Besnoitia darlingi (Brumpt, 1913) in Panama

CURT R. SCHNEIDER*

Gorgas Memorial Laboratory, Panamá, R. de P.

SYNOPSIS. *Besnoitia darlingi* (Brumpt, 1913) Mandour, 1965 has been rediscovered in the common opossum *Didelphis marsupialis* in the Republic of Panama. A strain (D3) has been established in laboratory white mice. Proliferative crescents of the D3 strain are 6.1 (range 6.0-9.0) by 2.1 (range 1.7-4.0) μ.

Mouse-adapted *B. darlingi* produced acute, fatal infections in white mice, hamsters, 14 marmosets (*Saguinus geoffroyi*), 2 squirrels (*Sciurus variegatoides* and *S. granatensis*), a woolly opossum (*Caluromys derbianus*) and a four-eyed opossum (*Philander opossum*). It probably produced chronic infections (cysts) in 2 wild-caught opossums (*Didelphis marsupialis*) and one lizard (*Ameiva ameiva*). Animals in which induced infection could not be found after inoculation with trophozoites of mouse-adapted *B. darlingi* were: 2 adult and 2 new-born guinea pigs, laboratory rats, 1 night monkey (*Aotus trivirgatus*), 2 rhesus monkeys (*Macacca mulatta*), one iguana (*Iguana iguana*) and 2 baby caimans (*Caiman sclerops*).

IN August 1964 an infection with *Besnoitia* was found in a common opossum *Didelphis marsupialis* taken in a small stand of original forest which remains in the largely cleared farming area of Quebrada Bonita, about 17 miles south of Colon on the Transisthmian Highway, Panama. A small number of whitish cysts, approximately 2 mm in diameter, were noted in the myocardium of this animal, which had inadvertently been placed in a freezing compartment overnight. The cysts contained the crescentic bodies characteristic of toxoplasmatids; the material, as expected because of the freezing, was non-infective for mice when inoculated intraperitoneally.

A search was initiated for more infections of this type. In March 1965 a second *D. marsupialis* with myocardial *Besnoitia* cysts was taken in the same locality. This time a strain, D3, was successfully transferred to white mice, in which it has subsequently been maintained. It is the purpose of this note to record certain morphologic and experimental data which help to characterize the strain.

* Present address: Parke, Davis & Co., 2800 Plymouth Road, Ann Arbor, Mich. 48106.

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MATERIALS AND METHODS

The strain was maintained in NIH all-purpose mice by pooling the peritoneal fluid from 8-10 mice with 7-day infections. To establish a suitable dilution the parasites in all pooled fluids were counted in a hemocytometer, using Hayem's solution as a diluting and fixing agent, and after shaking the pipets for 90 seconds.

Measurements under oil-immersion were made with an ocular micrometer using freshly-isolated proliferative organisms from a 7-day infection fixed in Hayem's solution. Earlier infections were not used because of scarcity of fluid and parasites before 7 days. A small drop of the trophozoite suspension was placed on a slide, covered with a No. 1 22-mm square cover glass and sealed with melted petrolatum. To ensure randomicity, organisms were selected for measurement as they came into view, omitting none, until 50 had been measured.

All wild animals used in the infectivity studies were obtained locally, mostly by purchase from itinerant vendors; with the exception of the single lizard, they were maintained at outside environmental temperatures. The lizard was kept at approximately 74-76 F, with a 60-watt lamp to warm the cage during the day.

RESULTS

Morphology. Two cysts in the 2nd infected *Didelphis* were large enough to be readily seen on casual inspection, 1 near the apex of the ventricle, the other embedded in the right intercostal muscle. Subsequent histologic examination revealed smaller myocardial cysts less than 50 μ in longest dimension with the characteristic thick walls, cyst-wall cytoplasm with nuclei, and non-compartmented contents of the genus *Besnoitia*.

Mean measurements of the lengths and widths of 50 trophozoites were, respectively, 6.1 (range 6.0-9.0) and 2.1 (range 1.7-4.0) μ.

Mouse infections. The first 2 mouse passages of the D3 strain were made in the absence of clinical signs of disease. Ten mice were included in each passage. Tranfers were made at 6- or 7-day intervals from mice in which the presence of 0.1 to 1.0 ml of peritoneal fluid containing sparse numbers of crescentic bodies was the only evidence of infection. Six of the 10 mice in the 1st passage survived, the average day of death for the other 4 being relatively extended (18 days). At the 2nd transfer, 3 mice survived and the average day of death for the others was 14.3 days. After the first 2 passages, the day of death after inoculation became established at an average 8.3, mortality approached 100%, and clinical signs characteristic of mouse besnoitiosis such as lowered temperature, rough fur and occasional diarrhea became evident within a day of death.

Hamster infections. When passed to hamsters from mice, the D3 strain produced a fulminating infection which was invariably fatal. Five hamsters inoculated with 662,000 proliferative parasites died in 6-7 days (average 6.6); 5 which received 150,000 parasites died in 5-12 days (average 7.4). The infections generally resembled those in mice with regard to inflammation of the intestine and mesentery and the production of peritoneal fluid containing parasites. However, fluid was not always present in moribund animals; 3 hamsters which received 150,000 parasites had only traces of exudate although saline washings of the peritoneal cavity revealed both leucocytes and parasites.

Opossum infections. Eight wild-caught opossums (*Didelphis marsupialis*) were inoculated intraperitoneally with 266,000 to 27 million trophozoites of the mouse-adapted D3 strain. Seven of the animals died in 6-46 days; the 8th was killed on the 47th day. Evidence of *Besnoitia* infection was seen in only 2 of these animals; stained sections of heart from the 47-day infection, and of heart, lung, spleen and pancreas from the 46-day infection, contained young *Besnoitia* cysts. The abundant cyst-wall cytoplasm, large number of cyst-wall nuclei, and loosely fibrous cyst walls, characteristic of young cysts, suggested that they were induced, and not natural, infections (Figs. 1-5). Deaths of the remaining opossums could not be related to the experimental inoculations with any certainty. Captive opossums are frequently in poor condition when brought to the laboratory and, under cage conditions, are apt to succumb within days or weeks. It is also of interest that 8 *Didelphis* taken in the same area as the experimental animals mentioned above were negative when examined for natural infections of *Besnoitia*.

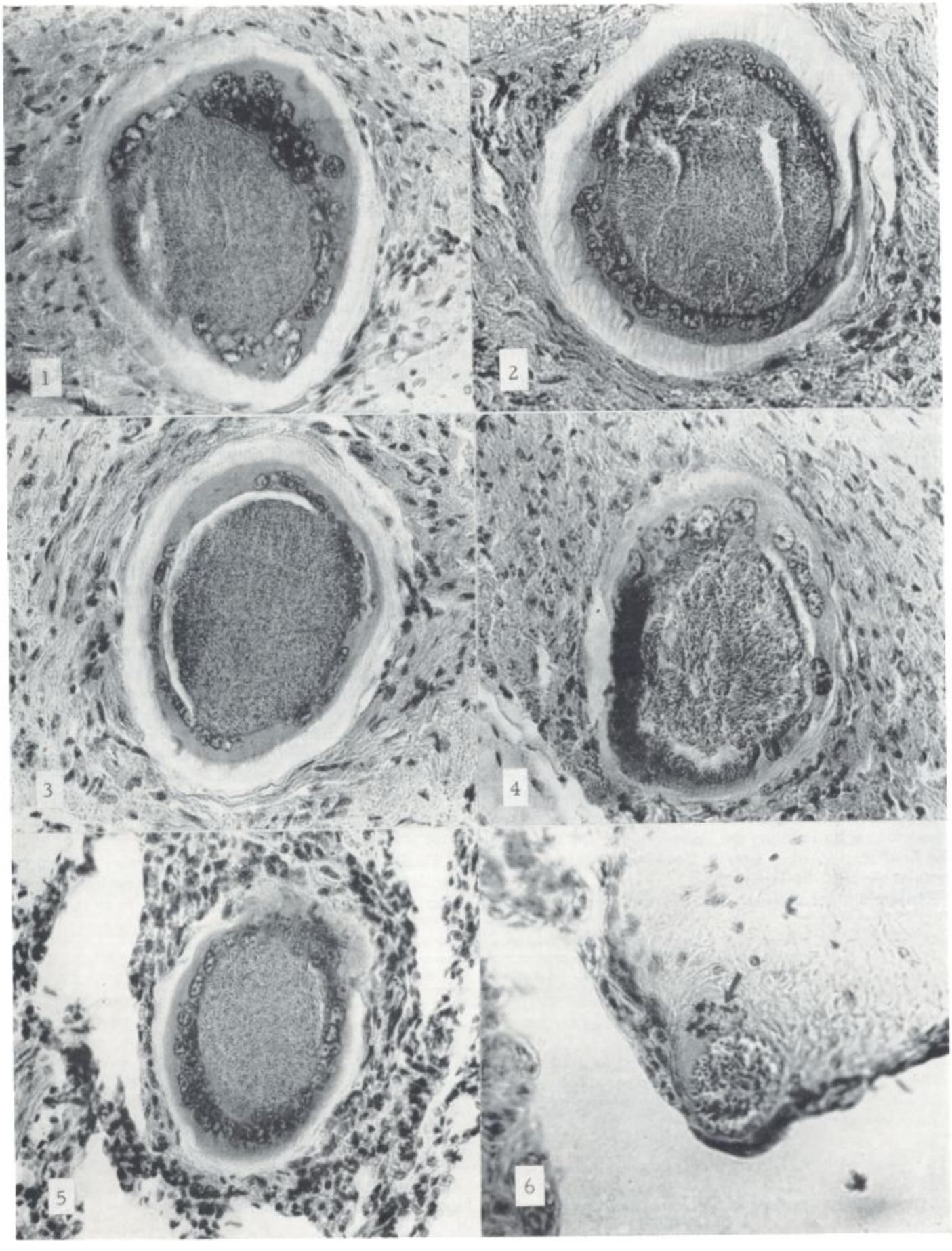
Probable lizard injection. One young adult *Ameiva ameiva*, wild-caught in Achiote, was inoculated intraperitoneally with 7.7 million D3 organisms from the 33rd mouse passage. Fifty-three days later the lizard was killed while in apparent good health. Direct examination of homogenized tissues showed a few typical *Besnoitia* crescents in the lung. Subsequently 3 of 4 mice inoculated with lung tissue and 1 of 4 inoculated with kidney developed acute besnoitiosis. Sparse cysts were seen in stained sections of lung (Fig. 6) and heart but not of liver, spleen, kidney or testis. It is impossible to rule out the possibility that this infection had been naturally acquired in the wild. However, the lizard was small, although sexually mature, and it has been observed in current work that only the largest and oldest lizards are found naturally infected with *Besnoitia*.

Guinea pig infections. Two adult guinea pigs remained asymptomatic following the intraperitoneal inoculation of large numbers of parasites. In 1, however, there was an indication that the parasites might have persisted in the tissues if they did not actually multiply: an infection was obtained in 1 of 9 mice inoculated with homogenized spleen from this guinea pig which had received 15 million organisms 18 days before.

Two newborn guinea pigs, 4 days old, remained asymptomatic after receiving intraperitoneal inoculations of 50,000 D3 organisms each.

Other experimental animals. Certain native wild animals responded to intraperitoneal injection of proliferative organisms of the D3 strain with acute, rapidly fatal infections. These included 2 squirrels (*Sciurus variegatoides* and *S. granatensis*), 14 marmosets (*Saguinus geoffroyi*), 1 wooly opossum (*Clauromys derbianus*) and 1 4-eyed opossum *Philander opossum*).

Some other wild-caught and laboratory-raised animals remained asymptomatic when challenged intraperitoneally with large numbers of fresh proliferative organisms. When the animals were killed after protracted periods, parasites could not be found in their tissues, either by direct microscopic examination of homogenized organs or by subinoculation of homogenates into mice. In the wild group were 1 night monkey (*Aotus trivirgatus*), 1 iguana (*Iguana iguana*), and 2 caimans (*Caiman sclerops*). Among refractory laboratory-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Figs. 1-5. *Besnoitia darlingi*, cysts from a 46-day-old experimental infection in an opossum, *Didelphis marsupialis*. Hematoxylin and eosin. × 335. Figs. 1-4, in myocardium. Fig. 5, in lung. Note the abundant cyst-wall cytoplasm, large number of cyst-wall nuclei, and loosely fibrous cyst wall in each.

Fig. 6. *Besnoitia darlingi*, pulmonary cyst from a 56-day-old experimental infection in a lizard, *Ameiva ameiva*. The cyst-wall cytoplasm contains dark-staining granules which may represent karyorrhexis of a cyst-wall nucleus (arrow). Hematoxylin and eosin. × 540.

raised animals were white rats and rhesus monkeys. No parasites could be found by mouse subinoculation in rats 20, 32 and 40 days after heavy inoculations. Two rhesus monkeys, 1 of which had been splenectomized, remained clinically asymptomatic after receiving massive inoculations intraperitoneally (25 and 100 million organisms); histologic studies and mouse inoculations with lung and spleen from the unsplenectomized monkey which had received the larger dose gave negative results.

DISCUSSION

In 1910, Dr. Samuel T. Darling described a cystic parasite from a common opossum *Didelphis marsupialis* caught in the Panama Canal Zone(3). He assigned the parasite to the genus *Sarcocystis* but was clearly troubled by certain characteristics which seemed to separate it from that genus. The cysts were whitish nodules, 1.5-2.0 mm in diameter, distributed throughout the voluntary muscles and fascia of the body as well as the heart, lungs, stomach, small intestine and its mesentery, pericardium, submaxillary gland and esophagus. They were not seen in the kidneys, liver, spleen, pancreas, bladder, gall bladder, gonads or eyes and their extrinsic muscles.

The genus *Sarcocystis* is parasitic only in striated and heart muscle, but because of the indiscriminate distribution of the opossum cysts Darling was in favor of emending the definition of the genus to include parasites able to develop in non-muscular as well as muscular tissue. At the same time he noted that the cysts apparently lacked the internal septa and division into compartments which are also characteristic of *Sarcocystis*. Moreover, although *Sarcocystis* cannot be transmitted by parenteral routes, Darling was able to infect 1 of 2 guinea pigs with trophozoites from a crushed opossum cyst by injecting the organisms into the muscles of the hind leg; 60 days later he found some intramuscular cysts at the site of injection.

Darling did not propose a specific name for the opossum parasite but Brumpt named it *Sarcocystis darlingi* in the 2nd edition (1913)(2) of his *Précis de Parasitologie* (p. 109). This specific name was accompanied by descriptive material (from Darling) and a bibliographic reference and is thus valid. It is seen that Brumpt disregarded Darling's own reservations about the correctness of *Sarcocystis* for this parasite.

Babudieri(1) was of the opinion that Darling's parasite ought to be assigned to *Fibrocystis*, a genus created by Hadwen(7) for the etiologic agent of "corn-meal" disease of reindeer and caribou. Babudieri's decision was based on the predilection of the parasite for non-muscular as well as muscular tissue in addition to its lack of internal compartmentation. These were precisely the points which had disturbed Darling. According to Babudieri, the correct name of the parasite was *Fibrocystis darlingi*.

Levine(10) observed that *Fibrocystis* is a synonym of *Besnoitia*, and when Darling's description is read with *Besnoitia* in mind, there is good concurrence. Recently, Mandour(11) noted this and stated that the proper combination of names ought to be *Besnoitia darlingi* (Brumpt, 1913).

Adult guinea pigs remained asymptomatic after inoculation with D3, although parasites were recovered after 18 days from 1 apparently healthy animal. Darling(3) inoculated his *Didelphis* parasite directly into the right hind-leg muscle of 2 guinea pigs (*not* the pectoral muscles, as reported by Brumpt) and, by examining many stained sections microscopically, found small "sarcocysts" at the site of inoculation in the animal killed at 60 days but not in the other, killed 146 days after infection. The question of guinea pig susceptibility has yet to be studied thoroughly, but it may be added here that repetition of Darling's work would require the use of organisms freshly isolated from an opossum rather than proliferative stages adapted to mice.

Finally, the rediscovery of *B. darlingi* raises a question regarding the validity of other species of American *Besnoitia*. Thus far, 6 species have been reported from the Western

TABLE 1. *Natural infections with* Besnoitia *species reported from the Western Hemisphere.*

Species	Host	Location	Author
bennetti	Burro	Mexico	Jones, 1957(9)
besnoiti	Cows	Venezuela	Vogelsang & Gallo, 1941(14)
darlingi	Opossum (*Didelphis*)	Panama	Brumpt, 1913(2)
jellisoni	White-footed mouse (*Peromyscus*); oppossum (*Didelphis*); vole (*Microxus*)	Idaho; Texas; Peru	Frenkel, 1955(4); Stabler & Welch, 1961(13); Jellison *et al.*, 1960(8)
panamensis	Lizards (*Basiliscus basiliscus*; *Ameiva ameiva*)	Panama	Schneider, 1965(12)
sauriana	Lizard (*Basiliscus vittatus*)	British Honduras	Garnham, 1966(6)

Hemisphere (Table 1). Frenkel(5), employing the dye test, found that sera from cows infected with *B. besnoiti* did not contain measurable antibody against *B. jellisoni* antigen. Since the rest are indistinguishable from each other both morphologically and (so far as is known) from the standpoint of animal infectivity, further immunologic studies are indicated in order to resolve the question. It seems worthwhile to point out that, in the event that any or all of these species (with the exception of *B. besnoiti*) are shown to be identical with *B. darlingi*, the latter name will have priority.

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