The Distribution of Lizard Besnoitiosis in Panama, and Its Transfer to Mice

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SYNOPSIS. In heavily endemic collecting sites in Panamá and Colón Provinces, Republic of Panama, 14.7% of Ameiva ameiva and 8.5% of Basiliscus basiliscus were injected with Besnoitia darlingi. Single infected specimens of A. leptophrys and A. festiva were also taken, these being new host species records for this parasite. Infections were found only in the older lizards.

Initially, virulence of the lizard parasites for white mice was low but increased with successive mouse passages. Concomitantly, the cyst-forming capacity of the strain diminished with successive mouse passages. No relation between initial virulence of the lizard parasites for mice and subsequent virulence after 16 or 17 mouse passages was recorded.

The original description of B. panamensis (a synonym of B. darlingi) is emended on the basis of extensive material to include cyst diameters of 200-500 μ; also, the liver, mesentery, and tunica propria of the testis occasionally contain cysts. Cysts are frequently macroscopic and on the surface of organs so that they can be seen on casual inspection.

B. sauriana Garnham, 1966 is a synonym of B. darlingi.

THE original description of Besnoitia panamensis (Schneider, 1965) was based on data which have been considerably augmented since 1965. The discovery of several highly productive sources of natural infections has permitted the accumulation of new information on infection rates in endemic localities, and on aspects of the parasite's biology and on transfer and adaptation to mice. The present paper presents some of these data.

MATERIALS AND METHODS

Lizards were shot and trapped in a number of sites in the provinces of Panamá and Colón. In addition, a large number of specimens preserved in formalin in the field were obtained from Mr. Charles W. Myers, University of Kansas Herpetology Unit, who made all identifications.

Freshly caught lizards were killed with chloroform and the viscera (heart, lung, spleen, and kidney) homogenized in a Ten Broeck tissue grinder with about 5 ml sterile saline. A drop of the homogenate was examined for Besnoitia directly under the microscope. At least 5 minutes, clocked by a timer, were spent on each examination; as many as 250 fields could be examined in this time. If no Besnoitia were recognized the animal was recorded as negative; it is felt that few, if any, true positives went unrecognized with this method, for even a single mature cyst could be expected to release many thousands of the distinctive "bent-spindle" cyst organisms into the surrounding fluid.

Formalin-fixed lizards were dissected and their lungs, heart, spleen and kidney were embedded in paraffin, sectioned and stained routinely with hematoxylin and eosin for histologic examination.

RESULTS

Collection sites. Two localities provided the bulk of the lizards in these studies: (1) Quebrada Bonita, on the Transisthmian Highway, and (2) the site of the Inter American Highway Bridge over the Pacora River, about 50 miles east of Panama City (Fig. 1). The latter site was worked most extensively since it was easier to get to from the city and because infected ameivas and basilisks could be found there.

In the dry season (January-April), the shoreline of the Pacora River just north of the bridge consisted of exposed gravel which supported low brush and tall grass. Much of the collection area had trails made by people and horses traversing the bush and dead grass. Both ameivas and basilisks were found here on sunny days, feeding in and around the human litter left from picnics.

Early in 1966 this excellent site was lost when developers bulldozed it and began extracting gravel from the river. A search of nearby areas for infected lizards was finally successful when it was found that the Viamonte Farm, less than a mile from the river, had a large population of heavily infected Ameiva. Further studies were done at Viamonte Farm.

Injection rates. A total of 634 lizards belonging to 9 species was examined (Table 1). More than half of these (320) were Ameiva ameiva, the species with the highest infection rate (14.7%). The next highest rate (8.5%) was for Basiliscus basiliscus. A single positive Ameiva

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leptophrys was taken in the small stand of forest remaining at Quebrada Bonita. One positive Ameiva festiva, taken near Almirante, Bocas del Toro Province, was found in a series of 180 specimens representing 8 species which were formalin-fixed in the field. These last 2 species were new host records for Besnoitia. The map (Fig. 1) also indicates the site of the Lago River where an infected Basiliscus basiliscus had been taken by Sneider.(12)

It is of interest that 40% of 25 A. ameiva captured at Viamont Farm were positive, a rate considerably higher than the overall rate of 14.7% from all areas collected.

Eleven Basiliscus plumifrons and 28 B. vittatus from Bocas del Toro Province were negative. Likewise, 19 Scoloporus malachiticus from Chiriqui Province and 42 Cnemidophorus lemniscatus from the neighborhood of El Real, Darien Province, were negative by this method.

**Relationship of host size to infection with Besnoitia.** Early in the work the impression was gained that only the larger (and older) lizards were infected with Besnoitia.

To prove this, a small series of Ameiva was taken from the heavily infected site at Viamont Farm and weights were recorded. Since lizards have frequently lost or are regenerating parts of the tail, length could not be used as a measurement of age. Very small lizards (less than 10 g) were not included in the data altho they were uniformly negative. It is seen in Table 2 that in a series of 22 lizards shot at random around the grounds of the tenant farmer's house, positive individuals of either sex fell into a higher weight group than did negative ones. These figures supported the impression that, altho all large lizards in an endemic area are not necessarily infected, infected lizards are generally large.

**Virulence.** The behavior of lizard Besnoitia when first transferred to white mice proved variable. Virulence for mice, measured in terms of the time (days) elapsing between inoculation and death, varied considerably with several isolates but could be described in general terms as low.

To make initial transfers, lizard tissues (usually one or 2
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Ooculated intraperitoneally with 0.5 ml of homogenate. The number of organisms in such inocula varied in 5 positive lizards, from 925,000 to 2,900,000. The fact that such large numbers of organisms sometimes did not prove fatal to mice is an indication of their relatively low virulence.

As successive mouse passages were made, virulence increased. This was incidentally noted in terms of the progressively greater dilutions of mouse peritoneal fluid required when transferring strains routinely at 4- or 5-day intervals. It could also be measured: Reed-Muench L.D<sub>50</sub> calculations made on 5 isolates at the 16th or 17th mouse passages were as follows: 2,100; 3,500; 8,700; 15,700 and 16,200. In the case of one strain (L<sub>50</sub>), by the time of the 47th mouse passage the L.D<sub>50</sub> had fallen to less than 30 organisms.

Cyst-formation. The cyst-forming capacity of B. panamensis tended to disappear soon after the initiation of repeated mouse passages. Uterine muscle was the site of preference for cysts. It is seen in Table 3 that the number of surviving mice positive for cysts when checked 6 months later fell off after the 6th or 7th mouse passage, although there was considerable variation (strain B<sub>50</sub> seemed to have a limited cyst-forming capacity after only the 3rd passage, whereas the capacity of B<sub>50</sub> was still strong after the 8th).

At the 10th to 12th passage, few or no mice survived during routine maintenance. In order to ascertain whether cyst-forming capacity had been lost, mice were made immune by the chemotherapy method of Frenkel(4), in which 60 mg of sodium sulfadiazine per 100 ml of drinking water is given to the newly infected mice. Such treatment permitted mice to survive large inoculations of virulent Besnoitia but when the uterine muscle was examined 6 months later (represented by the figures in parentheses in Table 3) they were uniformly negative for Besnoitia.

Curiously, in a few cases where all the data were recorded, the initial virulence of the lizards was found to have little or no relation to the virulence of the same isolate after a number of mouse passages (Table 4). For example, strain L<sub>50</sub> was initially rather virulent and an initial inoculum of 1,555,000 organisms (from pulmonary cysts) was fatal to 5 mice; yet this strain later had an L.D<sub>50</sub> of 16,200. This may be compared with strain L<sub>50</sub> in which a larger original inoculum killed none of 5 mice, but whose subsequent L.D<sub>50</sub> was approximately 3,500.

In general, the most outstanding feature of the strains with regard to initial virulence, subsequent virulence and cyst-forming capacity was their variability.

![Fig. 2. Left lung of Ameiva ameiva with heavy Besnoitia darlingi infection. Most of the cysts, easily seen with the unaided eye, were 200-300 μ in diameter; the largest one in the figure was about 500 μ.]
TABLE 4. Relative virulence of cystic stages from lizard and subsequent LD₅₀ values in mice.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Cystic organisms from lizard</th>
<th>Mouse-adapted organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size of initial inoculum: No. mice dead out of 5 inoc.</td>
<td>No. of mouse passage</td>
</tr>
<tr>
<td>L. 593</td>
<td>1,535,000</td>
<td>5</td>
</tr>
<tr>
<td>L. 604</td>
<td>2,000,000</td>
<td>0</td>
</tr>
<tr>
<td>L. 606</td>
<td>925,000</td>
<td>2</td>
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</tbody>
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Emendation of B. panamensis. In the original description of B. panamensis (Schneider, 1965), cyst measurements were stated to be 177 × 140 μ in the myocardium (with ranges of 115 × 90 to 254 × 156 μ) and 134 × 109 μ in the kidney (with ranges of 66 × 62 to 221 × 148 μ). Subsequent captures of numerous infected Ameiva ameiva and Basiliscus basiliscus showed that these figures were small and that, in many cases, B. panamensis cysts which are easily visible to the unaided eye can be found on the surface of tissues such as lung and mesentery (Fig. 2). In such cases, cysts were as large as 300-500 microns in diameter.

Moreover, altho the original paper described cysts as being found only in lung, heart, spleen and kidney (and these organs remain the most commonly affected, in my experience), cysts were found in several heavy infections in liver, mesentery and the tunica propria of the testis. Thus the original description of B. panamensis must be emended to include these new limitations.

DISCUSSION

Geographic distribution. In Panama, Besnoitia has been found in lizards(12) and opossums(13). In addition, this parasite had been reported from the following hosts and Latin American countries; in rodents from Peru(9), in 2 cows from Venezuela(15), in lizards from British Honduras (5,16) and in burros from Mexico(10,7). Dr. Bryce Walton of the Middle America Research Unit recently told me that 1 of 2 Ameiva ameiva captured by him in the Canal Zone was positive for Besnoitia. It is to be anticipated that other lizards and opossums will be found infected in areas thruout this large geographic range.

Initially, it was thought to be of some significance that, in Panama, the sites of heaviest endemicity were all places of human activity, whether door-yards, picnic areas, or cow pastures. For example, the Quebrada Bonita area is now largely fenced pasture used by grazing cattle, with a small stand of unfenced original forest occupying one hilltop; infected Ameiva and Basiliscus were taken near isolated houses and along the gravel road. It must be noted that one infected A. leptocephys was trapped in the Quebrada Bonita forest which is, so far as known, little used by either people or farm animals. Again, the heavily endemic site at the Pacora River (now destroyed by bulldozing and flooding) had been used as a picnic ground; here Ameiva ameiva, A. leptocephys, and B. basiliscus were captured feeding among human trash and detritus. Trails in the tall grass were made by browsing horses from a nearby farm, and were also used by people looking for privacy. The captures at Viamonte Farm, not far from the Pacora River, were made mostly near the farmhouse, altho many infected Ameiva ameiva were captured in the pastures, principally under wire fences where their holes may have been somewhat protected from damage by bovine hooves. The proximity of all these captures to areas of human activity was striking.

On the other hand, a few infected lizards were taken in areas which did not share these characteristics. The single infected basilisk taken on the Lagarto River was found in a heavily forested area away from houses. The one infected A. festiva was taken 3 miles west of Almirante (Bocas del Toro Province) in a cacao plantation where the only domestic animals noticed were pigs altho horses had access to the area. Finally, the infected lizard caught in the Canal Zone was taken in the town of Gamba, at the mouth of the Chagres River, where no farm animals are known.

Thus, the original hypothesis of an association between lizard infections and large animal (or even human) activity has not always been supported by field experience.

Nomenclature. A recent report(14) states that the opossum and lizard strains of Besnoitia appear to be conspecific and that B. panamensis Schneider, 1965, is a synonym of B. darlingi (Brumpt, 1935). It is not possible to make a decision regarding the validity of the American murine, equine and bovine strains which have been reported in the literature without new experimental evidence. But it would seem likely that the lizard strain from British Honduras is identical with the Panamanian strains. Garnham(6) gave the name Besnoitia sauriana to the British Honduran strain, separating it from B. panamensis because of its larger cyst size. But on this basis the present emendation of B. panamensis (or more correctly B. darlingi) relegates B. sauriana to synonymy.

The synonymy at present stands as follows:

Besnoitia darlingi (Brumpt, 1935) Mandour, 1965

Synonyms: Sarcozystis sp. Darling, 1910
Sarcozystis darlingi Brumpt, 1913
Fibrocystis darlingi Babudieri, 1932
Besnoitia panamensis Schneider, 1965
Besnoitia sauriana Garnham, 1966

In view of the reported presence of infections in Basiliscus vittatus from British Honduras, the failure to find Besnoitia in one collection of B. vittatus in Panama may reflect the relative inefficiency of searching for cysts in stained sections, where only the heavier infections are likely to be revealed. In the present study only a single infected lizard was discovered by this method out of 180 examined.

Transmission. The fact that natural infections were restricted to the older and heavier lizards suggests that the infections were associated with the passage of time. Perhaps infective stages are ingested by the host rather than transmitted by blood contamination from a blood-sucking arthropod. Using mice, Jellison et al.(8) found that both cystic and proliferative stages from mice can produce experimental infections when given by mouth. Another possibility
is that both blood transmission and ingestion are natural methods of transmission.

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