Hepatozoon procyonis INFECTIONS IN TEXAS *

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Abstract: The literature related to Hepatozoon infections is reviewed; observations from the study of spontaneous hepatozoonosis in 57 raccoons (Procyon lotor) and one white-tailed deer (Odocoileus virginianus) are presented, with special reference to pathogenesis.

INTRODUCTION

Hemogregarine parasites of the genus Hepatozoon have been recorded from a wide variety of vertebrate and invertebrate hosts. The range of vertebrate hosts includes reptiles, rodents, insectivores, marsupials, carnivores and ungulates. Infections of rodents and reptiles are most commonly reported. Tables I and II are partial compilations of Hepatozoon species described for rodents and reptiles, respectively.

Hepatozoonosis in carnivores has been less frequently reported but has been well documented in some parts of the world. Hepatozoon canis infections are found in dogs in India,[10,17,37,63] Africa,[60] Ceylon,[28] Iraq,[76] France,[67] Egypt,[27] and Malaya.[32,44] A hepatozoon, H. chattoni, of the jackal (Canis aureus),[53,58] was described in African hyenas (Crocuta crocuta).[40,43] Wild foxes (Vulpes bengalensis) in India were found to be naturally infected with a parasite morphologically identical to H. canis.[64] Hepatozoonosis (species undetermined) has also been reported in lions (Panthera leo)[12,13,43] and leopards (Panthera parda)[13,40] in Africa.

H. hoogstraali has been described from an insectivore, Hemicentetes semispinosus,[71] and Hepatozoon infections have been reported in genet cats (Gentta tigrina and G. rubiginosa)[13,38,40] palm civets (Paradoxurus hermaphroditus),[14] South American opossums (Didelphys didelphys),[72] and eight species of Australian mammals.[48] Rabbits and domestic cats have also been reported to have Hepatozoon infections, but detailed information is lacking.[56,59,69] In the Americas, Hepatozoon infections in carnivores occur principally in raccoons.[66,70] The species is H. procyonis, and it has been reported from Procyon lotor in Georgia and from P. cancrivorus in Panama.

Hepatozoon infections in ungulates are apparently rare: A hepatozoon found in impala (Aepyceros melampus) was presumed to be H. canis on the basis of similar morphology.[9] Presumed gametocytes of Hepatozoon sp. were found in monocytes of a reedbuck (Redunca arundinum) and in the lymphocytes of a giraffe (Giraffa camelopardalis).[9]

Hepatozoon infection is not reported to have harmful effects on rodents with the exception of H. muris[14,44,51,59] which reportedly caused anemia, hepatic lipidosis and death, and H. balfouri, which caused hepatic necrosis.[30] H. canis is reported to cause fever, anemia, icterus, and fatal infections in some dogs,[32,60,63] while other dogs are less severely affected.[28,67,77] Active inflammation was associated with hepatic infections in impala,[9] although hepatozoonosis in African carnivores is considered non-pathogenic.[40]

TABLE 1. **Hepatozoon** spp. of Rodents.

Host	Hepatozoon	Reference
Rattus norvegicus	H. muris	2, 20, 21, 33, 44, 48, 61
Rattus rattus	H. muris	44, 48
Rattus assimilis	H. muris	44, 48
Rattus conatius	H. muris	44, 48
Mus musculus	H. musculi	59
Mus decumanus	H. muris	4, 51
Mus rattus	?	1
Peromyscys boylii boylii	H. muris	78
Peromyscys truei gilberti	H. muris	78
Peromyscys maniculatus gambelii	H. leptosoma	78
Sigmodon hispidis	?	41
Mus sylvaticus	H. sylvatici	21
Gerbillus indicus	H. gerbilli	16
Jaculus jaculus	H. balfouri	4, 36
Cricetus frumentarius	H. criceti	52
Microtus agrestis	H. microti	21
Microtus californicys marposae	H. citellicola	52
Microtus oeconomus	?	55
Clethrionomys glareolus	H. erhardovae	42
Citellus beecheyi	H. citellicola	75
Citellus suslicus buttarius	H. mereschkowski	14
Sciurus carolensis	H. griseisciuri	20, 34
Sciurus vulgaris	H. sciuri	20, 21
Sciurus niger	?	66
Funambulus pennantii	H. funambuli	57
Atlantoxerus getulus	H. gaetulum	20
Petaurista petaurista	?	20
Meriones libycus erythrourus	?	20
Spermophilopsis leptodactylus	?	20
Tatera afra	?	42

TABLE 2. **Hepatozoon** spp. of Reptiles.

Host	Hepatzoon	Ref.
Boa constrictor	H. fusifex	6, 7
Drymarchon corais	H. rarefaciens	6
Crocodilus niloticus	H. pettiti	35
Crotaphopectis degeni	H. minchini	31
Tarentola mauritanica	H. burneti	45
Anolis carolinensis	?	11
Varanus tristis orientalis	H. breinli	50
Varanus varius	H. breinli	50
Varanus gouldii	H. breinli	50
Gecko verticillatus	H. mesnili	68
Sauromalus obesus	H. sauromali	47
Crotalus viridis	?	3, 54
Pituophis melanoleucus	H. rarefaciens	3, 54
Thamnophis sirtalis	?	3, 54
Thamnophis couchi	?	3, 54
Sceloporus occidentalis	?	3, 54
Madagascarophis colubrina	H. domerguei	54

*There may be other hepatozoon species infecting reptiles, but these are presently confused with other hemogregarines since it is necessary to ascertain both vertebrate and invertebrate hosts in order to place a hemogregarine in the genus *Hepatozoon*,[5] and in many cases, this has not been done.

H. procyonis was reported to cause no evidence of harm to host in *Procyon lotor*,[08] while an active myocarditis was reported in Panamanian raccoons.[70]

A wide variety of invertebrate hosts have been identified, including the following hematophagic arthropods: ticks,[7,18,19,63,77] fleas,[24,42] mites,[20,30,36,44,47,51,65] mosquitoes,[6,7,8,11] biting flies,[3,35] and lice.[16,59]

Schizogony occurs in the vertebrate hosts, with schizonts developing in liver,[6,20,30,40,44,47,51,55,63] bone marrow,[17,20,63,76] cardiac muscle,[12,13,39,40,66,70] skeletal muscle,[38] lung,[7,40,08] spleen,[29,43,63,76] lymph node,[9,43] and intestine[08] in various hosts.

Two types of schizonts have been described, one producing a few macromerozoites, and the other producing many micromerozoites. There is uncertainty as to which schizont produces gametocytes, but it is believed that one produces gametocytes and the other produces merozoites capable of perpetuating schizogony.[49,76] Gametocytes which are characterized by highly refractile cyst walls[10,17,20,34,37] are found in leukocytes in carnivores and most rodents, and in erythrocytes in marsupials, reptiles and some rodents.[48,53] Both schizonts and gametocytes have structures which are sudanophilic and PAS-positive.[24]

Sporogony, occurring in the arthropod host, is characterized by the development of large oocysts with sporocysts. (This is the feature which separates this genus from the genus *Haemogregarina*.[5]) Infection of the verebrate host reportedly occurs after ingestion of the parasitized arthropod although there is an unconfirmed report of transmission of *H. canis* by tick bites.[13] Following schizogony, trophozoites may undergo schizogony again [14,49] or form gametocytes which enter blood cells and are ingested by the arthropod host, where they undergo sporogony following zygote formation.[49,51,76]

Previously, detection of a *Hepatozoon* species in a new host was considered sufficient reason for designating is as a new species, since host specificity was considered to be an important taxonomic criterion.[14,34,42,44] However, recent work has shown that certain species of *Hepatozoon* are not host specific,[11,24,44,54] casting doubt upon the validity of some species designations.

MATERIALS AND METHODS

Sixty-five adult raccoons (*Procyon lotor*) obtained over a 2-year period from 14 Texas counties (Figure 1) were necropsied and sections of selected tissues were examined microscopically. In addition to routine hematoxylin-eosin staining, selected sections were stained by periodic acid-Schiff, Grocott's methenamine - silver, Gridley, Warthin-Starry, Giemsa, and azure-eosin procedures.

Examination of blood for gametocytes was accomplished by a leukocyte concentration technic.[34] The degree of parasitemia was estimated by placing 1 ml of whole raccoon blood into 5 ml of 2% formalin, preparing a smear stained with aqueous methylene blue, and counting the number of gametocytes visible in 5 minutes of microscopic examination (by one individual). Thick and thin Giemsa-stained blood smears from naturally infected raccoons were examined for the presence of gametocytes.

Twelve wild-caught infected raccoons were held in captivity for an extended period of time and concentrated leucocytes from peripheral blood were periodically examined for presence of gametocytes. One captive raccoon with a high parasitemia was splenectomized and periodic checks of gametocyte numbers were made. Two other raccoons with relatively low parasitemias were given daily injections of 30 mg prednisone* for 1 week.

Wild raccoons and nests in den trees were examined for the presence of ectoparasites in an attempt to find a common species which might be the arthropod host. Ticks (*Amblyomma americanum*) taken from wild-caught infected raccoons were fed to four white rats in an attempt to infect the rats. After 90 days, the rats were killed and necropsied. Ticks (*Dermacentor variabilis* and *A. americanum*) from infected raccoons were examined

* Meticorten (Schering Corp., Bloomfield, N.J.).

for *Hepatozoon* oocysts by making squash preparations and by embedding in paraffin and sectioning by conventional tissue sectioning techniques.

Blood and leukocyte fractions from two infected raccoons were placed on STB medium[25] and held at room temperature for 28 days. Smears were prepared from the surface of these, Giemsa-stained and examined microscopically.

OBSERVATIONS

Fifty-seven of 65 adult raccoons (88%) examined had developing gametocytes in microgranulomas in the myocardium. All adult raccoons examined from low, river bottom land had hepatozoonosis. Forty-four (68%) had schizonts in cardiac muscle. Thirty-nine (60%) had granulomas in skeletal muscle (most often diaphragm or tongue), and twenty-four (37%) had schizonts in skeletal muscle. Schizonts were found in splenic trabeculae of two animals.

On initial observation, 9 of 15 raccoons from high-prevalence areas had circulating gametocytes in enlarged monocytes, and rarely in neutrophils or free. Whenever gametocytes were present in the blood, organisms were present in microgranulomas in the myocardium, and vice versa. No gametocytes were detected in microscopic examination of routine (unconcentrated) blood films.
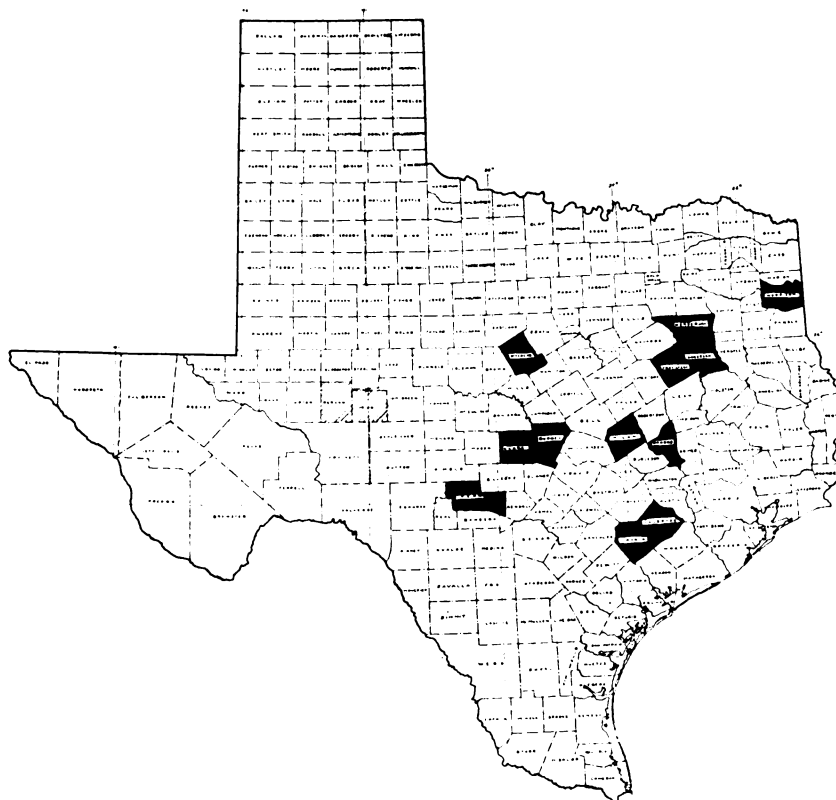

FIGURE 1. Distribution of observed hepatozoonosis by county in Texas. Cross-Latched counties had one or more confirmed case. The shaded areas were counties from which animals were examined and found not infected.

Effect on host

No clinical signs of illness attributable to *Hepatozoon* infection were observed in captive raccoons. Three raccoons which had circulating gametocytes on initial examination had a decrease in numbers of gametocytes (circulating gametocytes being rare or absent) after 3 months of captivity. The animal which underwent splenectomy had a threefold increase in circulating gametocytes 10 days after surgery. Ten months after splenectomy, no gametocytes were detectable. However, 12 months post-surgery (and following parturition) gametocyte numbers in the circulation were at about the same level as at initial examination. Necropsy revealed typical lesions of mild hepatozoonosis, with schizonts and gametocytes in the myocardium.

The two animals which received prednisone had a marked increase in gametocyte numbers. One raccoon was maintained in the laboratory for 16 months; initially and at necropsy, circulating gametocytes were present (but rare), and typical lesions, including schizonts, were present at necropsy.

The most common ectoparasite found on raccoons was the Lone Star tick, *A. americanum*. Nests in den trees were surprisingly free of parasites. The distribution of the Lone Star tick corresponds roughly with the observed distribution of infected raccoons, but feeding Lone Star ticks from infected raccoons to white rats did not produce infection in the rats. Furthermore, squash preparations and sections of ticks (both *D. variabilis* and *A. americanum*) did not reveal characteristic *Hepatozoon* oocysts.

No growth occurred on STB slants.

Lesions

No gross lesions referable to *Hepatozoon* infection were observed. Microscopically, in myocardium, skeletal muscle, and splenic red pulp, focal accumulations of macrophages were found containing phagocytized individual organisms within their cytoplasm. The organisms appeared as having a dark blue nucleus surrounded by a zone of clear light pink cytoplasm (Fig. 2). Lymphocytes, and rarely, neutrophils and eosinophils, were
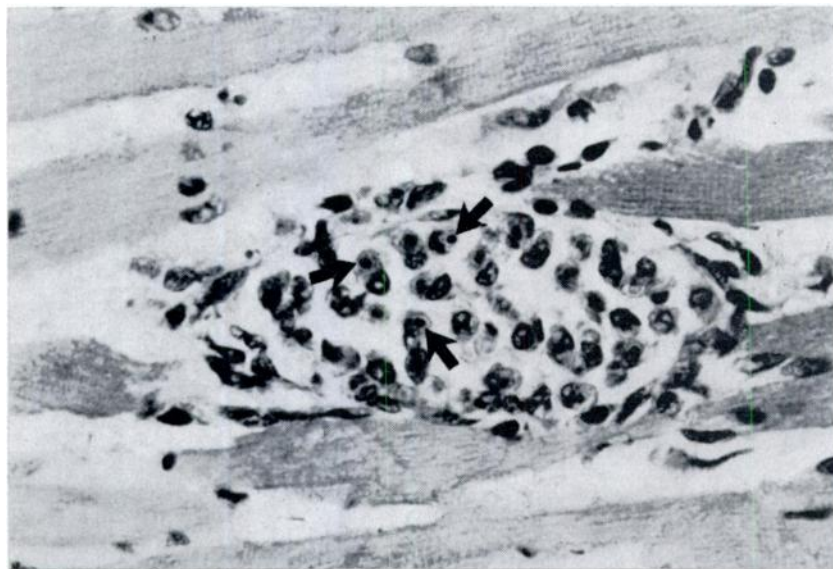

FIGURE 2. Focal granuloma in raccoon myocardium. Individual organisms are present in macrophages (arrows). H & E X 400.

found in association with these micro-granulomas. Multinucleated foreign-body giant cells were present in a few instances. Inflammation varied from intense, active inflammation to well - contained, apparently quiescent microgranulomas.

In a few instances, the granulomas were associated with focal myocardial necrosis and severe local inflammation, but in these cases, concomitant disease, usually canine distemper, was present.

Intact schizonts ($31.2\pm 7.8\ \mu$ x $22.7\pm 5.5\ \mu$) were not accompanied by any inflammatory response, and were found within myofibers (Fig. 3). The majority of these were X type schizonts[10] with many micromerozoites ($\overline{X} = 20 \pm 4.7$). Some mature schizonts were surrounded by a mild zone of inflammatory cells and newly-ruptured schizonts were accompanied by intense focal inflammation with phagocytes of merozoites and/or gametocytes (Fig. 4).

Organisms

Special staining techniques were utilized to facilitate differentiation of structures of the organisms. The nuclei of organisms within macrophages stained with hematoxylin-eosin and Giemsa, and was slightly PAS positive. Silver staining by the Warthin-Starry method allowed visualization of the capsule, revealing a characteristic "tadpole" morphology, with a recurved "tail" and a nucleus separated from each end by light-staining cytoplasm (Fig. 5A).

The staining characteristics of schizonts were similar, with merozoite nuclei staining with H&E, Giemsa and azure-eosin. The residual material was PAS positive, as was the cytoplasm of merozoites. However, no capsule or "tadpole" morphology was demonstrable in schizogonic stages.

Gametocytes found in circulating monocytes ($7.5\pm 0.5\ \mu$ x $3.9\pm 0.4\ \mu$) or in tissue macrophages have a refractile cyst wall which gives the gametocyte the characteristic "tadpole" morphology (Fig. 5B). Neither the cyst wall nor the tadpole shape is found in organisms in mature schizonts. There has been speculation that the host cell forms this "cytocyst" for the parasite.[3] Slow rotational movement of gametocytes in monocytes was frequently observed in concentrated leukocyte preparations.
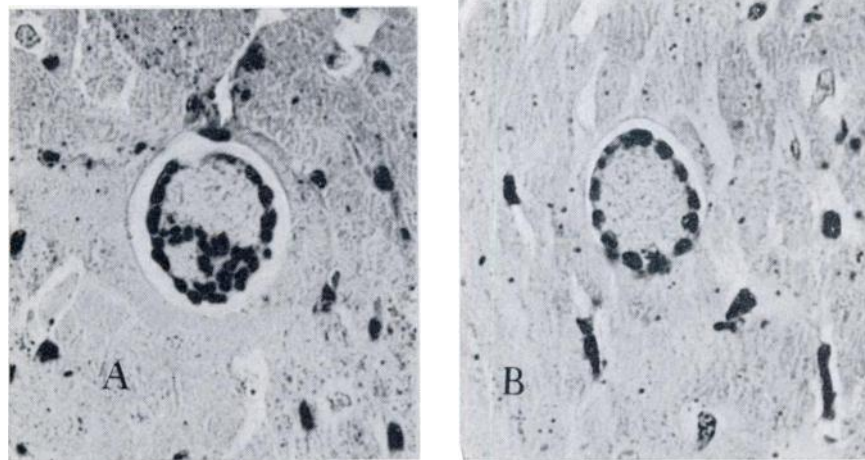

FIGURE 3. Schizonts. A. X-schizont, within myofiber with no inflammatory response. B. Y-schizont. Warthin-Starry X 400.

Infection in Other Species

Necropsy of a severely emaciated senile female white-tailed deer from Llano County revealed typical lesions of hepatozoonosis with gametocytes and schizonts in the myocardium. Other debilitating conditions (malnutrition, theileriasis, lungworms, pneumonia, and chronic nephritis) were present. No other infected deer have been found in over 500 necropsies of white-tailed deer.
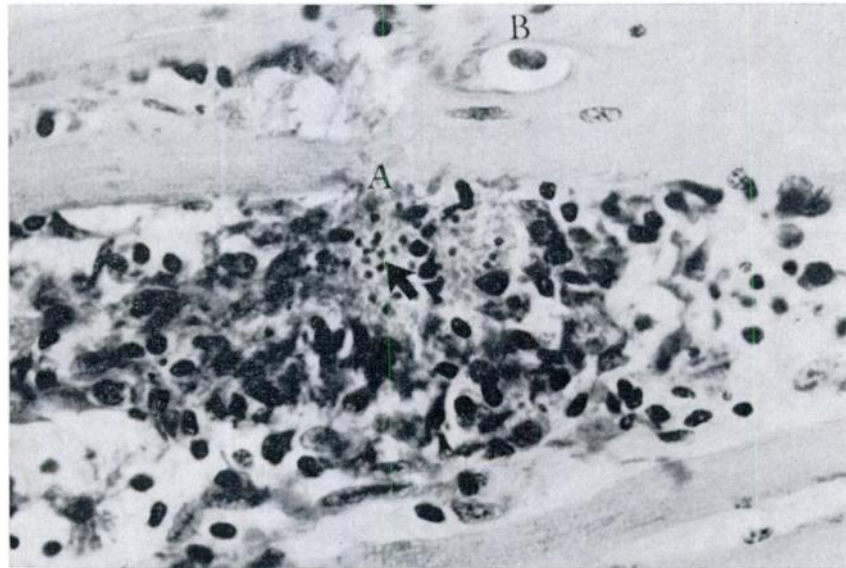

FIGURE 4. A newly-ruptured schizont with intense local inflammatory response around Merozoites (arrow). B. Immature schizont within myofiber with no inflammation.
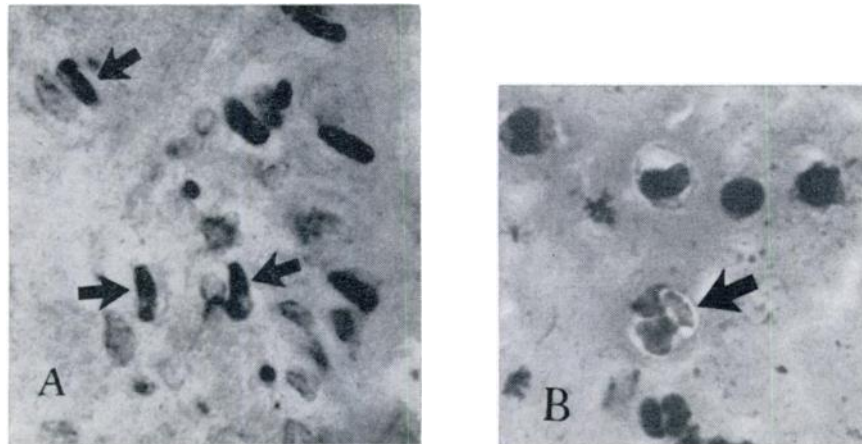

FIGURE 5. Gametocytes. A. In myocardium. Warthin Starry X 970. B. In concentrated leukocyte smear. X 970.

One opossum was examined which had organisms resembling *Hepatozoon* merozoites in microgranulomas in the myocardium. However, neither gametocytes nor schizonts were demonstrable, and it was impossible to definitely identify the organisms.

DISCUSSION

The high rate of infection in raccoons in specific areas, and the duration of infections within the population suggest that the virulence of the organisms is low, and that exposure is frequent. The low rate of parasitemia previously reported in raccoons[66] was observed. Consequently, blood films proved to be diagnostically unreliable. Both the leukocyte concentration technique and microscopic examination of myocardium are good diagnostic tools.

The mechanism by which splenectomy and corticosteroid administration temporarily increased the numbers of circulating gametocytes is uncertain. The stresses of surgery may have been more important than the removal of the spleen, and the increase in gametocytes following prednisone administration may have merely been a reflection of the leukocytosis which follows corticosteroid administration. On the other hand, splenectomy and corticosteroid administration may have caused immunosuppression, which allowed increased schizogony.

The disagreement of other authors as to the effect of hepatozoonosis on the host is understandable when some of the facts herein presented are considered: There was no evidence of inflammation or cellular damage associated with immature schizonts. There was, however, an intense inflammatory response associated with release of merozoites resulting in a marked lesion characterized by accumulations of macrophages containing phagocytized organisms. These organisms have been considered young schizonts,[63] but differential staining revealed that they are gametocytes (and/or merozoites). It was further demonstrated that all schizonts occurred in myofibers, and were not accompanied by inflammation. The infection was well-tolerated and caused no clinical signs of illness in raccoons which were held in captivity on a high nutritional plane, without constant reinfection, and in the absence of other diseases. However, in concomitant infection with canine distemper (or other debilitating disease), the severity of lesions of both diseases were enhanced, and in many previously reported cases in other species, the possibility of concomitant disease could not be discounted.

There is no reason to assume that the *Hepatozoon* found in the single white-tailed deer was a new or different species. The rarity of this infection in white-tailed deer, with the high prevalence in raccoons, strongly suggests accidental infection in the affected individual.

The arthropod host (or hosts) of hepatozoonosis in Texas is unknown; therefore, only limited studies have been possible. The true significance of hepatozoonosis on the wild raccoon population is uncertain, but the recognition of this infection is important for at least two reasons: (1) It may be an important predisposing factor rendering raccoons more susceptible to other diseases, and (2) the myocardial lesions of hepatozoonosis are somewhat similar to the lesions of American trypanosomiasis and toxoplasmosis, both of which have great public health significance. American trypanosomiasis has been reported in raccoons,[66,73] and there is no evidence that raccoons are immune to toxoplasmosis. Therefore, it is important to be able to differentiate hepatozoonosis from both of these diseases. This differentiation is easily accomplished by the demonstration of the characteristic gametocytes, either in concentrated leukocyte preparations or in sections of myocardium stained by the Warthin-Starry method.

Much of the pathogenesis of the lesions associated with *Hepatozoon procyonis* infection is obscure. The earliest recognizable phase of the life cycle within raccoon tissues is the schizont within a myofiber, and it is not associated with inflammation of damage to host tissues. The maturing schizont apparently becomes chemotactic, as evidenced by infiltration of mononuclear inflammatory cells into the adjacent tissues. When

merozoites are released from the schizont, intense granulomatous inflammation occurs, with subsequent phagocytosis of the merozoites. The fate of these phagocytized merozoites appears to be important to the course of the infection. Presumably, some of these macrophages containing gametocytes re-enter the circulation as monocytes with gametocytes. This appears to be a very small proportion of infected cells, since parasitemia is always relatively low, while numbers of parasitized cells in the myocardium are relatively high. By the same logic, it seems that few of these cells re-enter schizogony, since the ratio of granulomas to schizonts is approximately 20:1 in wild raccoons.

This state of equilibrium may develop as a result of either or both of two mechanisms; schizogony resulting in merozoites capable of reinfecting myofibers and initiating schizogony may be unusual; or phagocytosis of infective merozoites may prevent them from reaching cells when they are capable of initiating schizogony. This latter mechanism has been suggested in white rats.[51]

It seems then, that uncomplicated *Hepatozoon* infection results in the formation of multifocal microgranulomas in myocardium and skeletal muscle, with host and parasite in a state of equilibrium, with little or no damage to either occurring. Although macrophages remain at the site of merozoite release, there is little indication of continued active inflammation. However, when a raccoon with heptotozoonosis contracts canine distemper, the dual infection may localize within the microgranulomas, producing focal myocardial necrosis. This may, in part, explain the apparent hypersusceptibility of wild raccoons to canine distemper and the high mortality associated with canine distemper in wild raccoon populations.

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