Onderstepoort J. vet Res. 42 (4), 117–134 (1975)

OBSERVATIONS ON NATURALLY ACQUIRED HEPATOZOONOSIS OF WILD CARNIVORES AND DOGS IN THE REPUBLIC OF SOUTH AFRICA

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Abstract

McCULLY, R. M., BASSON, P. A., BIGALKE, R. D., DE VOS, V. & YOUNG, E., 1975. Observations on naturally acquired hepatozoonosis of wild carnivores and dogs in the Republic of South Africa. *Onderstepoort J. vet Res.* 42 (4), 117–134 (1975).

Hepatozoonosis was studied in hyaenas, lions, jackals, cheetahs and one leopard in the Kruger National Park and compared with the condition seen in dogs in the Republic of South Africa. *Hepatozoon* schizonts were found in the wild carnivores. The genesis of microschizonts was followed and is illustrated. The schizonts were sometimes very plentiful in the lung, myocardium and skeletal muscle, and were also encountered in the spleen, liver and lymph nodes. Gametocytes were present in leucocytes. The host response was usually very mild. Sporogenous development in ticks was observed in *Rhipicephalus simus* females removed from an infected hyaena and *R. sanguineus* adults fed on an infected jackal in the nymphal stage. Attempts to transmit *Hepatozoon* from a jackal to dogs by means of ticks gave inconclusive results.

The developmental stages of *Hepatozoon* seen in dogs were very similar to those encountered in wild carnivores, except for the presence of more prominent residual material in the mature microschizonts. Schizonts were found in the spleen, liver, lungs and lymph nodes. In most cases the host response to the schizonts was mild and the parasite appeared to be of little consequence to the animal. Nevertheless, severe lesions were sometimes found in uncomplicated cases as well as those complicated by intercurrent diseases such as babesiosis and viral infections. In dogs with severe infections there was considerable necrosis and a marked reticulo-endothelial response with granuloma formation in the spleen, liver and lymph nodes.

Résumé

McCULLY, R. M., BASSON, P. A., BIGALKE, R. D., DE VOS, V. & YOUNG, E., 1975. *Quelques observations sur l'hépatozoonose naturelle acquise chez carnivores sauvages et chez le chien en Afrique du Sud.* Onderstepoort J. vet. Res. 42, (4), 117–134 (1975).

Les auteurs ont fait une étude portante sur l'hépatozoonose chez 8 *hyènes,* 4 *lions,* 3 *rénards,* 2 *cheetahs et un seul léopard provenants du Parc National de Kruger, en la comparant avec la maladie chez le chien. Des schizontes de* Hépatozoon *ont été démontrés chez les carnivores sauvages. La génèse de microschizontes a été poursuivie. Dans le poumon, le myocarde et les muscles striés les schizontes ont été très nombreux, moins nombreux dans le rate, le foie et les ganglions lymphatiques. On a démontré des gamétocytes dans les leucocytes. Le parasite ne provoque q'une réponse faible de la part de l'hôte. Les auteurs ont démontré le développement sporogénique chez les femelles de* Rhipicephalus simus *prélevées d'un hyène infecté, ainsi chez les adultes de* R. sanguineus *nouries en stade nymphal du sang d'un rénard infecté. Des tentatives de transmission de* Hépatozoon *du rénard au chien à partir de tiques ont échouées.*

Les stades evolutifs de Hépatozoon *chez le chien ressemblent ceux des carnivores sauvages, sauf la prèsence de devantage de substances résiduelles dans les microschizontes maturés. On a constaté le présence de schizontes dans la rate, le foie, les ganglions lymphatiques. La réponse de l'hôte envers les schizontes était légère. Néanmoins des lésions sévères ont parfois été démontrées en certains cas indemnes de maladies intercurrentes aussi bien qu'en certains cas atteints de maladies intercurrentes, telles que la babésiose et les infections virales. Une nécrose marquée avec une reaction granulomateuse du système reticulo-endothelial ont pu être démontrées dans la rate, le foie et les ganglions lymphatiques de quelques chiens gravement atteints.*

Introduction

Hepatozoonosis, caused by haemogregarines of the genus *Hepatozoon,* is a protozoon parasitism of many species of mammals involving arthropods such as ticks mites, fleas and lice as intermediate hosts. *Hepatozoon canis* James, 1905 of dogs (*Canis familiaris*) was originally discovered by Bentley (1905), while the first case of hepatozoonosis reported in South Africa was a fatal infection in a dog diagnosed by Porter (1918). Christophers (1907) and Wenyon (1911) described the development of *H. canis* in the tick, *Rhipicephalus sanguineus.* Ingestion of ticks harbouring sporozoites of *H. canis* results in infection of the dog.

Wenyon (1926) lists 23 haemogregarines that have been seen in leucocytes of wild mammals. These include *Hepatozoon rotundatum* Patton, 1910 in the jackal (*Canis aureus*) of India, *H. canis adusti* Nuttall, 1910 in the jackal (*C. adustus*) of East Africa and *H. chattoni* Ledger, 1912 in the hyaena (*Hyaena crocuta*) of Senegal. Brocklesby & Vidler (1963; 1965) recorded the lion (*Panthera leo*), leopard (*Panthera pardus*), hyaena, jackal (*C. mesomelas*) and genet (*Genetta felina*) as hosts of *Hepatozoon* spp. in Kenya, whereas Krampitz, Sachs, Schaller & Schindler (1968) observed the parasites in lions and hyaenas in Tanzania. In South Africa the infection has been observed in wild carnivores, including hyaenas, jackals (*C. mesomelas*), lions and cheetahs (*Acinonyx jubatus*) (Basson, McCully, Kruger, Van Niekerk, Young, De Vos, Keep & Ebedes, 1971); and in ruminants such as the impala (*Aepyceros melampus*) (Basson, McCully, Bigalke & Van Niekerk, 1967), nyala (*Tragelaphus angasi*) and bushbuck (*T. scriptus*) (Basson *et al.*, 1971); giraffe (*Giraffa camelopardalis*) (Fantham, 1920) and reedbuck (*Redunca arundinum*) (Fantham, 1921).

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This work was supported in part by a research grant, Project Number 3A061102B710 from the Medical Research and Development Command, United States Army, Washington, D.C.

The opinions or assertions contained herein are not to be construed as official or as reflecting the views of the United States Army, Air Force or Department of Defence

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Received 5 August 1975—Editor

Hepatozoonosis was frequently diagnosed during the course of the histopathological examination of tissues of a number of wild carnivores that were shot for research material or died naturally in the Kruger National Park. Some of these findings have been reported briefly (Basson *et al.*, 1971). A more comprehensive description is presented in this article together with an account of the condition in dogs. The genesis of the microschizonts in wild carnivores is also outlined; the development of schizonts in dogs has been described previously (Christophers, 1906; Wenyon, 1911). In this investigation it was not possible to determine where the macroschizonts fit into the developmental cycle. Some observations on the vectors and the results of attempted transmission of the parasite are also recorded.

Materials and Methods

Histopathology

Various tissues were collected from 8 hyaenas, 3 jackals, 4 lions, 1 leopard and 2 cheetahs and fixed in 10% buffered neutral formalin. They were subsequently embedded in paraffin wax, sectioned at 3 μm thickness, using a sliding microtome, and routinely stained with haematoxylin and eosin (HE). Measurements of parasites were made with an ocular micrometer.

Specimens from 20 canine cases of hepatozoonosis were drawn from the files of the Pathology Department at the Veterinary Research Institute, Onderstepoort. This material had been collected over a period of several years and originated either from dogs necropsied at Onderstepoort, or from tissues that had been submitted in 10% formalin by veterinarians from various parts of South Africa for diagnostic purposes. Sections were prepared using similar methods.

Vector and transmission studies

Blood smears were prepared and ticks were collected from hyaenas. The ticks were identified and smears were prepared from their haemolymph. The smears were stained by Giemsa's method and examined for evidence of infection with *Hepatozoon*.

Transmission studies were carried out between an infected jackal and puppies using *R. sanguineus* ticks as vectors. Nymphs of *R. sanguineus* were fed on a jackal known to be harbouring *Hepatozoon* gametocytes in its blood. The ensuing adult ticks were fed on two 4-month-old puppies raised under tick-free conditions. After 7–10 days, engorged and semi-engorged female ticks were removed for attempted infection of 7-week-old puppies raised similarly. The ticks were examined for evidence of infection as described above. Two puppies were each dosed with intact and punctured ticks (792 and 793); one received triturated ticks orally (795) and the other the same suspension subcutaneously (794), while 2 served as unexposed controls (796 and 797). The puppies were examined for evidence of infection by scrutiny of blood smears and smears prepared from the white cell layer of centrifugalized blood samples, as well as by histopathological examination of various tissues after they had been killed and necropsied from 5–10 weeks post-infection.

Results

Hyaena (H. crocuta)

All hyaenas examined were positive for schizonts of a *Hepatozoon* sp. found in the liver, lymph nodes, spleen, lungs, myocardium and skeletal muscle. Schizonts were especially numerous in the 3 sites last mentioned where they were frequently seen developing within small veins and venules. Many muscles were found to be parasitized but those of the limbs (*M. gastrocnemius, M. triceps* and extensors), pectoral muscles, *M. longissimus dorsi* and diaphragm were more frequently so. The genesis of the microschizonts was studied by examination of a series of sections from these tissues. The developmental cycle appeared to be the same irrespective of the tissue the schizonts were in, but was best observed in either heart or skeletal muscle. The various stages of schizogony could be detected in a single animal. In this investigation it was not possible to determine where the macroschizonts fit into the developmental cycle.

The smallest developmental stage of a *Hepatozoon* sp. seen was a small trophozoite within the cytoplasm of a large cell in a blood vessel (Fig. 1). Thereafter both the cell and the trophozoite, which lay in a cytoplasmic vacuole, enlarged (Fig. 2–10). The cytoplasm of the parasite became foamy in appearance (Fig. 10) and, with further development, clumps of rather widely-dispersed chromatin, representing the future nuclei of merozoites, appeared in the developing schizont (Fig. 10 and 11). The clumps of chromatin dispersed towards the periphery of the spherical- to subspherically-shaped schizonts (Fig. 11–16). Formations resembling mitotic figures usually became apparent at this stage or shortly after. These became the nuclei of elongated structures, the developing merozoites (Fig. 14). Initially the merozoites were arranged peripherally where the nuclei had previously arranged themselves as a corona (Fig. 12, 14, 15, 16, 28). With further development, however, it was apparent that eventually the mature schizont would become completely filled with merozoites (Fig. 18–21). At this stage no residual body was visible, excessive cytoplasm apparently having been used up in the formation of the merozoites.

FIG. 1–10 The genesis of a *Hepatozoon* sp. microschizont in the myocardium of a hyaena
FIG. 1 Small trophozoite with a small dark nucleus adjacent to the nucleus of a large cell within a blood vessel initiates the formation of the schizont. HE × 1 200
FIG. 2 The trophozoite enlarges and its prominent nucleus can be seen. HE × 1 600
FIG. 3 A slightly larger trophozoite within a cytoplasmic vacuole adjacent to the nucleus of a large cell filling the lumen of a blood vessel. HE × 1 600
FIG. 4 The parasitized host cell which fills the lumen of the blood vessel has an enlarging trophozoite in its cytoplasm. HE × 1 600
FIG. 5 Two early schizonts. The one on the left shows a multinucleated host cell in a blood vessel and the one on the right shows a merozoite in an endothelial cell having an eccentrically located nucleus and abundant homogeneous cytoplasm. HE × 1 200
FIG. 6 The cytoplasm of the parasitized cell has a hydropic appearance. HE × 1 200
FIG. 7 Trophozoite within a large cytoplasmic vacuole of a parasitized host cell. HE × 1 200
FIG. 8 Similar but larger trophozoite. HE × 1 200
FIG. 9 Large binucleated host cell distinctly within the lumen of a blood vessel. HE × 1 200
FIG. 10 As the trophozoite enlarges into a schizont, its cytoplasm develops a bubbly, foamy appearance. HE × 1 200

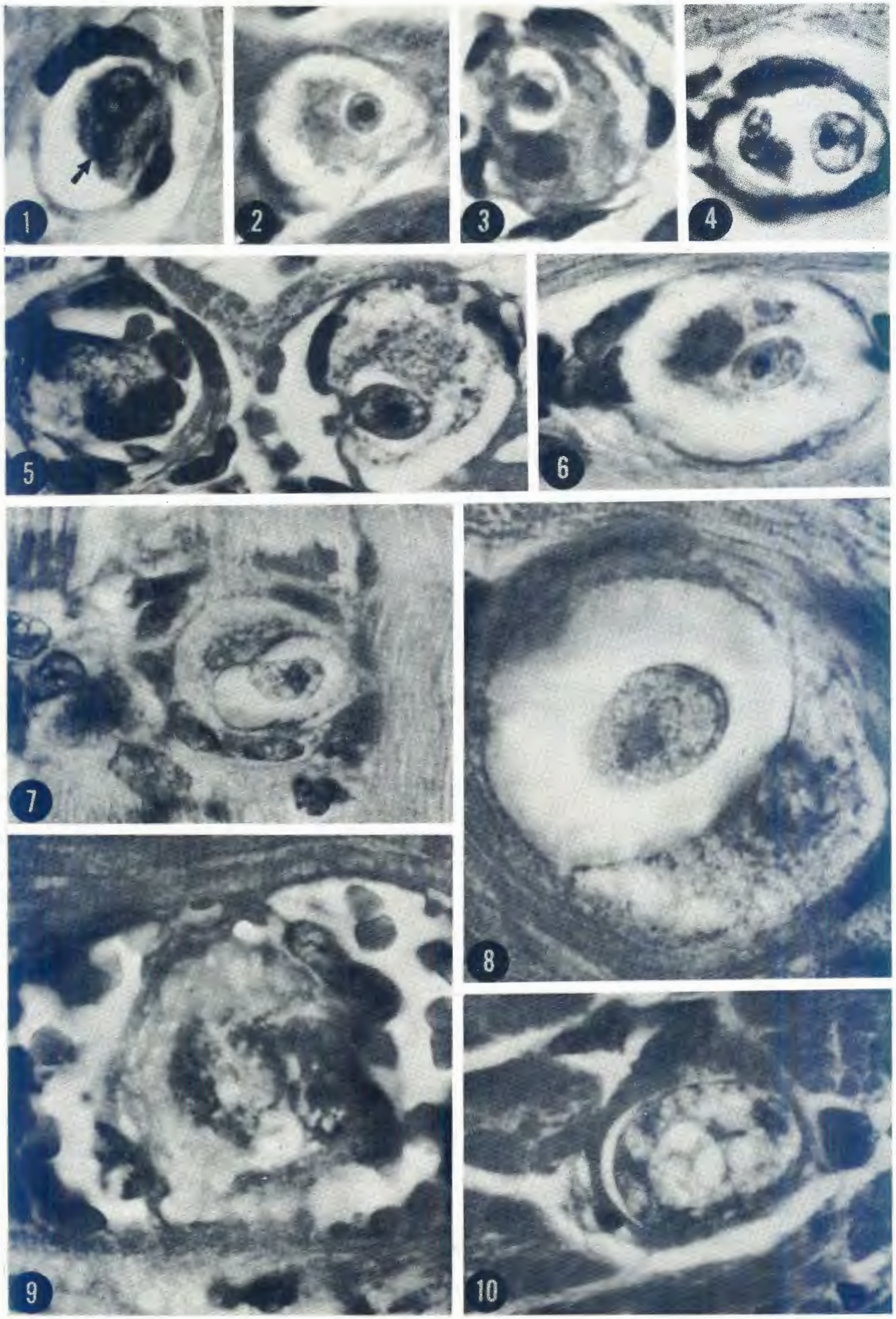
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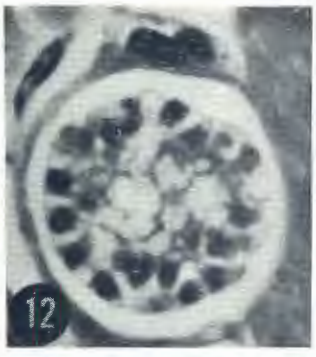
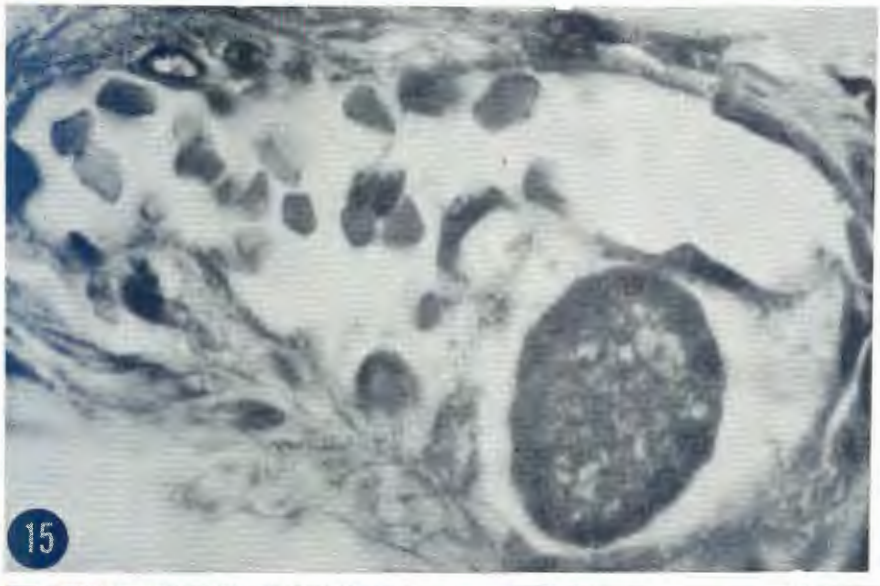
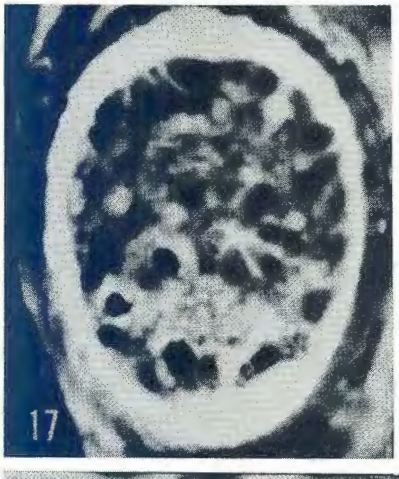
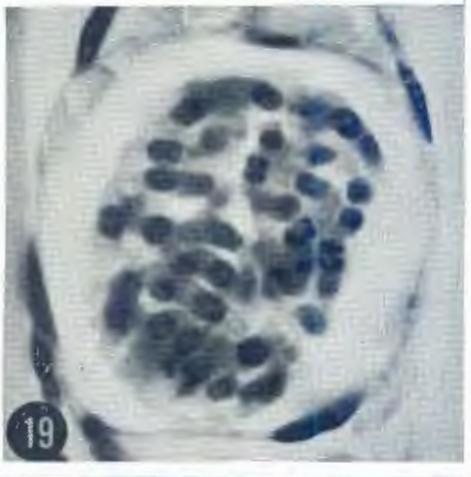
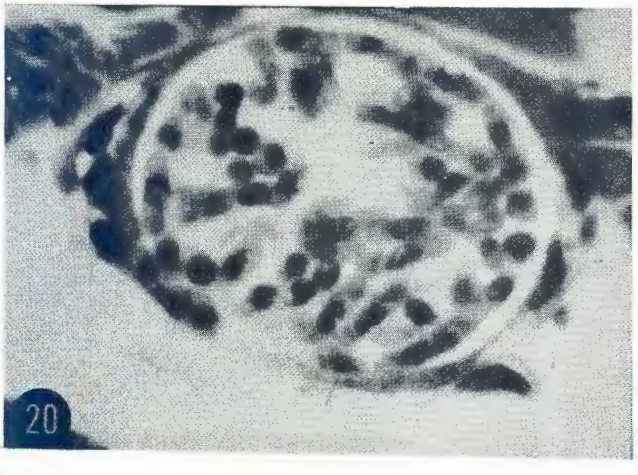
FIG. 11–21 Continuation of the genesis of a *Hepatozoon* sp. microschizont in the myocardium of a hyaena
FIG. 11–16 Poorly defined clumps of chromatin initially develop peripherally around the foamy central cytoplasm. These become more distinct nuclei as maturation progresses, at one stage resembling a morula. These schizonts are all bulging into the lumina of blood vessels, or, as in Fig. 15, situated in the wall just beneath the endothelium. HE. Fig. 11 & 12 × 1 600. Fig. 13–16 × 1 200
FIG. 17–21 As the schizont reaches maturity, discrete, elongated merozoites with a centrally-located nucleus are clearly distinguishable. Rupture of the wall of the schizont, as appears imminent in Fig. 21, should release the merozoites into the blood. HE × 1 600

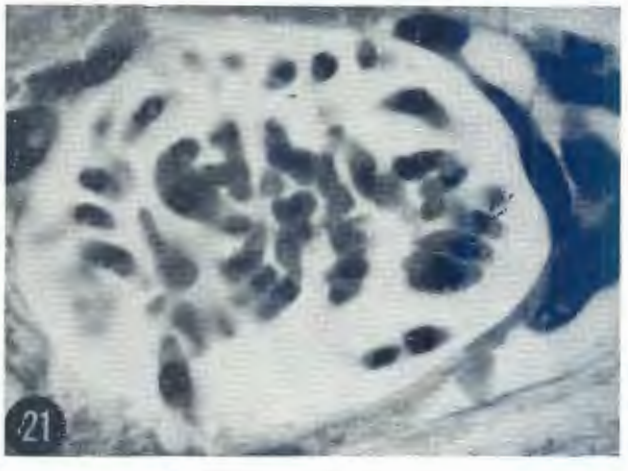












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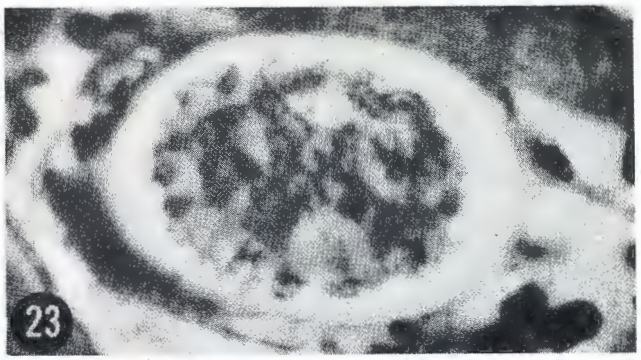
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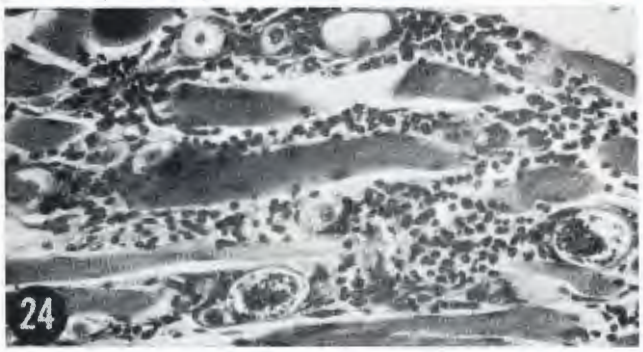
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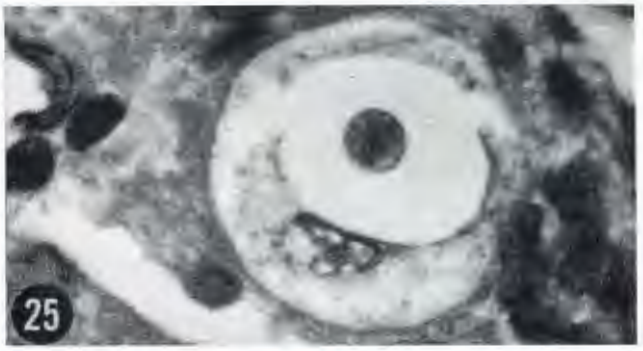
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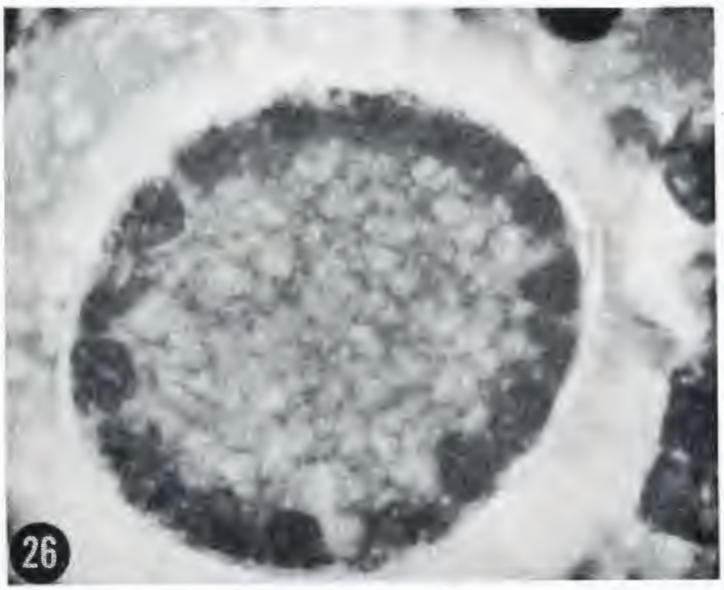
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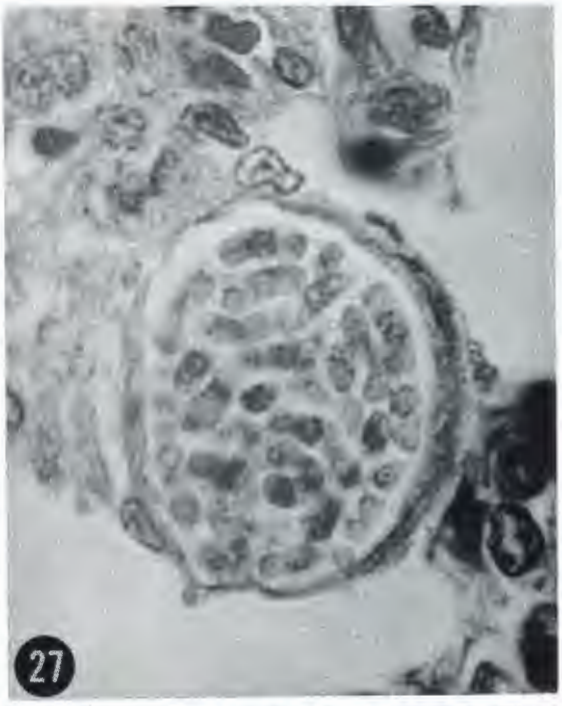
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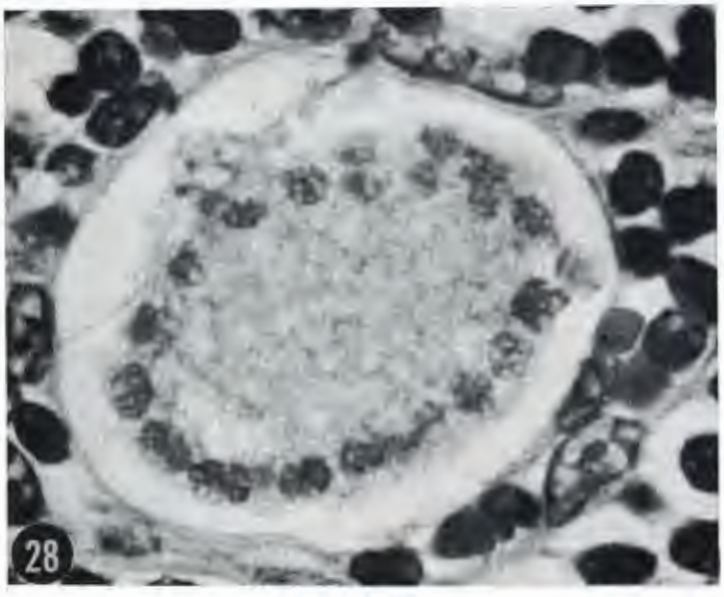
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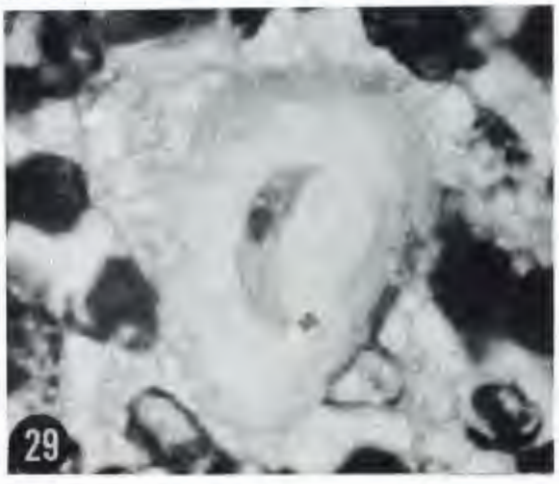
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Many schizonts developed in host cells within the walls of blood vessels. These cells sometimes occupied much of the lumen (Fig. 5 and 9); in other instances they caused only a slight bulge of the endothelium into the lumen of the vessel (Fig. 15). Some trophozoites were evidently within endothelial cells (Fig. 5) and it is possible that others developed in the primitive mesenchymal cells of the vessel wall. Several schizonts were sometimes present along the course of a single blood vessel (Fig. 22). Rupture of such mature microschizonts would result in the release of merozoite into the circulating blood. This appears to be imminent within some blood vessels (Fig. 21). Once released, micromerozoites would be free to occupy leucocytes in the circulation and develop into gametocytes. Gametocytes were occasionally seen in leucocytes in blood vessels, especially in skeletal muscle and lungs, but also in other tissues. They were also found in smears of the peripheral blood of hyaenas (Fig. 67 and 68) and showed no evidence of sexual dimorphism.

The schizonts in histological sections varied both in size and shape. Fifty microschizonts were measured: Twenty-nine in skeletal muscle had average measurements of 55×46 μm. The corresponding figures were 48×36 for 9 schizonts in the heart and 48×44 μm for 2 schizonts in the lung. The largest schizont contained mature merozoites and measured 74×43 μm. The mature merozoites were fairly consistent in size, measuring $14 \times 4{,}8$ μm on average.

A macroschizont containing a single large macromerozoite measuring $24 \times 4{,}8$ μm was present in a lymph node (Fig. 29).

Although there was little host response to most of the schizonts, a mild focal to somewhat diffuse mononuclear cell infiltration with an abundance of nuclear debris (Fig. 24) was sometimes seen in the skeletal muscle and myocardium. A few free merozoites were usually discernible in such foci. Degenerating immature schizonts, unaccompanied by any reaction, were also encountered (Fig. 23).

Kupffer cells were parasitized in the liver (Fig. 25). In the spleen the host cell nuclei in association with microschizonts were seldom apparent (Fig. 26). Microschizonts (Fig. 27) and gametocytes (not illustrated) were present in alveolar septa of the lung and were sometimes accompanied by pneumonitis characterized by an interstitial proliferation of primarily large mononuclear cells.

Stages in the sporogenous phase of the life-cycle of a *Hepatozoon* sp. were found in haemolymph smears prepared from partially engorged females of *R. simus* collected from hyaenas (Fig. 69 and 70). They were absent in *R. sanguineus* and *Haemaphysalis leachi.*

Lion (P. leo)

Early and mature stages of schizonts of a *Hepatozoon* sp. were present in the lungs, myocardium and skeletal muscle. In the myocardium very large parasitized cells with an abundance of homogeneous cytoplasm were observed interstitially where they nearly always appeared to be within blood vessels. The development of microschizonts, which was essentially the same as seen in the hyaena, is illustrated in Fig. 30–35. The subendothelial location of a schizont, which is bulging into the lumen, is shown in Fig. 33. The largest schizonts in the heart, lung and skeletal muscle respectively were 49×48 μm, 58×48 μm and 48×39 μm in size.

Leopard (P. pardus)

Developmental stages of *Hepatozoon* were also found in a leopard. Trophozoites and developing microschizonts were fairly plentiful in the walls of capillaries of the myocardium in the absence of cellular infiltration. Small numbers of free-lying merozoites were also seen. The skeletal muscle contained very few schizonts.

Cheetah (A. jubatus)

The development of microschizonts in the myocardium was essentially the same in the cheetah as in the lion. Several developmental stages of schizonts were sometimes present in the same microscopic field (Fig. 36). Schizonts were also observed within blood vessels of body adipose tissue (Fig. 37).

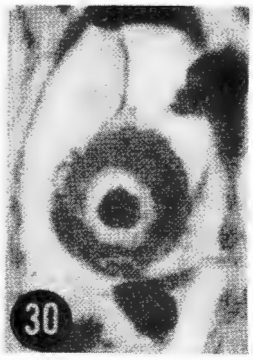
Jackal (*C. mesomelas*)

Hepatozoon schizonts with accompanying lesions were encountered in the skeletal muscles, lungs and bone marrow. The lung, muscles of the hind and fore limbs (e.g. extensors, flexors, *M. triceps*), pectoral muscles, diaphragm and *M. longissimus dorsi* were most heavily parasitized. Several adjacent microschizonts were observed within blood vessels of skeletal muscle (Fig. 38). Contrary to the findings in the other animals the accompanying myositis was invariably severe although somewhat focal. It consisted of infiltrates of numerous small round cells with much nuclear debris, which indicated necrosis of individual cells (Fig. 39). The bone marrow contained gametocytes as well as schizonts whereas only gametocytes were observed in the myocardium. Focal disseminated microgranulomatous reactions were noticed in the liver in the absence of parasites. The lesions were, however, very similar to those described in impala with hepatozoonosis (Basson *et al.*, 1971).

Dog (C. familiaris)

The tissues of 20 dogs were studied. The salient features are summarized in Table 1. Concurrent infections or other complicating conditions were present in 8 (40%) of the dogs examined. Three dogs had biliary fever due to *Babesia canis* infection and 3 cases were complicated respectively by canine distemper, a suspected viral infection and adenocarcinoma of the lung. In 2 of the others a viral infection was suspected. There was chronic anaemia in another but this may have been due to hepatozoonosis. The remaining 12 dogs (60%) had uncomplicated *H. canis* infections.

FIG. 22–29 Other pathological and parasitological aspects of infection with a *Hepatozoon* sp. in the hyaena
FIG. 22 Skeletal muscle. Multiple schizonts in the course of the single small vessel. HE × 500
FIG. 23 Heart. Degenerative schizont in myocardium. HE × 1 200
FIG. 24 Skeletal muscle. Focal interstitial myositis in response to multiple schizonts at various stages of development. A sarcosporidial cyst is present at the upper left of the picture. HE × 150
FIG. 25 Liver. Trophozoite in large cytoplasmic vacuole of Kupffer cell filling a hepatic sinusoid. HE × 1 200
FIG. 26 Spleen. Large microschizont with central foamy cytoplasm surrounded by a corona of nuclei of developing merozoites at the periphery. HE × 1 200
FIG. 27 Lung. Large microschizont with quite mature merozoites in blood vessel of an alveolar septum. HE × 1 200
FIG. 28 Lymph node. Large microschizont with central foamy cytoplasm and corona of nuclei of the developing merozoites. HE × 1 200
FIG. 29 Lymph node. This apparently represents a macroschizont because it contains a greatly elongated merozoite. HE × 1 200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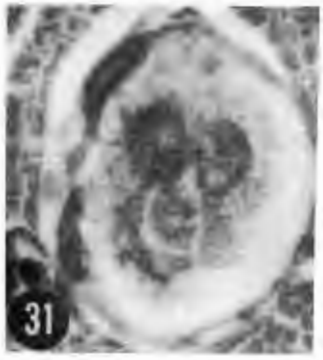
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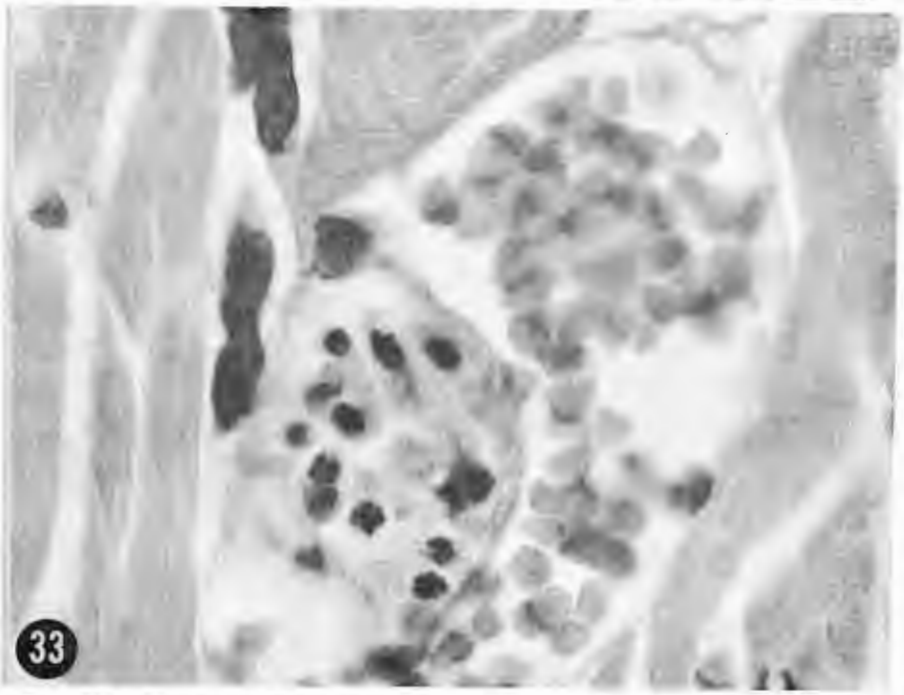
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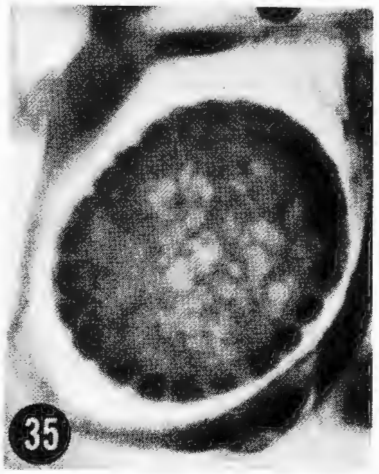
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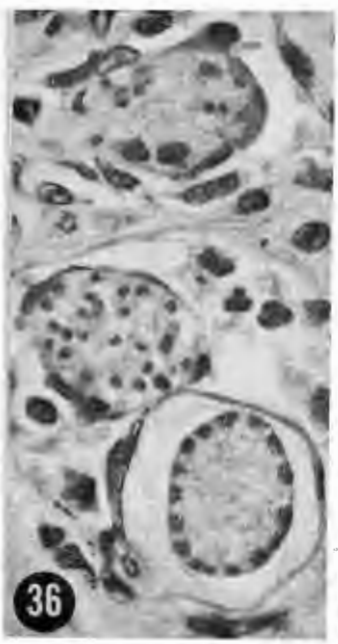
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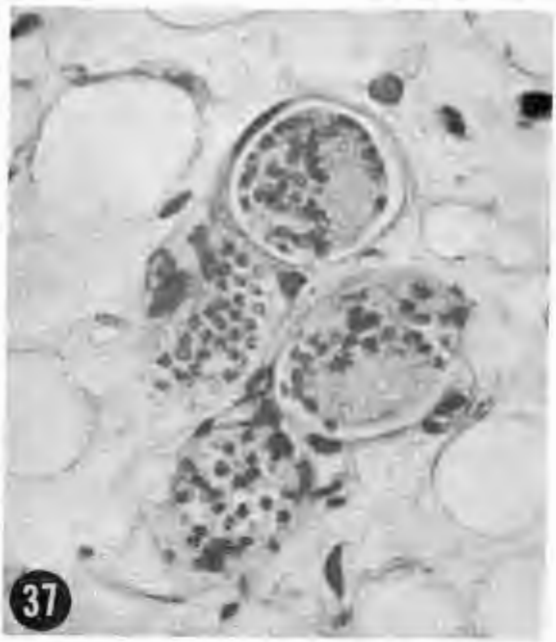
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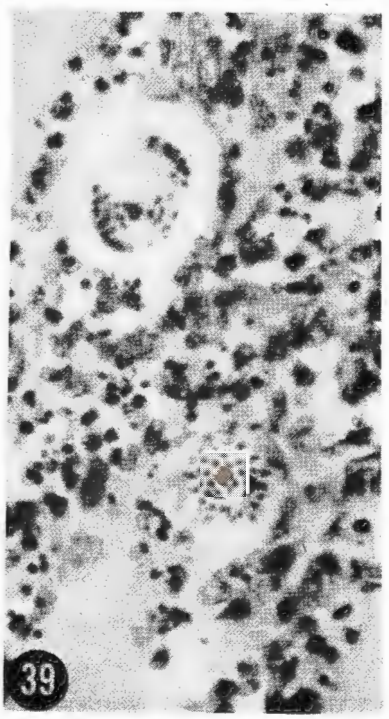
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TABLE 1 A summary of the most important parasitological and histopathological features of 20 canine cases of hepatozoonosis

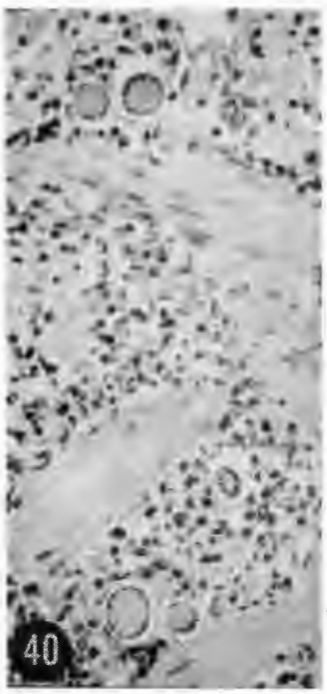
Case No.	Hepatozoonosis			Complicating disease(s)
	Parasitized tissues	Level of parasitism	Lesions	
63–969.....	Lymph node	+++	+++	Babesiosis
	Spleen	+	+	
	Lung	++	++	
66–598.....	Lymph node	++++	++++	Babesiosis
	Heart	++	++	*Spirocera lupi* infection
	Liver	+	+++(Centrilobular necrosis due to anaemia)	
	Skeletal muscle	—	+++(Due to babesiosis?)	
61733.....	Spleen	+++	Evaluation complicated by autolysis and *post mortem* bacteria	Babesiosis
68–1076.....	Lymph node	+++	+++	Distemper
62–3049.....	Spleen	++++	++++	Suspected viral infection
	Heart	—	—	
67–2573.....	Spleen	++	++	Adenocarcinoma in lung
	Kidney	+	+	
67–1029.....	Spleen.	++	++(Extramedullary haemopoiesis)	Anaemia
65–1928.....	Liver	++++	++++	None
	Lung	+	+	
61–999.....	Liver	++	++	None
65–1928.....	Liver	++	++	None
62827.....	Spleen	+++	+++	None
	Liver	++	++	
63–113.....	Lymph node	+	+	None
	Spleen	+	+	
65–481.....	Spleen	+	+	None
64–439.....	Spleen	+	+	None
61–737.....	Liver	++	++	None
64–041.....	Spleen	+	+	None
63–694.....	Liver	+	+	None
65–481.....	Spleen	+	+	None
66–3370.....	Spleen	++	++(Extramedullary haematopoiesis)	None
	Brain	+	+	
66–3233.....	Brain and meninges	+	+	

— = absent
\+ = rare
++ = fairly frequent
+++ = frequent
++++ = very frequent

There was considerable variation in the spectrum of the lesions and the number of parasites seen. A composite picture of the lesions in various tissues of these cases should serve to show the significance of this organism as a pathogenic agent.

Evidence of hepatozoonosis was most frequently present in the spleen (50%) where schizonts in various stages of development were noticed. The lesions varied from very mild (Fig. 40) to very severe reactions. In the most severely affected spleens, there was necrosis, some neutrophil infiltration and sometimes marked atrophy of the white and red pulp. These lesions were accompanied by the presence of large numbers of schizonts in various stages of development (Fig. 41–43). Many reticuloendothelial (RE) cells contained trophozoites, their numbers exceeding those of the schizonts. Suspected gametocytes were also noticeable. Numerous megakaryocytes and hypertrophic RE cells with abundant clear cytoplasm were regular features. Megakaryocytes were also frequent in the spleens in which there were few schizonts. This could be explained quite readily in the cases with concurrent babesiosis because of the anaemia, but they were also present in the uncomplicated cases, possibly for the same reason. Microschizonts containing micromerozoites (Fig. 43) predominated but macroschizonts with only 1–4 macromerozoites each were also seen (Fig. 52).

FIG. 30–39 *Hepatozoon* sp. schizonts in lion, jackal and cheetah
FIG. 30 Myocardium, lion. Trophozoite in cytoplasmic vacuole of large RE cell. HE×1 200
FIG. 31 Myocardium, lion. Multiple nuclei in large RE cell which is within a blood vessel. HE×1 200
FIG. 32 Myocardium, lion. A good example of an RE cell parasitized by a trophozoite which can be seen in a clear vacuole of an otherwise homogeneous cytoplasm. HE×1 200
FIG. 33 Myocardium, lion. Microschizont bulging the endothelium into the lumen of a vessel. Some of the merozoites are sectioned transversely. HE×1 200
FIG. 34 Myocardium, lion. At the top of picture there is an early parasitism of an RE cell with a single large trophozoite. Midway down is a developmental stage with multiple nuclei of merozoites and at the bottom an almost mature microschizont. HE×150
FIG. 35 Lung, lion. A microschizont in an alveolar septum showing the peripheral location of nuclei of future merozoites. HE×1 200
FIG. 36 Myocardium, cheetah. Three microschizonts at different stages of development within a blood vessel. HE×750
FIG. 37 Adipose tissue, cheetah. Four developing microschizonts in small blood vessels. HE×750
FIG. 38 Skeletal muscle, jackal. Small round cells in response to multiple schizonts within blood vessels. HE×500
FIG. 39 Skeletal muscle, jackal. Intense interstitial myositis in response to microschizonts, from some of which merozoites have escaped into surrounding tissue. HE×500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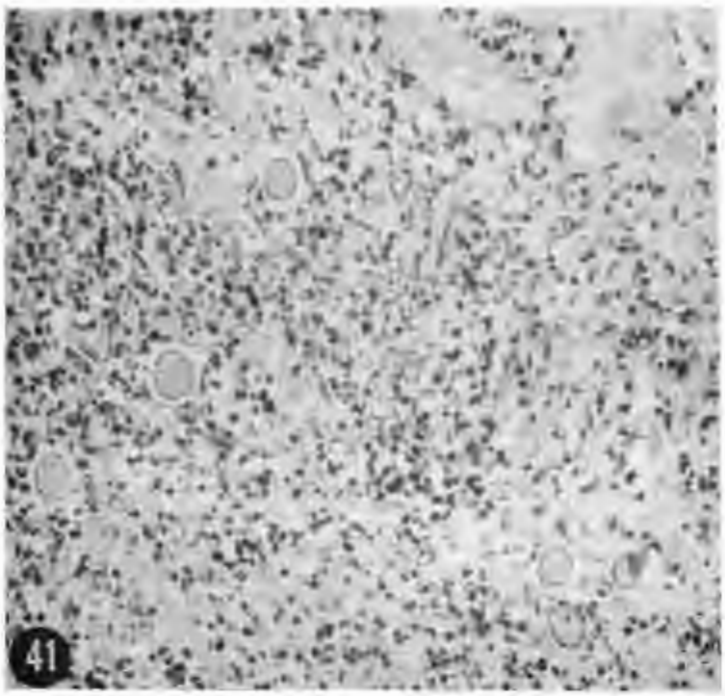
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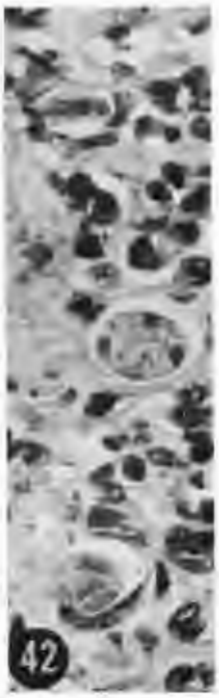
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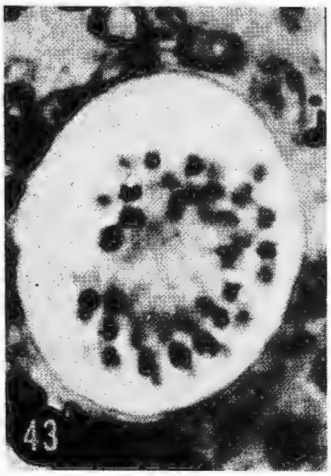
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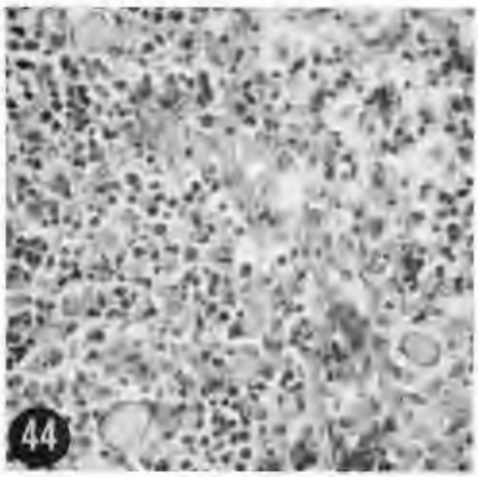
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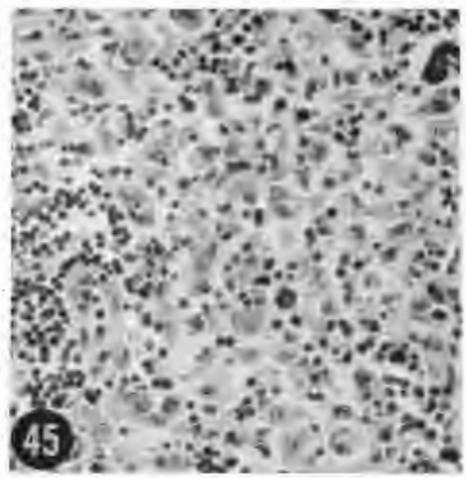
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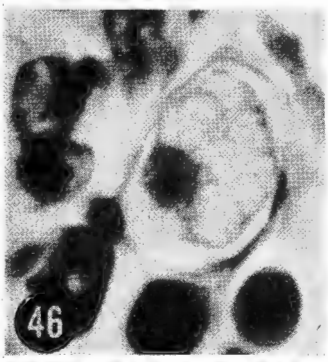
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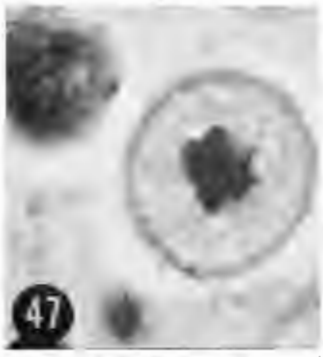
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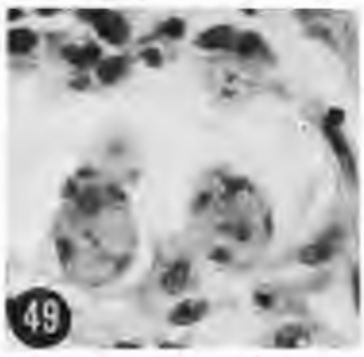
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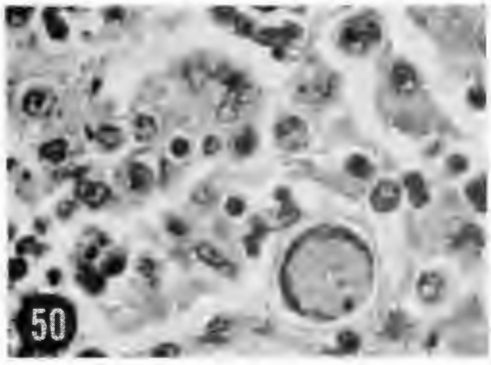
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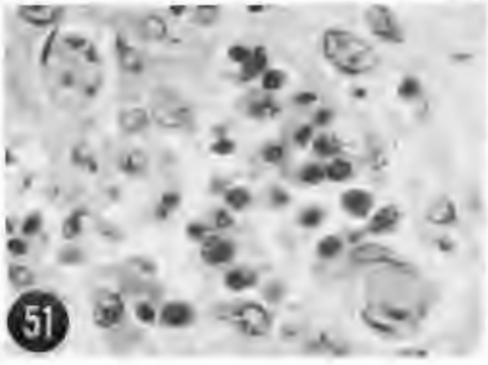
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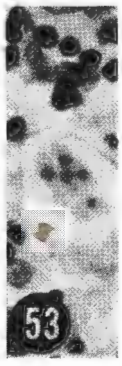
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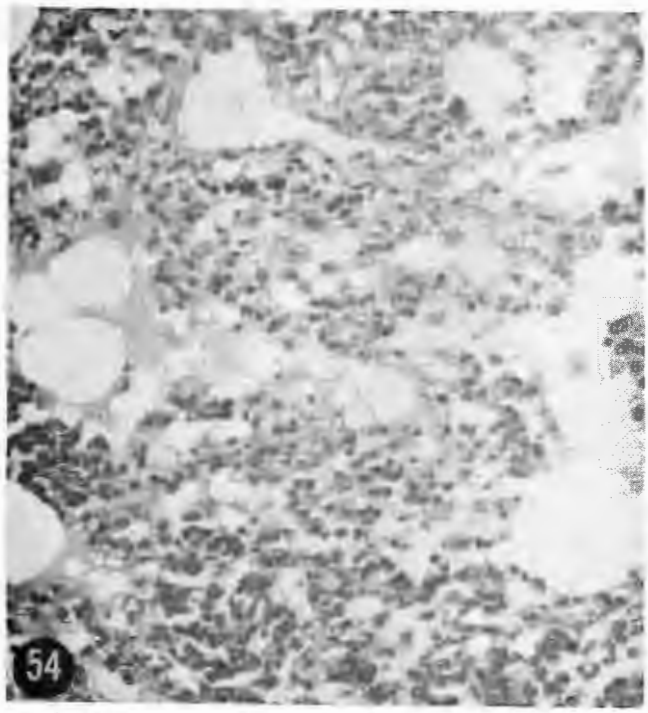
FIG. 40–53 *H. canis* in the dog
FIG. 40 and 41 Spleen. The many microschizonts, a number of which can be seen in the red pulp, give an indication of the severity of this infection. Notice nuclear debris in red pulp in Fig. 41. HE × 200
FIG. 42 Spleen. Early schizonts are seen here. HE × 500
FIG. 43 Spleen. A degenerating microschizont with pyknosis of the nuclei of the merozoites. HE × 1 200
FIG. 44 and 45 Lymph node. Marked RE hyperplasia is a prominent feature. HE × 200
FIG. 46 Lymph node. Foamy cytoplasm of a host cell together with a single enlarging parasite. HE × 1 200
FIG. 47 Lymph node. Foamy cytoplasm of a parasitized host cell. HE × 1 200
FIG. 48 Lymph node. Large signet ring-like parasite with foamy residual cytoplasm. HE × 1 200
FIG. 49 Lymph node. Three developing schizonts in lymphatic sinus. HE × 500
FIG. 50 Lymph node. RE hyperplasia adjacent to a developing schizont. HE × 500
FIG. 51 Lymph node. Developing schizonts with RE hyperplasia and plasma cell infiltrates. HE × 500
FIG. 52 Lymph node. A macroschizont showing three merozoites and a residual body. HE × 500
FIG. 53 Lymph node. A mature microschizont with no residual body visible. HE × 500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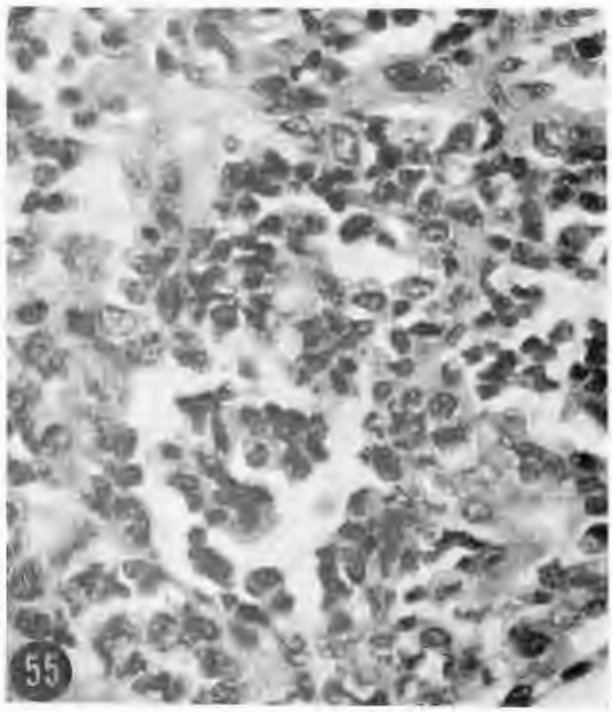
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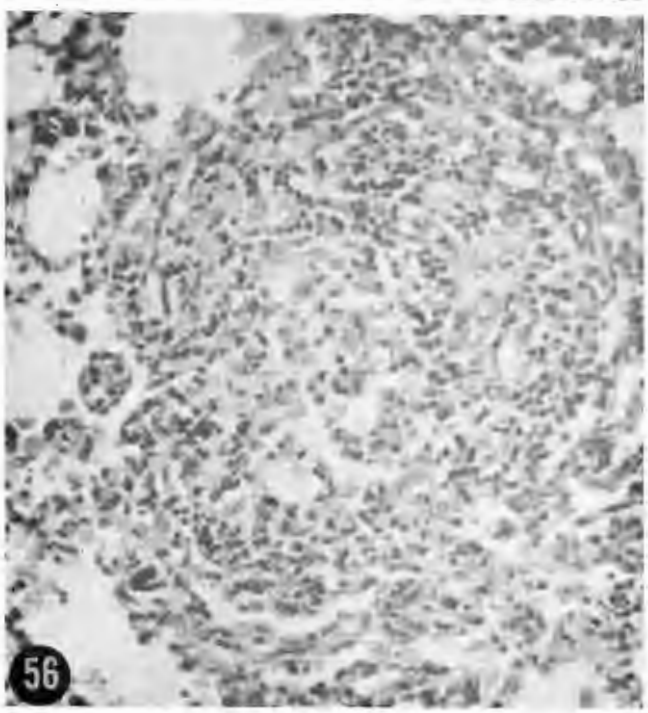
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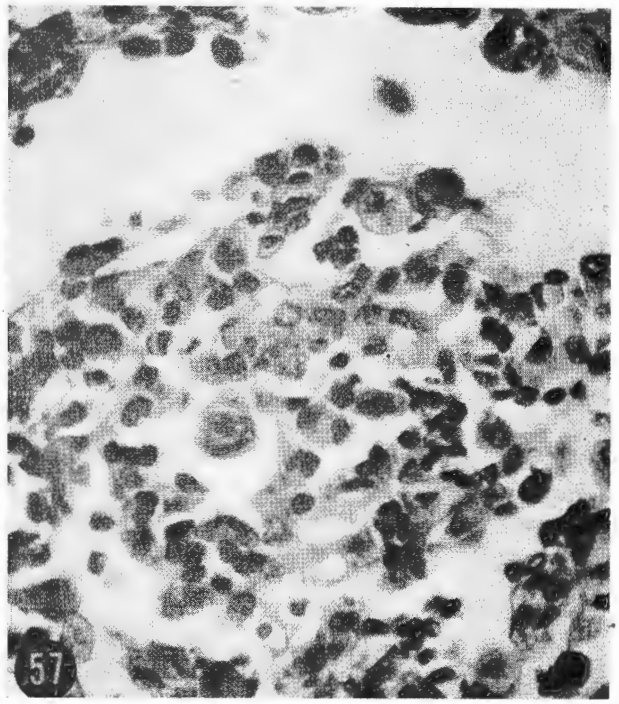
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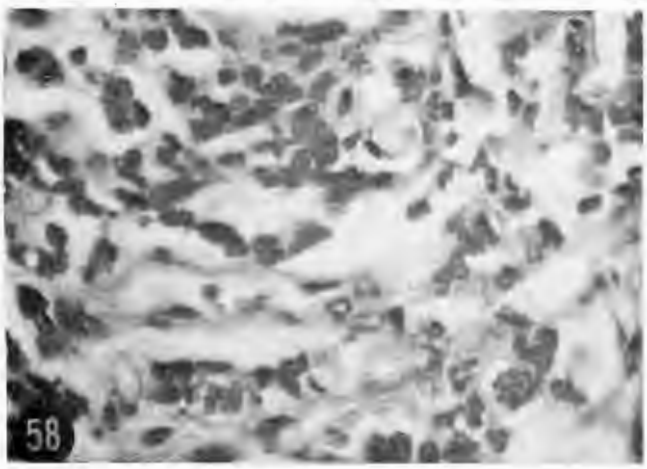
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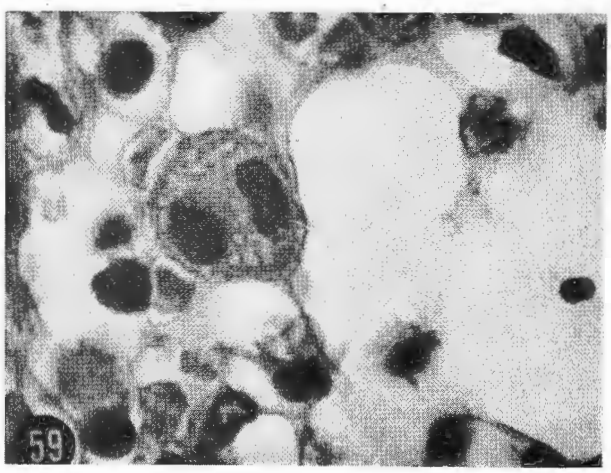
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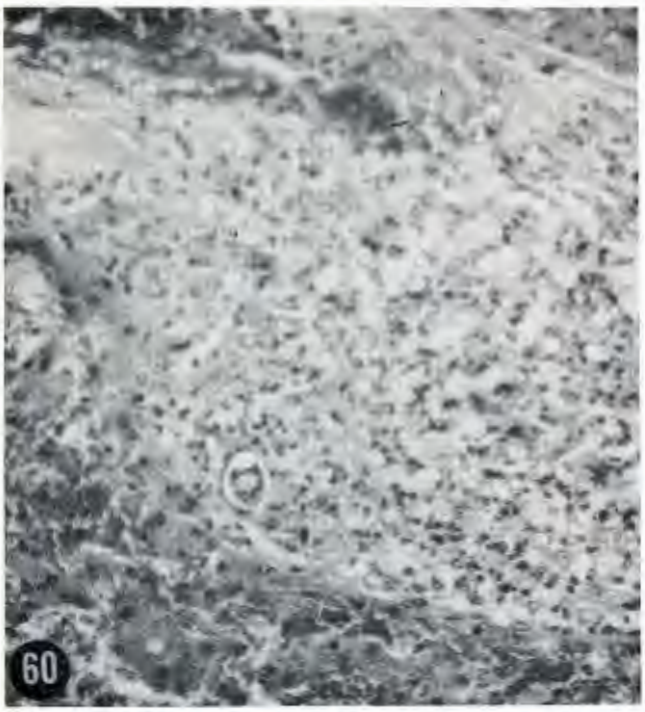
FIG. 54–59 Reaction to *H. canis* in the lung of the dog
FIG. 54 A rather diffuse pneumonitis is present with some oedema and round cells in alveoli. HE × 200
FIG. 55 The interstitial pneumonitis consists mainly of an infiltrate of round cells and RE hyperplasia. HE × 500
FIG. 56 Focal granulomas such as this one were occasionally seen. HE × 200
FIG. 57 Multiple RE cells, some of which are parasitized. HE × 500
FIG. 58 Pneumonitis with fine strands of collagen and many small round cells. HE × 500
FIG. 59 Some of the large RE cells were binucleated. HE × 1 200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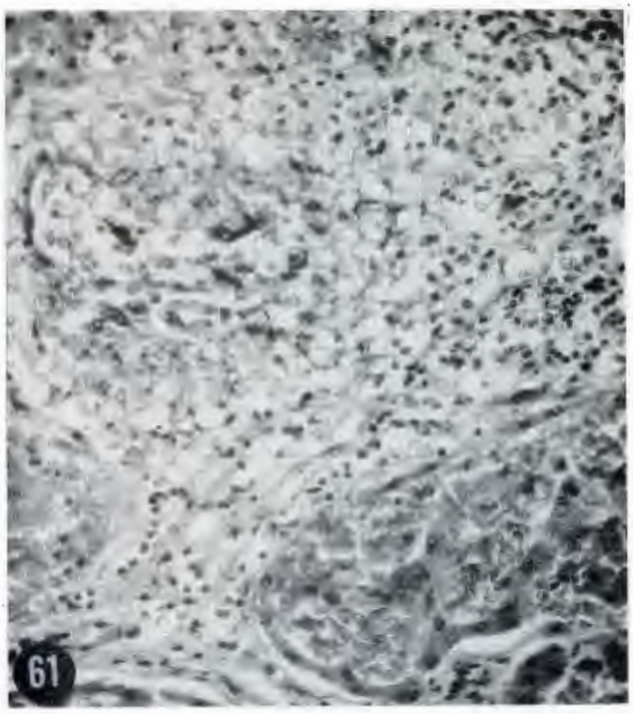
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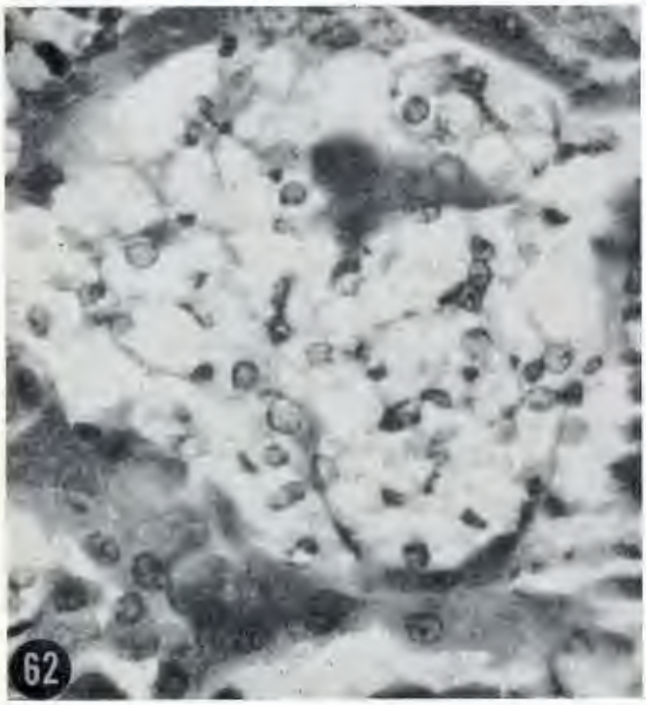
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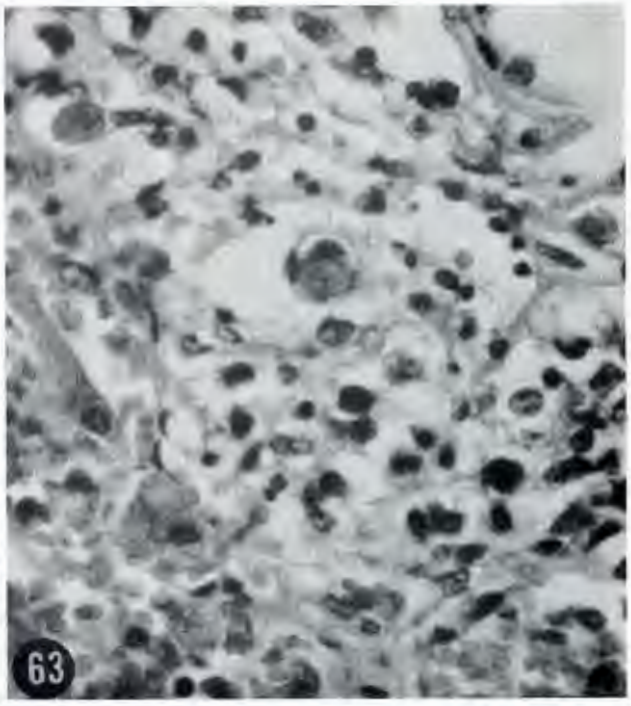
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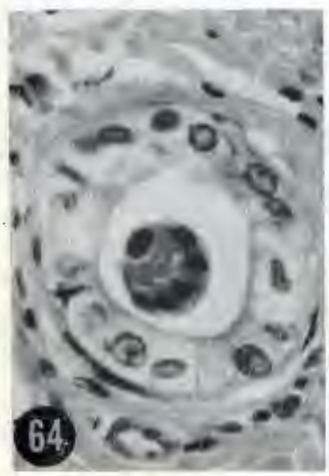
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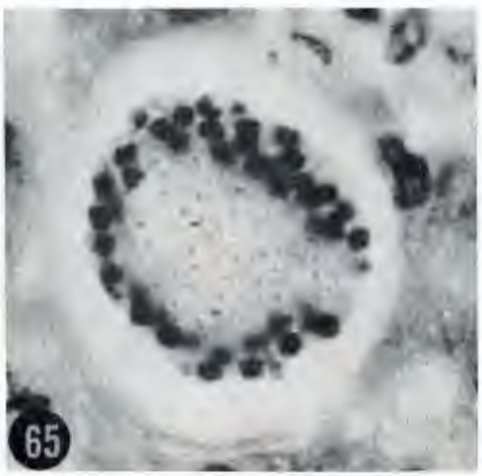
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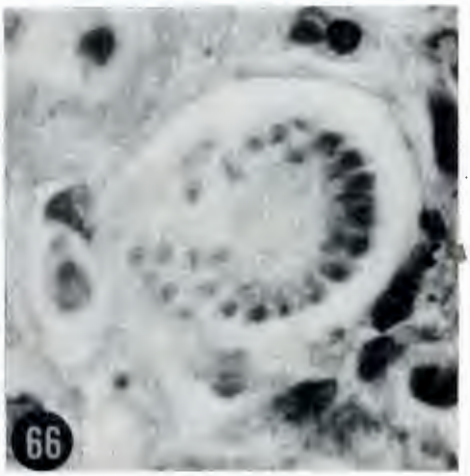
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FIG. 60–66 *H. canis* in the liver of the dog
FIG. 60 A microschizont is seen adjacent to an area of necrosis and heavy inflammatory infiltration of a portal area. HE × 200
FIG. 61 The striking RE hyperplasia and small round cell infiltrate are impressive in this portal area. HE × 200
FIG. 62 Sinusoids were sometimes filled by Kupffer cell hyperplasia as shown here. HE × 500
FIG. 63 Schizonts were scattered throughout the cellular infiltrates wherever they were present. HE × 500
FIG. 64 A schizont with pyknotic nuclei in the lumen of a bile duct. HE × 500
FIG. 65 A degenerating microschizont in sinusoid. Notice the pyknosis of the nuclei and strange appearance of the residual cytoplasm. HE × 1 200
FIG. 66 A microschizont showing a rim of rather mature merozoites at the periphery. HE × 1 200

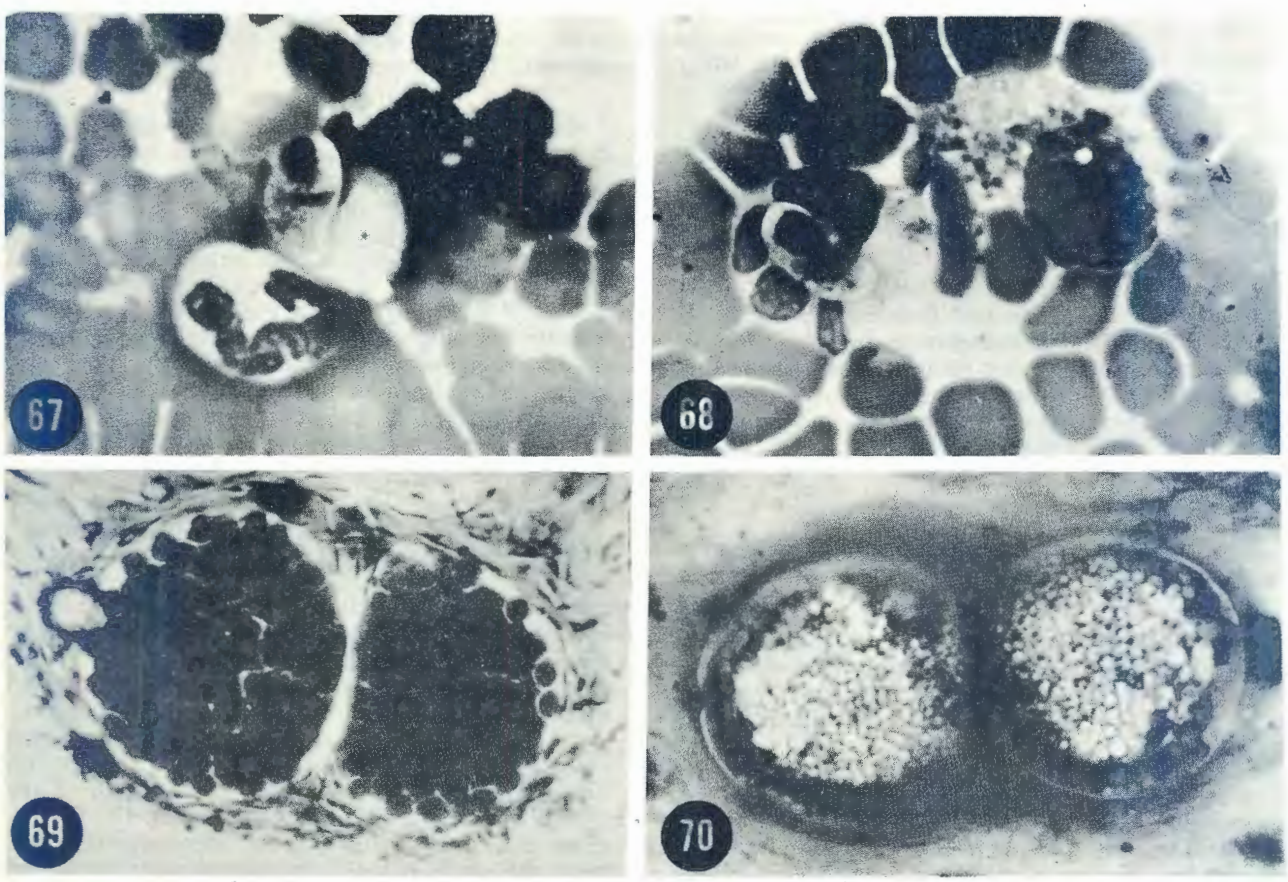

FIG. 67–70 *Hepatozoon* in blood smears and ticks
FIG. 67 and 68 Gametocytes of *Hepatozoon* in blood smears of a jackal and hyaena respectively. Giemsa × 1 300
FIG. 69 Haemolymph smear of *R. simus* showing 2 oocysts in which formation of sporocysts is taking place. Giemsa × 200
FIG. 70 Haemolymph smear of *R. simus* showing 2 sporocysts. Giemsa × 1 000

Hepatozoon was seen fairly frequently in the lymph nodes and the spectrum of the lesions varied accordingly. Some lymph nodes exhibited numerous schizonts at various stages of development (Fig. 46–53) as well as many trophozoites, severe oedema and extensive RE cell hyperplasia in the medullary sinuses (Fig. 44) and to a lesser degree in the medullary cords (Fig. 45). In the sinuses in particular, there were large groups of proliferating RE cells and many contained early stages of schizonts. A few RE cells were multinucleated. Once parasitized, the RE cells enlarged and tended to become discrete and more spherical (Fig. 47) Some of these cases in which there were extensive necrotic lesions in the lymph nodes were complicated by babesiosis and there was erythrophagocytosis of parasitized erythrocytes. However, the extensiveness of the necrosis in the lymph nodes of uncomplicated cases of hepatozoonosis coupled with the large number of parasites, especially immature schizonts, indicates that the lesions were due mainly to this disease. Some of the lymph nodes showed perilymphadenitis.

The myocardium was not as frequently involved as in the wild carnivores. In one case with extensive myocardial necrosis due to babesiosis, there were many areas with interstitial mononuclear cell infiltrates in which schizonts of *Hepatozoon* were present.

The lungs of the dogs showed mild to severe pneumonitis (Fig. 54, 55 and 58), focal granulomas (Fig. 56), frequently associated with early stages of schizogony, and variable numbers of mononuclear cells in alveoli. Alveolar oedema was present (Fig. 54) and alveolar septa contained parasitized RE cells (Fig. 57). An occasional binucleated cell was present amidst the inflammatory infiltrate of the alveolar septa (Fig. 59).

Lesions in the liver of the dogs varied from those of a mild to a quite extensive hepatitis. In those with lesions of a mild nature there were Kupffer cell hyperplasia, focal granulomas and mononuclear cell infiltrates in the portal areas. In one particularly severe case prominent RE cell hyperplasia and an intensive infiltration of various leucocytic cells, including plasma cells, lymphocytes and polymorphonuclear leucocytes, were observed in the portal areas (Fig. 60, 61 and 63). Marked hyperplasia of Kupffer cells was literally obliterating some of the sinusoids (Fig. 62). Their clear cytoplasm had a hydropic appearance. *Hepatozoon* gametocytes were recognized within leucocytes in sinusoids and blood vessels. Severe accompanying lesions were the exception rather than the rule in these cases. Other noteworthy features in the liver included a schizont in a bile duct (Fig. 64), a degenerating microschizont (Fig. 65) and a mature microschizont with peripheral nuclei (Fig. 66).

A few schizonts were found in association with mononuclear infiltrates in perivascular spaces of the brains and meninges of 2 dogs.

Both Christophers (1906) and Wenyon (1911) indicate that schizonts are frequent in the bone marrow of dogs with hepatozoonosis. Our material did not include this tissue. There was also no skeletal muscle and an inadequate amount of myocardium for satisfactory comparison with the situation encountered in wild carnivores.

Transmission studies

The attempt to transmit *Hepatozoon* from a naturally infected jackal to dogs by means of *R. sanguineus* gave inconclusive results.

Sporozoites and sporoblasts of *Hepatozoon* were found in smears of the haemolymph of some of the engorged and semi-engorged female and male *R. sanguineus* which had been fed on an infected jackal in the nymphal stage. This proved that the ticks had acquired the infection and that development of the parasite had progressed to a stage where the ticks were presumably infective. When an attempt was made to use these ticks to infect susceptible puppies, only one animal provided evidence of successful transmission. A single gametocyte was found in a smear made from the white cell layer of Puppy 793 thirty four days after it had been dosed with intact and punctured adult ticks. The puppy was necropsied the following day but no further evidence of infection could be detected. Smears of blood and the white cell layer from the other 3 puppies, which received respectively intact and punctured adults orally and triturated adults subcutaneously or orally, were consistently negative for *Hepatozoon* as were the viscera of these animals when examined histopathologically 56 (Puppy 792), 63 (Puppy 794) and 78 (Puppy 795) days after attempted infection. The unexposed controls were also negative for the parasite.

Discussion

This investigation has raised the possibility of the pathogenicity of *Hepatozoon*. In many of the dogs examined, *H. canis* was diagnosed as an incidental finding of small numbers of schizonts in the spleen, lymph node, lung and liver with virtually no evidence of a reaction on the part of the host, the parasite being apparently of little consequence to the animal. Conversely, the extensive lesions observed in association with numerous schizonts in the spleen, liver and lymph nodes, especially in some of the cases complicated by babesiosis, leave little doubt that this parasite may be pathogenic. These findings are in line with those of Christophers (1906) who reported that 100% of the neglected dogs in Madras, India, might be parasitized with *H. canis* and that heavy infections were present in 25% of these. Fatal cases of hepatozoonosis in dogs have been reported by Porter (1918) and Rau (1925). From the present study of the various tissues collected from several species of wild carnivores, it would appear that *Hepatozoon* was not very pathogenic to wild animals which have survived the rigours of a natural environment. Of the wild carnivores the jackal proved to be the most severely affected.

One of the more significant aspects regarding the histopathology of *H. canis* infections of carnivores is the fact that this organism must be considered in the differential diagnosis of other protozoal diseases and some fungal organisms. Its morphological similarity to *Toxoplasma gondii* has been stressed. Schizonts of *H. canis* were also recently mistaken for a fungus. Oduye (1972) claimed that he found *Coccidioides immitis* in a dog in Nigeria, but the organisms illustrated in the kidney, pancreatic lymph node, liver, spleen and prostate are in fact schizonts of *H. canis*. The illustrations further show lesions in the liver, spleen and lung similar to those present in the most severe canine cases described in this paper.

Another aspect of hepatozoonosis of carnivores which requires elucidation is the host-specificity of the parasites concerned. Wenyon (1926) expressed the belief that the parasites of wild cats, jackals, and hyaenas that have been given different specific names may actually not be distinct species. Levine (1961) refers to them all as *H. canis*. It has been suggested that studies on the developmental cycle in the invertebrate hosts may help clear up the confusion which exists (Redington & Jachowski, 1971). It may therefore be relevant that the sporogenous phase of the hyaena parasite could only be found in the tick *R. simus* and not in *H. leachi* and *R. sanguineus* obtained from the same animal, whereas *Hepatozoon* of the jackal seemed to develop quite satisfactorily in *R. sanguineus*. Attempts to infect dogs with tissues of ticks obtained from infected jackals, however, gave inconclusive results. This was contrary to the experience of Rau (1925), who recorded successful dog-to-dog transmission with tick and spleen suspensions. The only morphological difference observed between the schizonts of *Hepatozoon* of wild carnivores and dogs was the relative indistinctiveness of the foamy residual material in the developing schizonts of the former as compared to the latter. This difference seems too small to warrant species differentiation.

Only by more comprehensive vector and cross transmission studies will it be possible to clarify these problems on the pathogenicity and host-specificity of the parasites. Because of the propensity of schizonts to form in the myocardium and skeletal muscle of wild carnivores, these tissues should be carefully studied in both naturally and experimentally infected dogs. Moreover, there is some evidence that severe parasitism leads to anaemia. Further studies would be required to determine whether this anaemia was caused by direct involvement or toxic suppression of the bone marrow.

Acknowledgements

The authors wish to express their gratitude to the following: Mr A. M. du Bruyn and his staff for taking and printing the photomicrographs; Mr J. L. de B. van der Merwe and his technicians for the preparation of the microscopic sections.

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