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Eumycetoma caused by *Curvularia lunata* in a dog

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Abstract

Curvularia lunata was cultured from black granules found in granulomatous tumefactions excised from the subcutis of a three year old Medium Schnauzer dog. Draining sinuses were present in some of the tumefactions. Accordingly the diagnosis of eumycotic mycetoma was made. This diagnosis was confirmed by histopathological examination. During the four years following the first surgical intervention, several more similar tumefactions were excised on three different occasions. The dog died of chronic renal failure at the age of 8 years. There was no bone involvement or visceral diffusion of the fungus. The granules were examined by scanning electron microscopy. Immunoglobulins in the dog's serum, assessed by a qualitative test, proved to be equal to immunoglobulins in the serum of a control dog. Precipitating antibodies against *C. lunata* were not found. The dog was treated for 150 days with itraconazole. In spite of good initial results, recurrence of the fungal lesions were observed after the treatment's interruption. Further treatment with itraconazole for 45 days proved ineffective. No side effects of the drug were observed. This is, to the best of our knowledge, the first case in which *C. lunata* is identified as the causative agent of an animal eumycetoma.

Introduction

Mycetomas are localized, indolent tumors caused by fungi (eumycetomas) or actinomycetes (actinomycetomas). They result from traumatic implantation of soil organisms into the tissues. Skin and subcutaneous tissue are usually involved [1], but cases of visceral lesions in animals have been described [2].

Mycetomas are characterized by the presence of granules which are dense aggregates of hyphae and other vegetative components of the etiologic agent. They are sometimes embedded in a cement-like matrix. Draining sinuses are usually present [3].

Antimycotic chemotherapy of eumycotic mycetomas is usually unsuccessful and surgery is the treatment of choice [1].

Various species of fungi have been isolated from cases of human eumycotic mycetomas [1]. In animals, at least 18 reports on mycetomas have been published. These do not include those describing granulomas from which typical granules were absent, bovine nasal granulomas or subcutaneous tumefaction caused by dermatophytes, the definition of which as mycetomas is not uni-

versally accepted [4]. The cases reported until 1987 have been reviewed [3, 5, 6]. Two more cases were published in 1988 [2] and 1989 [7]. The causative agents of animal eumycetomas, when identified, were classified as *Pseudallescheria boydii* (8 cases), *Curvularia geniculata* (4 cases) and a *Torula* spp. (1 case). *C. lunata* has been isolated from a case of phaeohyphomycosis in a cat [8] and from human mycetomas in Sudan [9] and UK [10]. To the best of our knowledge, this is the first report of this fungus as an etiological agent of a mycetoma in an animal.

Case history

A three year old male medium Schnauzer dog was referred to the clinic of one of the authors in mid 1985 for evaluation of two subcutaneous tumefactions. The dog lived in a yard in which a great variety of cacti are grown.

One of the tumefactions measured about 1 cm. It was situated in the right gluteal region, was nonadherent and had no draining sinuses. The second, measured about 5 cm. It involved the left inguinal region, was multilobulated, adherent to the underlying tissues and had two draining sinuses from which serous exudate mixed with black granules was discharged. The whole gluteal tumor and part of the inguinal one, which already had invaded the muscular tissue, were surgically excised.

C. lunata was the only microorganism isolated from the granules.

Chemotherapeutic treatment was not attempted at the time considering previously reported results which were consistently unsatisfactory, the risk of eventual side effects of available antimycotic drugs and the absence of signs indicating that the dog was suffering.

In 1987 a further tumefaction was excised from a phalanx of the right hindleg from which *C. lunata* was again isolated in pure culture.

In 1988 the tumefaction in the inguinal region has reached the size it had before the first surgical intervention. Three more tumefactions, measuring 2–4 cm, were excised from the left plantar region. A fourth tumefaction was observed at the right tarsal region but it was not excised as muscular and nervous tissue were involved. The causative agent of all the tumefactions was again identified as *C. lunata*. Signs of suffering appeared in the form of claudication and licking of the involved regions. Concomitantly itraconazole (Janssen Pharmaceutica, 2340 Beerse, Belgium) was kindly made available by Abic (Abic Ltd., Chemical and Pharmaceutical Ind., P.O. Box 2077, Ramat Gan, Israel) for a clinical trial. Liver and kidney functions were assessed and therapy was initiated. One tablet (100 mg) of itraconazole (5 mg/kg) was given daily. Although apparent healing occurred after three months, the treatment was continued for two more months and was followed by a second liver and kidney function test.

Four month after the cessation of the treatment, signs of relapse became evident. Itraconazole treatment for 45 more days had no effect. In 1989 two new subcutaneous tumefactions measuring 2–4 cm. developed on the tibial region of both hindlegs and were excised, as was part of the old tumefaction in the left inguinal region. *C. lunata* was isolated once more from the excised tissue.

In 1990 the dog died after a short period of malaise. Biochemical tests performed during this period showed severe renal insufficiency. Postmortal histopathological examination showed severe chronic kidney lesions. No fungal dissemination to organs including bones was observed. It was concluded that chronic kidney failure was the cause of death.

Laboratory examinations

Histopathology. The excised granulomatous tissue was fixed in formalin and 6 μm thick paraffin sections were stained with hematoxylin and eosin. The biopsy of the mycetoma was characterized by numerous granulomas in the centers of which brownish annular masses of hyphae, measuring

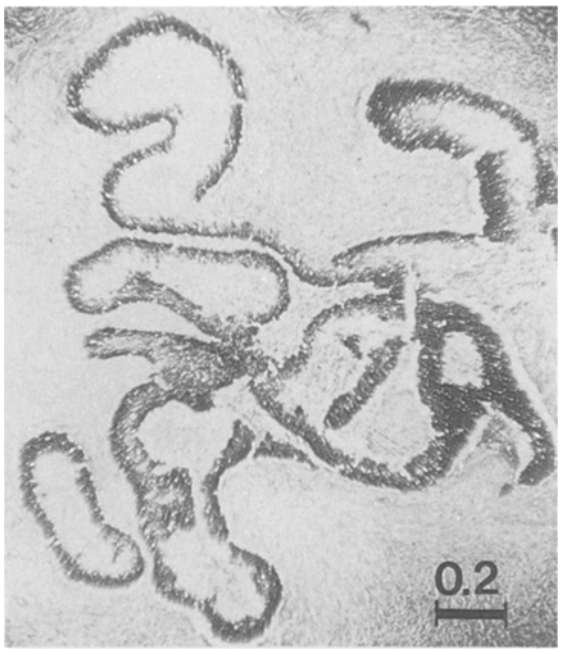

Fig. 1. Fungal colonies in the granuloma. Hematoxylin & Eosin. Bar length in millimeters.

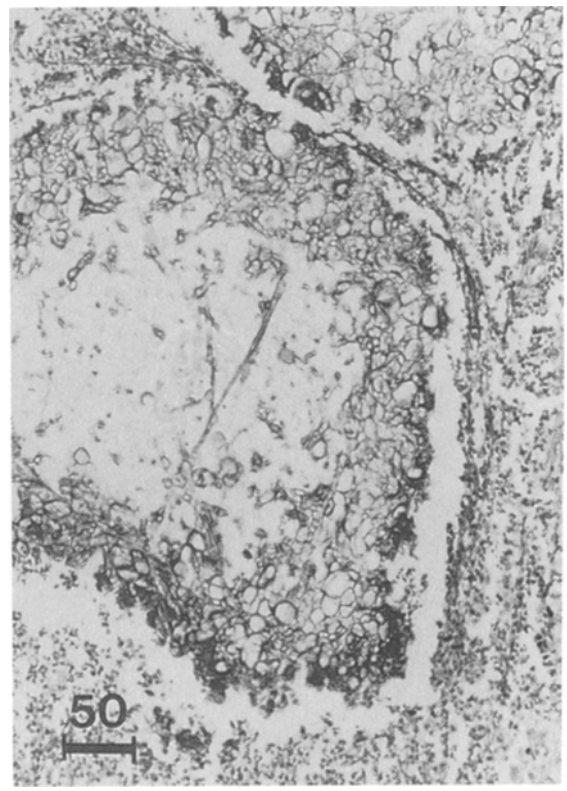

Fig. 2. Fungal colonies in the granuloma. Center partially filled with hyphae and surrounded by chlamydospores. Hematoxylin & Eosin. Bar length in μm.

0.5–2 mm (Fig. 1), were seen. These hyphae were surrounded by inflammatory cells, namely neutrophils, macrophages and a few giant cells. A thin-walled fibrous capsule enclosed the granulomas. The center of the granules was hollow and partially filled with septate branched light brown hyphae, 3–6 μm wide, loosely packed together, while at the border of the inflammatory cell reaction, a high number of tightly packed, round, dark brown chlamydospores with a diameter of 10–20 μm, were observed (Fig. 2).

Mycology. At the center of the excised granulomatous tissue hard, black granules of 0.5–2.0 mm were found (Fig. 3). Granules were immersed in a drop of 10% KOH and microscopically examined. They proved to be aggregates of irregularly shaped septate hyphae with swollen cells and chlamydospores having a diameter of 10–20 μm.

Specimens of the granulomas' centers were aseptically sampled and inoculated onto 5% sheep blood agar, nutrient agar, MacConkey agar and Sabouraud dextrose agar (SDA). All the media were produced by Difco (Difco Laboratory, Detroit, MI 48232, USA). The SDA plates were incubated at 28 °C and the other plates at 37 °C. Granules found in the lesion were washed and cultured on SDA at 28 °C. Fungal colonies were subcultured on potato dextrose agar plates and slide cultures were made on that medium.

Thorns from the cacti in the dog's surroundings were crushed and inoculated onto SDA and incubated at 28 °C.

Rapidly growing fungal colonies, initially white but turning greenish-black in about 4 days, developed on potato dextrose agar. Stromata or zonate growth were not observed. No other microorganisms were cultured from the granules. On microscopic examination of the slide-cultures,

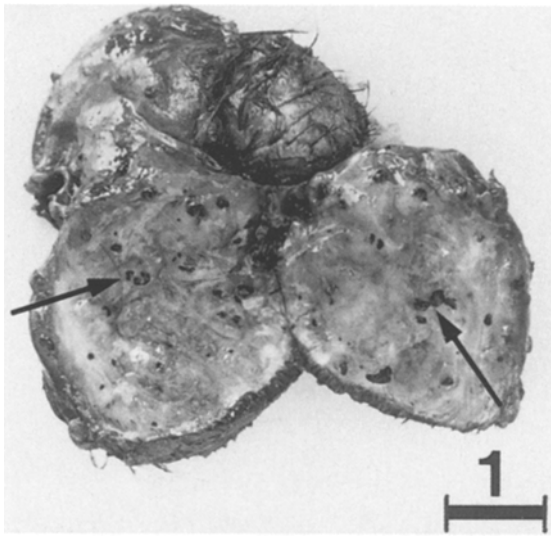

Fig. 3. Excised granulomatous tissue with black granules (Arrows). Bar length in centimeters.

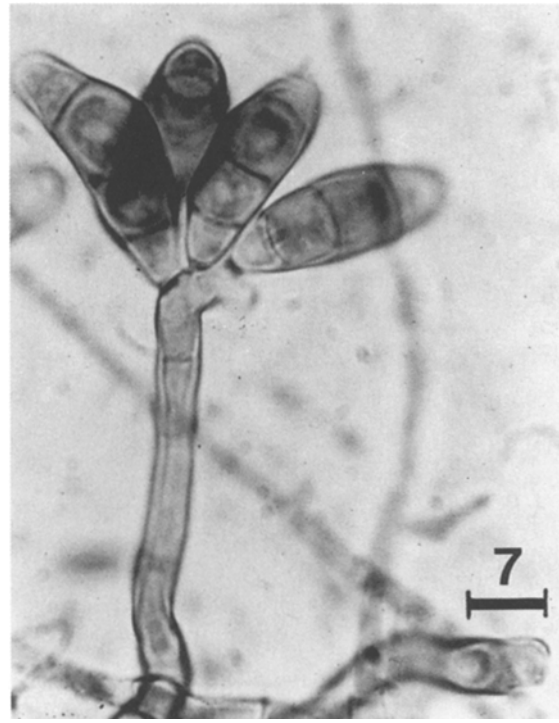

Fig. 4. Conidia of *Curvularia lunata* isolated from the granules. Bar length in μm.

conidiophores bearing brown, curved, 3-septate, smooth conidia measuring 16–24 μm in length and 6–10 μm in width were observed (Fig. 4). Identical results were repeatedly obtained each time the granules were cultured.

The fungal isolate was identified as *C. lunata* by Prof. C. K. Campbell (Central Public Health Laboratory, Mycological Reference Laboratory, 61 Colindale Avenue, London NW9 5HT, UK).

Fungal cultures of the decaying cacti thorns resulted in the isolation, among others, of fungi which, having the same characteristics as the one isolated from the granules, were identified as *C. lunata*.

Sensitivity testing. Sensitivity testing of *C. lunata* to itraconazole before the treatment was not possible as sensitivity discs were not available. The test was however performed after the second (unsuccessful) treatment. A disc (Neosensitabs, Rosco Diagnostica, Taastrup, Denmark) impregnated with 10 μgr. Itraconazole was disposed at the center of an SDA plate previously inoculated with 0.1 ml of a suspension containing 3×10^4 cfu/ml of *C. lunata* (corresponding to 0.5 McFarland units). The growth inhibition zone, measured after an incubation period of 48 hours at 28 °C, had a diameter of 30 mm.

Immunology. Cultures of *C. lunata* were crushed with sterile glass powder and centrifuged at 10000 g for 10 minutes. The supernatant was used as crude antigen in an immuno-double diffusion test to assess the presence of antibodies in the dog's serum. The presence of immunoglobulins was assessed by immunoelectrophoresis using canine antiserum (Biomakor, Kiriat Weitzman, 76326 Rehovot, Israel). The serum of a healthy dog was used as control in both tests. All classes of immunoglobulins in the dog's serum were equal to the control. No precipitating antibodies were found in the immuno-double diffusion test.

Hematology. Red blood cell count, mean cell volume, hematocrit, hemoglobin and total white

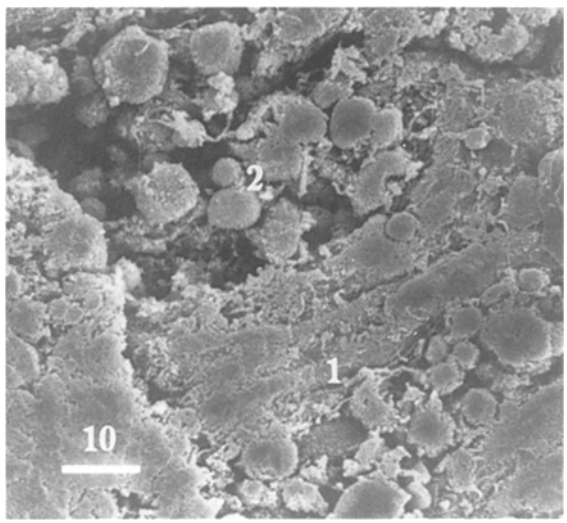

Fig. 5. Scanning Electron micrography of a granule: Hyphae (1) and chlamydospores (2) embedded in their matrix. Bar length in μm.

blood cell count, made on a Contraves Digicell 500 cell counter (Contraves AG, Schaffhausenstrasse 580, CH-8052 Zürich, Switzerland), and differential white cell counts, made microscopically, were normal during the whole period.

Biochemistry. Glucose, alkaline phosphatase, triglycerides, albumin, protein, τ glutamil transferase, alanine transferase and blood urea, measured by a Kone Progress Selective Chemistry Analyzer (Kone Progress, SF-02321 Espoo, Finland), were normal before and after the itraconazole treatment.

Scanning electron microscopy. The granules were fixed in 2% glutaraldehyde, dehydrated in ascending concentrations of ethanol, dried in CO_2 and covered with gold. Specimens thus prepared were examined under a GOUL-35C scanning electron microscope at an accelerating voltage of 20 KV.

On the pictures taken, chlamydospores and hyphae, embedded in their matrix, may be seen (Fig. 5).

Discussion

Few experiments have been carried out to investigate the pathogenesis of mycetomas [1], and the mechanisms involved are not fully understood [11].

The low number of reported eumycetomas in lower animals is in sharp contrast to the ubiquity of the isolated agents. This might be attributed to the fact that mycetomas are rarely diagnosed, especially if a surgical excision of a tumefaction results in remission and no histological or mycological examination is performed. The chronic evolution of the disease might contribute to this state of affairs, as two tumefactions, the appearance of which was divided by a long time lapse, might not be associated with one another.

The difficulty in the reproduction of the typical lesion [1, 11] indicates that the above mentioned reason alone is not enough to explain the rarity of mycetomas. The limited number of fungal species which cause mycetomas in animals suggests that pathogenicity factors typical of such species are involved. It is clear, however, that such factors can overcome the host's defenses only in rare cases. One such case might be the result of repeated and frequent traumatic exposure to the fungus inducing a state of low zone tolerance [12]. Such an exposure is more than probable in our case, considering the abundance of the causative agent on the cacti in the dog's environment.

Itraconazole is a relatively new antimycotic drug, belonging to the group of the triazoles. Its antifungal activity, similarly to other azoles, results mainly from the inhibition of ergosterol biosynthesis by binding to cytochrome P-450. The lack of side effects of itraconazole might result from its weak affinity to mammalian P-450, in contrast to another azole used for systemic antimycotic therapy, ketoconazole [13]. Another advantage of itraconazole is it's wide tissue distribution (approximately 20 times that of ketoconazole) and a better bioavailability [13]. The good initial therapeutic results in the case reported hereby might result from these characteristics of the drug. The reason for the drug's lack

of activity during the second treatment is unclear. The interpretation of the inhibition zone's diameter around the itraconazole sensitivity disc is difficult as no universally accepted standards for such interpretation exist. It is likely, however, that a 30 mm inhibition zone indicates that the *C. lunata* isolate was sensitive *in-vitro* to itraconazole. This would exclude resistance of the fungus to the antimycotic drug as cause of the second treatment's failure. It is possible that the first treatment's interruption was premature and that its continuation could have averted the recurrence of the fungal infection. We believe, therefore, that itraconazole shows some promise in the treatment of mycetomas and that further clinical trials with this drug should be made.

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