

Microsporidial Myositis

Edoardo Pozio

Laboratory of Parasitology, Istituto Superiore di Sanità, Rome, Italy

Abstract

Microsporidia are obligate intracellular protozoa of the phylum Microspora. These parasites are primitive eukaryotes, lacking mitochondria and centrioles. More than a thousand species induce infections among a wide range of vertebrate and invertebrate animals, including man. Their unique protective spore contains a long coiled polar filament (extrusion apparatus) and the sporoplasm which contains one or two nuclei [2]. The spore stage with its coiled polar filament is diagnostic for identification of microsporidiosis. This polar filament is extruded to penetrate the host-cell membrane allowing the infective sporoplasm to be injected directly into the cytoplasm. Microsporidian spore size varies from about 1 to 12 μm , but those found in humans have been of the smaller sizes (1-5 μm), necessitating electron microscopy for diagnosis at genus and/or species level.

Key words: microsporidia, *Pleistophora*, myositis, immunodepression, pathology.

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Five genera of microsporidia are documented in human hosts: *Encephalitozoon*, *Enterocytozoon*, *Nosema*, *Pleistophora* and *Septata* [16]. Among these parasites only those belonging to the *Pleistophora* genus have been documented as causative agents of myositis in humans.

Microsporidiosis in Animals

Microsporidial infections have been detected in over 30 mammalian species [4]. The etiological agent has been identified prevalently as *Encephalitozoon cuniculi*. Four other species were reported circumstantially [3]. Domestic (dogs and cats) and sylvatic (blue fox, polecat, suricate, clouded leopard and mountain lion) carnivores as well as ground shrew, goat and pet birds have been found to be naturally infected. Non-human primates (i.e. squirrel monkey) are susceptible to microsporidial infection. Among laboratory animals, the rabbit has been most studied. However, mice, rats, hamsters and guinea pigs have also been found infected. As a rule, microsporidiosis in immunocompetent animals is clinically silent due to the balanced host-parasite relationship. However, if immune competence is compromised, these protozoa can proliferate rapidly. At necropsy, macroscopic lesions have been observed only in old rabbits with chronic interstitial nephritis, frequently associated with chronic microsporidiosis. Microscopic lesions are generally focal in the brain and kidney. Puppies and foxes of all ages can develop

severe and often fatal microsporidiosis, characterized by wasting and encephalitis in the early phase, and chronic renal diseases in the later stage. Liver, lung and eye are often affected. In immunodepressed animals (i.e. athymic mice or cortisone suppressed mice), peritonitis, characterized by severe ascites, is the most common clinical sign [16].

Microsporidiosis in Man

As previously reported, five genera of Microsporidia have been detected in man. The diplokaria genus *Nosema* has been identified as the etiological agent of diarrhea, malabsorption, vomiting, acute respiratory distress and weight loss in an athymic child, who died at 4 months of age [10]. *Nosema* parasites inducing keratitis, was diagnosed in three immunocompetent subjects [1, 6, 15]. A perforated corneal ulcer due to *Nosema*-like protozoa, was diagnosed in an immunocompetent woman from Botswana [13]. The genus *Encephalitozoon*, characterized by the presence of a phagosome-like parasitophorous vesicle surrounding the development stages, was first diagnosed in a boy suffering from headaches, convulsions and loss of consciousness [11]. Two cases of encephalitozoonosis causing death, were observed in AIDS patients, one with a fulminant hepatitis, and the second with peritonitis, both due to *Encephalitozoon cuniculi* [17, 20]. *Encephalitozoon hellem*, the second species of the genus, has been identified

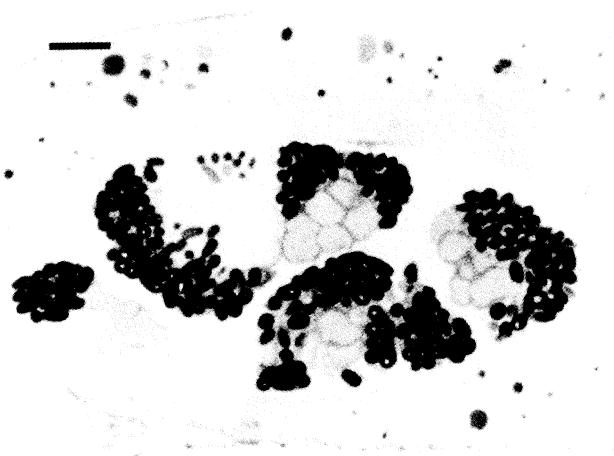


Figure 1. Muscle fiber of an AIDS patient containing meronts (faintly stained) and sporonts (intensely stained) of *Pleistophora*. Resin-embedded section (1 μm thick) with toluidine blue staining. Bar = 10.25 μm . (from reference 5, with permission).

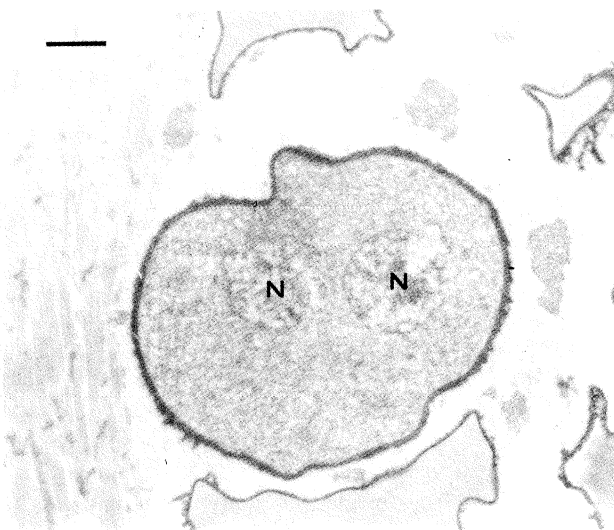


Figure 2. Meront of *Pleistophora* containing two nuclei (N) within a degenerated myocyte of an AIDS patient. Bar = 795 nm. (from reference 5, with permission).

as the etiological agent of systemic infections, and it was also detected in the urinary bladder, kidney, lung, sinuses, corneal and conjunctival epithelium of some dozen AIDS patients [16]. In AIDS patients, the most common microsporidian found is *Enterocytozoon bienersi*. This parasite has been detected in only two HIV-negative patients [14, 19]. This parasite, characterized by the formation of two types of plasmodial stages and a unique sporogonic sequence, is localized mainly in the tract from the duodenum through the distal ileum and more so in the distal duodenum and proximal jejunum. Infections have been also reported in the biliary tree. It induces a slow progressive weight loss, malabsorption of xylose and fat, 3-10 large-volume watery, non-bloody, non-mucoid stools daily. Diarrhoea appears gradually and may continue for months [16]. The most recent microsporidian species is *Septata intestinalis* inducing diarrhea in AIDS patients. This parasite is more widely distributed in the intestine ranging from enterocytes to lamina propria macrophages, causing necrosis and mucosal erosions. Systemic infections have also been reported [12, 18]. The genus *Pleistophora* will be reported in detail below.

Microsporidia Life Cycle

The sporal stage of microsporidian parasites is the infective phase. This resistant microsporidian stage when triggered by appropriate environmental stimuli extrudes the polar tubule (an extrusion apparatus consisting of a proteic tubule ending with an anchoring disk) that is capable to inoculate the sporoplasm into the host cell. The sporoplasm initiates the proliferative stage (merogony). Meronts multiply by binary and multiple fission into sporont stage (sporogony). Sporonts, characterized by the develop of a dense surface coat around the cell, multiply to sporoblasts which develop into spores. The contact parasite-host cell is of great importance in the taxonomy of these protozoan. *E. bienersi* develops in contact with the host cell cytoplasm. *Encephalitozoon* and *Septata* spp. are bounded, in a parasitophorous vacuole, by a membrane developed by the host cell. *Pleistophora* spp. is a distinct genus on account of pansporoblastic development i.e. a sporogony in which multiple spores develop from an individual sporont within the sporont plasmalemma, called the pansporoblastic membrane. In this genus, each sporont produces more than eight spores. Protozoan of this genus are found in insects, fish but rarely in other vertebrates. Only two human infections referable to *Pleistophora* genus have so far been reported in immunodepressed subjects, one of them with AIDS [5, 8, 9].

Patients

The first case was a 20-year-old homosexual black immunodepressed male, HIV-negative in a four-year follow-up. The origin of his immune dysfunction was unknown [9]. The second case was a 33-year-old Haitian male, admitted to the hospital with fever, a productive cough, diffuse myalgias and weakness. The HIV infection was

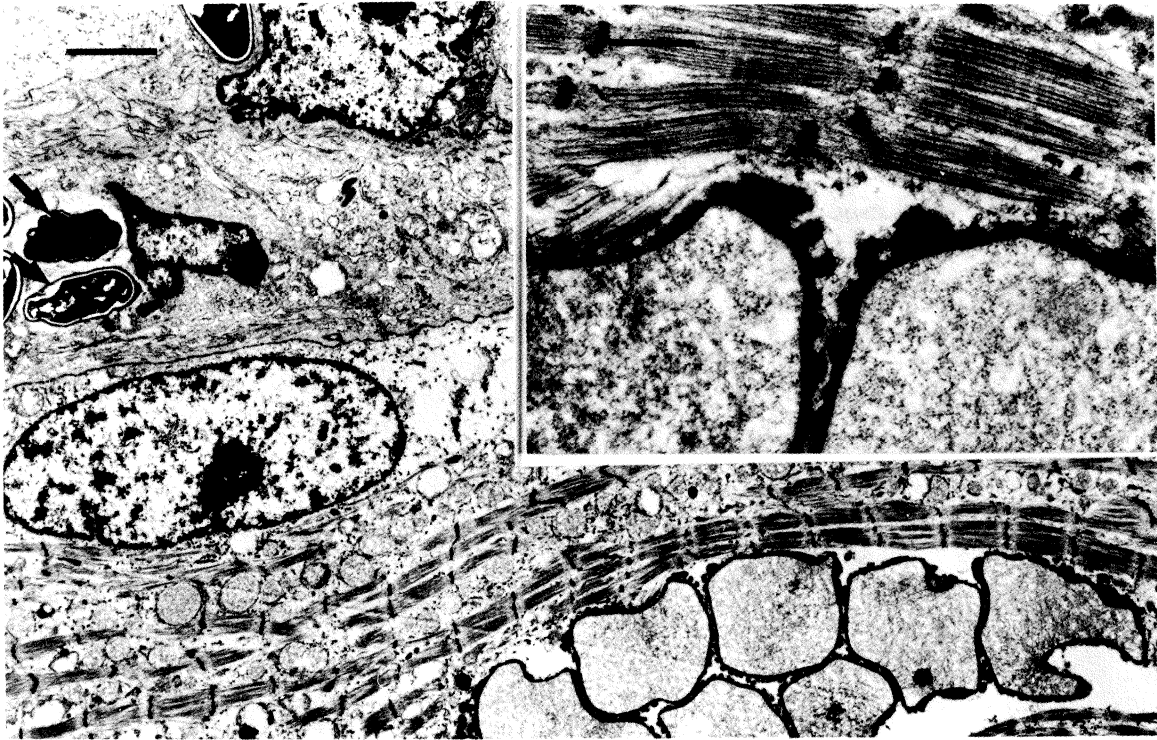


Figure 3. (A) Degenerating muscle fibers, containing *Pleistophora* meronts and two interstitial cells, containing *Pleistophora* spores into the cytoplasm (arrows) of an AIDS patient. Bar = 2.4 μ m. (B) Close-up of two *Pleistophora* meronts. Bar = 538 nm. (from reference 5, with permission).

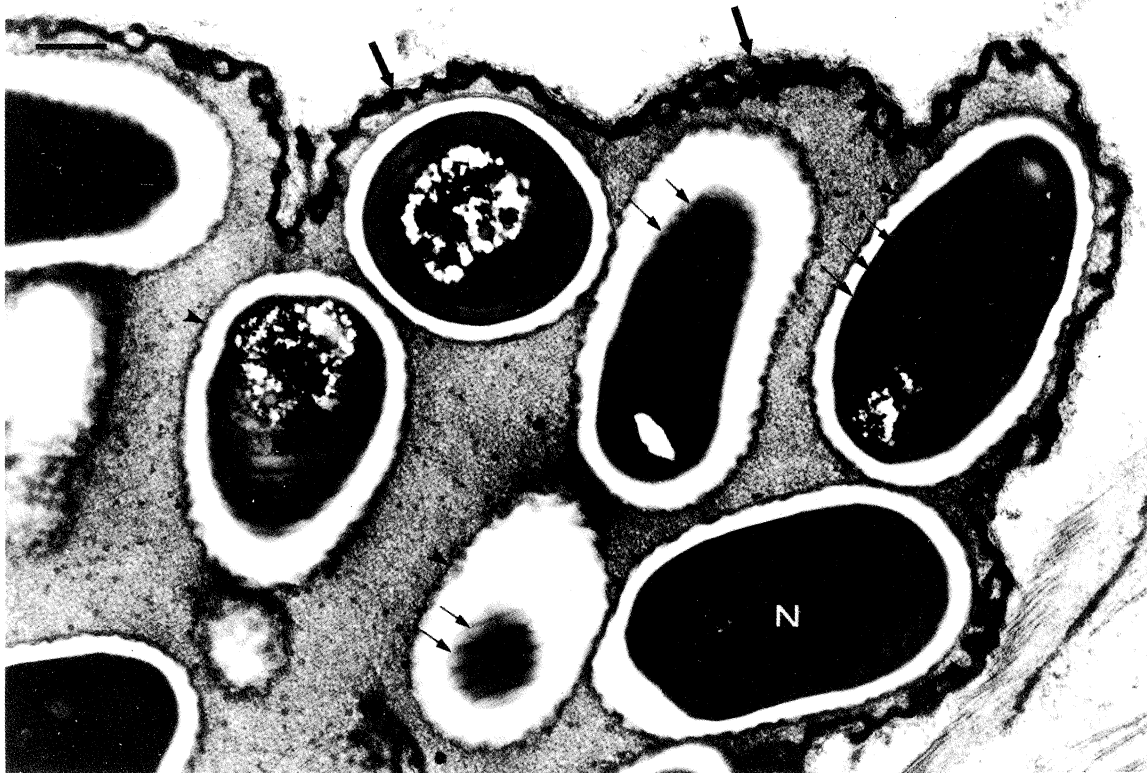


Figure 4. Muscle fiber with a *Pleistophora* sporont containing several sporoblasts of an AIDS patient. The sporont shows a thick, electron-dense, pansporoblastic membrane (large arrows). The sporoblasts have an electron-dense exospore (arrowheads) and contain a polar tube (small arrows) coiled 9-12 times and a nucleus (N). Bar = 570 nm. (from reference 5, with permission).

diagnosed three years before microsporidia myositis and developed to an AIDS case for *Pneumocystis carinii* pneumonia two years after [5].

Clinical Findings

The clinical findings of the two *Pleistophora* infected patients showed progressive and generalised muscle weakness with contractures, fever (37.4°C -39.4°C) and lymphadenopathy. Pain was also elicited with passive and active movement of the upper and lower extremities. Electromyogram and nerve conduction findings were consistent with a diffuse inflammatory myopathy. Strength and tone were normal and symmetrical in all muscle groups. Cranial nerve, sensory and cerebellar findings were normal as well as patient's reflexes and gait.

Morphological Findings

The focal nature of the infection was the most evident morphological aspect of the histological section (Figure 1). Meronts (Figure 1, 2 and 3), sporonts (Figure 1 and 4), sporoblasts (Figure 4) and spores (Figure 3) of *Pleistophora* were observed within degenerated muscle fibers characterized by loss of cross-striations.

Laboratory Features

No correlation was evidenced between laboratory data of the two patients with microsporidiosis myositis. The HIV-negative patient had 31% B cells (739 mm³), 11% T cells (262 mm³), 66/mm³ T-helper, 167/mm³ T-suppressor with a T-helper/T-suppressor cell ratio of 0.4. The white blood cells were 4,100/mm³ in the AIDS patient whereas leucocytosis (13,300/mm³) was evidenced in the HIV-negative patient. Eosinophilia was within the normal range 2-3% in both patients. The hematocrit values were low in both patients (21.0 and 28.2%, respectively). In the AIDS patient the serum CPK value increased from 485 U/L to 4,664 U/L (normal range 0-165 U/L) before hospitalization and ranged between 1,000 and 1,500 U/L during daily intravenous hydration. Serum aldolase was 21.1 U/L (normal range 1.7-4.9 U/L) and serum lactate dehydrogenase, 527 U/L (normal range 0-170 U/L). In the HIV-negative patient the muscle enzyme values were normal.

Diagnosis

At present, only a parasitological diagnosis for a suspected microsporidiosis in muscles can be done on muscle biopsies (quadriceps, deltoid, etc.) by light microscopy (LM) and by transmission electron microscopy (TEM). Muscle biopsy sections of paraffin-embedded myocytes should be stained with Ziehl-Neelsen stain (spores appear brilliantly acid fast) or with Gram's, Giemsa's and hematoxylin-eosin, or if resin-embedded (1 µm thick) should be stained with toluidine blue or trichrome. TEM identification must confirm the LM diagnosis. The most important morphological characteristic of spores (2.8 x 3.2-3.4 µm) is the coiled polar filament (with 11 coils), diagnostic for identification of microsporidia. Microsporidia inducing

myositis were never isolated *in vitro*. No specific antigen, nor monoclonal antibodies are available. Heterologous antigens produced with other microsporidia (i.e. *Encephalitozoon cuniculi*) were not utilized for a serodiagnosis with sera of the *Pleistophora*-infected patients. As observed from laboratory findings the morphological findings were different in the two patients. The infection in the AIDS patient was focal whereas it appeared disseminated in the HIV-negative patient, at hematoxylin and eosin staining of muscle biopsy. In one area of a muscle biopsy of the AIDS patient, all fibers were atrophic and contained intracellular basophilic organisms. In this patient a scanty inflammatory infiltrate, composed of lymphocytes, histiocytes, plasma cells and eosinophils was observed. On the contrary scarring and fibrosis of muscle with intense inflammatory reaction of plasma cells, lymphocytes and histiocytes was present in the HIV-negative patient. *Pleistophora* parasites were developing in myocytes but degenerating within macrophages.

Treatment

The HIV-negative case was treated with trimethoprim (20 mg/Kg body weight per day), sulfisoxazole (100 mg/Kg body weight per day) and given physical therapy. Five months after his hospitalization, the patient was afebrile and had a stable weight. The AIDS patient died before administration of anti-microsporidia therapy.

Epidemiology

No data are available on the epidemiology of microsporidia inducing myositis in immunodepressed patients. The unusual reports of this infection in humans, clearly suggest the zoonotic origin of the etiologic agent, even though the wide host spectrum of microsporidia of the genus *Pleistophora*, from insects to vertebrates, makes it practically impossible to identify the main host of this parasite. The two reports of this infection involving immunodepressed patients are in line with other microsporidia infections in humans capable of inducing a patent infection only in immunodepressed subjects, whereas serological investigations clearly have demonstrated frequent contacts between microsporidia (*Encephalitozoon* sp.) and humans [7].

Address correspondence to:

Edoardo Pozio, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Roma.

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