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The pathology and cross-infectivity of Sarcocystis spp. in mule deer white-tailed deer and elk

Daniel B. Pond
The University of Montana

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THE PATHOLOGY AND CROSS-INFECTIVITY

OF SARCOCYSTIS SPP. IN MULE DEER,

WHITE-TAILED DEER, AND ELK

By

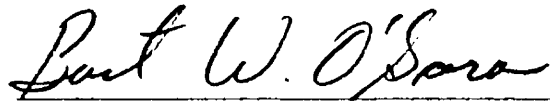Daniel B. Pond

B.S., University of Montana, 1982

Presented in partial fulfillment of the requirements for the degree of

Master of Science

UNIVERSITY OF MONTANA

1982

Approved by:

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Chairman, Board of Examiners

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Dean, Graduate School

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Date

UMI Number: EP37808

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Dissertation Publishing

UMI EP37808

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ABSTRACT

Pond, Daniel B., M.S., Fall 1982   Wildlife Biology

The Pathology and Cross-Infectivity of <u>Sarcocystis</u> spp. in Mule Deer, White-tailed Deer, and Elk (39 pp.)

Director: Bart W. O'Gara

<u>Sarcocystis</u> from mule deer, white-tailed deer, and elk was passed through coyotes, yielding 2 distinct types of sporocysts. Sporocysts of <u>S</u>. <u>hemionilatrantis</u> measuring 16.2 X 9.9 μm were common to all intermediate hosts, and another <u>Sarcocystis</u> sp. measuring 19.3 X 12.7 μm was passed only after eating elk meat. These sporocysts were fed to 12 mule deer fawns divided into 4 groups, each receiving sporocysts from 1 intermediate host with 1 control group. Those fawns receiving sporocysts developed acute sarcocystosis. Clinical symptoms started 11 days post infection and included anorexia, pyrexia, weight loss, wet cough, hair loss, and sloughing of the ears, tail, and glans penis. Only SGPT, SGOT, and CPK levels in the blood became elevated during the experiment. Three fawns died of congestive heart failure and 1 was moribund before being killed. The experiment was ended after 49 days. All fawns showed signs of congestive heart failure, lymphadenitis, meningoencephalitis, endocarditis, and hyperplasia of the spleen and liver. The primary lesions occurred in skeletal muscle and included perivascular necrosis with a mononuclear cell infiltration, edema, and degeneration of muscle tissue. Schizonts at various stages were seen in the skeletal and heart muscles of all fawns and in the adrenal glands or lungs of 3 others. No cyst formation was seen. Three pregnant mule deer does and 1 pregnant elk from the NBR were grossly infected with <u>Sarcocystis</u>. Cysts ranged in size from 0.2 mm X 0.4 mm to 0.4 mm X 1 mm. Light microscopy showed that cysts had prominent walls with septae compartmentalizing bradyzoites and metrocysts within the cyst. Electron microscopy revealed 1 cyst wall type common to both species of deer and elk and a second cyst wall type in elk only. Muscle digestion of 6 fetal mule deer and 1 fetal elk revealed a congenital infection in 1 deer fetus. Apparently <u>S</u>. <u>hemionilatrantis</u> infects mule deer, white-tailed deer, and elk; and a second unidentified <u>Sarcocystis</u> sp. infects elk.

ACKNOWLEDGMENTS

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INTRODUCTION

Sarcocystis is common in the musculature of many species of mammals, birds, and reptiles (Kalyakin and Zasukhin 1975). It is recognized as an important cause of disease in cattle, swine, and sheep (Dubey 1976). Sarcocystis has been reported in various North American free-ranging ungulates, including moose (Alces alces) (Kelley et al. 1950, Dubey 1980, Mahrt and Colwell 1980); bison (Bison bison) (Pond and Speer 1979, Dubey 1980); pronghorn antelope (Antilocapra americana) (Dubey 1980); mule deer (Odocoileus hemionus), white-tailed deer (O. virginianus), and elk (Cervus elaphus) (Pond and Speer 1979, Mahrt and Colwell 1980).

Prior to 1977, mule deer populations may have declined because of Sarcocystis in Oregon (Hudkins and Kistner 1977) and South Dakota (Anonymous 1977). S. hemionilatrantis involving coyotes (Canis latrans) and mule deer was found to be pathogenic to experimentally infected mule deer fawns (Hudkins and Kistner 1977, Koller et al. 1977). However, the impacts of Sarcocystis on wild populations are not clearly understood.

On the National Bison Range (NBR) near Missoula, Montana, the deer and elk populations have a high incidence of Sarcocystis (Pond and Speer 1979). The NBR is bordered by a 3-m high welded wire

1

fence effectively preventing immigration or emigration of big game animals. A survey of mule deer in 1980 revealed that 100% (N = 65) of the deer were grossly infected (Pond unpubl. data); in 1976, 80% of the mule deer, 50% of the white-tailed deer, and 50% of the elk were grossly infected (Pond and Speer 1979). Infected deer and elk found on the NBR must have received their infection from a source on the NBR.

The definitive host of S. hemionilatrantis is the coyote (Hudkins and Kistner 1977, Speer et al. 1980), the primary carnivore on the NBR. In 1976, 12 of 17 (70%) fresh coyote scats collected from 50 km of road on the NBR in a single day contained Sarcocystis sporocysts (Pond unpubl. data). Seventeen of 19 (89%) coyotes killed on the NBR in 1978 were also infected (Pond unpubl. data).

The high incidence of infected deer, elk, and coyotes on the NBR may indicate that there is 1 species of Sarcocystis found in these animals. The objectives of this study were to examine the development and histopathology of Sarcocystis infections in mule deer and determine if Sarcocystis from white-tailed deer and elk could be passed through coyotes and infect mule deer fawns.

MATERIALS AND METHODS

Infection of Coyotes

The original source of <u>Sarcocystis</u> was obtained from free-ranging mule deer, white-tailed deer, and elk collected on the NBR. All of these animals were grossly infected. The skeletal musculature of each animal was ground, divided into 500-g packets, and stored at 7°C.

Starting at 6 months of age, 4 coyote pups were used as definitive hosts throughout the experiments. All pups were housed together and had been weaned and maintained on dry commercial dog food. For 14 days, daily fecal samples were obtained from each coyote, floated on Sheather's sugar solution (sp. gr. 1.12), and examined for parasites prior to the start of each experiment. None of the coyotes exhibited coccidian oocysts or sporocysts during those 14-day periods.

The coyotes were initially fed 3 kg of ground mule deer each during a 1-week period. Fecal samples were checked daily for sporocysts or oocysts. Prepatent and patent periods were determined and all coyotes were again checked for 14 consecutive days after the last sporocyst was observed before the coyotes were considered <u>Sarcocystis</u> free. The same procedure was used when the coyotes were infected with <u>Sarcocystis</u> from white-tailed deer and elk.

Oocysts and sporocysts were collected daily by floating feces on Sheather's sugar solution in petri dishes (Hammond et al. 1968), cleaned of excess fecal debris, and stored separately in distilled water or 2.5% w/v potassium dichromate solution at 7°C for 1 week to 6 months. Morphological characteristics of 50 sporocysts from each of the 3 different intermediate hosts were compared using light microscopy.

Infection of Deer Fawns

Twelve 1-10 day old mule deer fawns were caught on the NBR and placed together in an outdoor pen isolated from carnivores. One additional mule deer fawn and a whitetail fawn were obtained from the Montana Department of Fish, Wildlife and Parks (MT FWP) and isolated from the NBR fawns. Fawns were bottle-fed raw cow's milk 3 times daily and were given grain, salt, and water ad lib after 2 weeks. At every feeding, fawns were stimulated to urinate and defecate with a warm wet paper towel to help prevent contamination for the first 3 weeks.

At 7-20 days of age, the 12 NBR fawns were randomly tagged and put into 1 group of 3, 2 groups of 4, and 1 control. These animals were orally infected with Sarcocystis according to Table 1. Sporocysts were suspended in milk and bottle-fed to each animal. All 4 groups remained housed together.

Table 1. Inoculation schedule of mule deer fawns
with Sarcocystis spp. from 3 intermediate
hosts.

Intermediate host origin of inocula	Number of mule deer inoculated	Number of sporocysts in inocula
Mule deer	3	1.0×10^5
White-tailed deer	4	5.5×10^4
Elk	4	1.2×10^5

The 2 fawns obtained from MT FWP were used as uninoculated controls and kept isolated from the NBR fawns. No rectal temperatures or blood were taken from those 2 animals. All fawns were observed 3 times daily throughout the experiment, and behavioral changes and clinical symptoms were recorded.

For 1 week prior to infection and throughout the experiment, rectal temperatures were taken twice weekly until onset of clinical symptoms, then temperatures were taken daily. Blood was drawn from the jugular vein into plain vacutainer tubes from each animal prior to infection and approximately every 2 weeks thereafter. Serum was separated and frozen at -20°C until tests were performed. Blood chemistries were performed on these sera using a SMAC II machine.

When fawns died or were killed after becoming clinically ill,

necropsies were performed and samples of the following tissues were fixed in 10% formalin after gross examination: brain, eye, skeletal muscle, tongue, heart, diaphragm, lung, kidney, liver, spleen, lymph nodes, adrenal glands, small intestine, and reproductive organs. These tissues were routinely dehydrated, embedded in paraffin, and sectioned at 5 μm. Sections were stained with either hematoxylin and eosin (H & E), or Jenner's stains.

Cross Placental Transmission

Three adult pregnant mule deer does and 1 pregnant cow elk were collected on the NBR. The deer and elk were approximately 190 and 235 days into their pregnancies. Skeletal muscle samples were obtained for in vitro tissue digestion (Box and McGuinness 1978) to confirm infection and study morphology of metrocytes by phase microscopy.

Specimens for electron microscopy were fixed in modified Karnovsky's or 3% (v/v) glutaraldehyde in 0.2 M cacodylate buffer, post-fixed in osmium tetroxide, prestained with 1% (w/v) uranyl acetate and 1% (w/v) phosphotungstic acid in 70% ethanol, embedded in Epon 812 or Spurr's medium, sectioned, stained with lead citrate, and examined with a Zeiss EM9-S2 electron microscope.

Six fetuses (3 sets of twins) were obtained from the 3 does and 1 fetus was obtained from the cow elk. Skeletal muscle samples

were taken from all fetuses for tissue digestion, and all major organs

were fixed in 10% formalin with representative samples of fetal

membranes and placentae. These tissues were routinely embedded

in paraffin, sectioned at 5 μm on a clinical microtome, and stained

with H & E.

RESULTS

Coyote Infections

Coyotes were successfully infected with Sarcocystis sp.

from each of the 3 intermediate hosts. These infections yielded

oocysts and sporocysts of 2 distinct sizes and patent periods (Table 2).

Sporulated and unsporulated oocysts were shed occasionally during the

first 5 days of infection. Sporocysts were typical of Sarcocystis in

that they contained 4 sporozoites and a granular residuum (Figs. 1-3).

Table 2. Sarcocystis sporocysts shed in coyote feces after eating mule deer, white-tailed deer, and elk meat.

Host origin	Prepatent period[1]	Patent period[1]	Size in μm[2]	S.D.[2]
Mule deer	9	33	16.2 × 9.9	1.95
White-tailed deer	10	35	16.3 × 9.9	2.02
Elk	9	30	16.1 × 9.8	1.79
	7	21	19.3 × 12.7	1.81

[1]Average number of days for 4 coyotes.

[2]Average of 50 sporocysts pooled from 4 coyotes.

Figure 1. Sporocyst of <u>Sarcocystis</u> <u>hemionilatrantis</u> passed in coyote feces after eating mule deer meat (1000✕).

Figure 2. Sporocyst of <u>S.</u> <u>hemionilatrantis</u> passed in coyote feces after eating elk (1000✕).

Figure 3. Sporocyst of <u>Sarcocystis</u> sp. passed in coyote feces after eating elk meat (1000✕).

Figure 4. Unsporulated oocyst containing 2 sporoblasts from a coyote fed white-tailed deer meat (1000✕).

Figure 5. Sporulated oocysts in an intestinal scraping of a coyote fed elk meat (400✕).
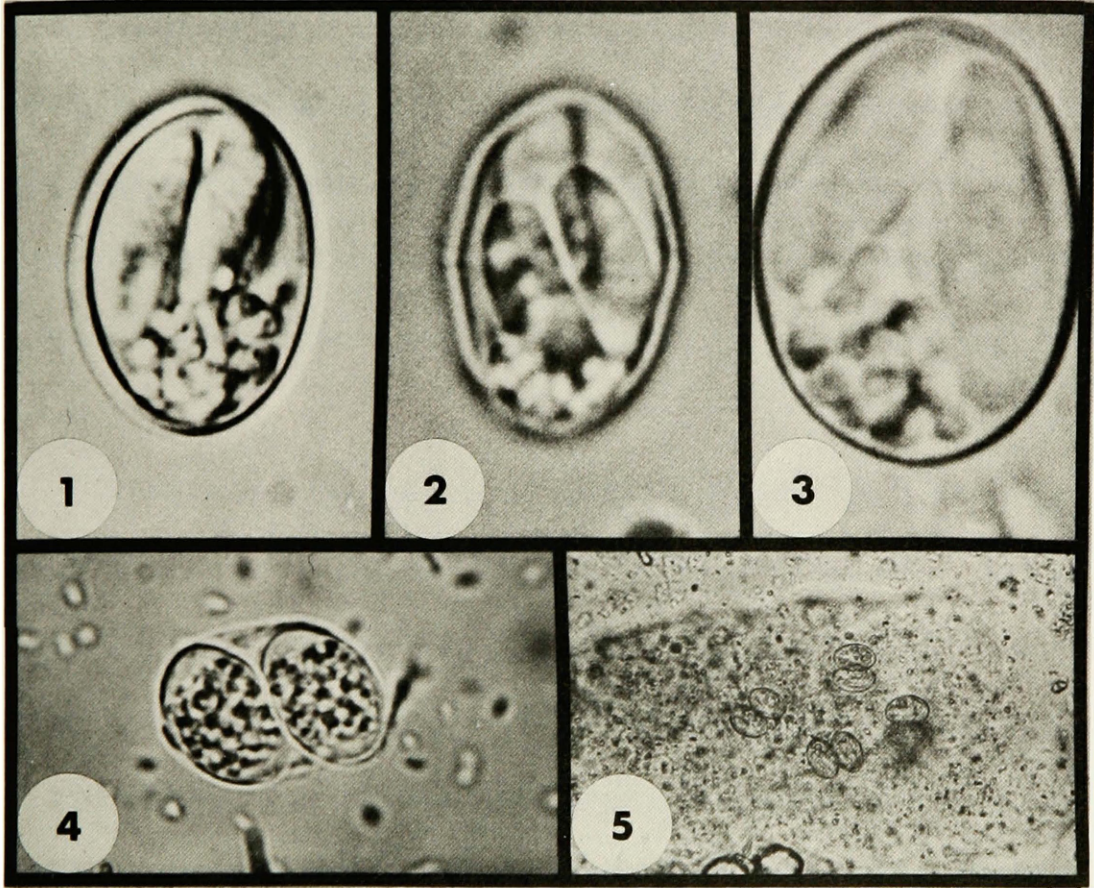
Fawn Infections

Clinical Symptoms

Clinical signs of illness first appeared post infection day (PID) 11 (Table 3). These early symptoms occurred in all 3 infected groups and consisted of a fever (Fig. 6), slightly pale mucous membranes, and a cough. Temperatures continued to remain elevated, coughing became worse, and infected fawns began to lose weight and appeared lethargic until PID 17 when all fawns began to improve. Temperatures slowly returned to normal, food intake increased, and the animals became more active, but the cough remained (Fig. 6 and Table 3).

Symptoms reappeared suddenly on PID 22 and were more severe. In some animals, food intake stopped completely. Temperatures remained high (Fig. 6) and coughing became more pronounced and wet. The general condition of all infected animals deteriorated until PID 33 when fawn 5 was found dead. Fawns 1, 7, 9, 10, and 11 developed sites of necrosis on the tips of their tails and ears, and hair loss and blackening of the skin appeared on their ears, tails, sides, and the backs of their heads. Hair on all animals was fragile and could be pulled out easily. Conditions deteriorated with a loss of coordinated movements and an unwillingness to rise. On PID 37, fawns 1 and 2 were found dead and fawn 3 was moribund. Fawn 3 was killed on PID 40. Fawns 7, 8, 9, and 10 grew steadily worse, losing

Table 3. Onset of clinical symptoms and death in mule deer fawns infected with Sarcocystis hemionilatrantis and Sarcocystis sp.

PID[1]	Intermediate host origin			Control[5]
	Mule deer[2]	White-tailed deer[3]	Elk[4]	
0-7	N[6]	N	N	N
8-14	Pyrexia, anorexia, cough, weight loss, reluctance to rise.	———————————————————|	Pyrexia, cough, slight weight loss.	N
15-21	Temperatures returned to normal, food intake near normal, cough remains.	———————————————————————————————|		N
22-29	Pyrexia, anorexia, weight loss, reluctance to rise, uncoordinated movements, wet cough.	———————————————————————————————|		N
30-37	Fawns 1 and 2 dead. Pyrexia, anorexia, dyspnea, reluctance to rise, tips of tails and ears necrotic, hair loss, cough.	Fawn 5 dead. ———————————————————————|		N
38-45	Fawn 3 killed.	Symptoms become more severe in fawn 7. Fawns 4 and 6 improve.	Symptoms become more severe in fawns 8-10. Fawn 11 improves.	N
46-49	All remaining fawns killed.			

[1]Post infection day. [3]Fawns 4, 5, 6, and 7. [5]2 mule deer, 1 white-tailed deer fawns.

[2]Fawns 1, 2, and 3. [4]Fawns 8, 9, 10, and 11. [6]N = no symptoms.

Figure 6. Rectal temperature changes of infected and control fawns.
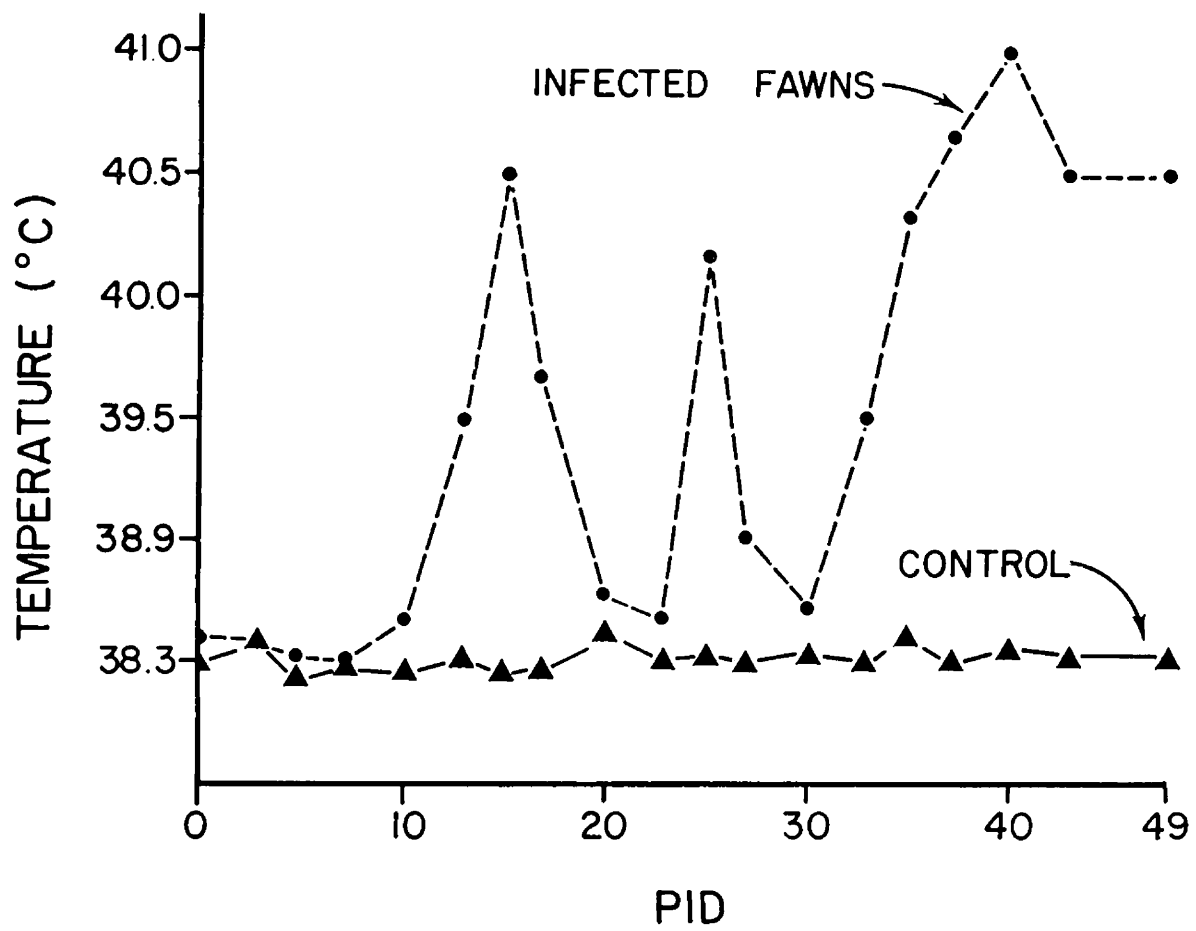
weight and hair. They exhibited skin necrosis, general lethargy, reluctance to rise, labored breathing with a wet cough, and high temperatures. Fawns 4, 6, and 11 slowly improved. The control from the NBR and the 2 fawns from MT FWP remained vigorous and continued to gain weight. On PID 48 and 49, the experiment was ended when all remaining fawns were killed (Table 3).

The 3 fawns that died appeared to have died suddenly. Fawn 5 was last seen alive bedded with its head up. About 8 hours later it was found dead on its side in the same bed. Fawns 1 and 2 were standing when last seen alive. Three hours later they were found dead spread eagled on the ground where they had been standing, giving the appearance of having collapsed and died.

Clinical Chemistries

Results of serum chemistries are listed in Appendix A. Most parameters remained near normal throughout the experiment. Serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and creatin phosphokinase (CPK) increased 2-3 times higher than the control during the experiment (Figs. 7-9).

Necropsies

Lesions associated with sarcocystosis were more severe in infected fawns that were killed at the end of the experiment than those that died earlier. Generally, the same lesions were seen in all fawns.

Figure 7. Changes in SGPT levels in sera of infected and control fawns.

Figure 8. SGOT changes in sera of infected and control fawns.

SGPT

	MD
	WT
	ELK
	CONTROL

Nu/dl

100

75

50

25

0

0   21   35   49

PID

SGOT

400

300

200

100

0

0   21   35   49

PID
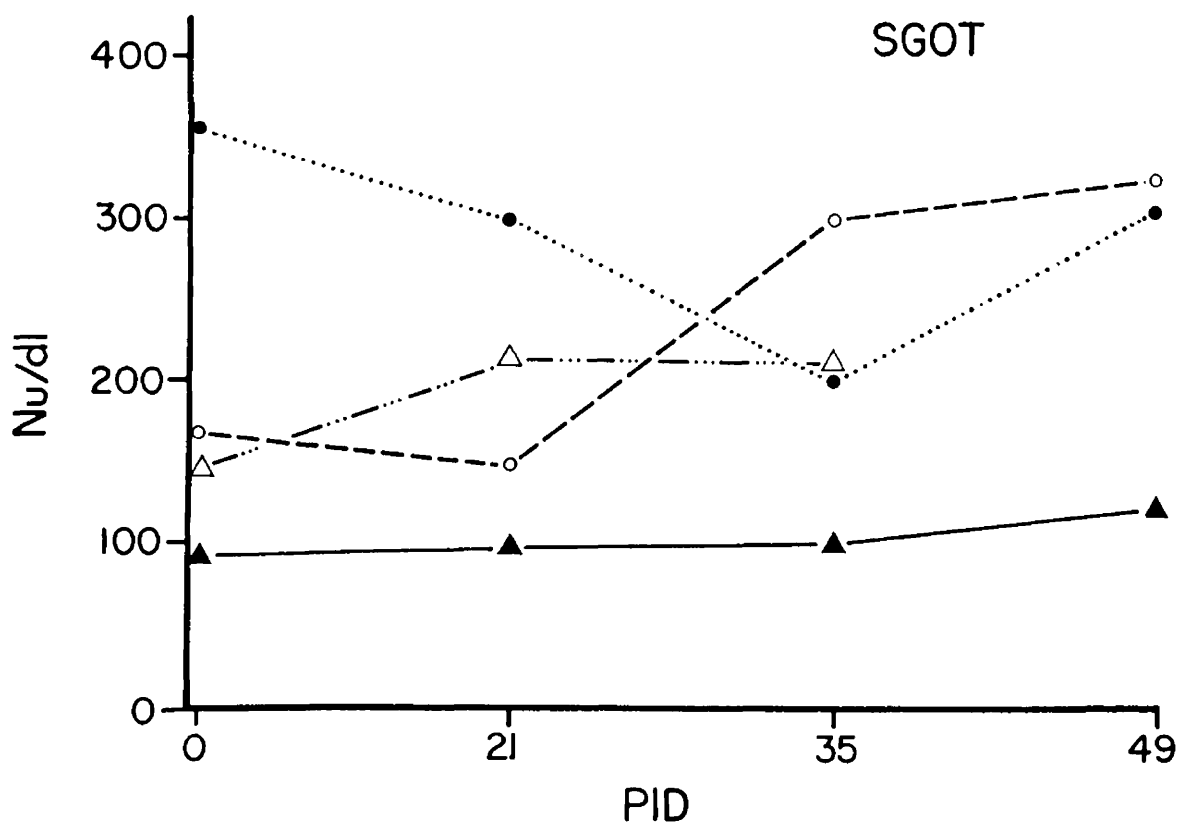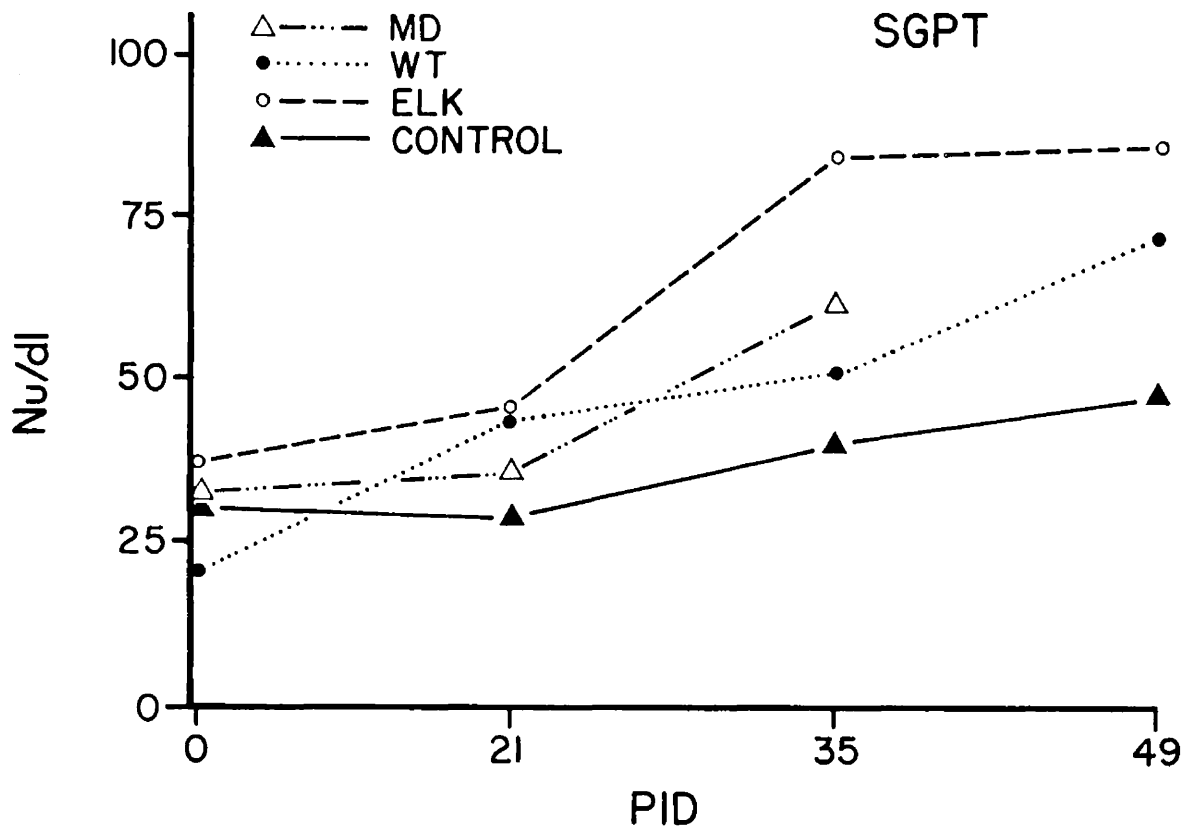
Figure 9. CPK changes in sera of infected and control fawns.
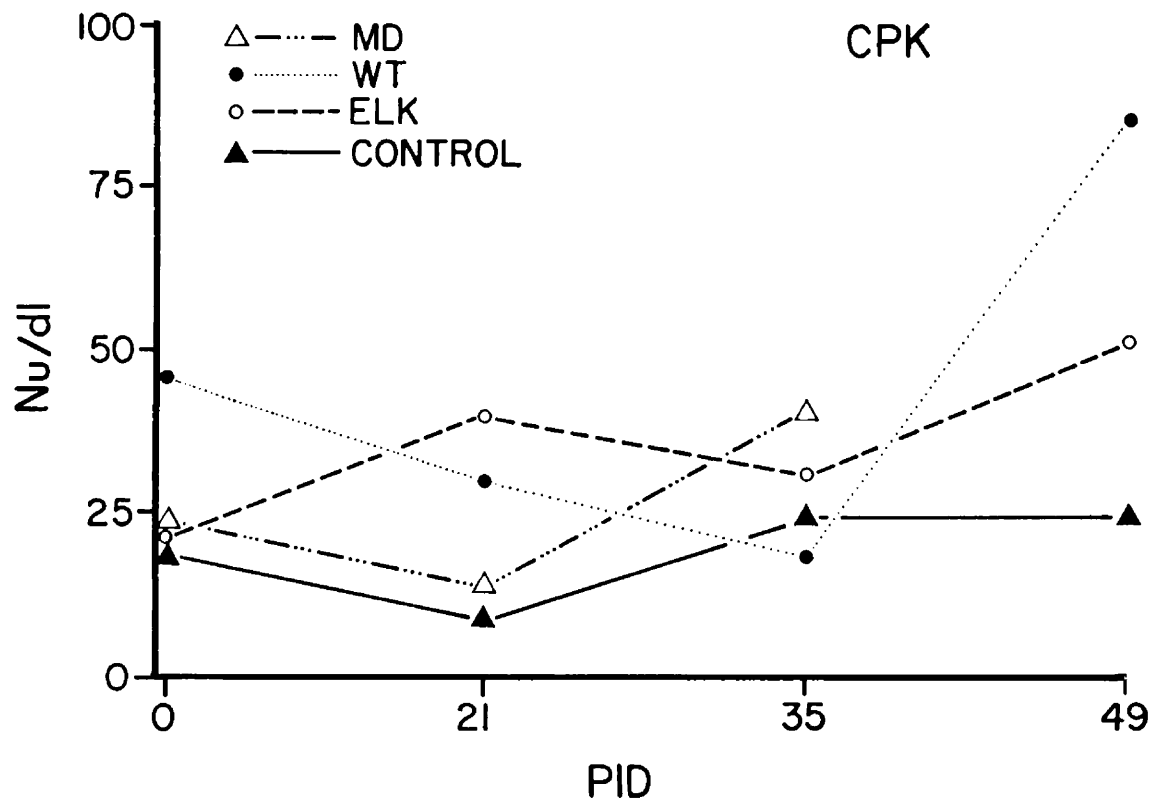
Infected fawns had a bilaterally symmetrical hair loss on their flanks, ears, and tail. Skin on the tops of their ears and their heads was black. The margins of the ears, tail, and glans penis were necrotic. All fawns appeared debilitated and thin with ribs showing and "hollow" appearing flanks.

Infected fawns were depleted of body fat and the sclera were icteric and congested. Skeletal muscles were pale and friable. Cervical, abdominal, and inguinal lymph nodes were enlarged and edematous. Kidneys were congested and friable, and hepatospleenomegaly was noted in all but 2 fawns. Adrenals were greatly enlarged, some with ecchymotic hemorrhages. The lungs of all animals were edematous and congested. Control fawns appeared normal in all respects.

The primary histopathologic lesions associated with sarcocystosis were located in skeletal and cardiac muscle. Lesions consisted of perivascular necrosis with mononuclear cell infiltration (Figs. 10 and 11). These lesions were associated with edema, degeneration, and focal necrosis of the muscle. Lymphocytes were found among necrotic muscle fibers in the more severely affected muscles. Macrophages containing developing schizonts were found between muscle fibers in the areas of focal necrosis (Fig. 12). Often schizonts were surrounded by a clear halo in areas where necrotic debris had been removed (Figs. 12 and 15). Occasionally, schizonts were found in blood vascular spaces (Figs. 13 and 14).

Figure 10. Perivascular necrosis and edema of skeletal muscle in Fawn 3 (H & E, 200×).

Figure 11. Infiltration of mononuclear cells, primarily lymphocytes, in the heart of Fawn 5 (Jenner's stain, 200×).

Figure 12. Mature schizont in an area of focal necrosis in the skeletal muscle of Fawn 8. Note that the area is almost clear of necrotic debris (H & E, 400×).

Figure 13. Mature schizont near a capillary bed in the heart of Fawn 2 (H & E, 400×).

Figure 14. Mature schizont in a capillary found in the heart of Fawn 9 (H & E, 600×).

Figure 15. Macrophage containing an immature schizont. Note the area around the macrophage has been cleared of necrotic debris (H & E, 1000×).

Figure 16. Immature schizont containing merozoites which have pushed the nucleus to the periphery of the cell (H & E, 1100×).

Figure 17. Mature schizont containing merozoites. Note that the cell nucleus is no longer visible (H & E, 1100×).
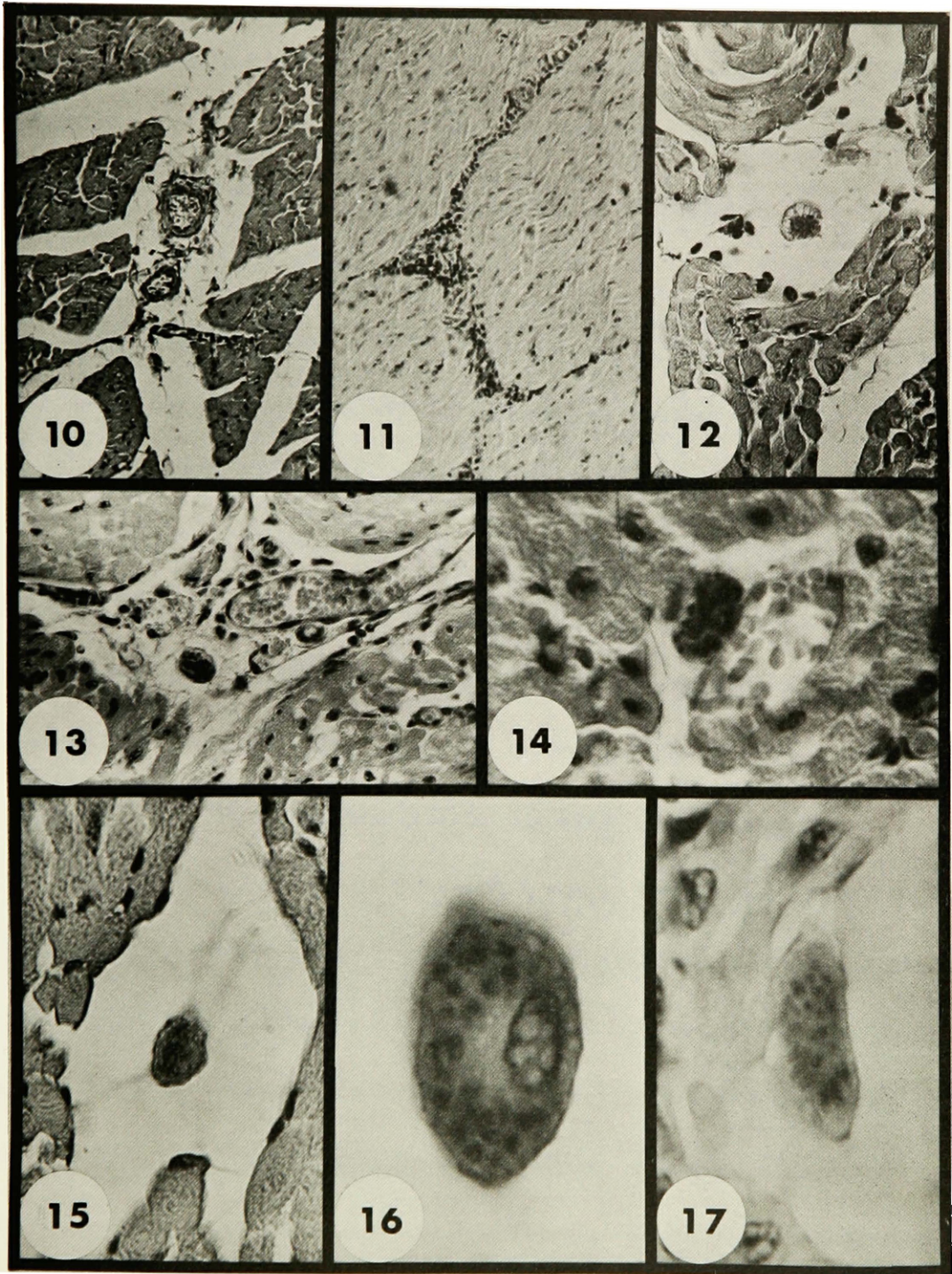
The earliest schizont stages were found in macrophages and consisted of a solid basophilic body (Fig. 15). As development proceeded, cytoplasm was filled with merozoites displacing the nucleus to the periphery of the cell (Fig. 16). Eventually, the nucleus disappeared and merozoites were surrounded by a thin cytoplasmic membrane (Fig. 17).

Schizonts containing merozoites were observed in the skeletal and cardiac muscles of all infected fawns, regardless of when they died. Muscle cysts or cyst formation was not observed.

Except in muscle tissue, schizonts were observed in the adrenals of 2 fawns (Figs. 18 and 19), and an immature schizont was found in the lungs of another (Figs. 20 and 21). Schizonts were not found in other organs. The adrenals of fawns killed PID 48 and 49 had a marked nodular hyperplasia indicating long-term stress.

All tissues showed a marked chronic inflammation with infiltration by mononuclear cells and a proliferation of fibroblasts. This inflammatory response was seen in all tissues examined even though no schizonts were observed. This inflammation resulted in lymphadenitis, reactive meningoencephalitis, endocarditis, and reactive hyperplasia of the spleen and liver. Many peripheral capillaries became hyperplastic and resulted in restricted blood flow causing areas of necrosis in the extremities.

The primary cause of death in the 3 fawns that died was

Figure 18. Schizonts (arrows) in the adrenal cortex of Fawn 7 (H & E, 1000X).

Figure 19. Schizont in the capsule of an adrenal gland in Fawn 9 (H & E, 600X).

Figure 20. Immature schizont in the lung of Fawn 5 (H & E, 600X).

Figure 21. Low-power view of Figure 20 showing the schizont (arrow) and the cellular infiltration and thickening of the alveolar walls (H & E, 150X).
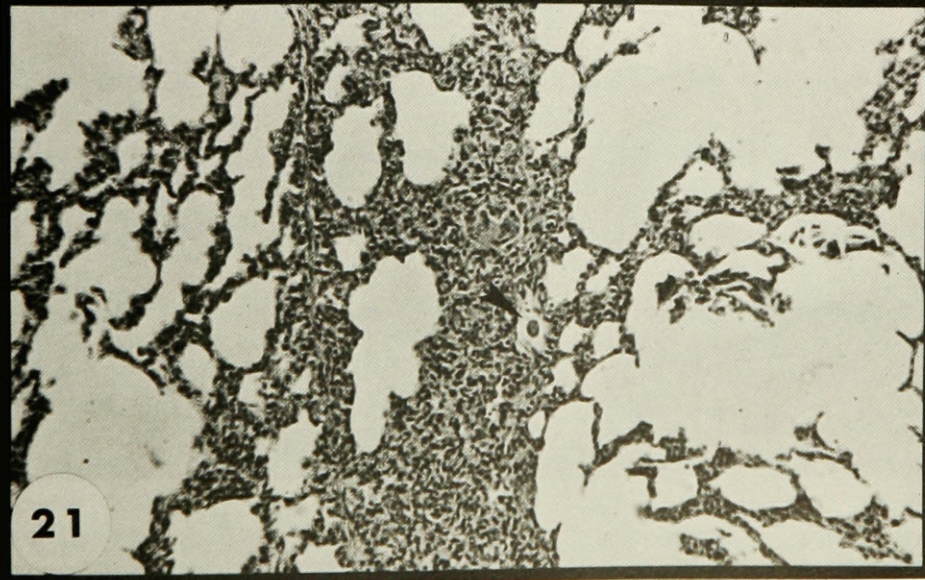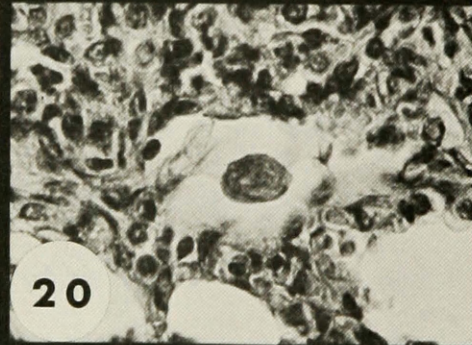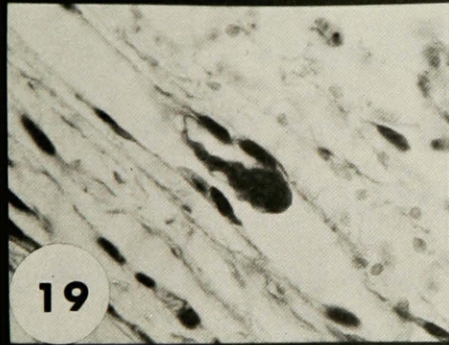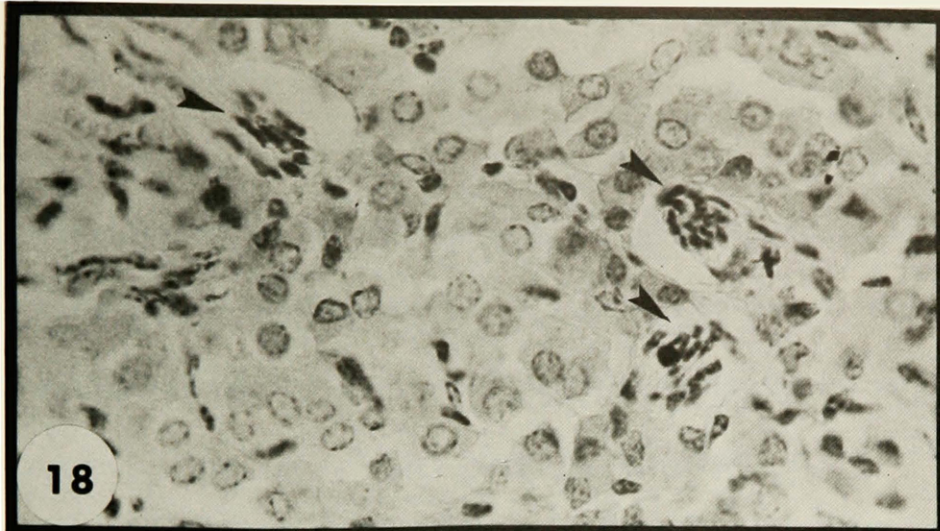
congestive heart failure (CHF). All other infected fawns showed signs

of CHF which appeared to be failure of the left side of the heart.

Kidneys were congested and the lungs were severely congested and

edematous. The alveolar spaces were filled with a fibrinous exudate

with a marked fibrosis and thickening of the alveolar walls (Fig. 21).

All control fawns appeared normal at necropsy. Only the

whitetail fawn showed any abnormality, which consisted of granulation

and scar tissue in an injury received prior to being caught.

Cross Placental Infection

Three adult pregnant mule deer and 1 pregnant elk were

grossly infected with Sarcocystis when shot (Fig. 22). Digestion of

muscle tissue from these animals confirmed infections. Metrocytes

were numerous in the digestion fluid (Fig. 24).

Sarcocysts appeared white and ellipsoidal in shape; however,

a few cysts in mule deer diaphragms were spheroidal. Sarcocysts

ranged in size from approximately 0.2 mm X 0.4 mm to 0.4 mm X

1 mm, although some were as long as 5 mm in the skeletal muscle of

mule deer (Fig. 22).

Examination of sarcocysts by light microscopy revealed a

similar structure among sarcocysts in deer and elk. The cyst is

bound by a prominent wall. Septae traverse the cyst separating it into

compartments, each of which contain bradyzoites and metrocytes (Fig.

23).

Figure 22.   Intercostal muscles containing sarcocysts in a
mule deer.

Figure 23.   Cross section of a sarcocyst in the diaphragm of
a mule deer. The cyst is compartmentalized by
septae and contains many bradyzoites and metro-
cytes (Paragon, 400×).

Figure 24.   Metrocytes in the digestion fluid of elk muscle
(1000×).

Figure 25.   Metrocyte found in the digestion fluid of a fetal
mule deer muscle (1100×).
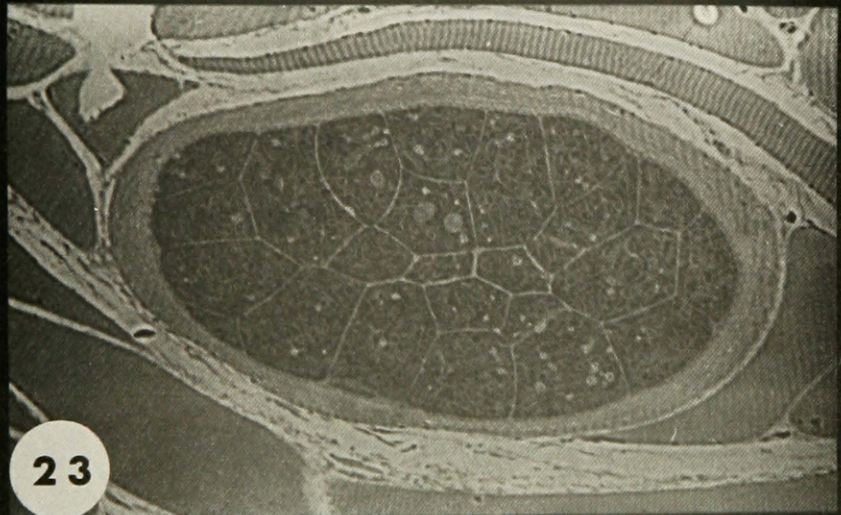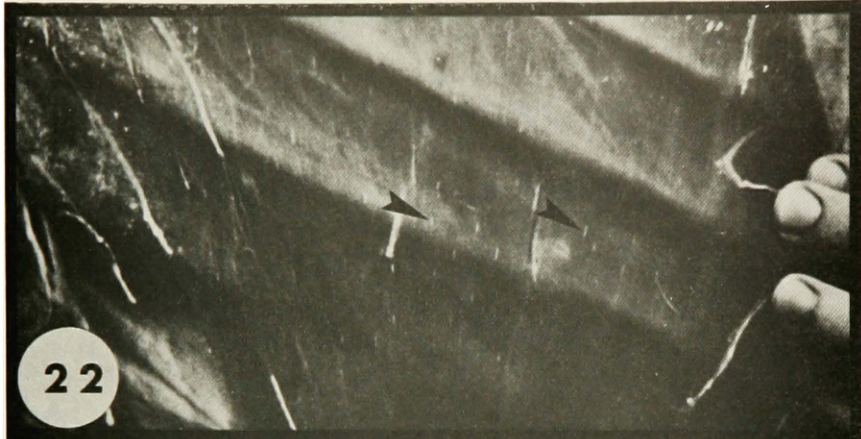
Electron microscopy revealed similarities between cyst wall structures in deer and elk. I found only 1 wall type in deer (Figs. 26 and 27) and 2 dintinct wall types in elk (Figs. 28 and 29). All cysts were similar in that bradyzoites containing micronemes, rhoptres, and an apical complex were observed (Fig. 26).

The cyst walls in the deer consisted of 2 zones. The inner zone was a moderately electron dense matrix made up of fine granules. This zone was continuous with the septae that traverse the cyst (Fig. 26). The granules of the outer zone were much coarser than the inner matrix. The outer zone had invaginations from the surface membrane surrounding the cyst. This membrane consisted of 2 thin membranes that could be differentiated by their electron density (Figs. 26 and 27). The areas where the 2 zones met were highly convoluted forming a reticular membrane (Figs. 26 and 27). Invaginations of the outer zones forming convolutions with the inner zones were interconnected, giving the cyst wall a honeycomb appearance (Fig. 27).

Two cyst wall types were found in elk. Type A cyst walls were much thinner than others (Fig. 28). The periphery of the cyst wall consisted of a uniformly convoluted membrane complex of 2 closely applied membranes. The inner portion of the cyst wall consisted of a moderately electron dense material which was very granular. This area was continuous with the cyst septae and an indentation in the wall was associated with the origin of the septae.

Figure 26. Electron micrograph of the cyst wall found in mule deer diaphragm[1] (Uranyl acetate/phosphotungstic acid, 8000×).

Figure 27. Higher magnification of the cyst wall found in mule deer showing honeycomb appearance[1] (Uranyl acetate/phosphotungstic acid, 9250×).

Figure 28. Type A cyst wall found in elk diaphragm[1] (Uranyl acetate/phosphotungstic acid, 6820×).

Figure 29. Type B cyst wall found in elk diaphragm[1] (Uranyl acetate/phosphotungstic acid, 8000×).

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[1]Abbreviations for Figures 26-29: AC, apical complex; BZ, bradyzoites; DB, degenerating bradyzoites; IZ, inner zone; OZ, outer zone; OM, outer membrane; SP, septa; SK, skeletal muscle.
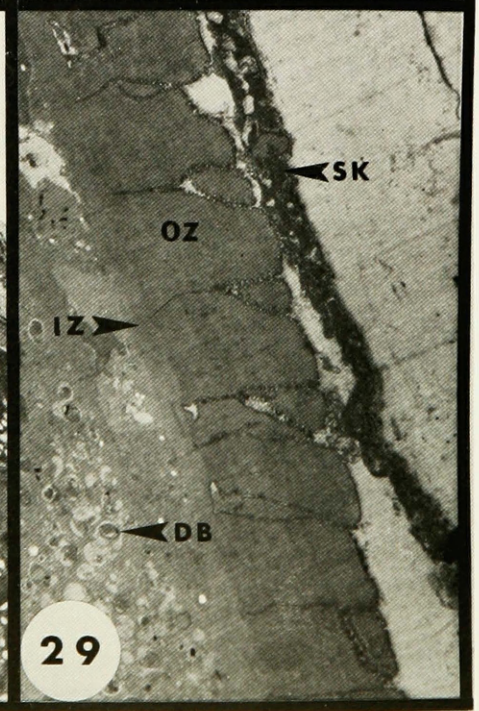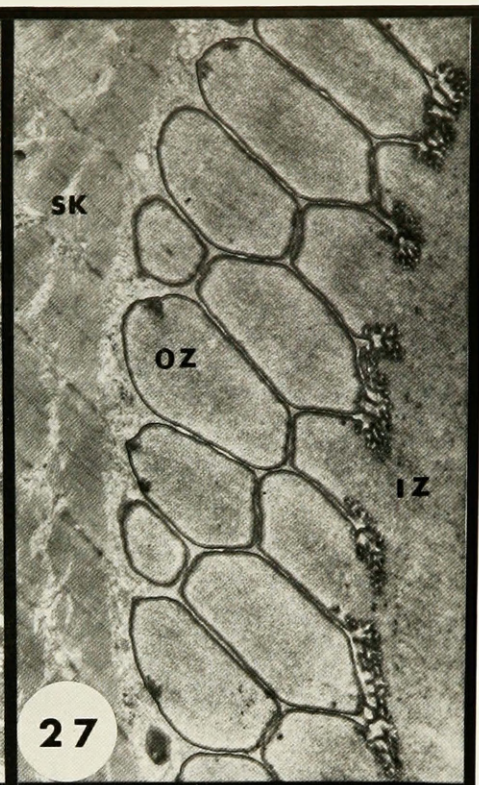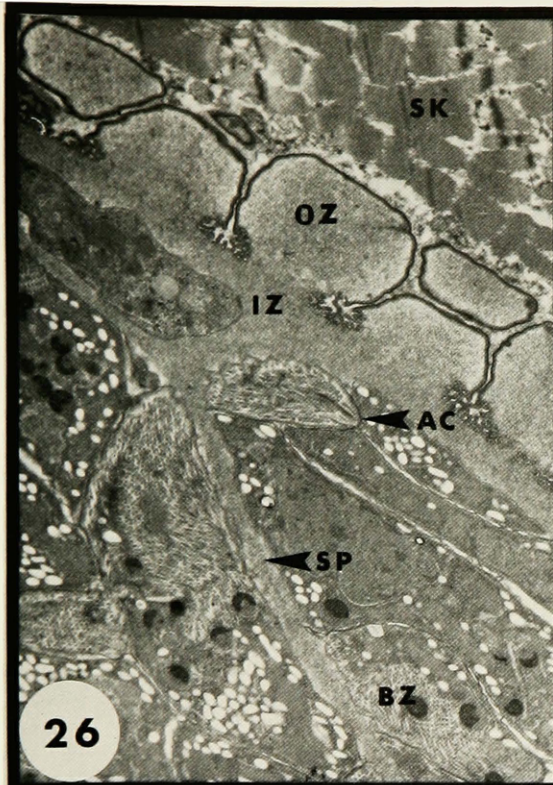
Cyst wall type B was similar to that in deer (Fig. 29). This wall type had 2 zones, an electron lucent inner zone which was continuous with the cyst septae and an outer zone which was more electron dense and contained microtubules. The outer zone had infoldings from the surface of the cyst to the inner zone and was thicker than the wall found in deer. Like the deer wall, the outer membrane was actually 2 closely applied membranes which were convoluted. The cyst shown in Figure 29 appeared to be an older cyst containing degenerated bradyzoites and other material.

Six fetal fawns and 1 fetal calf were examined. Gross and microscopic examination of representative tissues were unremarkable.

Digestion was performed on 1 complete hind leg from each fetus. One metrocyte, apparently identical to those found in the adults, was observed in 1 fetus (Fig. 25).

DISCUSSION

Deer and elk were prevented from moving in and out of the NBR by a 3-m high welded wire fence. Coyotes, the primary predator present, went under the fence. Since predation was not extensive, annual herd reductions by NBR personnel were often necessary to regulate deer and elk populations. The high incidence of Sarcocystis infections in coyotes, deer, and elk raised the possibility that 1 species of Sarcocystis infected all of these hosts. For the same reasons,

Mahrt and Colwell (1980) believed this may be true for Elk Island National Park, Alberta.

This study showed a direct connection between Sarcocystis found in mule deer, white-tailed deer, and elk on the NBR. Coyotes fed meat from these 3 cervids passed oocysts and sporocysts of 2 different prepatent and patent periods and sizes (Table 2). S. hemioni-latrantis was previously identified in mule deer from the NBR (Speer et al. 1980) having sporocysts 16.2 X 9.9 μm and a prepatent period of 9 days and a patent period of 31 days. Those findings were repro-duced in this study for all 3 intermediate hosts (Table 2).

Coyotes fed elk meat passed a second type of sporocyst (Table 2) that was 19.3 X 12.7 μm. Statistically it was different than S. hemionilatrantis (P < 0.01), S. bovicanis (16 X 11 μm), and other species found in domestic animals (Dubey 1976), and S. odocoileocanis (11.1 X 8.1 μm) in white-tailed deer (Crum et al. 1981). This Sarcocystis sp. has not been positively identified.

S. hemionilatrantis was extremely pathogenic to the mule deer fawns. All fawns receiving sporocysts developed acute sarco-cystosis (Table 3). The major clinical symptoms started approximately 11 PID and included anorexia, pyrexia, weight loss, wet cough, hair loss, and sloughing of the ears, tail, and glans penis (Table 3). Similar symptoms appeared 18 PID in 9-month-old mule deer fawns infected with S. hemionilatrantis (Koller et al. 1977). These symptoms

along with anemia, cachexia, enlarged palpable lymph nodes, abortion, and excessive salivation have been noted in acute bovine sarcocystosis (Corner et al. 1963, Fayer et al. 1976, Frelier et al. 1977, Clegg et al. 1978). Acute sarcocystosis causes anemia, anorexia, ataxia, and abortions in domestic sheep (Munday et al. 1975, Leek and Fayer 1978, Munday 1979).

Clinical symptoms in this study were most severe during 3 periods (Table 3). These crises corresponded with 3 cyclic body temperature peaks (Fig. 6). Fayer (1977) speculated that temperature peaks in cattle infected with S. bovicanis occurred when schizonts ruptured, releasing merozoites and resulting in inflammatory response. From their histological studies, Koller et al. (1977) believed that 1 or 2 schizogonous stages preceded death of infected fawns. I believe that fawns in this study died or were killed just after the rupture of third generation schizonts corresponding to the third temperature peak (Fig. 6 and Table 3).

The development and pathology of S. hemionilatrantis observed in this study was typical of Sarcocystis spp. Immature and mature schizonts were seen primarily in skeletal and cardiac muscle but were also seen in lung and adrenal tissues. The primary lesions were perivascular necrosis with a mononuclear cell infiltration, edema, and degeneration of muscle tissue. These lesions were observed in all tissues examined even though no schizonts were observed. Schizonts

have been seen in all organs in cattle (Fayer and Johnson 1974, Dubey 1976, Clegg et al. 1978) and sheep (Munday et al. 1975, Leek and Fayer 1978), usually in endothelial cells of arteries or capillaries. This indicates that the parasite was present as free merozoites released into the muscle tissue but no cysts had formed. These results duplicate Koller et al. (1977), although those authors saw cyst formation. Fawns used by Koller et al. were older and the experiment lasted longer than the present study, allowing the parasite to develop further.

Congested lungs and kidneys, lymphadenitis, meningoencephalitis, endocarditis, and hyperplasia of the spleen and liver, as seen during this study, have been noted in many studies (Corner et al. 1963, Munday et al. 1975, Frelier et al. 1977, Clegg et al. 1978, Leek and Fayer 1978) and is typical of acute sarcocystosis (Dubey 1976). CHF, the primary cause of death of fawns in this study, has not been noted in other studies. The heart failure was caused by the hyperplasia of the peripheral capillaries and arteries, damming the blood in the venous system and increasing the blood pressure/volume load on the heart (Robbins and Cotran 1979). The increased pressure and volume caused the lung and kidney congestion, muscle edema, and finally the heart failure. Pneumonia was a contributing factor in the deaths of fawns used by Koller et al. (1977), but those authors did not observe CHF. One reason for this difference may be that the fawns used in this

study were quite young and less tolerant of the circulatory failure resulting in CHF.

Serum chemistries did not show a cyclic elevational pattern like body temperature. The enzymes SGPT, SGOT, and CPK were the only parameters that were elevated during the experiment (Figs. 7-9 and Appendix A). The SGOT levels on 0 and 21 PID in the whitetail group were higher than at the end of the experiment (Fig. 8). This was due to exceptionally high levels in fawn 4 which raised the group average. I could find no reason why this animal had a high SGOT at that time. All of these enzymes rise because of damage to skeletal and cardiac muscle and liver tissues (Mauck and Davis 1980) which were observed in gross and microscopic examination of these and other tissues.

In naturally infected dairy calves, SGOT and LDH (lactic dehydrogenase) were elevated while serum albumin levels decreased (Frelier et al. 1977). Mahrt and Fayer (1975) observed an increase in SGOT, LDH, and CPK in calves artificially infected with S. fusiformis. They also noted an oligocythemic anemia and a leukocyte shift to the left which was probably due to a massive lymphocytic infiltration of infected tissues. Although no hematologic parameters were studied here, I assume similar conditions occurred in fawns infected with S. hemionilatrantis because of the observed lymphocytic infiltration.

One metrocyte was found in the digestion fluid of 1 fetal mule deer, demonstrating congenital Sarcocystis infection in a naturally infected wild ungulate. Hudkins and Kistner (1977) found a solitary cyst in a control fawn but assumed it was due to contamination. Congenital infections have been reported in cattle (Munday and Black 1976) and horses (Cunningham 1973). Other workers have reported finding schizonts only in placentae (Corner et al. 1963, Fayer et al. 1976). Schizonts were not seen in the placenta or fetal membranes of the fawn that was infected because the infection had already reached the cyst stage and formed metrocytes.

Sarcocysts found in the adult deer and elk were typical cysts (Dubey 1976). They were bound by a cyst wall with septae that compartmentalized the bradyzoites and metrocytes (Fig. 23). Ultrastructurally, 1 cyst wall type was found in both species of deer (Figs. 26 and 27) and 2 types in elk (Figs. 28 and 29). The type B cyst wall in elk (Fig. 29) was similar to those found in deer and is S. hemionilatrantis. Each of these cyst walls have an inner zone of fine granules that is continuous with the septae. The outer zone is convoluted, giving the walls a honeycomb appearance. This honeycombing is actually thick fingerlike projections into the muscle tissue. Similar projections can be seen in cysts of S. bovifelis and S. bovihominis and other species (Mehlhorn et al. 1976).

The type B cyst shown (Fig. 29) is probably older than those

seen in deer (Figs. 26 and 27). As the primary cyst wall develops, the outer membrane becomes more convoluted and microtubules form within the fingerlike projections (Mehlhorn et al. 1976). The outer membrane of type B is more convoluted than the deer wall and contains microtubules (Fig. 29). Numerous degenerating bradyzoites also indicate that this cyst is older.

The type A wall is probably a different species of Sarcocystis. Cyst walls alone cannot be used to differentiate between species (Mehlhorn et al. 1976). However, the added evidence of a different sporocyst and patent period than for S. hemionilatrantis in the coyote (Table 2) indicates 2 species of Sarcocystis in elk. Speer and Dubey (1982) found only 1 wall type in elk they examined. Their description of the cyst wall of S. wapiti sp. n. is very similar to wall type A; both have a thin outer convoluted membrane complex and a granular inner layer which invaginates and is continuous with the septae.

Some authors believe that Sarcocystis spp. are very host specific (Dubey 1980). These results indicate that S. hemionilatrantis has little specificity for mule deer by parasitizing white-tailed deer and elk. Figure 30 is a graphic representation of the life cycle of Sarcocystis spp. found in deer and elk. Although S. hemionilatrantis is pathogenic to mule deer, it may be nonpathogenic to white-tailed deer or elk. S. odocoileocanis is nonpathogenic to its secondary intermediate hosts (Crum et al. 1981). If S. hemionilatrantis is

Figure 30. Life cycle of <u>Sarcocystis</u> spp. in deer and elk on the
NBR.
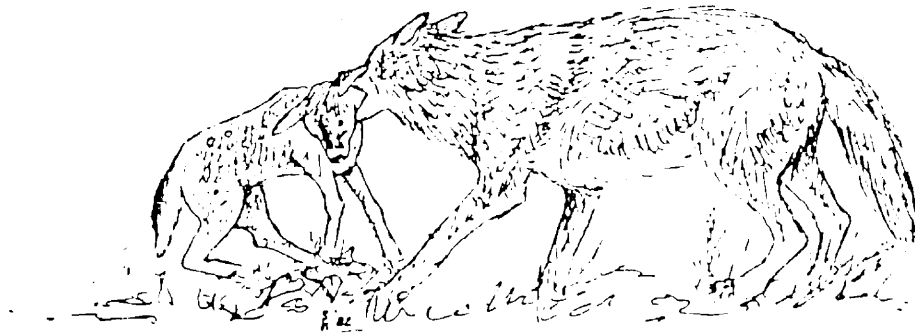
Coyotes feed on infected tissues
and cystozoites then penetrate
cells of the small intestine.

Sporogony occurs in the
villi of the small intestine.
Sporocysts are shed in the
feces after 10 days.

19

16

0

μ

Sarcocystis
sp.

Sarcocystis
hemionilatrantis
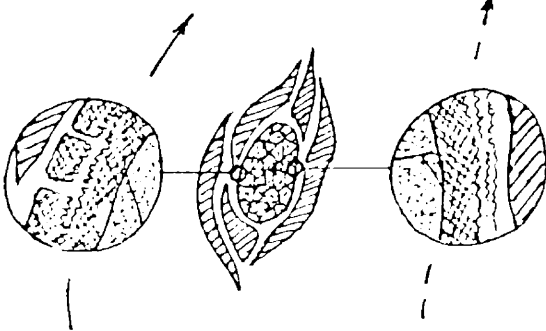
Sporozoites excyst and enter the
arteries and capillaries where
schizogony takes place. Muscle
cysts form about 60 days post
infection.
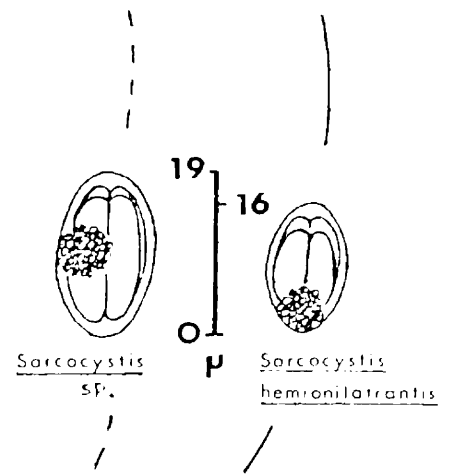
Deer and elk become infected
by ingesting contaminated
vegetation.
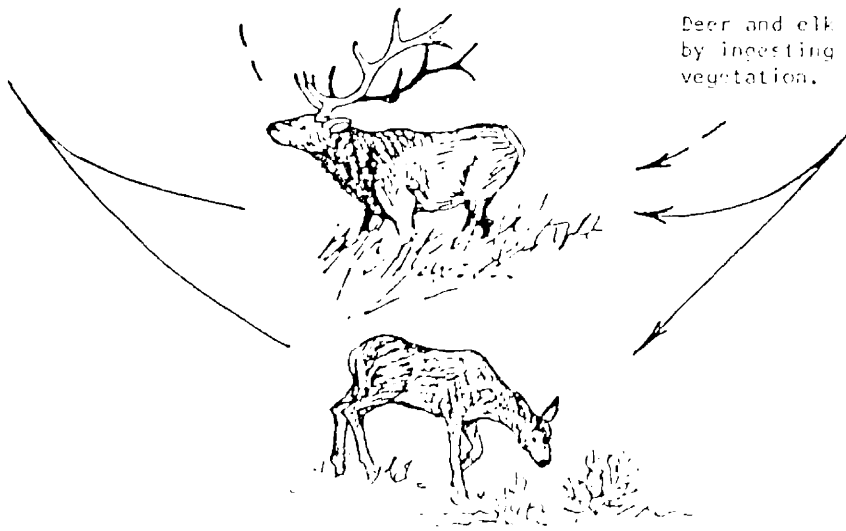
nonpathogenic to these 2 species they would act as important

reservoirs to infect coyotes and ultimately mule deer.

This study was carried out in the hopes of finding a clear

clinical and pathological picture of sarcocystosis in deer so it would

be more recognizable and easier to identify under natural conditions.

Wildlife managers should be aware of this common and pathogenic

parasite because S. hemionilatrantis may have direct and indirect

impacts on deer populations. Abortions, stillbirth, and congenital

infection would lower herd productivity. Acute sarcocystosis could

cause death or severe debilitation predisposing deer or elk to other

diseases or predation, thereby lowering population numbers.

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APPENDIX A

SERUM CHEMISTRIES OF MULE DEER FAWNS

INFECTED WITH SARCOCYSTIS

Table 1. Fawns infected with Sarcocystis from mule deer.

	DOI[1]	21 PID[2]	35 PID
NA meg/l	141.0	140.0	139.0
K meg/l	6.4	6.0	5.5
Cl meg/l	100.0	100.0	97.0
CO_2 meg/l	25.0	25.0	25.0
BUN mg/dl	42.0	17.0	31.0
Creat mg/dl	1.2	1.3	1.2
Tot Prot g/dl	5.5	4.7	4.8
Alb g/dl	2.6	2.5	2.2
Ca mg/dl	9.6	9.6	8.7
P mg/dl	7.6	7.8	8.8
Chol mg/dl	155.0	116.0	99.0
Tot Bili mg/dl	0.3	0.2	0.3
Dir Bili mg/dl	0.1	0.0	0.0
Alk Phos Nu/dl	341.0	270.0	100.0
LDH Nu/dl	185.0	265.0	168.0
SGPT Nu/dl	33.0	35.0	60.0
SGOT Nu/dl	146.0	215.0	209.0
CPK Nu/dl	22.0	15.0	42.0
GGT Nu/dl	197.0	272.0	178.0

[1] Day of infection.

[2] Post infection day.

Table 2. Fawns infected with Sarcocystis from white-tailed deer.

	DOI[1]	21 PID[2]	35 PID	49 PID
NA meg/l	122.0	142.0	142.0	143.0
K meg/l	5.9	5.6	5.4	7.0
Cl meg/l	102.0	101.0	98.0	100.0
CO_2 meg/l	25.0	26.0	25.0	27.0
BUN mg/dl	20.0	17.0	22.0	23.0
Creat mg/dl	1.3	1.1	1.1	1.1
Tot Prot g/dl	5.3	5.4	5.8	5.8
Alb g/dl	2.8	2.7	2.7	2.4
Ca mg/dl	10.2	10.9	10.3	9.7
P mg/dl	7.6	8.5	8.2	9.1
Chol mg/dl	123.0	192.0	166.0	133.0
Tot Bili mg/dl	0.4	0.2	0.4	0.2
Dir Bili mg/dl	0.1	0.0	0.0	0.0
Alk Phos Nu/dl	507.0	523.0	289.0	207.0
LDH Nu/dl	249.0	324.0	251.0	331.0
SGPT Nu/dl	19.0	44.0	51.0	73.0
SGOT Nu/dl	260.0	298.0	187.0	309.0
CPK Nu/dl	45.0	30.0	16.0	84.0
GGT Nu/dl	273.0	358.0	215.0	211.0

[1] Day of infection.

[2] Post infection day.

Table 3.   Fawns infected with _Sarcocystis_ from elk.

	DOI[1]	21 PID[2]	35 PID	49 PID
NA meg/l	152.0	147.0	146.0	148.0
K meg/l	6.2	6.4	6.3	5.4
Cl meg/l	106.0	104.0	104.0	103.0
CO_2 meg/l	28.0	23.0	23.0	27.0
BUN mg/dl	30.0	12.0	22.0	17.0
Creat mg/dl	1.2	1.3	1.2	1.0
Tot Prot g/dl	6.3	5.9	5.8	6.1
Alb g/dl	2.8	3.1	3.0	3.0
Ca mg/dl	10.8	10.8	10.8	10.1
P mg/dl	8.4	8.4	9.2	9.4
Chol mg/dl	121.0	174.0	190.0	136.0
Tot Bili mg/dl	0.4	0.2	0.3	0.2
Dir Bili mg/dl	0.1	0.0	0.0	0.0
Alk Phos Nu/dl	354.0	395.0	254.0	238.0
LDH Nu/dl	192.0	206.0	275.0	295.0
SGPT Nu/dl	37.0	46.0	83.0	84.0
SGOT Nu/dl	168.0	148.0	299.0	325.0
CPK Nu/dl	20.0	39.0	29.0	53.0
GGT Nu/dl	144.0	266.0	204.0	241.0

[1] Day of infection.

[2] Post infection day.

Table 4. Control fawns.

	DOI[1]	21 PID[2]	35 PID	49 PID
Na meg/l	154.0	134.0	146.0	143.0
K meg/l	5.6	5.3	7.3	5.5
Cl meg/l	108.0	96.0	103.0	98.0
CO_2 meg/l	28.0	25.0	27.0	30.0
BUN mg/dl	21.0	19.0	22.0	13.0
Creat mg/dl	1.2	1.1	1.4	1.1
Tot Prot g/dl	6.0	4.8	4.9	4.8
Alb g/dl	2.5	2.5	2.8	2.7
Ca mg/dl	11.2	10.6	11.1	9.4
P mg/dl	9.3	7.4	11.5	9.8
Chol mg/dl	102.0	154.0	152.0	125.0
Tot Bili mg/dl	0.4	0.1	0.4	0.2
Dir Bili mg/dl	0.1	0.0	0.0	0.0
Alk Phos Nu/dl	467.0	178.0	155.0	154.0
LDH Nu/dl	130.0	126.0	112.0	120.0
SGPT Nu/dl	31.0	29.0	41.0	49.0
SGOT Nu/dl	91.0	96.0	98.0	126.0
CPK Nu/dl	19.0	8.0	25.0	25.0
GGT Nu/dl	66.0	17.0	55.0	55.0

[1] Day of infection.

[2] Post infection day.