

## *Scedosporium inflatum* Osteomyelitis in a Dog

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***Scedosporium inflatum* Malloch et Salkin was found to cause osteomyelitis in a 6-year-old spayed female beagle. The previously healthy dog suddenly developed right-forelimb lameness. Bony changes consisting of proliferation with some lysis were noted on radiographic examinations. Microscopic observations of stained sections of tissue obtained by biopsy of the distal humerus revealed the presence of septate branching hyphae. Cultures inoculated with tissue from a later biopsy yielded a mold subsequently identified as *S. inflatum*. Tissue sections stained with specific *Scedosporium* fluorescent-antibody conjugate were positive, further substantiating the diagnosis. Although the dog was treated with oral itraconazole, no improvement in the animal's condition was noted, and it was euthanized. Autopsy revealed dissemination of the etiologic agent to the lungs.**

*Scedosporium inflatum* Malloch et Salkin is a newly described member of the dematiaceous hyphomycetous genus *Scedosporium* Sacc. ex Castellani et Chalmers (7). It differs from the clinically better known *Scedosporium apiospermum* (Sacc.) Castellani et Chalmers in its colony morphology, distinctive annellides with swollen bases, and more-rapid growth on standard nutrient media (9). It has been associated with approximately 20 cases involving infections in humans, 2 cases in horses, and 1 case in a cat (2, 6-9, 11, 12). However, to our knowledge, it has been neither recovered from nor implicated as the etiologic agent in an infection of a dog. This report provides the first description of such a case: osteomyelitis caused by *S. inflatum* in a 6-year-old beagle.

### MATERIALS AND METHODS

**Case report.** A previously healthy, spayed, 6-year-old female beagle was seen by a local veterinarian in February 1990 for acute onset of right-forelimb lameness. Since physical and radiographic examinations did not reveal a musculoskeletal or neurologic cause for the animal's condition, the lameness was attributed to a ligament strain, and the animal received nonspecific therapy consisting of aspirin and cage rest. However, as lameness worsened over the next 2 months, further radiologic studies were conducted; they revealed bony changes involving proliferation and some lysis. A biopsy sample was then taken from the distal humerus and submitted to a local diagnostic laboratory for analysis. When the biopsy report indicated chronic mycotic osteomyelitis with soft tissue and bone involvement, the animal was transferred to the facilities of one of us (J.W.B.).

Physical examination revealed a large swelling (4 by 4 by 5 cm) on the distal right humerus, causing non-weight-bearing lameness. Further progression of the osteomyelitis was noted through radiographic studies. A fungus was isolated from portions of a biopsy sample of the affected limb and was tentatively identified as a *Scedosporium* sp. The animal was empirically placed on oral itraconazole therapy, but as her condition did not improve, the dog was euthanized 4 months after the initial onset of the lameness. Autopsy

revealed that the etiologic agent had disseminated to the lungs.

**Mycologic studies.** Portions of the biopsy sample from the distal right humerus were streaked for isolation onto 100-mm-diameter petri plates containing 25 ml of Sabouraud glucose agar (SGA; Difco Laboratories, Detroit, Mich.) with penicillin and streptomycin and were also inoculated onto Mycosel (BBL/Becton Dickinson Microbiological Systems, Cockeysville, Md.) slants. All cultures were initially incubated at 27°C and observed daily for growth.

Isolates recovered from the biopsy specimen were subcultured to SGA slants incubated at 30, 37, 42, and 45°C and to Mycosel slants incubated at 30°C. Conidial morphology and ontogeny were investigated with 7- to 10-week-old potato dextrose agar (Difco) and cornmeal agar (Difco) in slide cultures. Colony morphology was studied after incubation for 14 days at 30°C on SGA.

**Microscopic studies.** Portions of the same biopsy specimen used in the mycologic studies were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with Grocott methenamine-silver stain and rabbit anti-*S. apiospermum* Fresenius fluorescein isothiocyanate stain. Control slides of *S. apiospermum* NYS M887-85 were stained in a similar manner for fluorescence studies (5).

### RESULTS

Microscopic examination of the Grocott methenamine-silver-stained sections prepared from portions of the biopsy specimen revealed the presence of branching fungal filaments and possible conidia and chlamydospores (Fig. 1).

Colonies isolated from the biopsy specimen were rapid growing, flat, moist, spreading, olive grey to black, and arachnoid to woolly. Initial microscopic examination of a portion of one of the developing colonies revealed oval, smooth-walled, single-celled conidia forming on annellides with distinct swollen bases (Fig. 2). The overall appearance of the colony and the microscopic morphology were consistent with those of *S. inflatum*.

This tentative identification was confirmed by more-detailed studies of the potato dextrose and cornmeal agar slide cultures. Conidia were hyaline to olivaceous and ranged in size from 2.5 to 4.5 by 3.0 to 12.5 µm. They were formed serially or at the tips of the conidiophores. The latter were

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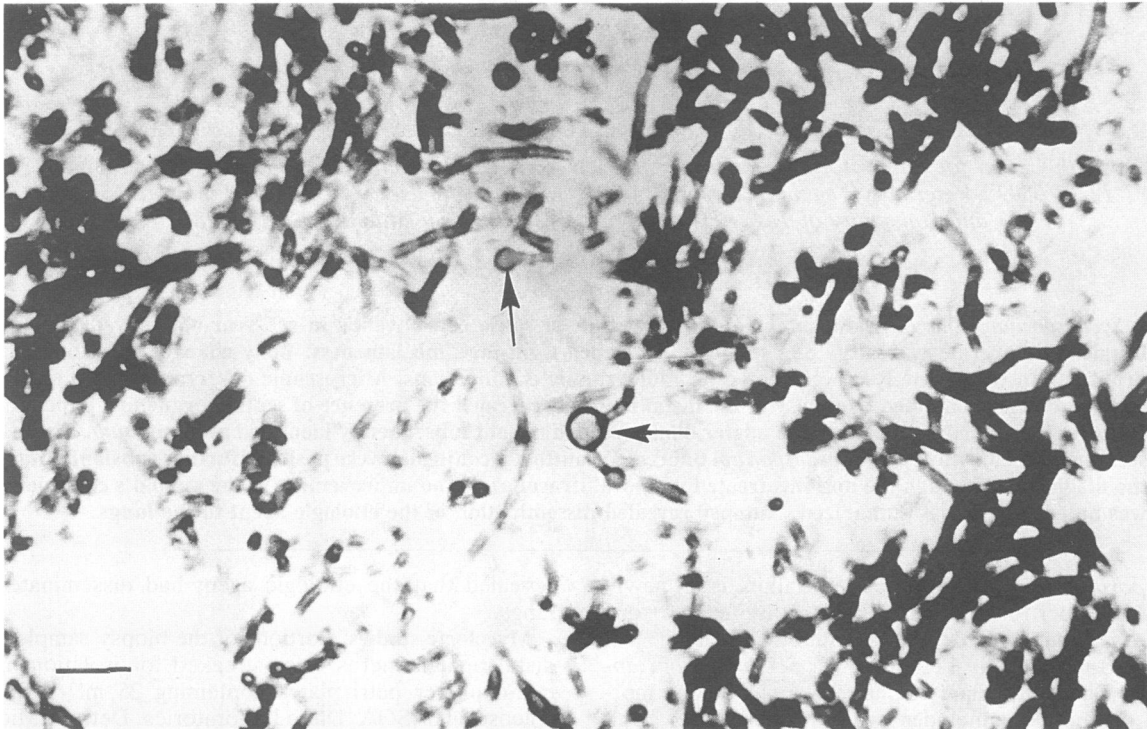


FIG. 1. Grocott methenamine-silver-stained section of a portion of the tissue biopsy sample showing branching filaments, possible conidia (arrow), and chlamydoconidia (arrow). Bar = 10  $\mu$ m.

annellides with distinct swollen bases and ranged in size from 1.0 to 3.0  $\mu$ m.

Subcultures on SGA grew at all temperatures except 45°C and did not grow on Mycosel medium. Although initially white to cream colored, colonies generally developed dark grey to black pigmentation within 7 days of incubation at 30°C.

Brightly fluorescing branching and unbranched hyphal filaments were observed in biopsy sections stained with fluorescein-tagged antibody to *S. apiospermum*. Similar structures were noted upon microscopic examination of the Grocott-stained sections.

#### DISCUSSION

The isolation of multiple colonies of *S. inflatum* from portions of the biopsy sample and the observation of fungal hyphae morphologically consistent with this fungus in Grocott-stained sections clearly establish the association of this dematiaceous mold with the osteomyelitis in the animal and its probable role as the etiologic agent of the infection. This conclusion is further substantiated by observation of positively fluorescing fungal filaments in tissue sections stained with fluorescein-tagged rabbit anti-*S. apiospermum* stain. Although not specific for *S. inflatum*, the positive results indicate cross-reactivity with *S. apiospermum*, a relatively common occurrence with closely related taxa (5).

At the time of the initial symptoms, the dog was not being treated for any specific clinical condition. No evidence of immunoincompetence was found in clinical tests, nor was any malignant disorder noted on necropsy. However, the acute onset of the lameness and prior reports of traumatic inoculation as the portal of entry for *S. inflatum* suggest that

the present infection was initiated through some form of wound injury to the affected limb.

It is interesting to note that since the original isolation of *S. inflatum* from a 6-year-old boy with osteomyelitis (7), approximately 80% of *S. inflatum* infections in humans have involved osteomyelitis or arthritis (2, 6, 7, 9, 11, 12). Since this dematiaceous pathogen is apparently resistant to clinically attainable concentrations of amphotericin B, miconazole, ketoconazole, and fluconazole, successful therapy has required early diagnosis and surgical intervention. However, even when such protocols are employed, the organism's predilection for bone, its rapid growth in vivo, and its resistance to antifungal agents have resulted in several cases in the amputation of the affected limb.

Hennebert and Desai described in 1974 a mold isolated from greenhouse soil, for which they established the monotypic genus *Lomentospora* based on the new species *Lomentospora prolificans* (4). They considered the new genus distinct from similar genera by having basipetally produced successive blastoconidia and sympodially proliferating conidiogenous cells which form long, flexuous, narrow rachids. Recently, Guého and DeHoog proposed to reduce the genus *Lomentospora* to synonymy with the genus *Scedosporium*. They erroneously contend that Hennebert and Desai had characterized *Lomentospora* "by [its] inconspicuously percurrent conidiogenesis leading to long, irregularly nodose rachids" (3). The only evidence presented by Guého and DeHoog to support their interpretation of conidiogenesis in *Lomentospora* and *Scedosporium* spp. is the similar appearance of the conidiogenous cells produced by the two genera in scanning electron micrographs.

In addition, Guého and DeHoog reported finding 100% DNA homology between isolates derived from those used to

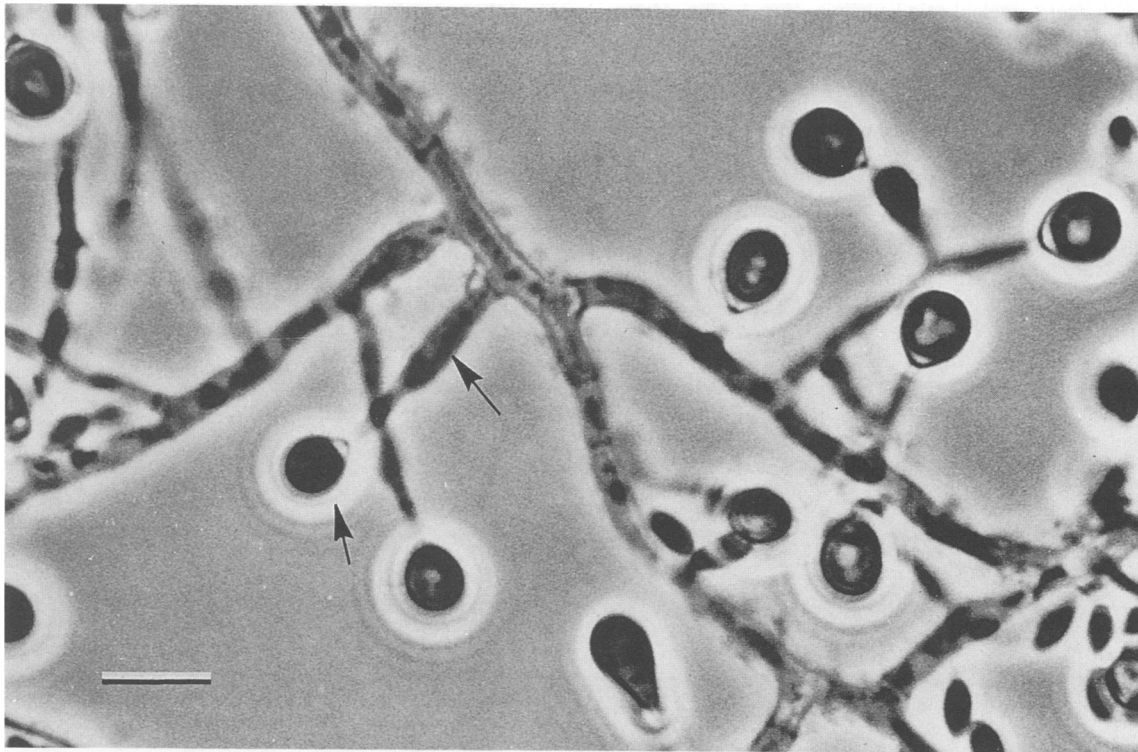


FIG. 2. Conidiogenesis of *S. inflatum* isolate recovered from biopsy specimen. Note conidia (arrow) situated terminally on annellides with swollen bases (arrow). Bar = 5  $\mu$ m.

prepare the type material of *L. prolificans* and *S. inflatum*. They stated that these molecular data "prove that the species are identical" (3). Since *L. prolificans* is the oldest validly published epithet, Guého and DeHoog proposed the new combination of *Scedosporium prolificans*.

However, a recent review by Bruns et al. (1) reported that results from DNA-DNA hybridization experiments often focus on percent hybridization between two genomes rather than the thermal stability of the hybrids. Consequently, while the overall composition of the genomes of the two organisms may be quite similar and thereby permit hybridization percentages of greater than 90%, these genomes may not be identical at a number of loci. Such identity is more effectively determined by the use of thermal-stability experiments.

A better measure of identity may be the use of restriction fragment length polymorphisms. Recent studies have shown that whole-cell DNAs extracted from cultures derived from the same material as is used in the preparation of the types of *L. prolificans* and *S. inflatum* do not have the same restriction fragment length polymorphisms when digested with one of several different restriction enzymes and analyzed by gel electrophoresis (1a). Therefore, these two isolates cannot be the same organism.

In addition, Guého and DeHoog did not conduct more-definitive investigations such as time-lapse developmental studies or transmission electron microscopic analyses of conidiogenesis with the isolates derived from those used to prepare the nomenclatural types. Either of these methods would have provided data that would have more clearly established the true nature of conidiogenesis in these hyphomycetes, i.e., holoblastic conidia forming on a sympodial proliferating conidiophore (Hennebert and Desai descrip-

tion) or enteroblastic conidial development resulting in percurrent proliferating conidiophores (Guého and DeHoog interpretation).

Consequently, while this does not negate the results obtained by Guého and DeHoog, it does call into question the synonymy of the genera *Lomentospora* and *Scedosporium* and the proposed new combination *S. prolificans*. We are conducting morphologic, physiologic, and molecular studies of these and other isolates of the two hyphomycetes as a means of clarifying the nomenclature and taxonomy surrounding these two fungi.

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