Copeia, 2002(1), pp. 39–43

Virulence of Lizard Malaria: Three Species of *Plasmodium* Infecting *Anolis sabanus,* the Endemic Anole of Saba, Netherlands Antilles

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***Anolis* in the eastern Caribbean islands are hosts to three species of malaria parasite (*Plasmodium*). Although the parasites are widespread on the islands, little is known about their effects on infected lizards. Presented here is an inventory of some costs suffered by *Anolis sabanus*, the endemic solitary anole of Saba, Netherlands Antilles, when infected with *Plasmodium azurophilum*, *Plasmodium floridense*, and an undescribed species of *Plasmodium*. Parasitemia (parasite density in the blood) for most infections was low for all three Saban parasites. Blood cell composition (percent of immature erythrocytes) and blood hemoglobin were altered by infection (severity varied depending on species of parasite). Not affected by infection were body temperature, proportion of lizards with broken tails, perching location, foraging success, male-male interactions in experimental manipulations, and body color or symmetry in body color. Overall, the three malaria parasites of Saban anoles have lower virulence than other lizard malaria parasites studied (one temperate and two tropical). Theory on the evolution of parasite virulence suggests transmission biology of the parasite may differ for the Saban parasites compared to the other studied species.**

THREE species of malaria parasites (*Plasmodium*) commonly infect *Anolis* lizards in the eastern Caribbean islands from Puerto Rico in the Greater Antilles to Grenada in the south (Staats and Schall, 1996a; Perkins, 2001). Telford (1975) described *P. azurophilum* as a parasite that invades both erythrocytes and two classes of white blood cells. Recent molecular studies reveal that these are two monophyletic species (Perkins, 2000, 2001), so here we use *Plasmodium azurophilum* for the species that infects white blood cells and refer to the undescribed cryptic species as *P.* sp. The third parasite is *Plasmodium floridense*, a typical malaria parasite that infects erythrocytes. Although Caribbean *Anolis* have long served as important models for studies in biogeography, speciation, environmental physiology, and population and community ecology (review in Roughgarden, 1995), the role of malaria in Caribbean anole biology has been pursued in only two studies. Schall (1992) found that *Plasmodium* affects the blood's cell composition and chemistry of *Anolis gingivinus* and *Anolis pogus* (=*Anolis* wattsi) on St. Martin and may mediate competition between the two similarly sized lizards. Schall and Pearson (2000) reported that body condition of *Anolis gundlachi* in eastern Puerto Rico was not altered by infection with the three malaria parasites. Contrasting with this paucity of information on the costs of malaria for *Anolis* are the substantial data on two other lizard malaria systems, *Sceloporus occidentalis* infected with *Plasmodium mexicanum* in northern California and *Agama agama* infected with *Plasmodium gianteum* and *Plasmodium agamae* in Sierra Leone, west Africa. Detailed studies show malaria is virulent in these two associations (reviews in Schall, 1990, 1996).

Here we present a broad inventory of the consequences of *Plasmodium* infection for *Anolis sabanus*, the solitary endemic anole of Saba, Netherlands Antilles. On Saba *P. azurophilum*, *Plasmodium* sp., and *P. floridense* all exploit only the anole as their vertebrate host (Staats and Schall, 1996b). The scarcity of data on the effects of natural parasitic infection for reptiles, or indeed for nonhuman parasites in general, hinders cross-species tests of hypotheses on the ecology and evolution of pathogens (Toft, 1991; Gulland, 1993); thus, our goal was to be as complete as possible in this survey of the costs of lizard malaria for the Saban anole.

MATERIALS AND METHODS

Saba, Netherlands Antilles, is a small (13 km^2) island in the northeastern Caribbean just south of St. Martin. The island is the tip of a massive undersea volcano and the island's topography is steep and complex, rising to 887 m elevation (Westermann and Kiel, 1961). Mean monthly temperature varies only from 26–28 C; rainfall is very variable among years but averages somewhat more from late August to December (130 mm/month) than in the other months (75 mm/month; Netherlands Antilles Meteorological Service data). Our study took

place during May to August but during a wet year.

A survey over the entire island found malaria prevalence varied by habitat, with 10–14% of lizards infected in the dry, windy areas, to 50–70% at some wet sites (Staats and Schall, 1996b). The cryptic species of *Plasmodium* was unknown during that previous study; hence, *Plasmodium* sp. and *P. azurophilum* were combined and equaled about half of the infections. All of the data reported here compare infected and noninfected lizards collected from the wet sites where malaria was most common in the lizards.

The methods used here are described in detail elsewhere (references given below). Briefly, lizards were captured by hand or a slipnoose at the end of a telescoping fishing pole. The height above substrate for the lizard's perching position was recorded, and that location was scored as in the sun or shade. Immediately upon capture (slip noose method only), some lizards were held by one toe while still on the noose, and a rapid reading thermometer was placed within the cloaca to record body temperature. The air temperature 1 cm above the perch site was also recorded, using the rapid reading thermometer with its bulb shaded from the sun. The anoles were maintained in mesh sacks until evening, when they were measured (SVL = snout–vent length), sexed based on the spotted dorsal color pattern and postanal scales in males, tail condition noted (intact, broken, or regenerated), and a toe clipped to extract a drop of blood to make a thin blood smear. The lizards were released the next morning at their point of capture. The smears were fixed in absolute methanol and stained with Giemsa, then scanned for 6 min at 1000× to detect and identify parasites (Schall 1996; Staats and Schall, 1996a). *Plasmodium floridense* and *Plasmodium* sp. were readily distinguished by size and number of nuclei in the dividing schizont stage (*Plasmodium* sp. is much larger with more nuclei) and the presence of malaria pigment (found only in *P. floridense*). *Plasmodium azurophilum* was found only in white blood cells. White cells are far less common in the blood than erythrocytes; hence, *P. azurophilum* typically has lower density in the blood. It is possible that very weak infections are more often overlooked than those of *P. floridense* and *Plasmodium* sp.

Parasitemia was determined by counting the number of parasites of *P. floridense* and *Plasmodium* sp. seen in microscope fields containing a total of 1000 erythrocytes. This method allowed determination of parasitemia for only the two species that infect erythrocytes. Also recorded was the number of immature erythrocytes, which differ in their shape, size, and staining color; immature erythrocytes contain less hemoglobin than mature cells (Schall, 1990). Blood hemoglobin was determined by the cyanmethemoglobin method (Schall, 1990).

Foraging success was assayed by capturing a lizard during 1000–1200 h, immediately placing it into a large (3000 cm^3 volume) zip-top plastic bag, and keeping it there for 24 h. Lizards were not used if they defecated upon capture. The bags were opened every 8 h to allow fresh air to circulate. Feces present in the bag after 24 h were collected, frozen, then later dried to constant mass and weighed (Eisen and Schall, 1997). The mass of the live lizard was also obtained and the relative fecal mass calculated as feces mass/lizard mass.

Male-male interaction was examined by placing an adult female *A. sabanus* and two adult males into a large net cage (3 × 3 × 2 m). Males were matched by SVL and tail condition, but one was infected with *Plasmodium*, and the other was not infected. Each male was marked with unique dots on the dorsal surface. The lizards were placed into the cage in the evening and observations were made for the next two days from 0800–1400 h. The observer was not aware which lizard was infected. Recorded were (1) number of dewlap displays apparently to the other male, (2) time in seconds spent displaying to another male, (3) number of dewlap displays apparently to the female, (4) time in seconds displaying to the female, (5) number of approaches to the female or other male, (6) number of times moved, (7) number of copulations and time spent copulating. These data were then examined by three individuals experienced in observing *Anolis* interactions and the dominant male identified if possible (Schall and Dearing, 1987).

Anolis sabanus is strongly sexually dimorphic in color; males develop brown spots on the dorsal surface, which are absent in the females (Lazell, 1972). The dorsal surface of lizards was photographed and the resulting 35-mm slides were projected onto a digitizing planimeter (Zeiss Co.) to measure the proportion of the dorsal surface covered with the spots (Ressel and Schall, 1989). The symmetry of the spots was determined by counting spots on the left and right side of the middorsal row of prominent scales (spots on the midline were scored depending on the proportion of the spot to the left or right of the midline) (Schall, 1996).

Nonparametric statistical tests were used throughout to compare infected versus noninfected lizards: Mann-Whitney *U*-tests for comparisons between two distributions, *G*-tests to

TABLE 1. HEMATOLOGICAL MEASURES COMPARING *Anolis sabanus* INFECTED OR NOT INFECTED WITH A MALARIA PARASITE. Given are number of immature erythrocytes (iRBC) per 10,000 erythrocyte and hemoglobin concentration in the whole blood (g/dl). Code for infection status: NI = Not Infected, Az = *P. azurophilum,* Fl = *P. floridense, P. sp. = undescribed species.*

Infection status	iRBC $\bar{x}$ (*n*, Range)	Hemoglobin $\bar{x}$ (*n*, Range)
NI	378 (27, 48–1494)	7.1 (128, 4.5–9.7)
Az	408 (27, 162–1039)	
Fl	536 (32, 168–1835)	7.1 (96, 4.6–11.2)
P. sp.	560 (24, 150–1428)	6.4 (15, 5.1–7.6)

compare counts, binomial test with expectation of equal number of wins for the male-male interaction experiments, and Wilcoxon signed rank tests to compare number of behaviors displayed by lizards in the male-male observations. For the analysis of blood parameters, only solitary infections (one parasite species) were compared with noninfected lizards. For other analyses, noninfected lizards were compared with lizards infected with any of the malaria parasites because partitioning results by species of *Plasmodium* did not alter the results.

RESULTS

Parasitemia.—Parasitemia was determined for a randomly selected sample of infections of *P. floridense* (mean = 0.87% of erythrocytes infected; range from not detected in 1000 cells counted to 6.6%, $n = 59$) and *Plasmodium* sp. (mean = 0.22%; range from not detected to 1.7%, $n = 32$). Both species can reach fairly high parasitemia, although such infections are rare. During the initial examination of blood smears to detect infected animals, we recorded an estimate of parasitemia. Therefore, we were able to search these preliminary notes to select five of the infections with highest parasitemia; these ranged from 3.7–11.3% for *P. floridense* and 1.7–21.8% for *P.* sp.

Blood cells and hemoglobin.—Immature erythrocytes were more abundant in the blood of lizards infected with *P. floridense* or *Plasmodium* sp. than for those not infected or infected with *P. azurophilum* ($P < 0.05$; Table 1). Hemoglobin content of the blood was not reduced for lizards infected with *P. floridense* compared with noninfected lizards ($P > 0.05$), but infection with *Plasmodium* sp. led to a significant 10% drop in hemoglobin ($P < 0.05$; Table 1). Only a very few solitary *P. azurophilum* infections were assayed for hemoglobin levels, precluding comparisons with other classes of infection status.

Body temperature.—Infection did not result in changes in behaviorally maintained body temperature of the lizards (infected lizards, mean = 28.9 C, $n = 69$, and for noninfected lizards, mean = 29.4, $n = 96$; $P > 0.05$). Air temperature at the perching site did not differ by infection status (28.8 C for noninfected vs 28.5 C for infected lizards; $P > 0.05$) nor did proportion of animals in sun versus shade ($P > 0.05$).

Tail breaks.—Although injured tails are common for the Saban anole for both males (35%, $n = 1420$) and females (31%, $n = 666$), there was no difference in injured tails (combining both recently broken tails and regenerated tails) for infected and noninfected lizards (partitioning data by gender of lizard, $P > 0.05$).

Perching location and foraging success.—Mean perching height did not differ between noninfected lizards (77.8 mm; $n = 85$) and lizards infected with a malaria parasite (80.9 mm, total $n = 34$, $P > 0.05$). Relative dry mass of feces produced during 24 h after capture did not differ between infected and noninfected lizards ($P > 0.05$, analysis split by gender of lizard, male noninfected mean = 0.0025, $n = 140$, combined infected mean = 0.0027, n = 71; female noninfected mean = 0.0041, $n = 62$; combined infected mean = 0.0028, $n = 28$).

Male-male interaction.—For 21 experiments, interactions of an infected and noninfected male were scored as a tie in five experiments, and infected lizards were winners in eight and noninfected winners in eight ($P > 0.05$). The number or duration of displays, approaches to another lizard, moves, number of copulations and duration of copulations did not differ significantly by infection status ($P > 0.05$).

Body color.—The number of spots on the dorsal surface of male lizards did not differ between infected and noninfected lizards (mean = 30 for each group, $n = 452$ noninfected and $n = 430$ for total infected lizards; $P > 0.05$). The area covered with the brown spots also did not differ among groups (mean = 31–36%, $P > 0.05$). The absolute difference in number of spots on the left and right sides of the middorsal scale row also did not differ by infection status (mean = 0.31–0.36, $P > 0.05$).

Discussion

The survey of potential costs suffered by the Saban anole lizards infected with three species of *Plasmodium* reveals low virulence for this parasite-host system. The low virulence is in striking contrast with the consequences of malaria infection for fence lizards, *Sceloporus occidentalis* (infected with *P. mexicanum*) in California, *Agama agama* (infected with *P. giganteum* and *P. agamae*) in west Africa, and possibly for *A. gingivinus* (infected primarily with *P. azurophilum* and *Plasmodium* sp.) on St. Martin (Schall, 1990, 1992, 1996).

The difference between the Saban parasites and the other cited lizard malaria systems is apparent when comparing the density of parasites in the blood. Parasitemia for the other lizard malaria parasites is typically higher than for any of the three *Plasmodium* infecting the Saban anole [3–11 times as high for *P. mexicanum* in California depending on comparison, 1–7 times for *P. agamae*, and 2–11 times for *P. giganteum* in west Africa (Schall, 1996; Schall and Bromwich, 1994)]. Parasite replication in the blood leads to destruction of erythrocytes, production by the lizard of replacement immature erythrocytes, and consequent reduction in blood hemoglobin levels (Schall, 1996). As expected, hemoglobin reduction is less severe for the Saban anole (no change for infections of *P. floridense* and a reduction of 12% for *Plasmodium* sp.) than for either the California (25%) or African (21%) lizards. The St. Martin anole, when infected with *Plasmodium* sp., suffers a 10% reduction in hemoglobin, similar to that seen for *Plasmodium* sp. infections on Saba.

Malaria infection results in significant behavioral changes in California lizards (and more limited evidence suggests this is true also for the African lizard hosts when infected), but we observed no similar effects for the Saban anole. Fence lizards in California infected with *P. mexicanum* are more likely to have an injured tail (for both males and females; Schall, 1996), but no such effect was found for the Saba malaria system. Infected male fence lizards are less able to maintain territories and compete for access to females than are noninfected males (Schall and Dearing, 1987; Schall and Houle, 1992). In experimentally staged male-male contests, 90% of the winners were the noninfected males. In contrast, infection did not alter the success of male *A. sabanus* in similar experimental male-male contests. The foraging success of free-ranging Saban anoles was not affected by malaria infection, but this is also true for fence lizards in California (Eisen and Schall, 1997), perhaps because maintaining a territory is not important for access to insect prey.

Parasitic infection could alter the appearance of showy traits in males such that females might obtain information on the genetic quality (resistance to parasites) of potential mates (Schall and Staats, 1997). Likewise, parasitic infection might disrupt the development of infected hosts, which can be measured as an decrease in the left-right symmetry in populations of bilaterally symmetrical animals (Møller, 1994). Fence lizards in California are sexually dimorphic in ventral color pattern; males develop blue and black markings to a far greater degree than females. Infected male lizards differ from noninfected individuals in the proportion of their ventral surface covered with black (Ressel and Schall, 1989) and infected animals are also less symmetrical in the size of their blue patches (Schall, 1996). In contrast, infection status did not alter the sexually dimorphic colors of infected males for *A. sabanus*.

Although lizards develop behavioral fevers in response to infection with some pathogens as an antiparasite tactic (Kluger, 1979), infection with malaria parasites does not cause changes in thermoregulation for infected lizards in Saba, California, or west Africa (Schall, 1990, 1996).

In summary, *P. azurophilum*, *P. floridense*, and *Plasmodium* sp. all appear to have low virulence for the Saban anole. This result is in stark contrast with detailed studies on temperate-zone lizard malaria in California, two malaria species of the west African tropical habitat, and perhaps even the same three Caribbean species on St. Martin, just 52 km from Saba. Several hypotheses have been presented to explain such variation in virulence among closely related parasite species, or even genetic strains within species (review in Schall, 2001). Two will be discussed here. The ''Small Worlds'' hypothesis argues that when host population sizes are small (or the parasite has access to only a small number of hosts because of low dispersal ability), avirulence is expected because the parasite must await the arrival of new young or migrants (Boots and Sasaki, 1999). Perhaps the available number of lizard hosts on islands is less than for mainland systems. This seems unlikely because *Anolis* are often very abundant on Caribbean islands and this is certainly so on Saba (pers. obs.). A more likely possibility is that other aspects of the transmission biology of the Saban malaria parasites differs from the mainland systems. Low virulence is expected when transmission intensity (in this case, number of bites by infectious vectors) is low (Ewald, 1994), there

is a period of impossible transmission resulting from seasonality (Gill and Mock, 1985), or the host normally has a long lifespan (Ebert and Mangin, 1990). All of these ecological forces would favor ''prudent'' parasites that maintain low parasitemia and thus extend their host's lifespan but also reduce their acute probability of transmission. The results presented here thus predict that the Saban anole is an unusually long-lived lizard, or vectors are rare or only seasonally active on the island. These predictions remain to be tested.

Acknowledgments

We thank the people of Saba for their kindness and friendship throughout this research. The research was conducted with the cooperation of the Saba Conservation Foundation. The van't Hof-Cornet family hosted us on the island. Our loyal field assistants were T. Bartolotta, M. Kaplan, R. Kim, and M. McKnight. The experiment was approved by the University of Vermont Animal Care and Use Committee (Protocol 94–19B), and the research was funded by grants from the National Science Foundation (DEB95–27115) and the National Geographic Society.

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