

THE ARMADILLO *EUPHRACTUS SEXCINCTUS* AS A SUITABLE ANIMAL FOR EXPERIMENTAL STUDIES OF JORGE LOBO'S DISEASE

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S U M M A R Y

The aetiological agent of Jorge Lobo's disease produced in *Euphractus sexcinctus*, an armadillo provenient from the Northeastern Brazil (Pernambuco) a nodular lesion measuring about 4 x 3 x 3 cm, after eleven months, at the site of the inoculation. The microscopic aspect of the lesion was the same as that in the human disease, but additional areas of liquefactive necrosis were also noted. This armadillo seems to be a suitable animal for experimental studies of Jorge Lobo's disease.

I N T R O D U C T I O N

Since the original description by Jorge Lobo of a deep mycosis in a patient from the Amazon Region¹⁰, many attempts have been made to reproduce the disease experimentally^{1,2,4,6,7,8,9,10,11,12}.

The Authors have used several animals as well as different inoculation sites with some success such as in the cheek-pouch of the hamster (*Mesocricetus auratus*)¹¹, and subcutaneous tissue of *Chelonia* from the Amazon Region (*Geochelone denticulata*, *G. carbonaria* and *Sternoides scorpioides*)¹².

Encouraged by the successful inoculation of leprosy in the armadillo *Dasypus novemcinctus*⁵ we decided to inoculate this species as well as *D. septemcinctus* and *Euphractus sexcinctus* with fungal cells of Jorge Lobo's disease.

Due to our little experience in keeping the animals in captivity most of our experimental animals died or were lost and we were only able to draw definite conclusions from one specimen of *Euphractus sexcinctus*.

MATERIAL AND METHODS

A rich suspension of fungal cells was prepared by scrapping and repeated cutting of

biopsy material in physiological saline obtained from a lesion of a patient who was described by us in 1970⁸ (case 1) whose infection has lasted for 25 years. 0.5 ml of this suspension was inoculated subcutaneously into the pelvic region of an adult, male, armadillo *Euphractus sexcinctus* (Fig. 1A).

The armadillo was from the Northeastern Brazil (State of Pernambuco where his species is common, and was kept in captivity in a cage which cemented floor and fed on bread, powdered milk and chicken's balanced diet.

The skin overlaying the lesion began to ulcerate due to rubbing on the cage's cemented floor. In order to prevent secondary infection it was decided to kill the animal eleven months after the inoculation.

When killed, the animal was apparently in very good health.

Fragments of the nodule were fixed in 10% formalin for histopathological studies in paraffin sections.

RESULTS

A palpable nodule was felt at the injection site five months after the inoculation. This nodule grew to the size of a small lemon, protruding at the skin surface (Fig. 1 B).

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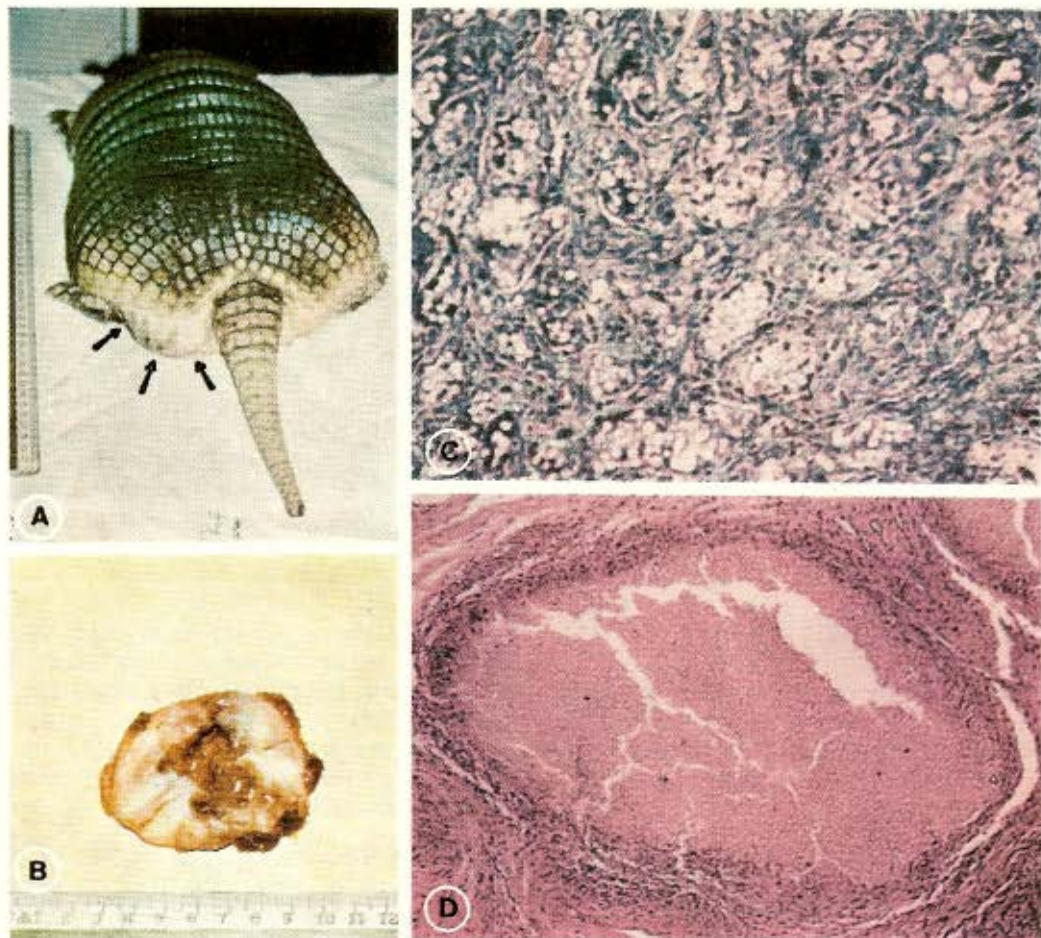


Fig. 1 — A) The armadillo *Euphractus sexcinctus* with a nodule (arrows) developed eleven months after a subcutaneous inoculation of Jorge Lobo's fungi. B) Surface of the section of the nodule with a large cavity at its center. The edge of the lesion is formed by a pulpaceous, shining tissue, with strands of fibrous aspect. C) Predominant microscopic aspect, with multiple confluent granuloma, with plump giant cells with numerous fungal cells in their cytoplasm, Gomori's trichrome stain (200 X). D) Lesion with large necrosis and numerous fungal cells scattered within the necrotic material (H. & E., 70 X).

The nodule, measuring about 4 x 3 x 3 cm was removed and was found to lie in the deep derma and subcutaneous tissue. There was no capsule and its margins were ill defined or limited by discontinuous strands of dense connective tissue. The overall aspect in H. & E. and Gomori's trichrome stain were of a lesion formed by a granulomatous process with a shining, pulpous, slightly lobulated tissue with greasy areas at its outermost layer. A central cavity with undulating margins was filled with a dense, viscous, brown yellow material which consisted of myriads of single chains of fungal cells either free or more com-

monly engulfed by macrophages or giant cells.

Outside the lesion, in the adipose tissue, there were foci of infiltrating mononuclear cells, chiefly lymphocytes and a few eosinophils.

The predominant feature was of extensive areas of confluent granuloma or granuloma which were beginning to form and whose centres were composed of a giant cell surrounded by histiocytes that mingled with those of neighbouring granuloma (Fig. 1 C and 2). Irregular thin strands of connective tissue separated very small pseudonodular

areas. The giant cells were either clearly seen or they became mere suspects due to the exaggerated number of fungi in their cytoplasm or they appeared to be ruptured allowing free parasites into the interstitial or intercellular spaces (Fig. 2 C and D). A few lymphocytes, rare plasma cells and eosinophils were found amongst the histiocytes. They were sometimes plump with many nuclei and their cytoplasm was either empty or contained one or more fungal cells. Necrosis occurred in certain areas either in its initial phase or involving deep, extensive inflammatory tissue (Fig. 10). In the initial phases, there was dissociation of the cells with signs of cellular damage accompanied by varying degrees of neutrophilic infiltration (Fig. 2 E and 3 D). Complete necrosis was also noted. In the space formerly occupied by inflammatory tissue there was an amorphous eosinophilic mass containing chromatinic remnants and myriads of fungal cells. The whole area was surrounded by connective tissue which did not form a marked capsule (Fig. 1 D, 2 E and 2 F). Liquefactive necrosis also occurred producing a cavity containing red blood cells mixed with other components when associated with hemorrhage (Fig. 2 E). In a few instances histiocytes and giant cells were found at the periphery of the cavities of the necrotic foci giving them a cystic appearance (Fig. 3 A, B and 3 C). Some necrotic foci contained a dense mass resembling cholesterol crystals mixed with fungi (Fig. 2 F).

The large reaction areas were bound together by connective strands in which there were isolated or grouped granuloma in small numbers, which tended to merge with each other. The small, more individualized granuloma, had a giant cell in their centre which was surrounded by many histiocytes with or without lymphocytes and plasma cells (Fig. 2A). At the outermost layer of these small granuloma there were cells and collagen fibers (Fig. 2B). In the larger or confluent granuloma, histiocytes were abundant and contained many nuclei as well as occasional fungi in their cytoplasm (Fig. 2B, C). Some lesions of this type showed signs of cellular damage and necrosis with tissue dissociation and cavitation and secondary neutrophilic infiltration (Fig. 2B, C e D and E).

The giant cells tended to be plump with

numerous nuclei and they were of Langhans or foreign body types. Their cytoplasm had abundant or innumerable fungal cells (Fig. 2 A, D and 3 C).

The fungal cells in sections of the necrotic areas were either single or exhibited simple budding or, more rarely, formed chains of 3 to 4 parasites. However, fresh microscopic examinations of the contents of cavities resembled a culture because of the immense numbers of individual cells and the presence of chains of 5, 6 or more fungal cells. It is to be noted that besides a simple budding, a few of them had 2 or more buds.

The connective tissue of septal or peripheral strands had portions with abundant histiocytes, as well as a few lymphocytes, plasma cells and eosinophils. The dense connective strands had more cellular elements and the collagen fibers which tended to hyalinization were more abundant. Fungi phagocytized by histiocytes, giant cells or even initiating granuloma could be seen in these areas.

There was no invasion of the subjacent muscular tissue.

Two lymph nodes were removed from neighbouring areas. One of them had histiocytes containing fungi in the marginal and medullary sinuses, and forming granuloma with giant cells.

DISCUSSION

The lesion of the armadillo described by us was larger in volume and contained more fungal cells than experimental infections seen by us in hamster¹¹ or *Chelonia*¹². In man, lesions in accidental infections² or those in a volunteer⁴ were not comparable to the lesion in *Euphractus sexcinctus*.

The pathology of the *Euphractus sexcinctus* lesions differed, in part, from that observed in man. The Authors agree that in human lesions there is a more productive component without suppuration or necrosis. However, BARUZZI et al.³ noted, in ten cases described amongst Caibi Indians, one with a focal necrotic lesion of small size, and neutrophilic infiltration. In natural infections of Jorge Lobo's fungus in the dolphin (*Tursiops truncatus*) CADWELL et al.⁶ observed extensive ne-

crisis accompanied by neutrophilic infiltration of a few nodules, including cholesterol clefts similar to the ones we describe in this paper. In hamsters and *Chelonia* the lesions we have reported^{11,12} were millimetric and of a productive character.

In view of the relatively low virulence of this fungus in man which in a quarter of a century causes a slowly progressive disease we conclude that the armadillo *Euphractus sexcinctus* is more susceptible than man to the fungus of Jorge Lobo's Disease. With this in

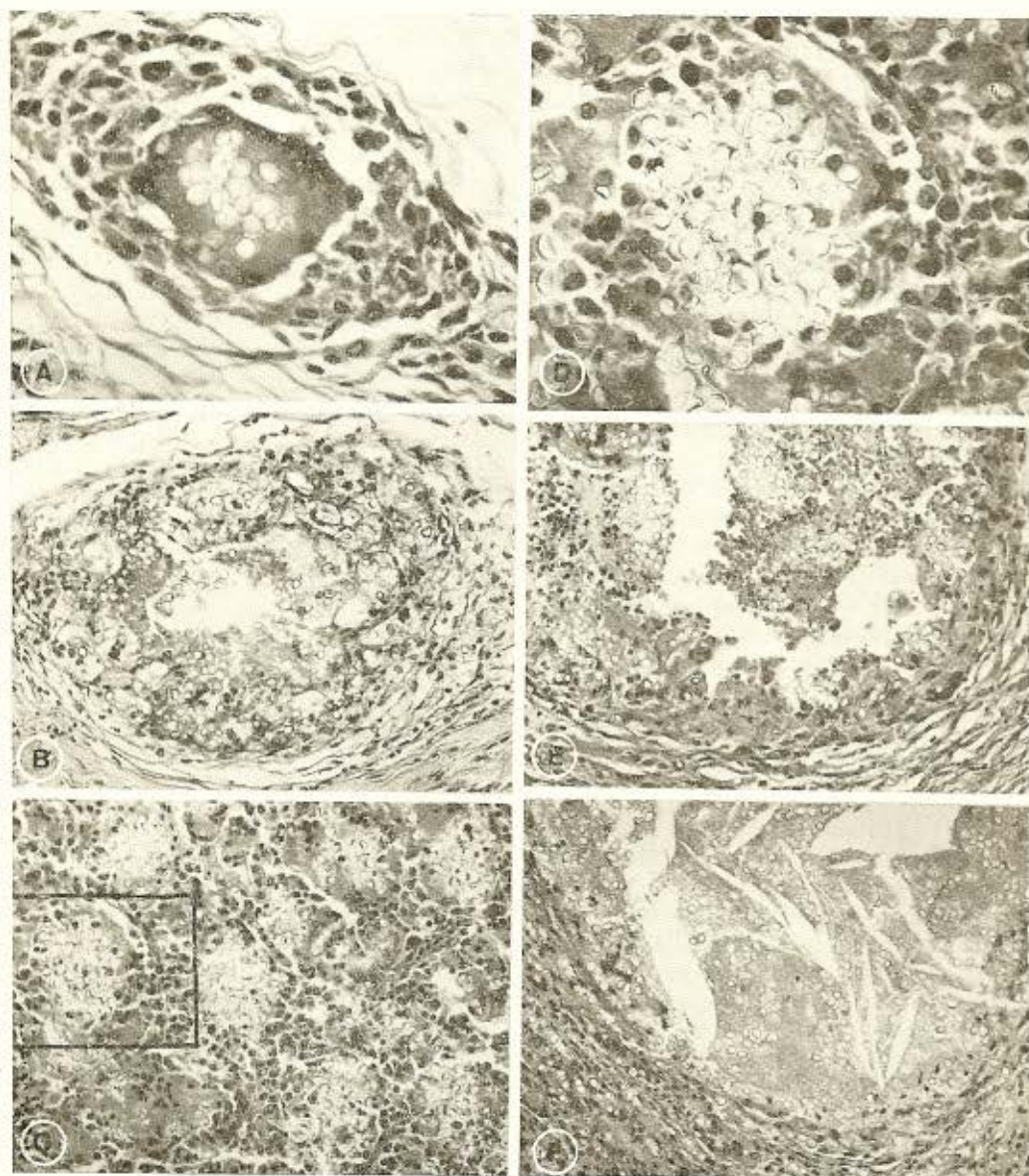


Fig. 2 — A) An isolated granuloma, with a Langhans giant cell with many fungal cells (H. & E., 320 X). B) A larger granuloma with its necrotic excavated center. Abundant parasitized histiocytes and free fungi randomly dispersed into forming cavity (H. & E., 170 X). C) Area with confluent granuloma with ill defined limits. Note giant cells with fungi (H. & E., 170 X). D) Detail of Fig. C with rupture of the giant cell and extravasation of fungi (H. & E., 320 X). E) Necrosis and cavity formation of another inflammatory area with associated hemorrhage (H. & E., 170 X). F) Completely necrotized lesion with cholesterol cleft-like crystals and numerous parasites. A strand of connective tissue with abundant histiocytes with phagoctyized parasites, can be seen at the periphery (H. & E., 170X).

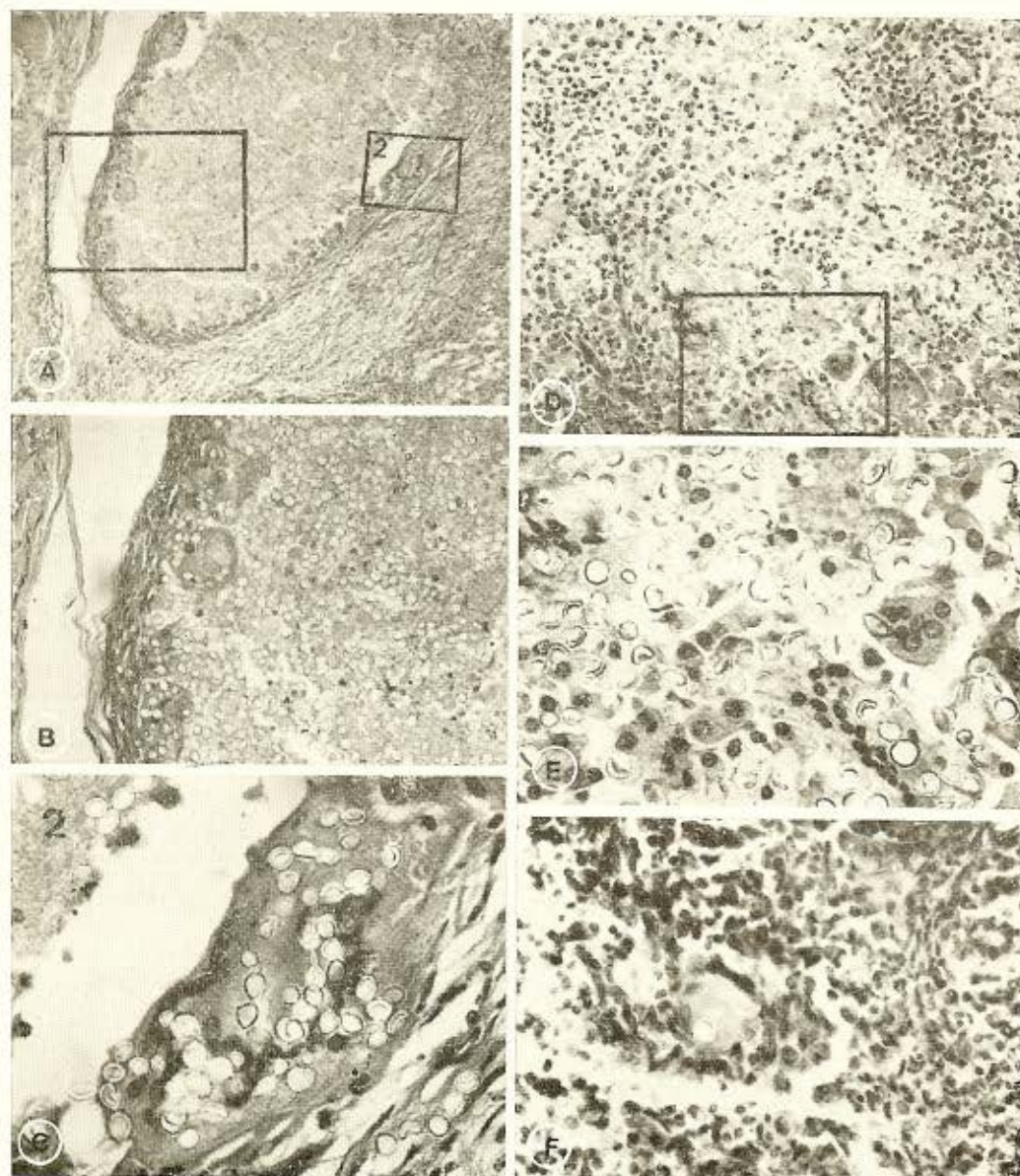


Fig. 3 — A) Necrotic area of pseudo-cystic character, with the presence of giant cells at its walls. Note at the arrow a dense connective strand. (H. & E., 50 X). B) Detail of the squared area of Fig. 1A pointing out the abundance of fungal cells into the cavity (H. & E., 170 X). C) Detail of squared zone of Fig. 2 A showing a parasitized giant cell (H. & E., 320 X). D) Necrosis and initiating cellular dissociation turning free the parasites in tissue spaces. Note neutrophilic infiltration (H. & E., 170 X). E) Detail of square of Fig. D pointing out to fungi (H. & E., 320 X). F) Lymph node with a forming granuloma, with a parasite inside a giant cell (H. & E., 320 X).

mind we think that *Euphractus sexcinctus* may be considered as a suitable animal for experimental studies of Jorge Lobo's disease.

RESUMO

O tatú *Euphractus sexcinctus* como animal

adequado para o estudo experimental da Doença de Jorge Lobo

O agente etiológico da Doença de Jorge Lobo produziu em tatú (*Euphractus sexcinctus*) proveniente do Nordeste do Brasil (Per-

nambuco), 11 meses após e no local da inoculação, lesão nodular medindo cerca de 4 x 3 x 3 cm, com aspecto microscópico semelhante ao da doença humana, acrescido de áreas de necrose coliquativa. O crescimento do nódulo foi rápido e proporcionalmente maior que o observado no homem e nos animais de laboratório já utilizados. Essa espécie de tatú parece ser adequado para o estudo experimental da Doença de Jorge Lobo.

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