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BLACK GRAIN MYCETOMA (MADUROMYCOSIS) IN HORSES

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ABSTRACT

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Black grain mycetoma occurred in two horses, one a cross-bred pony and the other a Thoroughbred racehorse. Lesions were limited to wart-like growths in one case and a deep-seated girth gall in the other. In both cases the granules in the affected tissues were similar in pigmentation and structure to those produced by *Curvularia geniculata* in dogs. Since all the specimens were received in formalin, the causative fungus could not be isolated. These are the first cases of maduromycosis in animals to be recorded in South Africa.

Résumé

GRANULOMYCÉTOME NOIR (MADUROMYCOSE) CHEZ DES CHEVAUX

Un granulomycétome noir s'est manifesté chez deux chevaux: un poney hybride et un cheval de course pur-sang. Les lésions se limitaient dans le premier cas à des excroissances verruqueuses et dans le second à une excoriation sous-ventrière profondément enracinée. Dans les deux cas les granules dans les tissus atteints offraient une pigmentation et une structure semblables à celles produites chez le chien par Curvularia geniculata *mais, comme les spécimens reçus avaient été conservés dans le formol, il n'a pas été possible d'isoler le champignon responsable de ces lésions. Ces cas sont les premiers exemples de maduromycose animale qu'on ait observés en Afrique du Sud.*

INTRODUCTION

Cases of Madura foot in man were first described by Godfrey (1846) in Madura, India. Carter (quoted by Bridges, 1957) in 1886 identified a fungus as the causative agent and introduced the term "mycetoma". The term "maduromycosis" was introduced to differentiate the true mycetomas, caused by fungi classified as Ascomycetes and Deuteromycetes, from those caused by *Actinomyces* and *Nocardia* (Pinoy, 1913). From a therapeutic point of view it is important that the eumycotic (maduromycotic) mycetomas be differentiated from the actinomycotic mycetomas, since, while the latter may be controlled successfully with antibiotics, the true fungi resist practically all forms of therapy except total surgical excision (Bridges, 1957; Emmons, Binford & Utz, 1970).

Some 11 species of fungi and 6 species of aerobic free-living Actinomycetes have been incriminated in human cases of mycetoma (Mahgoub & Murray, 1973). Within the affected tissues the organisms occur in the form of discrete microcolonies, termed granules, and with some experience the different types of granules can be assigned fairly accurately to the causative agents. The dark granules or black grains are formed exclusively by dematiaceous Hyphomycetes, while the light granules comprise Actinomycetes or Hyphomycetes with hyaline mycelium. Separation of the latter groups is based on the diameter of the filaments being less than 1 μm in Actinomycetes. Other details of the granules, such as size, shape and structure, are important for specific identification (Mahgoub & Murray, 1973).

In spite of the multiple and diverse aetiologies, mycetoma is regarded as a definite clinical and pathological entity (Emmons *et al.*, 1970). The disease, involving mainly the skin and subcutaneous tissues, is chronic and of long duration. The lesions usually occur on the extremities or on those sites which are frequently subject to trauma. Moreover, the lesions are invariably granulomatous and contain granules of the causal organism. The disease has long been known in man, and cases in animals were described as early as 1925 by Krishnamurti (cited by Davis & Shorten, 1936). With one exception, in Turkey (Akun, 1953), all the reported cases have so far occurred in North America. This is the first report of eumycotic mycetoma in animals in South Africa.

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MATERIAL AND METHODS

Formalin-fixed biopsy material of suspected tumours from 2 horses was submitted for routine histological examination. Horse 1, a racehorse, developed a girth gall which extended between the ventral thoracic muscles and appeared deep-seated. Horse 2, a cross-bred pony, showed wart-like growths on the skin of the neck, shoulders and front limbs above the metacarpal joints. Since the lesions were black in colour, a tentative diagnosis of melanosarcoma was made. No unpreserved material was available for the isolation and identification of the organisms involved.

Tissues were embedded in paraffin wax, sectioned at 5 μm thickness and stained with haematoxylin and eosin (HE). To demonstrate the fungus, sections were also stained with the periodic acid—Schiff reaction (PAS) (Pearse, 1961), Mayer's mucicarmine (MM) (Luna, 1968), Gomori's methenamine silver nitrate (GMS) (Luna, 1968) and the Humberstone modification of the Gram stain (Luna, 1968).

RESULTS

Horse 1

Macroscopic examination of the specimens from the racehorse showed small, irregular, black spots scattered in a greyish-white substance (Fig. 1). Histological examination revealed a marked subcutaneous granulomatous reaction surrounded by a fibrous capsule. Connective tissue septae extended from the peripheral capsule, dividing the granuloma into discrete pockets. Aggregations of a dark-brown fungus were found mainly at the edges of the pockets. The central area contained many neutrophils, occasional degenerative organisms and small numbers of other inflammatory cells. Colonies of the fungus were surrounded by histiocytes, Langhans-type giant cells, lymphocytes, fibroblasts and a few plasma cells (Fig. 2). Many of the histiocytes contained brown-black

pigments, while phagocytosed organisms were found in some of the giant cells. Fibrin strands sometimes occurred between the inflammatory cells.

The fungal colonies varied from small groups of dematiaceous cells to large, distinctive granules which often occurred in the form of horseshoe and scroll-like configurations (Fig. 3). The granules consisted primarily of vesicular cells and chlamydospores embedded in an eosinophilic amorphous matrix (Fig. 4). Rudimentary hyphae were only occasionally observed near the central zone of the granule (Fig. 5). The thick-walled chlamydospores often contained one or more septae. The fungus stained intensely black with GMS, positive with Humberstone's Gram stain, moderately positive with PAS and negative with MM.

Horse 2

Specimens from the cross-bred pony were homogeneously greyish-white on cut surface. Microscopic examination revealed that the fungus had developed in the host's tissue in the form of isolated cells and small granules which were not visible with the unaided eye.

In pigmentation and other morphological aspects the fungus was similar to the one which occurred in the first case (Fig. 6). There were no essential differences in tissue reaction in the 2 cases.

Discussion

The literature carries several reports of cases of granulomatous dermatitis in domestic animals, most of which were classified according to the categories used for similar infections in man. Akun (1953) associated the lesions in the nasal cavity of a horse in Turkey with chromoblastomycosis, basing his diagnosis on the presence of dark-coloured septate bodies in the affected tissues. Similar structures in lesions in horses as well as in dogs and a cat were reported by Seibold (1955), Bridges (1957), Bridges & Beasley (1960) and Brodey, Schryver, Deubler, Kaplan & Ajello (1967) in the United States of America. The infections were described as eumycotic (maduromycotic) mycetoma, because in their hosts the fungi developed as granules or, in some cases, as small groups of cells which were interpreted as granules. There was a definite correlation between the *in vivo* and *in vitro* characteristics of the causal species in those cases where cultural studies were made. In 2 dogs with lesions containing vesicular cells and chlamydospores organised into well-defined granules, the aetiological agent proved to be *Curvularia geniculata* (Bridges, 1957; Carter, 1861, cited by Bridges, 1957). The fungus which appeared single or as small groups of septate hyphae in lesions in a horse and a cat was isolated and identified as *Brachycladium spiciferum* (Bridges & Beasley, 1960). According to Ellis (1971), however, the proper name for this species is *Drechslera spicifera*.

Only 1 recognised agent of eumycotic mycetoma in man has as yet been incriminated as a cause of this disease in animals. The fungus, *Allescheria boydii*, was isolated from a chronic eczematous skin lesion of a dog by Kurtz, Finco & Perman (1970) and from a chronic uterine lesion of a mare by Reid, Jeffrey & Kaiser (1976).

Lesions of mycotic origin but without granules have also been described in cattle. Several investigators (Bridges, 1960; Creech & Miller, 1933; Davis & Shorten, 1936; Dikmans, 1934; Roberts, McDaniel & Carbey, 1963) have described this disease as nasal granuloma or maduromycosis of the bovine nasal mucosa. Muller, Kaplan, Ajello & Padhye (1975) do not believe that these cases of bovine nasal granuloma can be classified as mycetoma and suggested that they be regarded as cases of phaeohyphomycosis. This disease was defined by Ajello, Georg, Steigbigel & Wang (1974) as "infections of a cutaneous, subcutaneous and systemic nature caused by hyphomycetous fungi that develop in the host's tissue in the form of dark-walled (dematiaceous) septate mycelial elements". Muller *et al.* (1975), however, considered phaeohyphomycosis as a disease category that also includes infections caused by *D. spicifera*, a view shared by Kaplan, Chandler, Ajello, Gauthier, Higgins & Cayouette (1975).

The available literature indicates that no species other than *C. geniculata* has so far been isolated from black grain mycetoma in animals. Although the granules in the present cases varied considerably in size and shape, their structure and pigmentation are similar to those of *C. geniculata* as described and illustrated by Bridges (1957) and Brodey *et al.* (1967).

There is strong evidence in these cases that the infections occurred as a result of fungal implantation into the tissues at the site of an injury. This is illustrated in Horse 1, in which the initial girth gall developed into a mycetoma. The lesions in the case of Horse 2 occurred on the forequarters which are more prone to injury, especially under ranching conditions.

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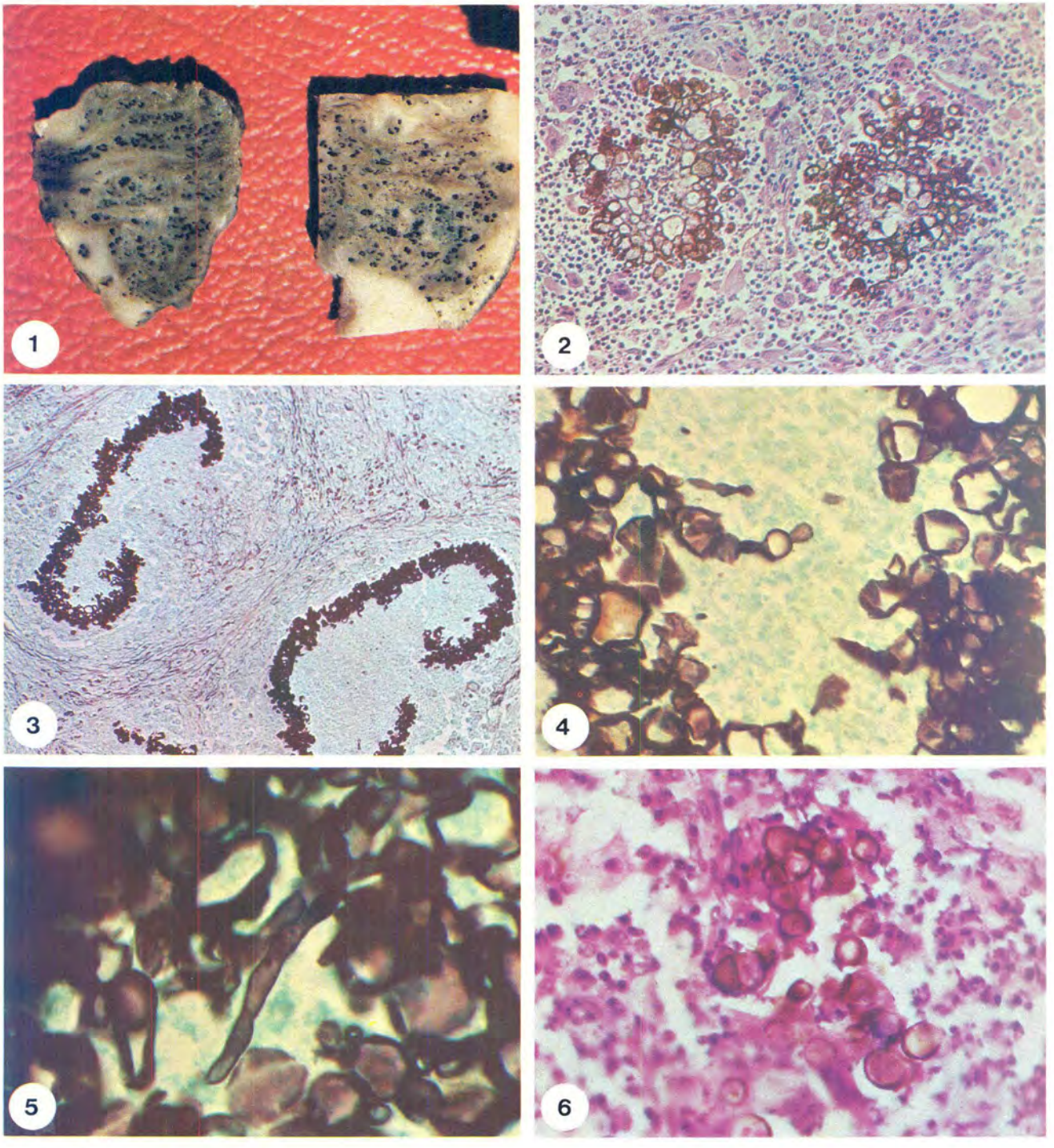

FIG. 1 Black grain mycetoma from a Thoroughbred racehorse
FIG. 2 Round colonies of the fungus, with necrotic centres and neutrophil infiltration. Langhans' type giant cells and histiocytes are visible around the periphery. Thoroughbred racehorse, HE × 65
FIG. 3 Scroll-like colonies of the fungus surrounded by a fibrous capsule. Thoroughbred racehorse, GMS × 25
FIG. 4 Vesicular cells and chlamydospores. Thoroughbred racehorse, GMS × 160
FIG. 5 Segmented hypha in the central zone of a granule. Thoroughbred racehorse, GMS × 400
FIG. 6 A small granule and some isolated fungal cells from the lesion of the cross-bred pony, HE × 160

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