULTURE

Cells were incubated at 37°C and 30°C. No growth occurred at 37°C, but growth was seen at 30°C. Over a period of 21 days, the infection increased until about 30 percent of the cells were infected (Fig. 1C). Transfer of infection with the use of cultured spores was possible at 30°C but not at 37°C. PCR of the cultivated organism demonstrated that the rRNA sequence was the same as that obtained from muscle tissue. A culture of this organism was deposited in the American Type Culture Collection (ATCC-PRA109).

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## DISCUSSION

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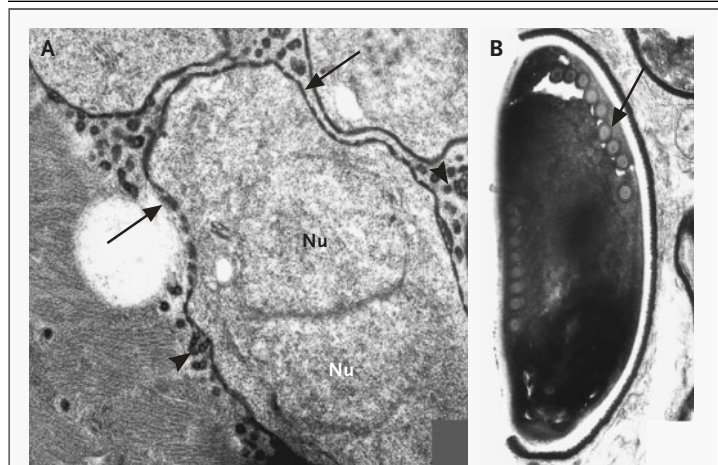
We document a deep-tissue infection with *B. algerae*, a mosquito pathogen, in a patient with diabetes and rheumatoid arthritis. The infection was fatal despite treatment with albendazole and itraconazole. Large numbers of mature spores were present in all muscle-biopsy specimens; after treatment, however, almost no proliferative forms were present, suggesting that treatment may have prevented replication. The diagnosis of a microsporidian infection was established by the demonstration of characteristic spores and proliferative stages consistent with *B. algerae*.<sup>5,7</sup> Both *B. algerae* and *B. vesicularum* produce extensive vesicular appendages that extend into the host-cell cytoplasm, but only *B. vesicularum* produces branching protoplasmic extensions.<sup>5,7</sup> The diag-

nosis was confirmed by molecular phylogeny with the use of the small-subunit rRNA gene.

Microsporidia are obligate intracellular parasites that are ubiquitous in the environment and infect almost all animal phyla (invertebrate and vertebrate). They are eukaryotes containing a nucleus with a nuclear envelope, an intracytoplasmic-membrane system, vesicular Golgi,<sup>1</sup> and “remnant” mitochondria.<sup>9</sup> Microsporidia possess prokaryotic-sized ribosomes lacking a 5.8S subunit.<sup>4</sup> They were once considered to be primitive protozoa; however, molecular phylogenetic analysis has led to the recognition that these organisms are more closely related to the fungi than to other protozoa. The phylum Microsporidia (Microspora) includes more than 144 genera<sup>1</sup> of which the following contain species that are known to infect humans: nosema (*N. corneum*, renamed *V. corneae*, and *N. algerae*, renamed *B. algerae*<sup>5</sup>), pleistophora,<sup>10-12</sup> enterocytozoon, encephalitozoon, septata (reclassified as encephalitozoon), trachipleistophora,<sup>13</sup> brachiola,<sup>7</sup> and microsporidium. Infection has been described in persons who are immunocompromised as a result of HIV infection<sup>14</sup> or organ transplantation<sup>15-19</sup> as well as in immunocompetent persons.<sup>1</sup>

Three microsporidian genera have been implicated in myositis. Cali et al. described a *B. vesicularum* infection in a patient with the acquired immunodeficiency syndrome (AIDS) and a CD4 cell count of 35 per cubic millimeter who presented with a five-month history of fever, progressive muscular weakness, and leg pain.<sup>7</sup> Treatment with albendazole and itraconazole resulted in clinical improvement. Myositis associated with *T. hominis* was described in a patient with AIDS and a three-month history of worsening muscle pain, fevers, and a creatine kinase level of 1410 U per liter.<sup>13</sup> The patient's condition improved after treatment with albendazole, sulfadiazine, and pyrimethamine. Pleistophora have been described as the cause of myositis in a patient with AIDS (*P. ronneafiei*<sup>12</sup>) and in an HIV-negative patient with a low CD4 cell count.<sup>10-12</sup> The patient with AIDS died, whereas the HIV-negative patient was free of symptoms four years after the initial presentation.<sup>10-12</sup>

Our patient was taking immunosuppressive agents for rheumatoid arthritis, had no evidence of HIV infection, and presented with muscle pain and progressive weakness associated with elevated creatine kinase levels. The causative organism was identified as *B. algerae*. This organism has been reported to cause a superficial corneal lesion, but no



**Figure 2. Findings on Transmission Electron Microscopy of the Initial Muscle-Biopsy Specimen.**

In Panel A, a diplokaryon, a thickened plasmalemma (arrows), and vesicotubular extensions (arrowheads) are evident in proliferative forms of *Brachiola algerae* in muscle ( $\times 14,000$ ). Nu denotes nucleus. Panel B shows a mature *B. algerae* spore with a single row of nine polar filaments (arrow) in cross section. Mature spores in this biopsy specimen had only single rows of 8 to 12 polar filaments in cross section ( $\times 18,300$ ).

systemic infections have been described.<sup>3</sup> Other nosema species have also been reported in corneal infections in HIV-negative patients.<sup>20</sup> The finding that *B. algerae* can grow at temperatures of 26 to 37°C in several kinds of tissue-culture cells, including those of insects, amphibians, and mammals,<sup>8</sup> suggests that the pathogen could infect mammals. However, this broad temperature range could also be an important limiting factor restricting the site of infection.<sup>5,21</sup>

Because of its ability to infect many mosquito genera that are found worldwide, including culex, anopheles, and aedes, *B. algerae* has been investigated for use as a pesticide.<sup>22</sup> The infection is transmitted horizontally but not transovarially. Infected mosquitoes have reduced reproductive capacity, longevity, and susceptibility to malarial parasites. *B. algerae* has been used experimentally to infect many other genera of insects, including those feeding on infected mosquitoes.<sup>22</sup>

Intravenous, peroral, or intranasal inoculations of *B. algerae* into immunologically competent mice failed to produce persistent infections.<sup>21,23</sup> However, subcutaneous inoculations into the ears, tail, or footpads of such mice — sites in which temperatures are lower than 37°C — produced localized

infections.<sup>21,23</sup> Similarly, in athymic mice, superficial inoculation caused disseminated disease,<sup>23</sup> whereas intravenous, peroral, or intranasal inoculation failed to establish infection. These studies suggest that an immunodeficient host could become susceptible to *B. algerae* if the site of infection, before dissemination, was in a superficial location with a temperature that was lower than core body temperature.

Our patient had begun treatment with infliximab, a monoclonal antibody with high binding affinity and specificity for tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), six months before myositis developed. Infliximab therapy is associated with an increased risk of infection or reactivation of infection with pathogens such as *Mycobacterium tuberculosis*, *Histoplasma capsulatum*, *Listeria monocytogenes*, and pneumocystis.<sup>24-27</sup> The ability to mount an effective host response to many intracellular pathogens depends partly on the production of type 1 helper T-cell cytokines, including TNF- $\alpha$ .<sup>28</sup> Protective immunity against *E. cuniculi*, a microsporidian, depends on the induction of type 1 helper T-cell cytokines such as interleukin-12 and interferon- $\gamma$ .<sup>29,30</sup> The role of TNF- $\alpha$  has not been studied in animal models of microsporidiosis; however, elevated fecal levels of TNF- $\alpha$  have been detected in patients with AIDS and intestinal microsporidiosis.<sup>31</sup> Treatment of mu-

rine peritoneal macrophages in vitro with TNF- $\alpha$  inhibited the replication of *E. cuniculi*.<sup>32</sup> Therefore, anti-TNF- $\alpha$  therapy may have an adverse effect on microsporidiosis.

The present case report illustrates that insect pathogens such as *B. algerae* are capable of causing disseminated disease in humans. Studies in animals suggest that local replication in body parts with lower temperatures (e.g., ears, nose, and skin) may be necessary for the microorganism to adapt to mammalian body temperature before it begins disseminating throughout the body.<sup>21,23</sup> *B. algerae* infects many mosquito species. Small numbers of spores have been found in the feeding tubes of mosquitoes infected with *T. hominis*, but there are no published reports of *B. algerae* in the salivary glands of mosquitoes.<sup>33</sup> The most heavily infected organ is the gut and malpighian tubules, but infection is found throughout the insect, including muscle and fat cells.<sup>6</sup> Although the inoculation of spores during feeding cannot be excluded, we believe it is more likely that infection with *B. algerae* in this patient was a consequence of crushing an infected mosquito while it was feeding, thereby mechanically inoculating the spores into the skin-bite wound.

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