Journal of Herpetology, Vol. 20, No. 3, pp. 318–324, 1986

Prevalence and Virulence of a Haemogregarine Parasite of the Aruban Whiptail Lizard, *Cnemidophorus arubensis*

JOS. J. SCHALL

Department of Zoology, University of Vermont, Burlington, Vermont 05405, USA

ABSTRACT. — ***Cnemidophorus arubensis*, an endemic teiid lizard of Aruba island, Netherlands Antilles, is parasitized by a haemogregarine protozoan. The proportion of animals infected (prevalence) was greater for males than females and for adults compared to juveniles. Brightly colored males were more likely to be infected than blandly colored males of the same body size. Percent of erythrocytes infected with parasite gametocytes, and parasite prevalence, were similar in both wet and dry seasons. Infected and noninfected lizards were similar for several hematological, physiological, anatomical, and behavioral measures of parasite virulence. The Aruban haemogregarine appears to have an avirulent effect on *Cnemidophorus arubensis*.**

Most lizard populations harbor a broad variety of parasites, including protozoans, helminths, and ectoparasitic arthropods (see Telford [1970] for an example of a model survey). Despite the abundance of lizard-parasite associations, reliable data on the effects of parasites on their saurian hosts are scarce. Some parasites, though, do play a significant role in the biology of lizards. For example, infection with the malarial parasite *Plasmodium mexicanum* results in severe hematological, physiological, behavioral, and reproductive consequences for its vertebrate host, *Sceloporus occidentalis* (Schall et al., 1982; Schall, 1983*a*, *b*). *P. mexicanum* may be a typical protozoan blood parasite in its effects on its host, or it may be an unusually virulent species. The lack of comparative data on other species of parasites makes general statements on the virulence of lizard parasites highly speculative.

The haemogregarines are among the most common protozoan parasites of lizards. These organisms are related to *Plasmodium* and may resemble the ancestral form of the malarial parasite (Manwell, 1977). Haemogregarines most often utilize two hosts, a blood-feeding invertebrate and a vertebrate, such as a lizard. Most haemogregarines of lizards undergo asexual reproduction in the liver and produce sexual cells, or gametocytes, which infect host erythrocytes. Some haemogregarines are known to cause severe liver damage in mammalian hosts (Miller, 1908; Hoogstrahl, 1961; Furman, 1966); their effects on lizards are unknown.

Here I examine the patterns of prevalence (% of hosts infected) and the virulence of a haemogregarine that infects the Aruba island whiptail lizard, *Cnemidophorus arubensis*. Comparisons in prevalence are made between wet and dry seasons, between male and female hosts, and among host size classes and color patterns of males. The latter is interesting because adult male *C. arubensis* are polymorphic in color, ranging from bright blue with white spots to brown with brown stripes (Schall, unpubl. obser.). Hamilton and Zuk (1982) have proposed that bright colors in male animals may vary with parasite burden and provide females with information on the fitness of potential mates. Finally, I examine several measures of parasite virulence; most of these measures are similar to those used in the studies of malaria in *Sceloporus* and thus provide comparative information on the virulence of protozoan parasites of lizards.

Materials and Methods

Cnemidophorus arubensis is a common endemic teiid of the island of Aruba, Netherlands Antilles. Aruba experiences a pronounced wet-dry seasonal cycle; the rainy period commences in September and ends in February. Lizards for this study were observed and collected during October 1980, March 1984, and November 1984, thus covering both wet and dry seasons. As indicated in the Results section, not all kinds of data described below were gathered for each sample period.

Aruban whiptails were collected by noosing or in funnel traps baited with tomato and canned tuna. That same evening, a blood sample was extracted from the postorbital sinus of each lizard, using a heparinized capillary tube. Thin blood smears were made, fixed in absolute methanol, and later stained for 50 minutes with Giemsa 1:10 at pH 7.2. Hemoglobin concentration of the blood was determined using a colorimetric method (Brown, 1984). Five ml of cyanmethemoglobin reagent and 0.02 ml whole blood were mixed and read at 540 nm on a Bausch & Lomb Mini 20 spectrophotometer. Readings were compared to those taken with dilutions of a hemoglobin standard (Hycel, Inc.).

Each collected animal was sexed, measured (SVL), and scored for dorsal color pattern. The March 1984 sample was scored from living animals using four color classes: (1) bright blue with pale blue spots on flanks; (2) blue-brown with white spots; (3) brown with blue spots; (4) brown with brown stripes. The October 1980 sample was scored from preserved animals which had experienced color fading; therefore, color classes 2 and 3 had to be combined. *C. arubensis* is easily sexed by the presence on males of spur-like anal scales. A sample of animals was preserved in 10% formalin solution, and later dissected to determine mass of the liver, inguinal fat bodies, and testes (for males). Liver and fat mass were corrected for animal size as organ mass/body mass. The mass of internal organs of vertebrates typically do not scale linearly with body mass (Calder, 1984), so only the largest lizards of each sex were used for analysis (♂♂, mass > 20 g; ♀♀ mass > 9.5 g).

Live whiptail lizards were brought into the laboratory and maintained in large pens, 2 m × 1 m, or 2 m × 0.5 m in size, outfitted with a substrate of soil, sand, rocks, logs, and cardboard hiding places. Overhead were heat lamps and full-spectrum fluorescent fixtures. Dogfood, and occasionally crickets, were provided daily.

After starving the animals for two days, resting O_2 consumption, maximal O_2 consumption, and running stamina were measured using methods adapted from Bennett and Gleeson (1976) and Bennett (1980). For determination of resting O_2 consumption, male lizards were placed in a 232 ml airtight glass chamber within a darkened incubator set at 39°C, the typical body temperature of active *Cnemidophorus* (Schall, 1977). Tubes entering and leaving the chamber passed through a peristaltic pump, then through columns of drierite to remove water vapor and ascarite to remove CO_2, and finally to a Beckman E2 oxygen analyzer. The system remained open for at least one hour, and then closed to record O_2 consumption. Approximately 400 ml of air circulated through the closed system; the exact volume depended on the volume of the enclosed animal. When a very tight linear drop in O_2 content of the contained gas indicated the animal was at rest, readings commenced and were taken over at least an hour.

Maximal oxygen consumption was indexed using animals that had been starved several days. Each lizard was individually placed into a cloth sack, then stored for several hours in an incubator set at 39°C. The lizards were then taken individually into an environmental chamber set at the same temperature.

Each animal was chased around a cardboard running track (5.7 m around × 17 cm wide) for 30 sec at its maximal running speed. The observer promptly placed the lizard into a 235 ml glass vessel outfitted with sampling ports. After 2 min, a sample of gas could be extracted and compared to a sample taken just before the trial began. Results for both resting and maximal oxygen consumption are corrected to STP conditions and reported as ml per g of lizard per h ($\dot{V}O_2/g \cdot h$).

Each animal was returned to the incubator the next day for several hours of rest in individual cloth sacks and then run at maximal speed around the track. Distance run by the lizard was recorded after 30 sec and 2 min of running. This measure of locomotive stamina, though simple, is reproducible among animals (Bennett, 1980).

Blood smears were scanned for presence of parasite gametocytes in the blood cells. False negatives were possible if the infection had not yet begun to shed gametocytes into the blood. Parasitemia in blood is expressed as parasites/10,000 erythrocytes (RBC). We also recorded the percent of immature cells (iRBC) among the erythrocytes. These are easily distinguished when stained with Giemsa by their color and morphological features (Schall, 1983*a*).

Results

Parasitemia and Prevalence.—Haemogregarine gametocytes are very obvious in *C. arubensis* erythrocytes that have been stained with Giemsa. As no haemogregarine has ever been described from *C. arubensis*, I describe this species' gametocytes here. It is probably a species of *Hepatozoon*, but should not be given a species designation without knowledge of the full life cycle (Manwell, 1977). Gametocytes are sausage-shaped, the cytoplasm stains very lightly purple, and the centrally located nucleus stains deeply purple. The host cell nucleus is displaced to accommodate the parasite. Mean sizes of 10 gametocytes measured were: length 15.0 μ (SD = 1.3) and width 6.0 μ (SD = 0.64). Lengths ranged from 12.8 μ to 16.8 μ.

Parasitemia of gametocytes in erythrocytes was low. Of 60 randomly selected infected lizards, 39 showed parasitemia <1/10,000 red blood cells (RBC) and the highest seen was only 394/10,000 RBC. Samples from the wet season of 1980 and dry season of 1984 showed very similar parasitemia: 19/30 infections in the wet season and 20/30 in the dry season had parasite loads <1/10,000 RBC. There was no significant difference between seasonal samples for those infections with measurable parasitemia (Mann-Whitney U test; $P > 0.05$).

Haemogregarine prevalence did not differ between wet and dry season samples (Table 1; χ^2 tests, $P > 0.05$). Parasite prevalence was higher for male than female lizards (Table 1). In males, prevalence increased with body size, but leveled off as lizards reached adult size (Fig. 1). There was no significant difference in body size for infected and noninfected females (Fig. 1 and U tests, $P > 0.05$). Because of the leveling off in prevalence in larger males, there was no overall difference in prevalence based on male body size (U tests; $P > 0.05$)

Female *C. arubensis* are all brown with brown stripes, whereas males, including the largest animals, can be of any of the color classes. However, because juvenile males resemble females, I selected only males >100 mm SVL for the following analyses. Differences among individual lizards in growth rate are unknown; by choosing only the largest males I hoped to compare samples of infected and noninfected lizards that were of similar age distributions. For the 1984 sample (scored from live animals), infected adult male lizards had a significantly different color distribution than that of noninfected animals ($\chi^2 = 7.84$, $P < 0.05$, N = 83). Infected animals fell more often into color classes 1 and 2 (60%), and noninfected ones into

classes 3 and 4 (69%). Thus, more colorful males were more likely to be infected with the haemogregarine, regardless of their body size. The 1980 sample was scored from 91 preserved adult males. Once again, more colorful males were more likely to be infected (36% of infected males were class 1, and only 17% of noninfected males were color class 1), although the difference between infected and noninfected males was not significant ($\chi^2 = 5.08$, $P = 0.079$).

Hematological Effects.—Percent immature erythrocytes (iRBC) was scored for four groups: noninfected lizards collected in 1984 dry season ($\bar{x}$ iRBC = 0.70%, SD = 1.03), noninfected animals collected in 1980 wet season ($\bar{x} = 0.97\%$, SD = 1.02), infected lizards with parasitemia <1/10,000 RBC ($\bar{x} = 0.78\%$, SD = 1.21), and infected lizards with parasitemia >1/10,000 RBC ($\bar{x} = 0.66\%$, SD = 0.96). Sample sizes were 30 for each group. All four groups displayed similar, low-level percentages of immature cells, indicating the parasite does not cause measurable host erythrocyte destruction (Kruskal-Wallis test, $P > 0.05$).

Hemoglobin concentration in whole blood was determined only for the March 1984 sample and did not differ significantly between sexes of *C. arubensis* ($\bar{x}$ for males = 8.4 g/100 ml, N = 58, and for females 8.9 g/100 ml, N = 56; *U* test, $P > 0.05$); thus, data were combined to compare infected and noninfected lizards. These two groups did not differ in hemoglobin content of the blood ($\bar{x}$ for both groups = 8.7 g/100 ml, infected N = 36, noninfected N = 114; *U* test, $P > 0.05$). Parasitemia and hemoglobin concentration were not correlated (Spearman correlation, $P > 0.05$). Several animals collected in March 1984 were very thin and slow moving. These animals had low hemoglobin concentrations (~5 to 6 g/100 ml blood), but this group contained both lizards infected and not infected with the haemogregarine.

Organ Weights.—Mean relative fat mass and liver mass differ between sexes (*U* test, $P < 0.05$), so comparisons of infected and noninfected lizards were conducted separately for each sex. Both infected and noninfected males had relative fat mass at 0.8% of body mass, and both groups of females stored fat equal to 0.5% of body mass (*U* tests, $P > 0.05$). Similarly, there was no difference between groups in relative liver mass (for males 2.6% of body mass, for females 4.0% of body mass; *U* tests, $P > 0.05$). Lastly, mean relative testis weight was larger for noninfected whiptail lizards (0.2% of body mass vs. 0.1%, but the difference was also not significant (*U* test, $P > 0.05$).

TABLE 1. Numbers of *Cnemidophorus arubensis* infected with a haemogregarine parasite for two sample periods, October 1980 (wet season on the island of Aruba) and March 1984 (dry season).

		Infected	Not infected
Females	1980	11 (18%)	49
	1984	11 (18%)	49
Males	1980	21 (36%)	38
	1984	20 (33%)	41

Physiological and Behavioral Effects.—Table 2 reveals that haemogregarine infection does not have substantial impact on an important physiological function, consumption of oxygen by tissues, and its behavioral correlate, running stamina. However, infected *C. arubensis* displayed significantly higher consumption of oxygen when at rest.

DISCUSSION

The effect of parasites on their hosts ranges from severely pathogenic, in which host fitness is substantially reduced, to a benign association approaching commensalism. The evolutionary origin of variation in parasite pathogenicity, which occurs even among closely related species of parasites, is of considerable theoretical interest (Ewald, 1983). However, a general explanation for this variation may be elusive. Of more prosaic interest is the realization that parasites can add an

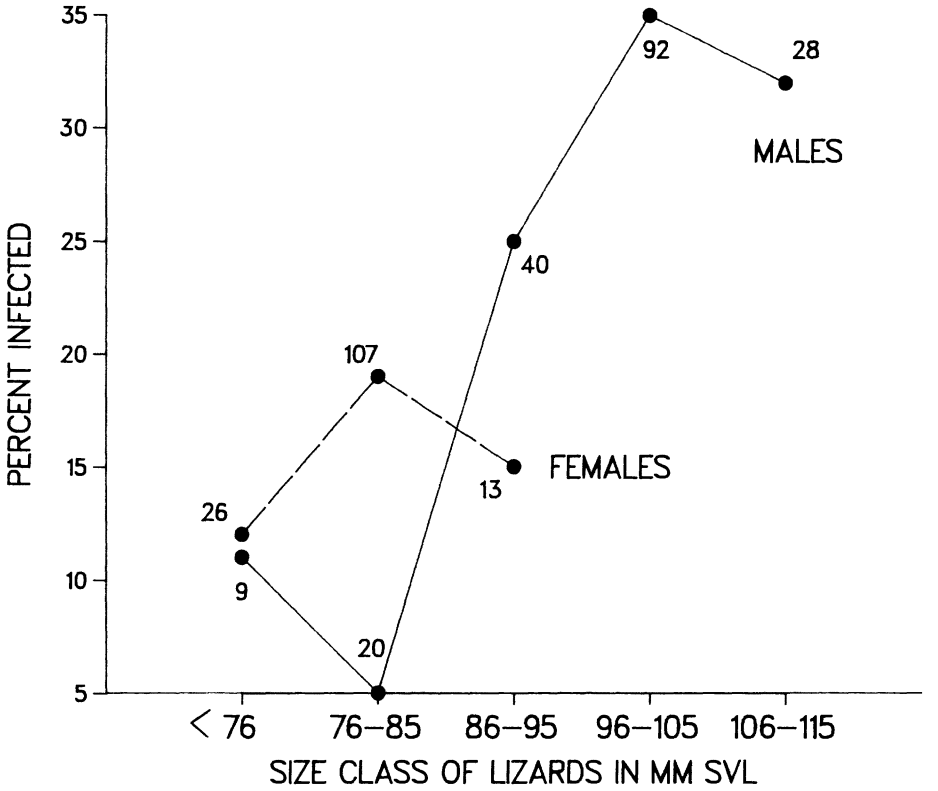

FIG. 1. Percent of male and female *Cnemidophorus arubensis* infected with a haemogregarine parasite by body size class. The figure demonstrates sexual dimorphism in which adult males are substantially larger than females.

unexplained source of variation to data (Schall, 1983*a*). The measured effects of *Plasmodium* on fence lizards demonstrate that the presence of parasites can seriously compromise results from ecological or physiological studies.

Despite the fact that haemogregarines can be severely pathogenic to some hosts (Maxwell, 1977), the Aruban haemogregarine appears to have little, if any impact on *Cnemidophorus arubensis*. The lack of hematological pathology is not surprising because the parasite does not undergo reproduction in the blood cells, but only casts relatively few gametocytes into erythrocytes. However, as the Aruban haemogregarine presumably undergoes proliferation in the liver of the lizard, I expected the parasite to have measurable impact on its host. Damage to the liver was measured only in a crude way by simply weighing the organ. Pathology at the histological or biochemical level, though, should have been evident from the amount of fat tissue stored or from a reduction in behavioral performance. Although any destruction of liver tissue is critical for a vertebrate, such damage would be of primary significance for the Aruban whiptail lizard because of its diet. *C. arubensis* is primarily a herbivore and consumes several kinds of highly toxic plants (Schall, 1986). Vertebrates neutralize toxic substances in their diets via mixed function oxidase systems in the liver (Rhoades, 1979), so disruption of liver function could severely alter the whiptail's ability to process toxic plant material in its diet.

TABLE 2. Physiological and behavioral performance of *Cnemidophorus arubensis* infected and not infected with a haemogregarine parasite. Oxygen consumption when animal was at rest, for two min after 30 sec of maximal activity, and the difference between the two are given as ml $O_2/g \cdot h$. Also presented is the distance run in meters in an oval running track in 30 sec and 2 min when chased. Means are followed by SD and sample size.

	Not infected	Infected	*U*-test
Resting $\dot{V}O_2$	0.265 (0.067) 6	0.294 (0.077) 6	$P < 0.05$
Maximal $\dot{V}O_2$	0.960 (0.243) 74	0.936 (0.233) 14	ns
Increment $\dot{V}O_2$	0.544 (0.136) 6	0.498 (0.133) 6	ns
Distance run in 30 sec	24.06 (6.40) 61	23.57 (6.64) 14	ns
Distance run in 2 min	46.16 (10.36) 61	45.37 (12.42) 14	ns

The presence of haemogregarine gametocytes, which are the only infective stage, in very similar numbers in both wet and dry seasons suggests that transmission takes place year-round. If so, this fact would help in discovering the vector. However, *C. arubensis* blood cells might live a long time (autoradiography of radioisotope labelled erythrocytes reveals they can live at least 48 days; Maizels, 1980), so those seen in one season could simply be aging cells cast into the blood some months earlier.

The prevalence distribution among size classes seen in Fig. 1 approximates one that emerges from simple models of the dynamics of parasite infection in which the chance of infection per time period is constant, there is no mortality caused by the parasite, and infection is life-long (Cohen, 1976). The chance of becoming infected with the Aruban haemogregarine must be very stable over time for *C. arubensis* because parasite prevalence was very similar for wet and dry season samples taken almost four years apart.

Hamilton and Zuk (1982) propose that sexually dimorphic traits remain important in female choice of mates even after long periods of sexual selection because they provide information about the ability of males to elude parasitic attack. Thus, infected males could appear scrofulous, or simply less colorful, when parasitized. In the Aruban whiptail lizard, however, infection with haemogregarines and bright colors are actually positively associated in males. Brightly colored *C. arubensis* males appear more aggressive and frequently engage in chases (Schall, unpublished observations). Perhaps these animals are simply more frequently exposed to attack by the biting arthropod that is the vector of the haemogregarine. Alternatively, the more colorful animals might simply be older and have had a longer exposure to the vector. I attempted to eliminate this possibility by using only the largest males in the analysis. Although Hamilton and Zuk have proposed an intriguing hypothesis, proper testing requires a sophisticated understanding of the particular parasite-host system.

Acknowledgments.—Rene W. Schall assisted greatly with the field work. My laboratory assistants were invaluable: Cynthia Lord, Steve Thompson, Lauri Harvie, Steve Ressel, Ross Nayduch, and Terri Gutterson. The research was funded by grants from the American Philosophical Society, the Whitehall Foundation, and NSF (BSR-8306925).

LITERATURE CITED

BENNETT, A. F. 1980. The thermal dependence of lizard behavior. Anim. Behav. 28:752–762.

———, AND T. T. GLEESON. 1976. Activity metabolism in the lizard *Sceloporus occidentalis*. Zoology 49:65–76.

BROWN, B. 1984. Hematology: Principles and Procedures. Lea & Febiger, Philadelphia. 405 pp.

CALDER, W. A. 1984. Size, Function, and Life History. Harvard Univ. Press, Cambridge. 431 pp.

COHEN, J. E. 1976. Schistosomiasis: a human-host parasite system. *In* R. M. May (ed.), Theoretical Ecology: Principles and Applications. Pp. 237–256. W. B. Saunders, Philadelphia.

EWALD, P. W. 1983. Host-parasite relations, vectors, and the evolution of disease severity. Ann. Rev. Ecol. Syst. 14:456–486.

FURMAN, D. P. 1966. *Hepatozoon balfouri* (Laveran 1905) sporogonic cycle, pathogenesis, and transmission by mites to jerboa hosts. J. Parasitol. 52:373–382.

HAMILTON, W. D., AND M. ZUK. 1982. Heritable true fitness and bright birds: A role for parasites? Science 218:384–387.

HOOGSTRAHL, H. 1961. The life cycle and incidence of *Hepatozoon balfouri* (Laveran 1905) in Egyptian jerboas (*Jaculus spp.*) and mites. J. Protozool. 8:231–248.

MAIZELS, C. S. 1980. The cell kinetics of the peripheral blood of the Aruban whiptail lizard, *Cnemidophorus arubensis*. MS thesis, Immaculate Heart College, Los Angeles. 58 pp.

MANWELL, R. D. 1977. Gregarines and haemogregarines. *In* J. P. Kreier (ed.), Parasitic Protozoa III. Pp. 1–32. Academic Press, New York.

MILLER, W. W. 1908. *Hepatozoon perniciosum* (n.g., n. sp.) a haemogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Laelaps echidninus*). Bull. Hyg. Lab., Treas. Dept., Washington 46.

RHOADES, D. F. 1979. The evolution of chemical defense against herbivores. *In* G. A. Rosenthal and D. H. Janzen (eds.), Herbivores, Their Interaction with Secondary Plant Metabolites. Pp. 4–55. Academic Press, New York.

SCHALL, J. J. 1977. Thermal ecology of five sympatric species of *Cnemidophorus* (Sauria: Teiidae). Herpetologica 33:261–272.

———. 1983*a*. Lizard malaria: Parasite-host ecology. *In* R. B. Huey, E. R. Pianka, and T. W. Schoener (eds.), Lizard Ecology: Studies of a Model Organism. Pp. 84–100. Harvard University Press, Cambridge, Mass.

———. 1983b. Lizard malaria: Cost to vertebrate host's reproductive success. Parasitology 87: 1–6.

———. 1986. Toxic plant compounds and the diet of the herbivorous whiptail lizard, *Cnemidophorus arubensis*. *In* J. Wright (ed.), Biology of *Cnemidophorus*. Los Angeles Co. Mus. Nat. Hist. *In press.*

———, A. F. BENNETT, AND R. W. PUTNAM. 1982. Lizards infected with malaria: Physiological and behavioral consequences. Science 217: 1057–1059.

TELFORD, S. R. 1970. A comparative study of endoparasitism among some southern California lizard populations. Amer. Midl. Natur. 83:516–554.

Accepted: 18 October 1985.