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BESNOITIA PANAMENSIS, SP. N. (PROTOZOA: TOXOPLASMATIDAE) FROM PANAMANIAN LIZARDS

Curt R. Schneider
Gorgas Memorial Laboratory, Panama, Republic of Panama

ABSTRACT: A new species of *Besnoitia*, *B. panamensis* (Protozoa: Toxoplasmatidae) is described from two species of Panamanian lizards, *Basiliscus basiliscus* (L.) and *Ameiva ameiva praesignis* (Baird and Girard). This is the first report of *Besnoitia* in Panama and the first description of the genus in a poikilothermic host. Infectivity of the new species for the mouse, hamster, and marmoset is reported and discussed.

The existence of *Besnoitia* in Panama has been recognized only since 24 September 1963, when a female basilisk lizard, *Basiliscus basiliscus* (L.), from the Rio Lagarto, about 6 km from the river's mouth (Colón Province), was found to be infected. Subsequently, five infected specimens of the large teiid lizard, *Ameiva ameiva praesignis* (Baird and Girard), locally known as the "borriguero," were taken at Quebrada Bonita, on the Transisthmian Highway about 17 miles south of Colón. Sections of heart, kidney, lung, and spleen revealed large, thick-walled cysts containing numerous organisms which resembled toxoplasmatids. Morphological studies and the results of animal infections suggest that this parasite represents a new *Besnoitia*, no species of which has hitherto been described from a poikilothermic host.

MATERIALS AND METHODS

The infected lizards formed part of a larger collection, most of which was taken by rifle with .22 caliber long-rifle dust shot. Some lizards were caught in banana-baited traps, perhaps attempting to eat insects feeding on the bait. Thin blood smears were routinely prepared from shot specimens in the field at the time of collection. Since all collecting sites were within easy driving distance of Panama City, lizards were brought promptly to Gorgas Memorial Laboratory for further processing. Organs were examined by grinding portions with a little saline in a hand-operated Ten Broeck tissue grinder and examining the resulting suspension under the microscope. *Besnoitia* trophozoites thus released from their cysts appeared as small, crescentic bodies which moved by flexion and closely resembled *Toxoplasma*. Tissues were also fixed in formol–Zenker, sectioned at 6 μ, and stained with hematoxylin–eosin or tetrachrome. Some of the saline–tissue suspension was also used without further manipulation to inoculate experimental animals.

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Genus *Besnoitia* Henry, 1913

Besnoitia panamensis sp. n.

Hosts: *Basiliscus basiliscus* (L.) and *Ameiva ameiva praesignis* (Baird and Girard).

Locality: Rio Lagarto and Quebrada Bonita, Colón Province, Republic of Panama.

Specific diagnosis

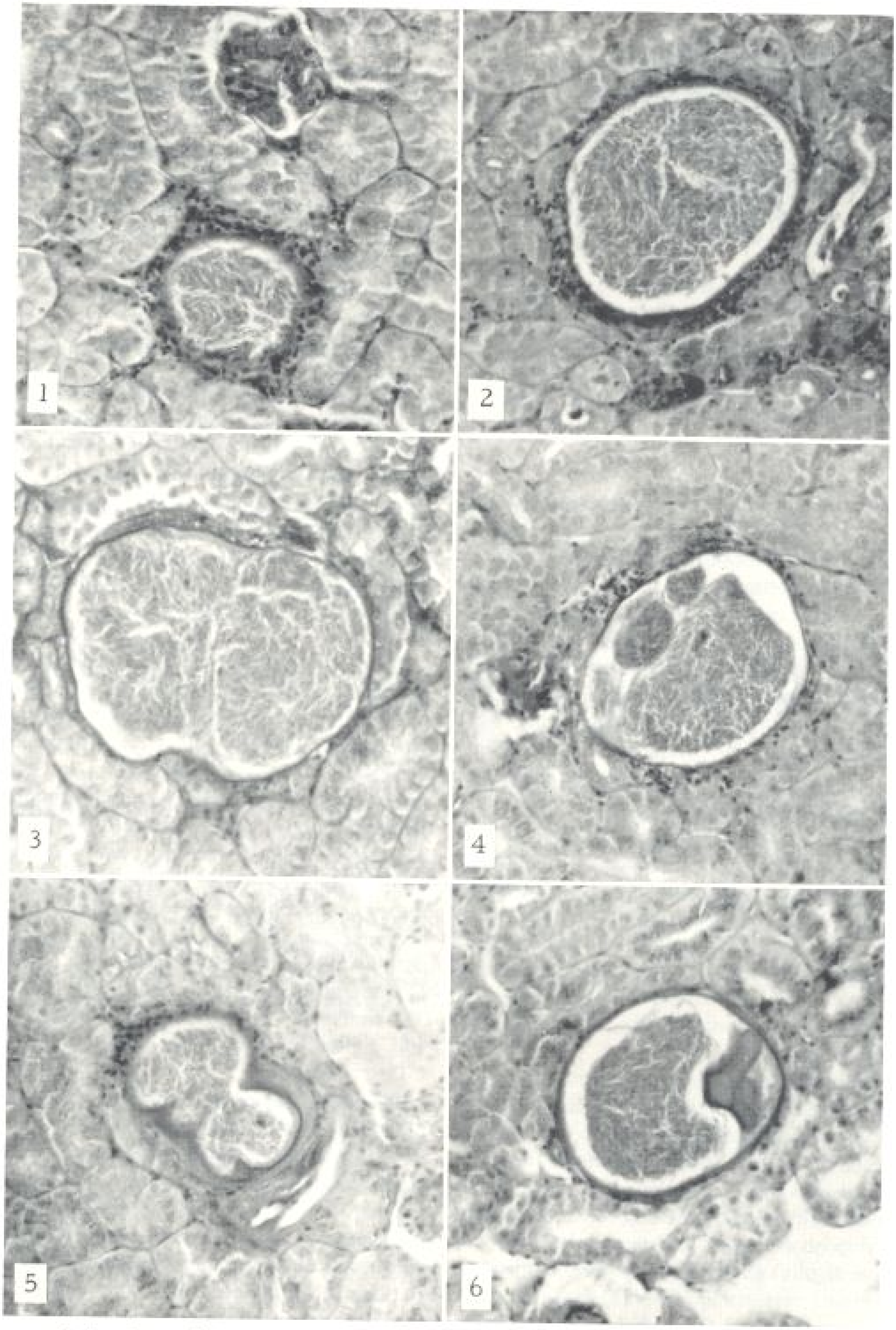
Cysts oval or circular in cross section. May appear lobed. Found in lung, kidney, heart, spleen. Cysts in heart measure (in microns) 177 by 140 (range, 115 by 90 to 254 by 156). Cysts in kidney measure 134 by 109 (range, 66 by 62 to 221 by 148). Outer layer of cyst wall 2.5 to 3.0 μ thick. Mature cysts contain thousands of crescentic bodies. Intracystic crescentic bodies banana-shaped; measure (in microns) 7.4 by 1.5 (range, 7.0 by 1.4 to 7.7 by 1.6). Free trophozoites in peritoneal exudate of experimental mice crescentic, spindle-shaped, pyriform or oval, measure 6.7 by 2.3 (range, 3.6 by 1.4 to 10.9 by 2.7).

Parasite infective for mouse, hamster, and marmoset. Not infective for rat, guinea pig, rabbit, pigeon, or rhesus monkey.

Type specimen: A section of kidney of *Basiliscus basiliscus*, stained with tetrachrome and containing numerous cysts, in the collection of the Parasitology Section, Gorgas Memorial Laboratory, Panama, R. P.

DESCRIPTION OF CYST

Cysts in lizard tissues are oval or circular in sectional outline (Figs. 1 to 4) but also appear not infrequently lobed (Fig. 5). A series of 30 selected at random in the kidney and 30 in the heart of the basilisk lizard (the most heavily infected of the positive lizards) gave measurements averaging 156 by 124 μ. Only oval or circular sections were measured. Cysts in the heart were somewhat larger (177 by 140 μ) than those in the kidney (134 by 109 μ). Such measurements, of course, cannot represent the true three-dimensional size or shape of the cysts, but they indicate an order of magnitude which is useful in making interspecific comparisons.



FIGURES 1–6. Cysts of *Besnoitia panamensis* in kidney of basilisk lizard. Tetrachrome stain. × 220. **1**. Small cyst surrounded by pronounced cellular infiltrate. Note glomerular tuft. **2**. Large cyst, accompanied by diminished cellular infiltrate. The clear space around the mass of proliferating bodies represents an artifact of shrinkage due to fixation. **3**. Large bilocular cyst. **4**. Multilocular cyst. **5**. Lobulated cyst with slight cellular infiltrate. **6**. Cyst containing infolding of outer wall.

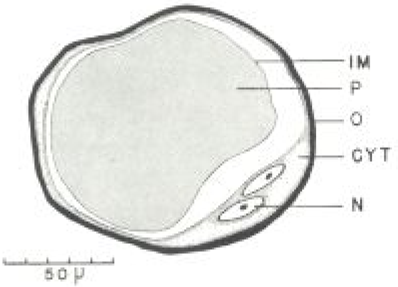


FIGURE 7. Semidiagrammatic drawing of a typical cyst of *Besnoitia panamensis*. The space between the cyst-wall cytoplasm and the inner membrane represents fixation shrinkage. CYT, cyst-wall cytoplasm; IM, inner membrane; N, cyst-wall nucleus; O, outer layer; P, mass of crescentic bodies or trophozoites.

The cyst is composed of three distinct parts (Fig. 7). These include (1) an outer layer of dense, hyaline substance, the inner surface of which sometimes appears to stain darkly, (2) a layer of cyst-wall cytoplasm, occasionally seen to contain nuclei, and (3) a thin inner membrane which is in intimate contact with the mass of trophozoites and frequently remains associated with the latter during fixation shrinkage. The thickness of the outer layer is irregular, usually measuring about 2.5 to 3.0 μ but sometimes no more than 1 μ. The cyst-wall cytoplasm with nuclei is not seen in every section.

On occasion, a cyst appears to include an irregular mass of dark-staining hyaline material (Fig. 6). This is believed to represent a tangential section through an area where the outer wall is flattened or infolded, the included matter being actually continuous with the outer layer.

Most of the cysts are unilocular (Figs. 1, 2, 5, 6), but the occurrence of bilocular cysts (Fig. 3) is not uncommon, and multilocular cysts (Fig. 4) are sometimes seen.

Some cysts are accompanied by a very mild cellular infiltration consisting of round cells, probably lymphocytes, and macrophages. Connective tissue cells may be present and occasionally an eosinophil (Figs. 1, 2, 4, 5). The cells may surround the cyst in section (Figs. 1, 2) or may be limited to a portion of the outline (Fig. 5). They probably represent a reaction to cyst rupture, although morphological evidence of this was not unmistakable in the present material. Frenkel (1961) has described and figured a much more intense cellular infiltration of ruptured cysts of *Besnoitia jellisoni* in hamster retinas.

Cysts have been seen in the lizards most commonly in lung and kidney, less frequently in heart and spleen. They have not been observed in sections of intestine, liver, gonad, or brain of infected lizards, nor have Giemsa-stained blood films taken from these revealed any other stages of the parasite.

DESCRIPTION OF TROPHOZOITES

Intracystic trophozoites from lizard material are quite uniform in size and shape. In sectioned tissues they measure (in microns) 7.4 by 1.5, with a range of 7.0 by 1.4 to 7.7 by 1.6. They are banana-shaped or crescentic with rounded ends (Fig. 8u, v), almost straight (Fig. 8w) or boomerang-shaped (Fig. 8x, y). The cytoplasm stains densely with eosin but the nuclear material takes up hematoxylin only faintly.

The organisms found free in mouse peritoneal fluid exhibit some variation in length and width. The average of 21 living organisms was (in microns) 6.7 by 2.3, with a range of 3.6 by 1.4 to 10.9 by 2.7. They are, in general, crescentic in shape (Fig. 8j, k, p, s) or spindle-shaped (Fig. 8a, b, c, g, h, i). One end may be pointed and the other rounded (Fig. 8d, e, f). While alive, the organism may contract somewhat and become pyriform (Fig. 8l, m, n) or oval (Fig. 8r). It may move in a helical path by flexing the body accordingly (Fig. 8o) or one side may become flattened (Fig. 8q). The body may flex strongly in a comma-shape and remain this way for several minutes at a time (Fig. 8t).

Besnoitia panamensis exhibits the gliding motion described for toxoplasmatids in general. In peritoneal fluid, the parasites are freed traumatically from their host cells. It is not unusual to observe a disintegrating leukocyte carrying as many as ten moving parasites attached to it. Parasites can also attach firmly to the glass microscope slide. The quiet trophozoite initiates motion by raising one end and waving it about in an exploratory fashion while the other end remains fixed to the substratum. Then the organism detaches itself and glides away in an arc, a straight line, or a helix. Halting, it may revolve slowly on its long axis or flex itself suddenly.

EXPERIMENTAL INFECTIONS

Mice

The intraperitoneal inoculation of ground lizard tissues containing trophozoites of *Besnoitia panamensis* into adult CFW white mice has repeatedly resulted in fatal infections. The mice appear normal until the 4th to 8th day, when they develop roughed fur and apathy. Fecal material is unformed and body temperature drops. Death occurs shortly thereafter, usually on the 5th or 6th days. At the time of death the intestine is inflamed and the vessels of the mesentery are dilated. The spleen is enlarged and shows light-colored ischemic areas. If an autopsy is done promptly, intact, live *Besnoitia* can be demonstrated by direct examination of spleen and mesentery. Other organs appear normal and parasites have not been found by microscopic examination of brain, liver, heart, kidney, adrenals, or cervical lymph nodes of infected mice.

On the 4th or 5th day after infection a peritoneal exudate is formed, 0.5 to 2.0 ml of fluid being recoverable from a single mouse. The fluid is rich in granulocytes and round cells and contains free trophozoites of *Besnoitia*. The largest number of parasites is to be found in the early exudate; if the mouse survives beyond the 5th day the number of demonstrable trophozoites in the peritoneal fluid diminishes rapidly. The strain is currently maintained in this laboratory by passing fluid drawn on the 4th or 5th day, diluted 1 : 4 in normal saline, by intraperitoneal inoculation to new mice.

Hamsters

Two young hamsters weighing 72 and 82 g were inoculated intraperitoneally with peritoneal fluid from infected mice. After 21 days, with no signs of disease or distress, both animals were killed. The peritoneal cavity contained no fluid or exudate. Brain, heart, lung, liver, spleen, kidney, and intestine were sectioned. The lung only proved to contain a sparse number of *Besnoitia* cysts in varying stages of development. All other organs were histologically negative. In the lung, the parasites were found in giant cells with nuclear hypersegmentation and an even-staining blue cytoplasm, resembling megakaryocytes. Ten cysts (the total number in one longitudinal section of lung) measured an average of 102.9 by 78.5 μ (range: 61.5 by 57.4 to 172.2 by 106.6). These measurements are, in general, smaller than those given for natural infections in the lizard. The hamster lung cysts are probably, at the age of 21 days, still capable of further growth.

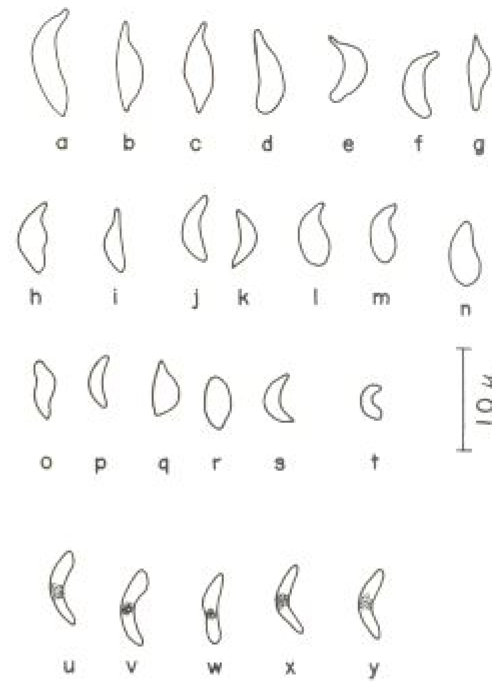


FIGURE 8. Camera lucida drawings of trophozoites of *Besnoitia panamensis*; a–t, from living specimens in freshly-drawn peritoneal fluid, of mouse; u–y, from sectioned material, tetrachrome stain, from *Basiliscus basiliscus*.

Marmoset

A wild-caught marmoset, *Saguinus geoffroyi*, was inoculated intraperitoneally with a suspension of ground kidney and lung tissue from an infected *Ameiva* lizard. Six days later the monkey was found moribund in its cage and was killed. Some 2 ml of peritoneal fluid were recovered by tapping the peritoneal cavity before opening the animal. This fluid contained large numbers of free trophozoites of *Besnoitia*. Mice subinoculated intraperitoneally with this material died in 6 to 8 days, exhibiting a peritoneal exudate containing many parasites.

Animals which have been inoculated with positive lizard tissues but without successfully establishing signs of infection include guinea pigs, both adult and newborn, pigeons, rabbits, a rhesus monkey, and a white-faced monkey (*Cebus capucinus imitator*).

DISCUSSION

A definitive record of the nomenclature of the genus *Besnoitia* has been given by Jellison (1956); it need not be reviewed here.

Four species of *Besnoitia* are accepted by Levine (1961). They include *B. besnoiti* (Marotel, 1912) Henry, 1913, of cattle in southern France, Portugal, and portions of southern Africa; *B. bennetti* Babudieri, 1932, of equines in southern France, Sudan, South Africa, Mexico, and the United States; *B. tarandi* (Hadwen, 1922) Levine, 1961, of reindeer and caribou in Alaska; and *B. jellisoni* Frenkel, 1955, from the white-footed deermouse, *Peromyscus maniculatus*, in Idaho. *B. jellisoni* has also been reported from an opossum (*Didelphis*) in Texas (Stabler and Welch, 1961) and from a rodent (*Microxus*) in Peru (Jellison et al., 1960).

With regard to infectivity for experimental animals, *B. panamensis* most closely resembles *B. jellisoni* in that mouse and hamster are susceptible whereas rabbit, adult guinea pig, pigeon, rat, and rhesus monkey are not. On the other hand, *B. besnoiti* readily infects the rabbit, in which experimental infections can be maintained (Pols, 1960).

B. jellisoni is reported to parasitize hamsters heavily, often proving fatal in 8 to 12 days (Frenkel, 1956). In contrast, *B. panamensis* produced only sparse pulmonary cysts 3 weeks after a heavy intraperitoneal inoculation of young hamsters, with no accompanying clinical signs of disease.

In addition, *B. jellisoni* is fatal to newborn guinea pigs; *B. panamensis* produced no evidence of disease upon injection into 2-day-old guinea pigs.

Attention may be drawn to the difference in the size of cysts in the natural hosts. Those of *B. jellisoni* in the deermouse achieve a very large size indeed, up to 2 mm in diameter when mature (Frenkel, 1956). The cysts of *B. panamensis* in the lizard (*Basiliscus* and *Ameiva*) are much smaller, ranging from 66 by 62 to 254 by 156 μ, and were never visible to the naked eye. (Cyst size is probably determined by a number of factors including the attributes of the surrounding host tissue as well as age. The wild infections were thought to include mature cysts, but the question could better be studied in the laboratory under controlled conditions.)

It is entirely possible that an immunologic analysis of *B. jellisoni* and *B. panamensis* will show them to be closely related. Until such information is available, however, it would appear more useful to give specific status to the Panamanian form.

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