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 *Besnotia* 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.

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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.

Infection 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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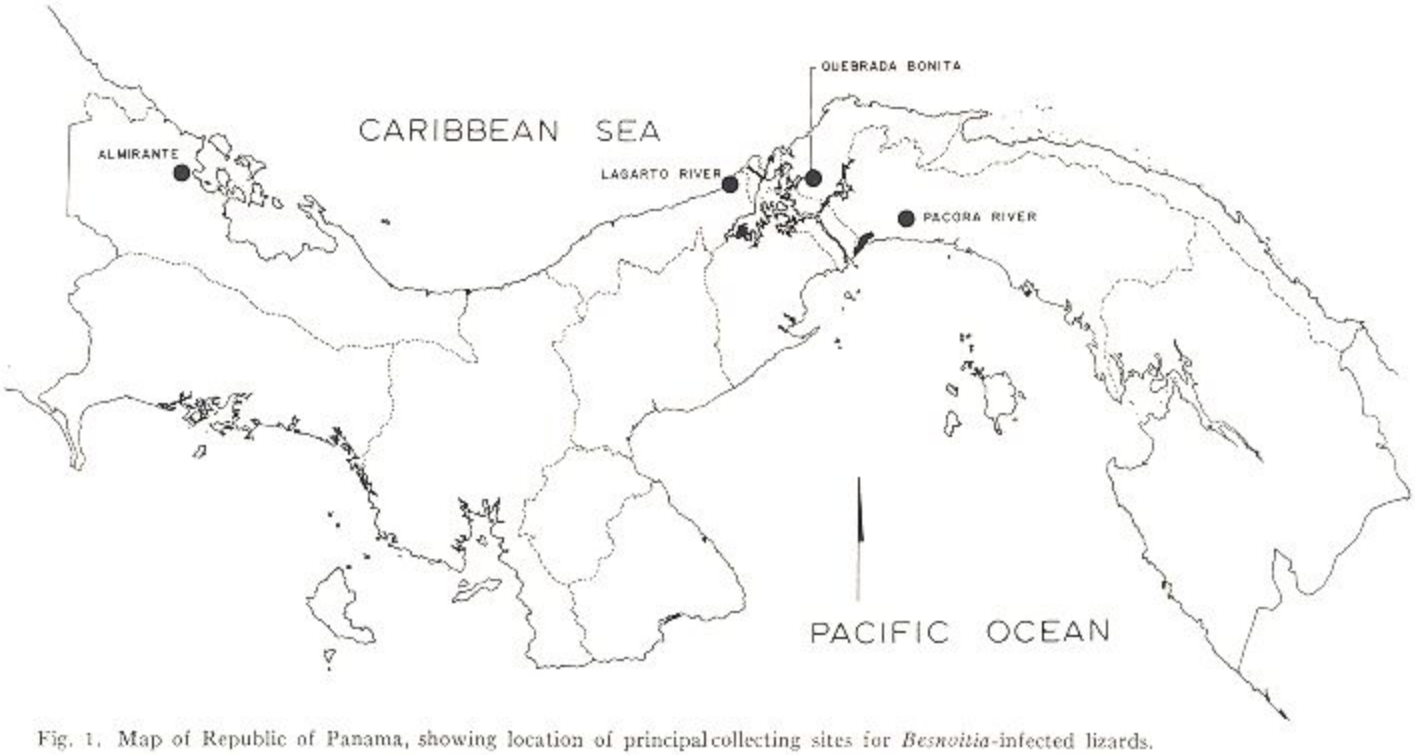

Fig. 1. Map of Republic of Panama, showing location of principal collecting sites for *Besnoitia*-infected lizards.

TABLE 1. *Infection rates of 9 species of Panamanian lizards with* Besnoitia.

Species	Total examined	Examined fresh		Stained sections		Total		Percent positive
		Neg.	Pos.	Neg	Pos.	Neg.	Pos.	
Ameiva ameiva	320	270	47	3	0	273	47	14.7
A. festiva	41	3	0	37	1	40	1	2.4
A. leptophrys	17	3	1	13	0	16	1	5.9
A. quadrilineata	26	—	—	26	0	26	0	0.0
Basiliscus basiliscus	130	119	11	—	—	119	11	8.5
B. plumifrons	11	—	—	11	0	11	0	0.0
B. vittatus	28	—	—	28	0	28	0	0.0
Cnemidophorus lemniscatus	42	—	—	42	0	42	0	0.0
Sceloporus malachiticus	19	—	—	19	0	19	0	0.0
Total	634	395	59	179	1	574	60	

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 Lagarto River where an infected *Basiliscus basiliscus* had been taken by Sneider(12).

It is of interest that 40% of 25 *A. ameiva* captured at Viamonte 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 *Sceloporus malachiticus* from Chiriquí Province and 42 *Cnemidophorus lemniscatus* from the neighborhood of El Real, Darién 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 Viamonte 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

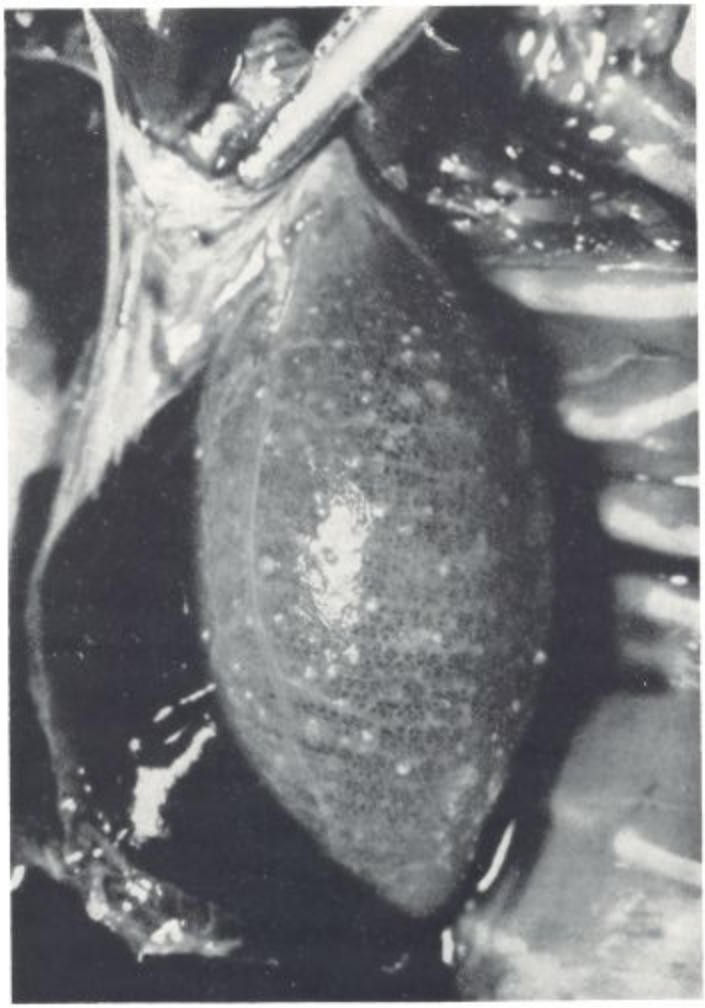

Fig. 2. Left lung of *Ameiva ameiva* with heavy *Besnoitia darlingi* infection. Most of the cysts, easily seen with the unaided eye, were 200-300 μ in diameter; the largest one in the figure was about 500 μ. × 5.

TABLE 2. *Relationship of weights in grams of* Ameiva ameiva *lizards taken at random in a single locality to natural infection with* Besnoitia.

	Negative			Positive		
Sex	Number	Weight	Range	Number	Weight	Range
Male	8	68.9	26.4–134.0	7	131.4	85.3–174.9
Female	7	36.7	12.0– 79.0	3	70.4	68.2– 73.1
Total	13	49.5		9	111.1	

lungs) containing large numbers of cysts were homogenized in a tissue grinder with 5 ml of sterile saline. Mice were inoculated 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 LD_{50} 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_{62}), by the time of the 47th mouse passage the LD_{50} 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, altho there was considerable variation (strain L_{604} seemed to have a limited cyst-forming capacity after only the 3rd passage, whereas the capacity of L_{596} 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 lizard organisms 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_{593} was initially rather virulent and an initial inoculum of 1,555,000 organisms (from pulmonary cysts) was fatal to all of 5 mice; yet this strain later had an LD_{50} of 16,200. This may be compared with strain L_{604} in which a larger original inoculum killed none of 5 mice, but whose subsequent LD_{50} 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.

TABLE 3. *Disappearance of cyst-forming ability with continuing mouse passages of* Besnoitia. *Numbers represent the number of mice with uterine cysts in relation to the number which survived the initial infection. Examinations done 6 months after initial infection.*

	No. of mouse passage												
Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
L 593	—	4/4	2/3	5/5	3/4	3/3	0/1	—	—	—	—	(0/4)	—
L 596	3/4	4/5	1/3	1/3	2/2	3/3	5/5	3/3	—	—	—	(0/4)	—
L 604	4/5	3/4	0/5	1/4	0/3	0/4	1/3	0/5	1/4	0/2	(0/5)	—	—
L 606	3/3	2/3	4/4	3/3	4/5	1/2	—	—	—	—	(0/5)	1/1	—
L 608	2/2	3/5	1/2	2/3	5/5	1/2	—	—	1/1	(0/5)	—	—	0/1

( ) = Mice which became immune after chemotherapy.

TABLE 4. *Relative virulence of cystic stages from lizard and subsequent LD_{50} values in mice.*

Strain	Cystic organisms from lizard		Mouse-adapted organisms	
	Size of initial inoculum	No. mice dead out of 5 inoc.	No. of mouse passage	LD_{50}
L 593	1,555,000	5	17	16,200
L 604	2,900,000	0	16	3,500
L 606	925,000	2	16	8,700

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 *Besnoita*. 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. leptophrys* 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. leptophrys*, 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 Gamboa, 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, 1913). 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, 1913) Mandour, 1965

Synonyms: *Sarcocystis* sp. Darling, 1910
Sarcocystis 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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