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CEPHALOSPORIOSIS IN THREE CAIMANS

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Abstract: A fatal diffuse granulomatous pneumonia and focal necrotizing hepatitis were found at necropsy of three 6-month old caimans. Centers of the widely disseminated, discrete lesions in the lungs had a branching fungus with septate hyphae, chlamydospores, and elliptical and cigar-shaped conidia. On culture the fungal organism was identified as Cephalosporium sp. Characteristics of the genus are discussed.

INTRODUCTION

The cephalosporia are classified with the Deuteromycetes (Fungi Imperfecti)1.6 which possess septate hyphae and reproduce asexually. Conant et al6 consider cephalosporia to be contaminants because of their ubiquity and the ease with which they may be inadvertently introduced into culture media. Members of the genus Cephalosporium are ordinarily considered to be opportunistic and saprophytic.1,6,8,18 Cephalosporia have long been known to be pathogenic for certain insects, trees, and plants.13 Perhaps less well appreciated is the fact that occasionally, pathogenic forms of Cephalosporium have been isolated in man, including maduromycosis,1.6 onychomycosis,7 blepharitis,10,17 endophthalmitis, keratitis, 3,20 and arthritis, 10 Airborne molds containing cephalosporia have been incriminated in allergies in sensitive individuals.4 Cephalosporiosis has also been reported from a tumefaction in the mouth of a cat² and from a swelling in the digestive tract of a snake.14

CLINICAL HISTORY

A group of 12 South American crocodilians, Caiman sclerops, were placed on an experiment concerned with inducing Vitamin E deficiency. In the course of the study several of the caimans died of various causes not directly related to tocopherol inadequacy. A necropsy was performed on three of the animals.

GROSS PATHOLOGY

The lungs of all three caimans were studded with miliary nodules varying in size from approximately 2 mm to 1 cm. The lesions were discrete, grayish-white, and gave a knobby appearance to the pulmonary surface. On section the lesions appeared to have a necrotic dark red center. Swabs from the nodules were streaked on blood agar and Saboraud dextrose agar for microbiologic isolation.

Circumscribed areas of grayish-white discoloration, measuring from 1-8 mm in diameter, were observed in the livers of all three animals. Grayish, thick-walled cysts were present in the kidneys and livers. Additionally two of the animals had tiny white nodules in the muscle layers of the small intestine. Swabs of the cut sections of all of these lesions were submitted for culture.

No other lesions were seen in the musculoskeletal, nervous, cardiovascular, or integumentary systems.

Representative specimens of lung and other organs were fixed in 10% buffered neutral formalin, embedded in paraffin, and cut at 6μ . The sections were stained with hematoxylin and eosin (H and E), and Gomori's methenanine silver, Gridley's fungus, Brown-Brenn, and acid-fast stains.

HISTOPATHOLOGY

Numerous focal granulomas were observed in the lungs. The lesions had cores of caseation necrosis surrounded by heterophils, lymphocytes and large epithelioid

cells. Within many of the granulomas were masses of tangled hyphae, some of which had bulbous enlargements representing chlamydospores (Fig. 1). Hyphae were also present in the lumens of thrombosed vessels, the walls of which were being invaded by the fungi. In areas of most intense growth there were slender conidiophores, and dislodged elliptical, sickle- or cigar-shaped conidia were scattered around the terminal end of the conidiophore. The hyphae were from 2 to 4μ in width, and were finely segmented and branched. The organisms were not easily seen with H and E but with Gomori's methenanine silver, Gridley's fungus, and Brown-Brenn stains, clear morphologic detail could be discerned (Figs. 2 and 3). Brown-Brenn stain also revealed colonies of Gram-negative cocci and Gram-positive bacillary bacteria both within and around several bronchioles.

A report of histopathologic pulmonary

findings on the first 2 animals was rendered prior to the receipt of microbiologic results. A preliminary diagnosis of granulomatous mycotic pneumonia due to aspergillosis, with secondary bacterial invasion, was made.

Foci of necrosis were observed in the liver, which also had a peculiar distribution of numerous melanophores common to reptiles and amphibians. Aggregates of Gram-negative cocci and Gram-positive rod-shaped bacteria were noted within the necrotic lesions, which were not encapsulated and contained numerous heterophils and lymphocytes. The livers additionally had encapsulated areas lined by tall, foamy cells surrounding a material that was amorphous and stained very poorly with H and E.

Nematode parasites in cross and longitudinal sections surrounded by fibrous connective tissue and a mixture of macrophages, lymphocytes, and heterophils were

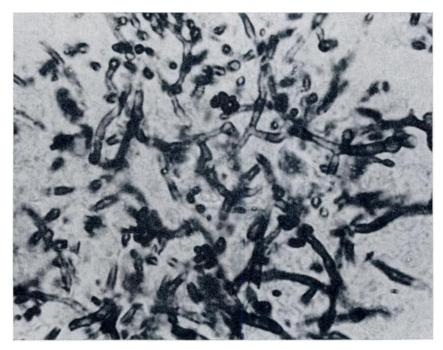


FIGURE 1. Cephalosporium sp. in a granuloma. Note branching, septate hyphae with chlamydospores. Gomori's methenamine silver, 1000X.



FIGURE 2. The fungus, showing chlamydospore formation, at the edge of a granuloma. Gomori's methenamine silver, 1000X.

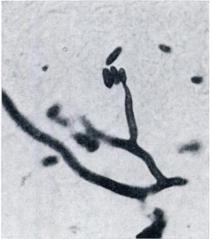


FIGURE 3. Displaced cigar-shaped conidia at end of slender conidiophore. Gomori's methenamine silver, 1000X.

located in the submucosa and muscular layers of the small intestine.

The kidneys had numerous cysts, enveloped by fibrous walls, which contained an amorphous material and resembled those observed in the livers. The lining of the cysts was comprised of elongated cells with basally oriented nuclei. At times giant multinucleated cells were observed in the linings. The cysts contained an acellular precipitate barely discernible with H and E. These cystic lesions resembled those of visceral gout, but specific confirmatory staining for urates was not undertaken because remaining tissue was all fixed in formalin.

Acid-fast organisms were not observed in any of the tissues.

RESULTS OF MICROBIOLOGIC CULTURES

Growth of fungi began to appear after 4 days in the cultures of the lungs from all three animals. Thereafter growth was rapid, and the colonies produced a tan pigment. Slender, hyaline mycelia with septate hyphae measuring about $3-5\mu$

were seen. Sickle-shaped non-septate conidia were borne at the ends of thin conidiophores of variable length. The morphologic features of the organism were compatible with those of *Cephalosporium*, but no attempt was made to identify the species.

Pseudomonas sp. and Proteus sp. were cultured on blood agar from the lungs and livers of two of the animals.

DISCUSSION

Opportunistic fungi are more frequently associated with disease in persons debilitated by such diseases as diabetes, uremia, leukemia, or lymphoma, occurring singly or concomitantly. A state of reduced resistance may also be created by drugs such as corticosteroids, multiple antibiotics, anti-cancer agents, and others. Suppression of lymphoreticularmyeloid activities, normally operative in defensive mechanisms, may also be induced by irradiation. It is probable that the caimans in this report were poorly adapted to their captive environment and had a

lowered resistance to disease, allowing bacteria and fungi to grow virtually unimpeded by host defenses.

Whether the fungi or the bacteria were the primary invaders in the tissues of these caimans is an unresolved issue. The fact that fungi were within granulomas and produced intramural invasion of vessels indicates their assumption of a pathogenic role

Isolation of the fungal agent in this instance stresses the increased awareness that has evolved over the last two decades of the need for precise cultural identification of fungi in tissues. Since histopathologic examination alone is not adequate to identify some fungi unequivocally, mycologic studies have raised the strong possibility that cases reported as mucormycoses may, in actuality, have been due to other phycomycetes. 11,15 Mycologic culture thus made it possible to apply the

more appropriate term, phycomycosis, to those diseases erroneously ascribed on microscopic examination of lesions to members of the family Mucoraceae. The isolation of Curvularia geniculata, formerly not known to be pathogenic, from a canine mycetoma, added this organism to the list of saprophytic fungi which can assume pathogenicity.

The branching, septate hyphae observed in the lungs of these caimans initially led to the erroneous diagnosis of aspergillosis. However, the round conidia, distinctive conidiophore, larger hyphae, and absence of chlamydospores in *Aspergillus sp.* should have been adequate to distinguish the latter from *Cephalosporium*.

This report further emphasizes the necessity for cultural isolation procedures from infectious lesions and documents the contribution of an organism of ordinarily low pathogenicity to the fatal termination of disease.

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