AN INTRAERYTHROCYTIC PROTOZOAN PARASITE OF THE GARTER SNAKE Thamnophis sirtalis

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BRIEF NOTES, SURVEYS AND COMMENTS

AN INTRAERYTHROCYTIC PROTOZOAN PARASITE OF THE GARTER SNAKE Thamnophis sirtalis

Garter snakes *(Thamnophis sirtalis)* were collected during April, May, September and October, 1965, from Long Point Provincial Park and vicinity on the north shore of Lake Erie, in southern Ontario.

During July, a number of the captive snakes became lethargic and died. Histological sections from many of these showed a heavy infestation with a species of lungworm identified by Dr. R. C. Anderson as *Rhabdias* sp. Bacteriologic cultures of visceral organs revealed *Proteus* and *Arizona* species. Other snakes appeared to die only from weakness and inability to compete for food.

Blood was obtained from the most lethargic snakes either by cardiac puncture or by severing the end of the tail with a scalpel. Examination of blood smears revealed that many of the snakes had a heavy infection with a hitherto undescribed intraerythrocytic protozoan parasite. Blood smears were stained by the May-Grunwald Giemsa technique. Hematocrit determinations were made on each blood sample, using an International Micro-Capillary reader.

Whole blood from both infected and non-infected snakes was injected via the wing vein in 0.25 ml and 0.50 ml amounts into healthy, three day-old Leghorn chicks. Smears of peripheral blood were made from four consecutive weekly bleedings of the chicks.

Blood from infected and non-infected snakes was injected also into the allantoic chambers of ten-day embryonated eggs. Daily observations were made to detect embryo mortality. Blood smears were made from the embryos at nineteen days of incubation.

Several snakes, found to be infected by examination of blood smears, were killed and portions of lung, heart, liver, intestine and kidney were fixed in buffered formalin. Histological sections cut from the paraffin-embedded tissues were stained with hematoxylin and eosin.

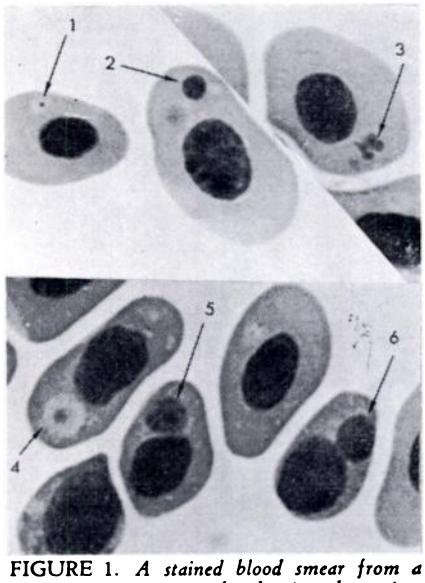

FIGURE 1. *A stained blood smear from a garter snake showing the variety of forms of the intraerythrocytic parasite.*

Results and Discussion

In smears of snake blood, the parasite appeared as a round to oval body varying in size from 1 μ to 4 μ in diameter. It was located about halfway between the nucleus and the periphery of the erythrocyte. The variety of forms of the parasite suggested the possibility that a process of schizogony had taken place, similar to that in piroplasmosis. The stages observed included clear or faintly basophilic forms approximately 1 μ in diameter, each of which contained a centrally located single basophilic body (Figure 1-1). Also seen were slightly larger and more darkly basophilic forms with purple colored central bodies (Figure 1-2). Enlargement of the parasite apparently occurred and several purple bod-

ies, assumed to be chromatin material, appeared around the periphery (Figure 1-3). This form of the parasite varied in staining from blue to purple and the numbers of chromatin dots in a single parasite varied from 6 to about 16 (Figures 1-4, 5, 6). If these dots represented merozoites resulting from a process of schizogony, it is strange that no such structures were found singly either in the cytoplasm of the erythrocytes or free in the blood.

Study of the blood smears from the inoculated chicks revealed no evidence of transmission of the parasite.

The snake parasite failed to cause mortality in the chicken embryos and no evidence of propagation was found when allantoic fluids and blood smears were examined at nineteen days of incubation.

Hematocrit determination did not show a correlation between infection rate (as determined by peripheral blood smears) and the presence of anemia. The average rate of infection of snakes, based on several field collections, was 30%.

The pathogenicity of this parasite for garter snakes is unknown. From observations made in this study, it would appear to be relatively non-pathogenic. However, other factors such as seasonal incidence, abundance of vectors and concurrent infections, may affect the degree to which this parasite manifests itself.

A search of the literature has failed to reveal any previous description of this organism in garter snakes. However, several papers have been published dealing with similar organisms in both reptiles and birds.

Dut Toit (1937, Onderstepoort Jour. Vet. Sci. 9:289-299) described a piroplasm of a lizard, which he named *Sauroplasma thomasi* as a new genus and species under Piroplasmidae. Usually in the lizard one round parasite, 0.6 to 4.5 μ in diameter, was seen per erythrocyte. Smaller anaplasmoid forms were described. These consisted of chromatin granules or signet ring forms with a nucleus at one side and the centers vacuolated.

Chatton and Blanc (1914. C. R. Soc. Biol. 77:496-498; 1916. C. R. Soc. Biol. 79:39-43) described a parasite, *Pirhemocyton tarentolae* in the erythrocytes of a gecko. These parasites appeared as anaplasmoid bodies, 1 μ in diameter, or larger forms 3 to 4 μ in diameter, with central chromatin dots. They were associated with globular, refractile bodies which were present in infected corpuscles, but which were separate from the parasites. Bearup (1951. Proc. Linn. Soc. N.S.W. 76:26) and Mackerras (1961. Aust. Jour. Zool. 9:61-122) described similar parasites in *Phyllurus platurus* in Australia. However, in these cases they described no clear zone of cytoplasm around the central chromatin body.

Dutton et al. (1907, Ann. Trop. Med. Parasit. 1:285-370) mention an unidentified round parasite of snakes. From their colored sketch, it appears to resemble the parasite of garter snakes described in this paper.

The piroplasm, *Aegyptianella pullorum* of chickens was first described by Carpano (1928. Boll. Serv. Tech. Sci. Min. Agr. Egitto 86:1-12). Morphologically it resembles our snake parasite. The early stages of the parasite are described as oval or rounded granules less than 1 μ in diameter. Later, schizonts developed that were round or oval in shape, and 1 to 2 μ in diameter, with 1 to 4 centrally located chromatin bodies. Six to 20 deeper staining merozoites then formed. Large, round or oval forms rich in chromatin and thought to be gametes were demonstrated.

The possibility also exists that the parasitic forms observed in the snake erythrocytes were stages of the life cycles of one of the genera of primitive coccidia, namely *Lankesterella, Shellackia* or *Karyolysus* (Kudo, 1966, Protozoology. 5th Ed. Charles C. Thomas, Springfield). No histological evidence was found, how-

ever, in any of the organs of the snakes examined, to suggest intermediate stages of a life cycle of such a parasite.

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HEMATOZOA OF THE MALLARD DUCK *Anas platyrhynchos* L. IN SOUTH DAKOTA

The only study of hematozoa of birds in South Dakota is that of Weib (M.A. Thesis, Univ. of South Dakota, 1962). Although his survey included 29 species of birds, belonging to 12 different families, it did not cover any waterfowl. In the present study 169 wild mallard ducks *Anas platyrhynchos* L. were examined for blood parasites during the latter part of January, 1965. The ducks were part of a large waterfowl concentration which winters annually at Lake Andes National Wildlife Refuge, Lake Andes, South Dakota. Blood smears were made from the birds during the time of the annual winter banding of waterfowl.

Blood for the smears was obtained by needle puncture of the lower leg vein located medially on the metatarsal region of the leg. The smears were air dried fixed in absolute methyl alcohol, and stained with Wright's stain according to Lillie's modification (Histopathologic Technic and Practical Histochemistry, 1954). They were examined under low power (100X) for 15 minutes and under high power (450X) for 15 minutes. Two blood smears were made from each duck so the blood of each bird was examined for a period of at least one hour. Subsequently, slides recorded as positive were scanned completely.

In this study 75 (44.4%) of the 169 ducks were found infected with at least one species of blood parasite. Five birds were infected with two different parasites. However, the parasitemia levels were low with no more than 10 parasites on any positive slides and usually only from one to three.

Leucocytozoon simondi Mathis and Leger was found in 47 ducks, 20 birds were infected with *Parahaemoproteus nettionis* (Johnston and Cleland), and seven birds were infected with microfilariae. Double infections consisted of two cases of *L. simondi* and *P. nettionis,* two cases of *L. simondi* and microfilariae, and one case of *P. nettionis* and microfilariae. A majority of the gametocytes of *L. simondi* were elongate forms but some round ones were observed. Two distinct types of microfilariae were seen. A short, broad unsheathed form with a distinctly striated cuticle was found in six birds, whereas a slender, sheathed form was present in one bird.

A relatively high infection rate for *L. simondi* (27.8%) was observed here. This suggests that the reservoir potential of this mallard population is sufficient to establish infections of the insect vectors in the areas in which these ducks will migrate in the spring. This is of interest with regard to the epizootiology of *L. simondi* infections, since it appears that infected wild ducks, at least in certain areas, are responsible for supplying an adequate parasite source to start epizootics (Fallis & Bennett, Can. J. Zool. 44:101-112, 1965). Also, this study was made at the time of the year when the parasitemias were probably lowest (see Chernin, Am. J. Hyg. 56:101-118, 1952), so that even a higher rate might have been found with a longer examination period.

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