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Incidental Finding of a Microsporidian Parasite from an AIDS Patient

RODNEY J. McDOUGALL,[1]* MARK W. TANDY,[1] ROBYN E. BOREHAM,[1] DEBORAH J. STENZEL,[2] AND PETER J. O'DONOGHUE[3]

Microbiology Department, Drs. Sullivan, Nicolaides and Partners, Taringa, Brisbane, Australia 4068[1]; Analytical Electron Microscope Facility, Queensland University of Technology, Brisbane, Australia 4001[2]; and Parasitology Section, Department of Agriculture, Adelaide, South Australia 5000[3]

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Light microscopic examination of feces from a human immunodeficiency virus-positive patient with chronic diarrhea, anorexia, and lethargy revealed the presence of numerous refractile bodies resembling microsporidian spores. They were subsequently identified as belonging to the genus *Nosema* on the basis of their ultrastructural characteristics. However, the microsporidia were enclosed within striated muscle cells, suggesting that they were probably ingested in food; thus, this represented an incidental finding rather than a true infection.

Microsporidia are obligate intracellular spore-forming protozoa belonging to the phylum *Microspora* (20). These organisms have a wide host distribution, and five genera have been implicated as causing human disease: *Nosema*, *Encephalitozoon*, *Pleistophora*, *Microsporidium*, and *Enterocytozoon* (17). Features used in identification include spore size, nuclear configuration of spores and developing forms, the number of polar tubule coils, and the parasite-host interaction (17). Microsporidia were first implicated as a cause of human disease in 1959 (13) and as a complication of AIDS in 1985 (14). *Enterocytozoon bieneusi* has been well documented as a cause of chronic diarrhea in human immunodeficiency virus-infected patients (4, 15).

The diagnosis of intestinal microsporidiosis generally involves ultrastructural studies of intestinal biopsies (15), although Giemsa-stained smears from duodenal biopsies have also been reported to provide a quick and easy method for the diagnosis of intestinal infections (16). Recently, it has been reported that spores may be detected in human fecal specimens by Giemsa staining (22), while others have recommended the use of a new chromotrope-based stain with stool specimens and examination by light microscopy (23). These workers claimed that this was sufficient for a diagnosis of microsporidiosis and that electron microscopy was not as sensitive as light microscopic examination of either stool or biopsy material (23). However, others (8, 14) assert that a duodenal or jejunal biopsy is necessary to establish that there is an intestinal infection. They also state that electron microscopic examination is required for definitive diagnosis, that it is more sensitive than light microscopy, and that a fecal smear is not useful because of the small size of the spores. This paper presents a case of a spurious infection in a human by a microsporidian parasite. The organisms were detected during a study undertaken to compare light microscopic methods that would provide a rapid and simple technique in the clinical laboratory for the detection of intestinal microsporidiosis.

A stool specimen was collected from a 48-year-old human immunodeficiency virus-positive male on zidovudine (AZT) treatment who had complained of diarrhea, nausea, and anorexia for several months. The specimen was fixed in 10% formalin. Saline and iodine wet preparations (9) and a formalin-ethyl acetate concentrate made by using the Evergreen Fecal Parasite Concentrator Kit (Evergreen Scientific, Los Angeles, Calif.) were examined by light microscopy. Direct specimen and fecal concentrates were also stained with a chromotrope-based (23), Kinyoun acid-fast, and safranin (9) stains. *Giardia lamblia* cysts, *Entamoeba coli* cysts, and numerous small ovoid bodies measuring 5 to 8 by 3 μm and occurring singly and in clusters were detected in the direct unstained preparations. These bodies appeared to contain a vacuole at one end, but no other features could be

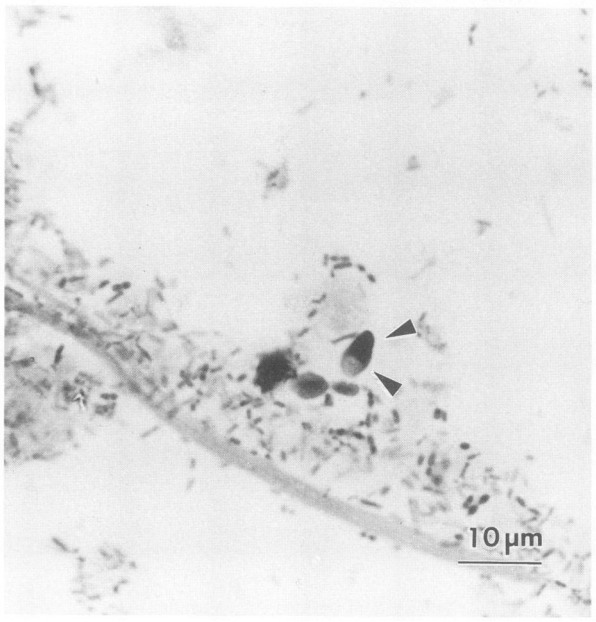

FIG. 1. Light micrograph of microsporidian organism with posterior vacuole (arrows) in fecal material, stained with Kinyoun acid-fast stain.

* Corresponding author.

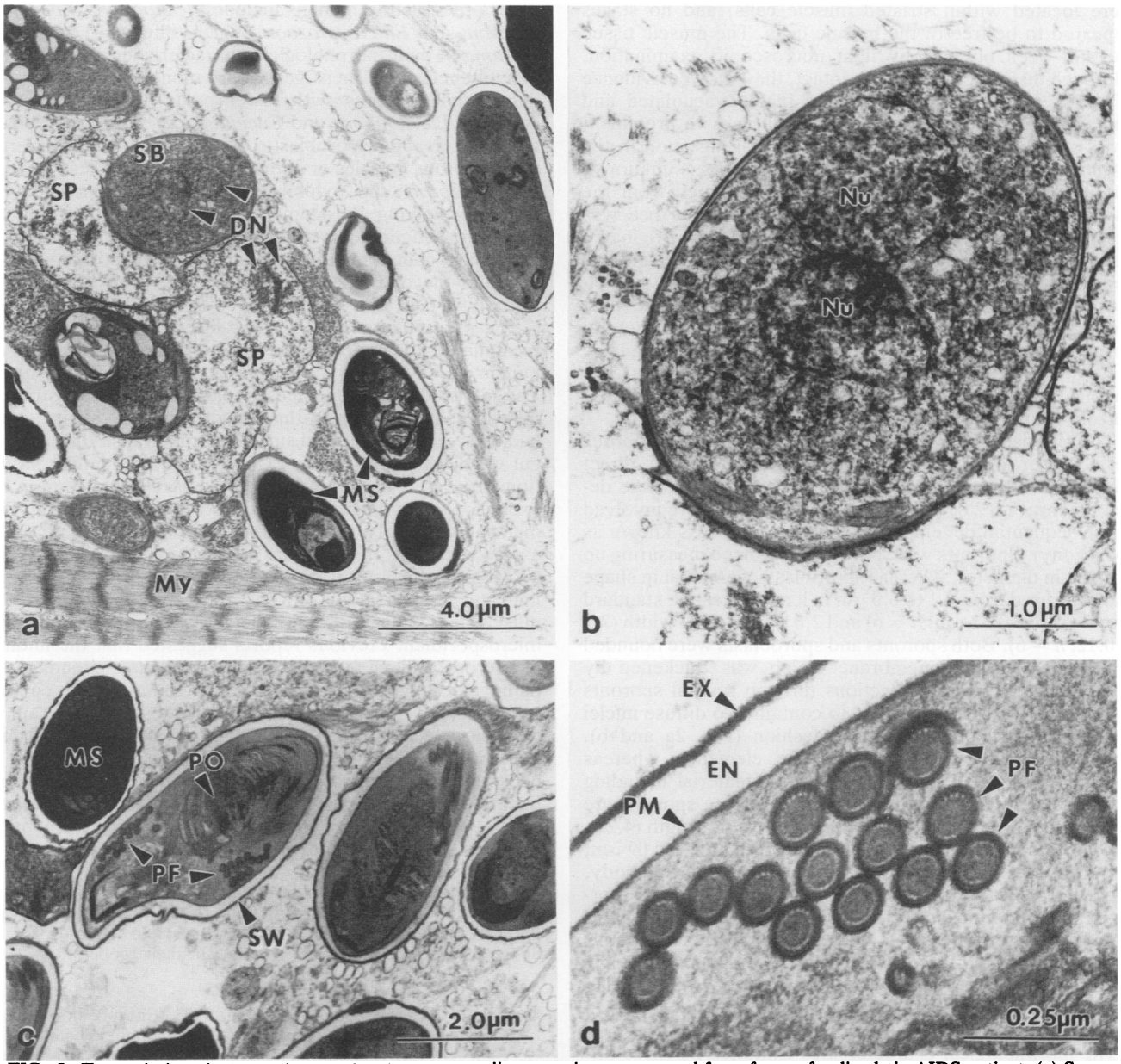

FIG. 2. Transmission electron micrographs of microsporidian organisms recovered from feces of a diarrheic AIDS patient. (a) Sporonts (SP), sporoblasts (SB), and mature spores (MS) of *Nosema*-like microsporidian parasite detected in striated muscle cells. The muscle cells were highly vacuolated in appearance and contained fragmented strands of cross-striated myofibrils (My). Note the paired nuclei abutted in diplokaryotic arrangement (DN) within the sporoblast and sporont. (b) Enlargement of sporoblast showing paired nuclei (Nu). (c) Mature spores (MS) containing prominent polaroplast (PO) and polar filaments (PF), and bounded by a well-developed spore wall (SW). (d) Section through periphery of a mature spore showing wall with an electron-dense exospore layer (EX), electron-lucent endospore layer (EN), and thickened plasma membrane (PM), as well as isofilar polar filament (PF) with 14 coils.

discerned. Concentration by the formalin-ethyl acetate technique did not appear to enhance their recovery. The organisms stained bright pink-red with the chromotrope-based, acid-fast, and safranin stains with the posterior vacuole remaining unstained (Fig. 1). The morphology and staining properties of the organisms suggested their identification as a microsporidian, but their appearance was different from that of *E. bieneusi* spores, which are significantly smaller (measuring approximately 1.5 by 1.0 μm), do not show a demonstrable posterior vacuole, and may stain with a stripe girding the spores diagonally or equatorially (23).

A portion of the formalin-fixed specimen was examined by electron microscopy in order to identify the organism. This material was post-fixed with 3% glutaraldehyde followed by 1% osmium tetroxide, dehydrated in an ascending series of alcohol solutions, and embedded in Spurr's epoxy resin (10). Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined by transmission electron microscopy.

Three different developmental stages of a microsporidian parasite (sporonts, sporoblasts, and spores) were detected by electron microscopy (Fig. 2). All three stages detected

were located within striated muscle cells, and no stages appeared to be free of the muscle cells. The muscle tissue had not been obvious by light microscopic examination. Although the muscle cells were intact, they showed evidence of degeneration in that they were highly vacuolated and contained fragmented strands of myofibrils with prominent cross-striations (Fig. 2a) suggesting partial digestion in the intestinal tract. The source of the muscle cells is unknown, but it is highly improbable that they originated from the tissues of the patient, even though chronic diarrhea was present. It is more likely that the muscle cells originated from animal tissues which had been ingested as food and had been excreted only partially digested due to rapid gastrointestinal transit. Unfortunately, the identity of the host muscle cells could not be determined by ultrastructural observations, and there was insufficient material available for tissue typing studies using immunological markers. The patient was lost to follow-up after treatment with metronidazole for the *G. lamblia* infection.

The microsporidia were not enclosed within sporophorous vescicles or parasitophorous vacuoles but lay in direct contact with the host cell cytoplasm (apansporoblastic development; Fig. 2a). All three stages detected were involved in the sequential development of spores (process known as sporogony). Sporonts were polygonal in shape measuring up to 8 μm in diameter, whereas sporoblasts were oval in shape and measured from 4.1 to 6.0 μm in length (mean ± standard error = 5.0 ± 0.32 μm; $n = 6$) and 2.6 to 3.4 μm in width (2.9 ± 0.12; $n = 6$). Both sporonts and sporoblasts were bounded by a single plasma membrane which was thickened by electron-dense material. Sections through several sporonts and sporoblasts revealed them to contain two diffuse nuclei abutted in typical diplokaryotic fashion (Fig. 2a and b). Sporonts contained few cytoplasmic elements, whereas sporoblasts were filled with amorphous material including ribosomes and endoplasmic reticulum. Mature spores were oval in shape, measuring from 3.6 to 5.2 μm in length (4.2 ± 0.25 μm; $n = 10$) and 1.8 to 2.7 μm in width (2.2 ± 0.09 μm; $n = 10$). Each spore contained a posterior vacuole and body, an elaborate polarplast (both lamellar and vesicular portions), an anterior anchoring disc, and an isofilar polar filament (90 to 100 nm in diameter) with 14 to 15 coils arranged in two to three ranks (Fig. 2c and d). The spores were bounded by a well-developed wall (Fig. 2d) consisting of an electron-dense exospore layer (20 nm thick) and an electron-lucent endospore layer (100 nm thick).

The morphological characteristics exhibited by these different developmental stages allowed the identification of the parasite as a member of the genus *Nosema*. This genus is characterized by apansporoblastic development, disporoblastic sporogony, sporonts with thickened plasma membranes, and the presence of diplokaryotic nuclei in all developmental stages (5, 11). They differ from other genera in the family *Nosematidae* (such as *Ichthyosporidium* and *Hirsutosporos*) in spore morphology, exospore ultrastructure, and nuclear arrangement during sporogony. *Nosema* infections have been reported in a wide variety of host species, mainly insects, fish, crabs, and shrimps (7, 21). Several cases of *Nosema* infections have previously been described in humans, one involving disseminated infection and the remainder involving corneal infections (1–3, 12, 18, 19).

The spores detected in the present study were similar in size and appearance to previous descriptions except for the unusual arrangement of the polar filament coils in two to three ranks instead of a single file. Other microsporidian genera recorded in humans include *Pleistophora*, *Encephalitozoon*, and *Enterocytozoon*, which differ markedly from *Nosema* in their morphological characteristics and developmental cycles (2). These genera are monokaryotic and produce smaller spores with fewer polar filament coils. In addition, *Pleistophora* and *Encephalitozoon* spp. are characterized by pansporoblastic development within large sporophorous vesicles or parasitophorous vacuoles whereas *Enterocytozoon* spp. exhibit unique apansporoblastic development involving merogonial and sporogonial plasmotomy and the production of well-matured sporoblasts. *E. bieneusi* is the most commonly reported human microsporidian infection and the one suggested as a cause of gastrointestinal disease (4, 6, 8, 16).

The present report of *Nosema*-like organisms in human fecal material is not considered to represent another case of human infection but is regarded as an incidental finding only. The parasites were located in partially digested striated muscle cells, suggesting that infected animal musculature had been ingested and had survived rapid transit through the gut of the diarrheic patient. Considering that similar situations may arise in the future, it is advisable that caution be exercised when diagnosing microsporidian infections in human patients solely on the basis of coprological examination, as the presence of organisms in stool specimens does not necessarily confirm an intestinal infection. Examination by light microscopy alone did not reveal the presence of the muscle cells, nor did it allow definitive identification of the microsporidian. Previous reports suggested that the finding of microsporidia in feces from AIDS patients may indicate a pathogenic role (22, 23). Until this is confirmed, we concur with the view of others (8, 14) that a biopsy examination is necessary to establish the presence of intestinal infection and, in view of this present case, that electron microscopy is necessary for definitive identification. However, the use of light microscopic staining techniques such as that described by Weber et al. (23) may be useful as screening techniques for intestinal microsporidian infection.

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