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ADIASPIROMYCOSIS IN STRIPED SKUNKS IN ALBERTA, CANADA

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ABSTRACT: Pulmonary adiaspiromycosis was diagnosed in seven of 25 striped skunks (*Mephitis mephitis*) in east-central Alberta. The infection varied from mild, where only microscopic lesions were seen, to severe, where gross lesions of grayish-white nodules were observed in the lung parenchyma. Mild lesions were restricted to the lung, while severe lesions extended to the tracheobronchial and mediastinal lymph nodes. Histologically, the lesions were characterized by a centrally located fungal spherule, surrounded by granulomatous inflammation. The morphology of the fungal spherules was consistent with that of *Emmonsia crescens*. By electron microscopy, the fungal cells had an outer thick fibrillar wall and an inner cytoplasm filled with large lipid vacuoles with relatively few mitochondria, ribosomes or glycogen inclusions. The absence of endosporulation and budding suggested that each fungal cell in the lung represented a separate inhaled spore. Infection was by inhalation, nevertheless adiaspores may disseminate to the regional lymph nodes.

INTRODUCTION

Adiaspiromycosis, previously known as haplomycosis, is a mycotic pulmonary disease of many small wild mammals caused by fungi of the genus *Emmonsia* (Jellison, 1981; Jones and Hunt, 1983). In 1962 the agents of adiaspiromycosis were reclassified under the genus *Chrysosporium* (Carmichael, 1962). In this paper we used the alternative genus designation *Emmonsia*. The fungal infection has been reported in wild rabbits, rodents, and carnivores (including skunks) in Montana and Argentina (Jellison, 1950; Jellison and Lord, 1964). Surveys have indicated that adiaspiromycosis is found commonly in the white-footed mouse (*Peromyscus maniculatus*) in western Canada (Dowding, 1947; Bakerspigel, 1956). Ultrastructural studies of adiaspiromycosis in experimental animals and a naturally infected human have been reported (Malinsky et al., 1972; Watts et al., 1975; Hejtmanek, 1976). The present report describes light and electron microscopic findings in lungs and lymph nodes of striped skunks naturally infected with *Emmonsia* sp.

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MATERIALS AND METHODS

Twenty-five striped skunks were trapped in east-central Alberta during the spring, summer, and fall of 1983. The animals were killed at different times after capture. Tissue samples obtained at necropsy were fixed in neutral buffered 10% formalin and embedded in paraffin. Six μm sections were stained with hematoxylin and eosin. Selected lung tissues were stained with periodic acid-Schiff reagent (PAS) and Grocott's methenamine-silver techniques. For detailed observation of the mycotic agent, formalin-fixed lung tissue was selected from a representative case and processed for electron microscopy (Weakley, 1981).

RESULTS

Pulmonary fungal spherules were noticed incidently in seven of 25 skunks. The infection varied from mild, where only a few spherules were seen with the light microscope, to severe where gross lesions could be recognized. Severely infected lungs had multiple, grayish-white nodules, 0.5 to 1 mm in diameter, spread throughout the lung (Fig. 1).

Histologically, the morphology of the fungal spherules was consistent with *Emmonsia crescens*. The fungal cells were round, 60 to 150 μm in diameter, had a thick wall, and some appeared "double contoured." A thin refractile or an irreg-

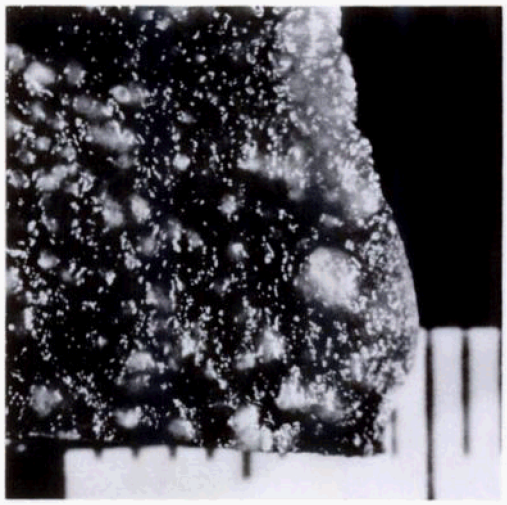

FIGURE 1. Formalin-fixed lung of mature striped skunk heavily infected with adiaspores. Note multiple nodules distributed throughout the lung.

ular eosinophilic membrane appeared to extend from the outer surface of the wall, making direct contact with the inflammatory cells (Fig. 2). A few fungal cells were empty, but most had a central mass composed of small globules and fine granular pale basophilic material. The central mass formed a dense basophilic material at its attachment to the wall (Fig. 2). A few spherules had crescent shaped structures located eccentrically in the cell. They were composed of small round basophilic particles in an eosinophilic or light basophilic finely granular background. These basophilic particles presumably represented nuclear material surrounded by eosinophilic cytoplasm. No budding or endosporulation was seen. The wall and inner globules stained purple by PAS and black by Grocott's methenamine-silver techniques.

The inflammatory reaction around the fungal cells varied among spherules in the same lung section (Fig. 2). Some were surrounded by a few macrophages and lymphocytes, whereas a number of other spherules were surrounded by well developed granulomatous inflammatory tissue composed of epithelioid cells, macrophages, lymphocytes, plasma cells, a few scattered neutrophils, and fibrous tissue (Fig. 2). Multinucleated giant cells were also seen in a few granulomas. Fragments of fungal cell walls were seen in a few instances, mostly associated with well developed granulomatous reaction along

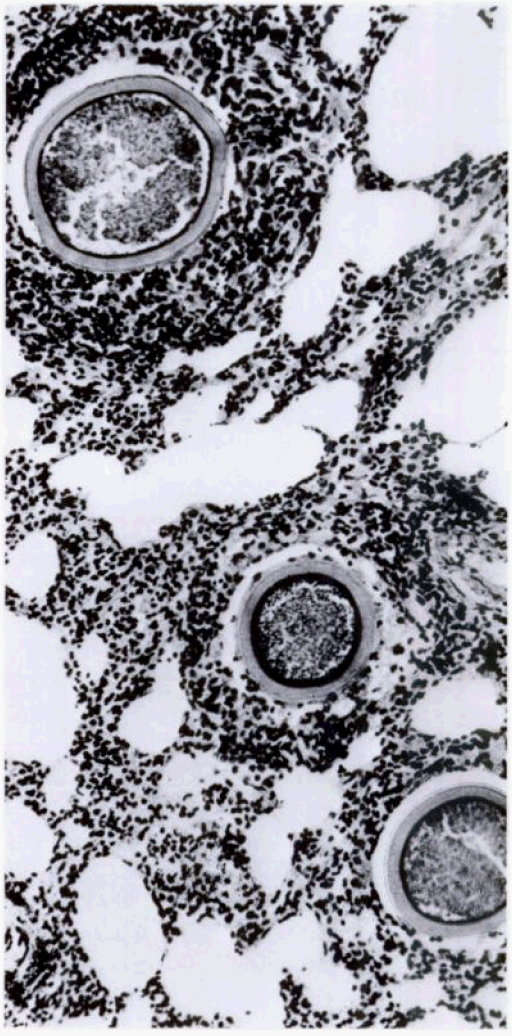

FIGURE 2. Histological section of three fungal spherules in the lung of a striped skunk. Note the thick fungal cell wall with globular central mass and variable inflammatory reaction around fungal cells. H&E, ×109.

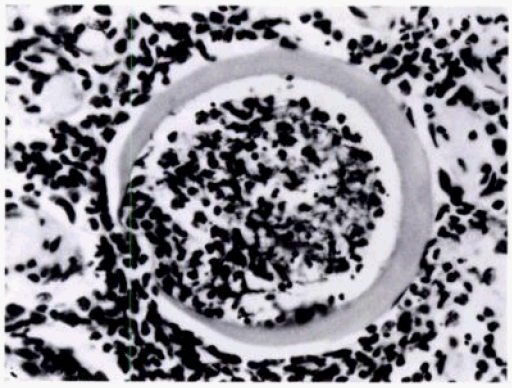

FIGURE 3. Histological section of a fungal spherule in the lung of a striped skunk. Note focal thinning and destruction of the wall with marked inflammatory cell infiltration. H&E, ×435.

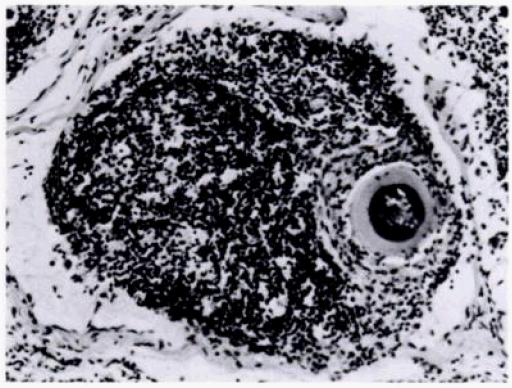

FIGURE 4. Histological section of a fungal spherule in the mediastinal lymph node of a striped skunk. Note the mild granulomatous inflammatory reaction around the spherule. H&E, ×109.

with a few scattered neutrophils. The early signs of fungal destruction by inflammatory cells were reflected by focal destruction of the thin refractile outer layer. The process continued by focal thinning and digestion of the wall leading to complete lysis of the fungal cell wall and destruction of the cell cytoplasm with marked leukocytic infiltration (Fig. 3). Most inflammatory cells that had infiltrated the fungal cytoplasm were necrotic or at various stages of degeneration.

In one lung section, a fungal cell was seen in the terminal bronchiolar lumen with no inflammatory reaction accompanying it. In a heavily infected skunk, fungal spherules were seen in the tracheobronchial and mediastinal lymph nodes with mild to moderate granulomatous inflammatory reaction around the fungal cells (Fig. 4).

By electron microscopy, the spherule wall was 6.5 to 7 μm thick, and was composed of fibrils of different electron densities that could be divided into three arbitrary layers. Starting from the cytoplasm, the first layer had predominantly fine pale fibrils that extended up to half of the wall thickness. The second layer had thicker fibrils and appeared more electron dense, thus separating the two layers. The outer layer appeared as irregular heterogeneous fibrils of high electron density, attached to the primary fungal cell wall (Figs. 5, 8). The central part of the fungal cell cytoplasm contained many lipid vacuoles, a few rod shaped mitochondria, and free ribosomes (Figs. 5, 6). The mitochondrial cristae were compressed in the form of thin tubules, running parallel to the mitochondrial long axis. The peripheral cytoplasm was composed of numerous mitochondria, with closely packed cristae (Fig. 7). Glycogen granules, free ribosomes, and a few lipid vacuoles were also seen. Small dark clumps, consistent with glycogen granules, were dispersed in the central cytoplasm (Fig. 5).

The granulomatous inflammatory reaction was composed of macrophages, lymphocytes, plasma cells, and collagen fibers (Fig. 8). Most macrophages had a well developed endoplasmic reticulum and were often filled with large phagolysosomes (residual bodies) of heterogeneous electron density. The rough endoplasmic reticulum of a few plasma cells was distended with small Russell bodies (Fig. 8). Occasionally, focal thinning and digestion of the fungal cell wall by inflammatory

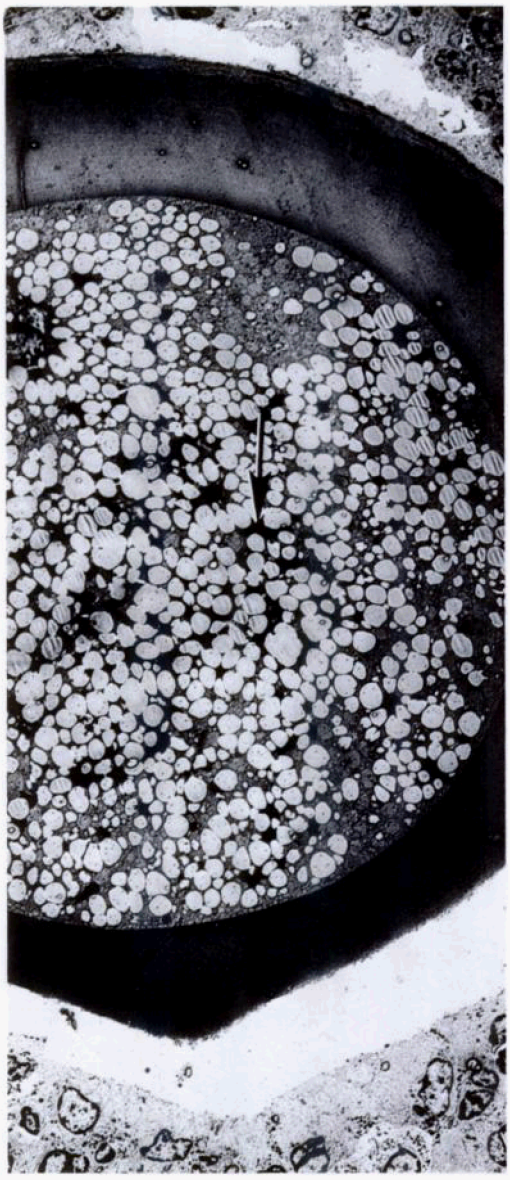

FIGURE 5. Electron micrograph of a fungal spherule in the lung of a striped skunk. Note the thick outer wall, large central lipid vacuoles, glycogen clumps (arrow) and outer mitochondria. Uranyl acetate and lead citrate, ×1,250.

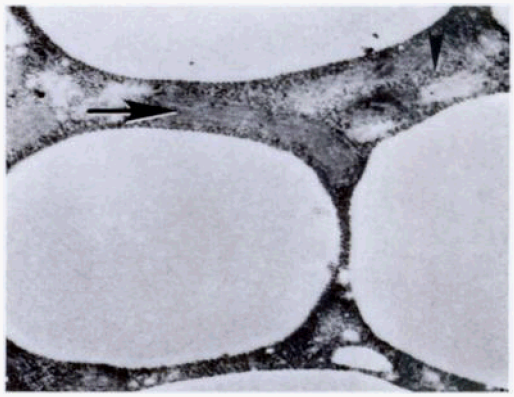

FIGURE 6. Higher magnification of the central fungal cell cytoplasm in the lung of a striped skunk. Note large lipid vacuoles, compressed mitochondria (arrow) and ribosomes (arrowhead). Uranyl acetate and lead citrate, ×18,000.

cells was observed, and necrotic debris was seen adhering to the spherule wall.

DISCUSSION

The results of this investigation indicate that adiaspiromycosis is prevalent in striped skunks in east-central Alberta. The absence of endosporulation and budding indicated that each fungal cell represented a single infective spore (aleuriospore).

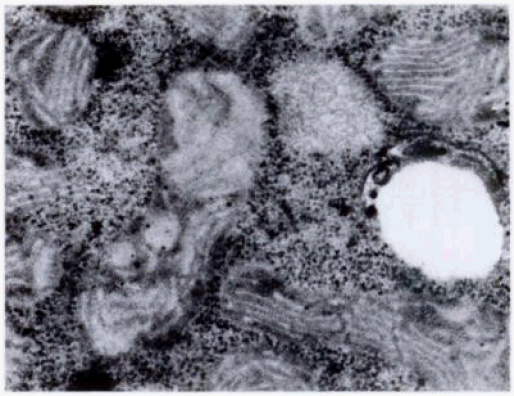

FIGURE 7. Higher magnification of the peripheral fungal cell cytoplasm in the lung of a striped skunk. Note many mitochondria, lipid vacuoles and dark glycogen granules. Uranyl acetate and lead citrate, ×30,000.

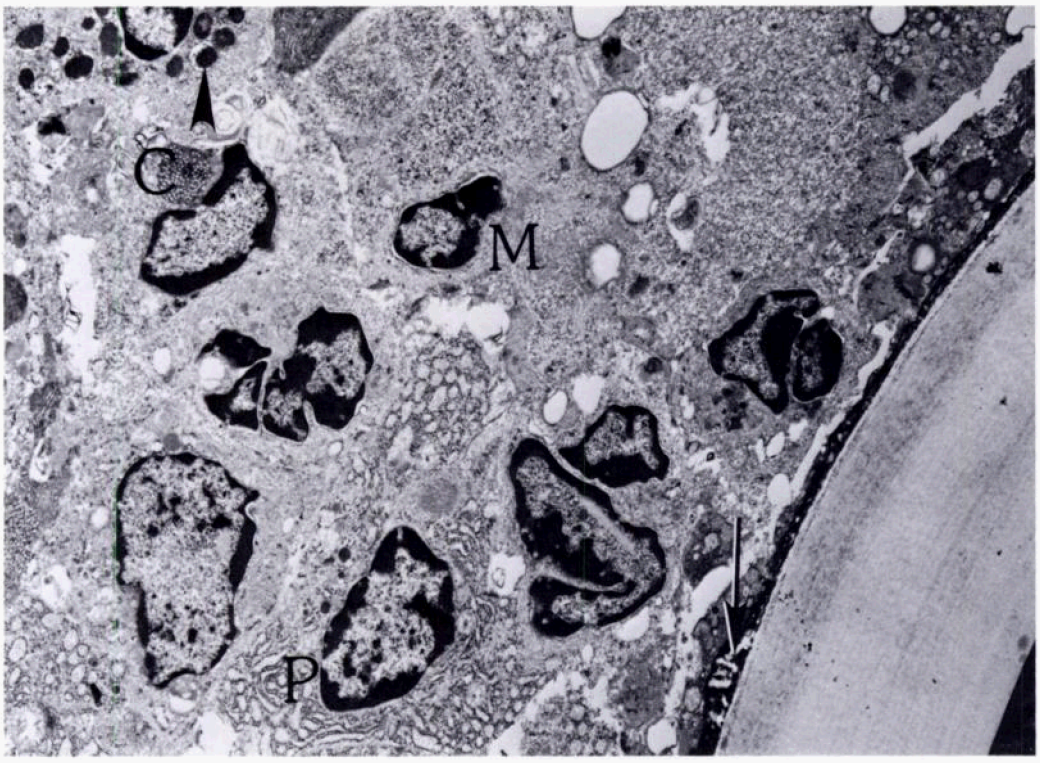

FIGURE 8. Electron micrograph of a fungal spherule wall in the lung of a striped skunk with inflammatory reaction around it. Note the different wall layers, outer heterogeneous dark layer (arrow), plasma cells (P), Russel bodies (arrowhead), macrophage (M) and collagen fibers (C). Uranyl acetate and lead citrate, ×5,775.

Infection was limited to the respiratory system, and free spherules were seen in the terminal airways. Therefore, the route of infection was likely through inhalation of spores. In this report, one animal had fungal lesions in the mediastinal lymph nodes. To our knowledge, this is the first report of adiaspiromycosis in the thoracic lymph nodes of a wild animal. A previous study (Al-Doory et al., 1971) reported a similar lesion in the axillary lymph node, in addition to the lungs, of a dog with osteoarthritis of the right forelimb. This investigation provides evidence that adiaspores can disseminate via lymphatics to the regional lymph nodes. Therefore, the infection is not restricted to the lungs, especially in heavily infected animals.

The absence of significant inflammatory reaction around some spherules in the lung might be a reflection of time. In experimental infections of mice with *E. parvum* (Ashburn and Emmons, 1945) and *E. crescens* (Malinsky et al., 1972) a fully developed granulomatous inflammatory reaction did not occur until several weeks after the spores reached the lungs. Moreover, all spherules present in the lungs of skunks may not be from a single exposure, but may represent repeated inhalation of aleuriospores. Thus, these adiaspores might be in different stages of maturity and elicit different inflammatory responses. Alternately, the variable reaction may reflect a low antigenicity of the adiaspore wall in this host.

Destruction of the spherules by inflammatory cells appeared to start focally by destroying the outer thin refractile layer. The process then continued by focal lysis and thinning of the wall leading to the destruction of the fungal cytoplasm. Electron microscopy provided further evidence that the outer layer of the fungal wall is covered by an irregular heterogeneous dark layer presumably formed by interaction between the host tissue and the fungus. This layer might act to protect the fungal cells from hydrolytic enzymes of the inflammatory cells and also from humoral defenses of the host.

A fenestrated layer lining the inner aspect of the middle zone of the fungal wall was demonstrated by light and electron microscopy in the lung of a man naturally infected with *E. crescens* in Guatemala (Watts et al., 1975). In the present study, no fenestrated layer was seen; this variation was probably due to morphological differences between the two fungal strains in Guatemala and Canada.

Nuclei, mitochondria, lipid vacuoles, endoplasmic reticulum and microbodies were recognized by electron microscopy at different stages of adiaspore maturity in experimentally infected mice (Malinsky et al., 1972). In this report no endoplasmic reticulum or nuclei were found in the spherules examined by electron microscopy. Absence of endoplasmic reticulum and nuclei in the examined sections can be attributed to one or both of the following reasons: (1) the examined spherules might have been sectioned at an angle that missed the nuclei and other organelles, and (2) the examined spherules may represent an advanced stage of maturation that was not reported in the experimental infection of mice.

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