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Besnoitiasis in Indiana opossums

Sherman W. Jack, William G. Van Alstine, Joann Swackhamer

During the spring of 1988, 6 opossums (*Didelphis marsupialis*) were necropsied. All were found dead along roads in Tippecanoe County, Indiana, and had lesions consistent with vehicular trauma (i.e., multiple lacerations, abrasions, and fractures). Four sexually mature opossums had numerous firm, white, up to 3-mm nodules distributed in skin, skeletal muscle, lung, heart, kidney, and adrenals (Fig. 1). Several nodules were squashed between 2 glass microscope slides, stained,³ and examined by light microscopy. The nodules contained myriads of 2 x 8- μ m and occasional 3 x 6- μ m coccidial zites but no leukocytes. The distribution and cytology of the lesions suggested *Besnoitia* spp.

Affected tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μ m, and stained with hematoxylin and eosin (HE) and Masson's trichrome stains. Histologic findings were similar to previous reports^{1,7} and confirmed the diagnosis of besnoitiasis. The nodules represented parasitic cysts that consisted of a single severely hypertrophied cell containing myriads of 2 x 8- μ m bradyzoites surrounded by a scant rim of eosinophilic host cell

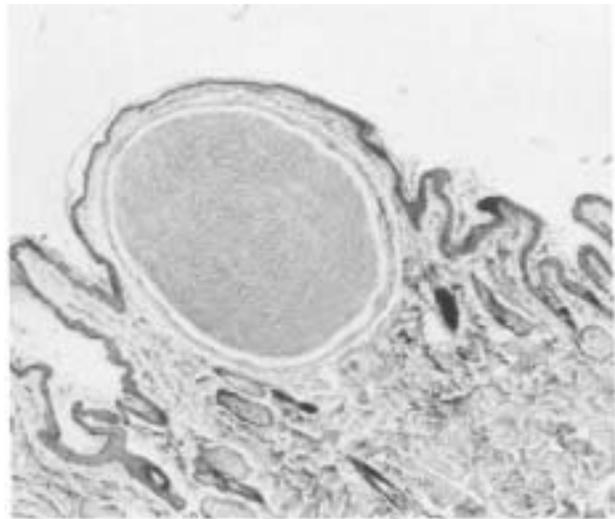


Figure 2. *Besnoitia* cyst in the skin of an opossum. HE.

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cytoplasm with occasional and sometimes multiple elongated host cell nuclei. Most cysts were surrounded by a 2-5- μ m rim of eosinophilic acellular connective tissue (collagen) with no inflammatory cellular reaction (Figs. 2, 3). Occasional

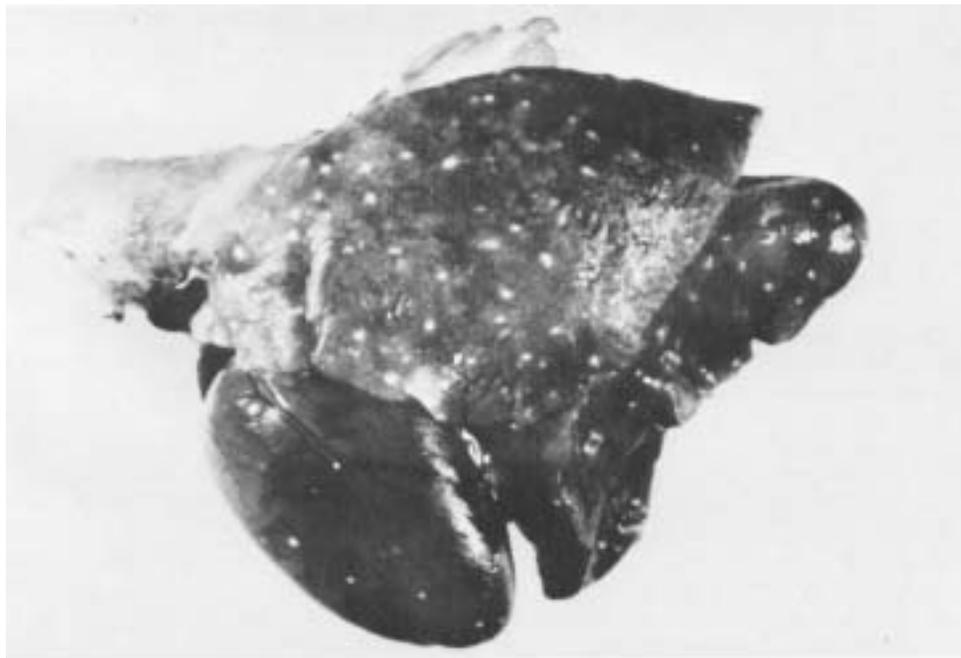


Figure 1. Heart and lungs of a female opossum.

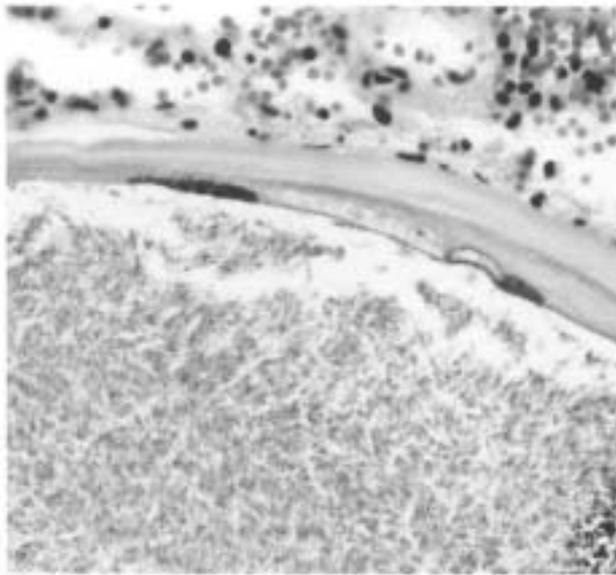


Figure 3. *Besnoitia* cyst in the lung of an opossum. HE.

cysts were collapsed, mineralized, and surrounded by moderate numbers of mononuclear leukocytes.

Nodules from lung were postfixed in 1% osmium tetroxide-1.5% potassium ferrocyanide, embedded in Epon, sectioned at 90-110 nm, stained with lead citrate and uranyl acetate, and examined under an electron microscope. Ultrastructural examination of cysts revealed numerous intracellular bradyzoites suspended in electron-lucent matrix within a parasitophorous vacuole (Fig. 4). Individual bradyzoites had outer and inner membranes and a tapered apical terminus, irregular scattered electron-opaque internal bodies, and abundant moderately electron-dense micronemes near the rounded terminus. The membrane of the parasitophorous vacuole was moderately electron-dense with a fimbriated inner surface. Host cell nuclei were elliptical with abundant euchromatin and a prominent nucleolus. The host cell cytoplasm contained occasional mitochondria, a few Golgi complexes, and abundant rough endoplasmic reticulum dilated by moderately electron-dense material. The host cell membrane was irregular, with numerous papillary projections that blended into the surrounding loose fibrillar connective tissue matrix. The extracellular matrix adjacent to the host cell had a loosely woven pattern that blended into a more condensed linear banding pattern with marked periodicity, consistent with collagen (Fig. 4).

Besnoitia darlingi has been recognized for over 70 years in opossums and certain Central American reptiles.^{1,2,7} *Besnoitia* spp. are heteroxenous coccidians of the family Sarcocystidae. Other organisms in this family include *Sarcocystis*, *Hammondia*, and *Toxoplasma*.⁶ *Besnoitia* cysts are differentiated from other Sarcocystidae based on their characteristic thick wall that is composed of an outer fibrous component surrounding a thin rim of host cell cytoplasm.⁶ Organisms were described in peritoneal macrophages and fibroblasts of mice inoculated intraperitoneally with *Besnoitia jellisoni*.³⁻⁵ However, in naturally occurring besnoitiasis, the cysts are believed to form within fibroblasts.^{6,7} Cats are

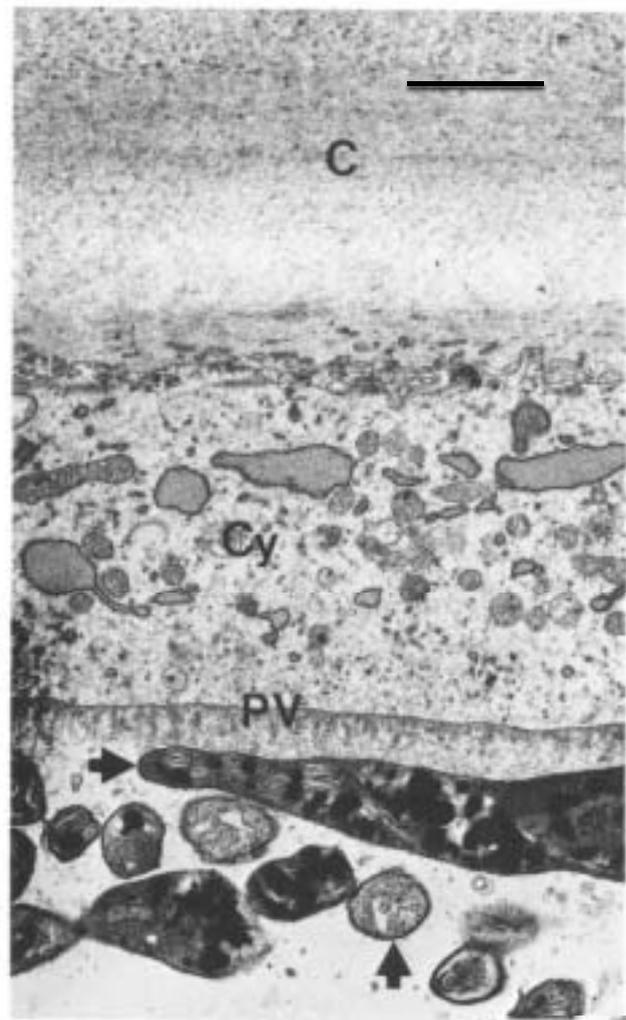


Figure 4. Electron micrograph of *Besnoitia* cyst wall from opossum lung with several bradyzoites (arrows), a parasitophorous vacuole (PV), host cell cytoplasm (Cy), and extracellular matrix (C). Bar = 2 μ m.

the definitive hosts of *Besnoitia*, and thus harbor the sexual stages and pass oocysts. Oocysts are not infective for the definitive host but must pass through an intermediate (usually herbivorous) host,⁷ the opossum in this case. In intermediate hosts, *Besnoitia* spp. replicate by endodyogeny; horizontal transmission may occur through cannibalism or carnivory.⁶

Besnoitia spp. incite minimal inflammation in intermediate hosts. A privileged position within a hypertrophied host cell may account for lack of leukocyte response. Inflammatory cellular reaction was limited to those cysts that were collapsed or mineralized or had released bradyzoites into surrounding tissues. Previous reports have suggested that the parasitized cell is a fibroblast. Our observations tend to confirm this finding. The parasitized cell appears metabolically active and is probably producing the dense fibrillar extracellular cyst wall.

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