Preliminary studies with a dog (Canis familiaris) and a coyote (Canis latrans) showed that these carnivores can serve as definitive hosts in the life cycle of Sarcocystis fusiformis of cattle (Bos taurus) and a microscopic Sarcocystis of mule deer (Odocoileus hemionus hemionus). Preliminary studies to determine effects of the cervine Sarcocystis on deer were inconclusive.

Further studies confirmed that coyotes were definitive hosts for this cervine Sarcocystis. Sporocysts passed from infected coyotes were used to inoculate eleven mule deer fawns, two sheep (Ovis aries) and a calf. Three fawns, two sheep and a calf were maintained as uninoculated control animals. All control animals as well as the inoculated sheep and calf remained healthy and did not develop Sarcocystis. All inoculated fawns underwent an acute infection and nine of eleven died. A new species name, Sarcocystis hemionilatransis, was proposed for this parasite.
Experimental Cervine Sarcocystis Infections in Mule Deer

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EXPERIMENTAL CERVINE SARCOCYSTIS INFECTIONS IN MULE DEER

I. INTRODUCTION

The decline in mule deer numbers became apparent in the early 1960's in several of the Western States (McKean and Luman 1964). One of the major deer populations affected in Oregon was the Steens Mountain herd, located in southeastern Oregon, Harney County (42°30'N, 118°40'W).

In an attempt to elicit factors responsible for the population decline in the Steens Mountain herd, the Oregon Department of Fish and Wildlife (formerly the Oregon State Game Commission) initiated a study late in 1968 with the primary objective of identifying causes for the low mule deer numbers in the Steens Mountain mule deer herd.

Annual inventories of the Steens Mountain deer herd December fawn:doe ratios during 1951-58 averaged about 80 fawns per 100 does in this population, a relatively high survival rate. Similar inventories taken since 1959, however, show about 40 percent decline in fawns from the 1951-58 figures, averaging 50 fawns per 100 does (Trainer 1975). It was inferred that decreased recruitment was primarily responsible for the decline in mule deer numbers.

Pertinent findings of the Steens Mountain study to date indicate adequate buck service in the herd and adequate reproduction rates. Trainer (1975) reported a fetal rate of 131 fetuses per 100 does. However, fawn:doe ratios during August-September, December and March 1968-74 averaged only 86, 54 and 29 fawns per 100 does, respectively (Trainer 1975). This 78 percent loss of fawns from birth to March of the following year has been documented by radio-monitoring and herd composition data. A mean of 44 percent of the total mortality
evident occurred between June and mid-July, 32 percent between mid-September and December and 24 percent during December to March. It was further determined that mortality did not occur between mid-July and late September in 1973 or 1974; hence, the summer loss of fawns was confined to the first 45 days of life.

The final report on the Steens Mountain study has not been completed since the project will terminate June 30, 1977. However, some preliminary findings can be summarized as follows (Trainer 1975). Through radio-monitoring, the June-July mortality has been identified, as has been the December-March. It has not been possible, however, to adequately assess the September-December mortality. It was determined that predation, primarily by coyotes, was involved in 55 percent of the early summer and 79 percent of the winter fawn mortality.

Thirty-five percent of the total mortality in fawns, occurring during the first 45 days of life was attributed to disease (17%), starvation (12%) and accidents (6%), with the remaining 10 percent of the mortality attributed to unknown factors. Review of data indicated that this level of disease mortality in newborn fawns was comparable to those reported in domestic animals and was considered of minimal consequence in regards to population dynamics (Kistner 1974, Kistner and Hudkins-Vivion 1975).

The disease related segment of the Steens Mountain study is directed towards identification of both direct causes of mortality or debilitating factors. Kistner (1974) identified colibacillosis as the only consistent cause of early direct disease-related mortality. Although colibacillosis is indeed an infectious disease entity,
he felt that the percentage (50% of the total mortality attributed to disease) was not excessive in free-ranging populations as compared to domestic animals. Investigations searching for debilitating factors also included examinations for infectious diseases, mineral deficiencies of does and fawns, and parasitism. Kistner (1974) summarized some of the findings as follows:

"Serum antibodies were found in a few deer against hemorrhagic disease (bluetongue and epizootic hemorrhagic disease viruses), but did not appear to reflect an epizootic in any year. Markedly lower levels of liver copper and iron were found on the study area (Steens Mountain) as compared to the control area (Starkey Management Unit) in older fawns and adult deer. Molybdenum levels did not appear high enough to interfere with copper availability on either area."

Kistner and Hudkins-Vivion (1975) reported that endoparasite burdens from Steens Mountain fawns were relatively light, and considered them unimportant as a debilitating factor. Another aspect of the disease investigation involved collection of muscle samples to search for evidence of white muscle disease. Kistner (1974) did not find lesions of white muscle disease, but did observe large numbers of Sarcocystis sp. cysts in the musculature of fawns collected in the spring of 1974. Follow-up field studies revealed a significant increase in Sarcocystis infection levels coinciding with the major periods of fawn mortality (Kistner and Hudkins-Vivion 1975), i.e. the autumnal/winter periods.

Recent research with Sarcocystis in domestic animals has demonstrated a life cycle similar to that of some coccidial protozoans in that two vertebrate hosts are involved (Wallace 1973, Fayer and Johnson 1974, Ford 1974). Specific carnivores fed tissues infected
with *Sarcocystis* developed coccidian sexual stages in the small intestine, with sporulated sporocysts subsequently shed in the feces (Rommel and Heydorn 1974, Rommel et al. 1972, Fayer et al. 1976). The life cycle of *S. fusiformis* of cattle was established in that carnivores served as the definitive host and herbivores were the intermediate host (Fayer and Johnson 1974). Sporocysts shed by laboratory-reared dogs produced illness and mortality in experimentally infected calves. Early schizogonous stages were found in various tissues and later typical cysts were found in musculature of the calves.

The purpose of the present study was to attempt completion of the life cycle of the *Sarcocystis* sp. found in mule deer and to determine if this represents a pathogenic agent to mule deer, either in the form of direct mortality or as a debilitating agent.
II. LITERATURE REVIEW

The state of the literature concerning Sarcocystis was perhaps best summarized by Scott (1930).

"Many contradictory opinions have been expressed concerning these parasites, divergent results have been obtained in their study and erroneous conclusions drawn, frequently from inadequate data....The discovery of truth involves certain important principles of scientific method. In scientific procedure, there is the need of rigidly accurate observations, carefully planned experiments and logical reasoning in drawing conclusions. A critical study of the development of knowledge of the Sarcosporidia reveals that one and all of these principles have from time to time been violated. Such procedure leads to confusion of ideas, contradictory results, speculative hypotheses and erroneous conclusions."

Such conditions had not appreciably improved until the last decade. For this reason, it was deemed appropriate to touch upon a few key points concerning the early reports on Sarcocystis and concentrate mainly upon a review of the most recent work.

Miescher in 1843 was the first to report discovery of a sporozoa parasitic in striated muscle. He described milk-white filaments, visible to the naked eye, in the striated muscles of domestic mice. The filaments were cylindrical tubes, parallel to the muscle fibers and contained innumerable elongated or kidney-shaped forms and a smaller number of globular forms. V. Hessling (1854) verified this observation when he reported finding "Miescher's tubes" in the striated muscle of deer, cattle and sheep. Rainey in 1858 published an account of the development of what he believed to be Cysticercus cellulosae in the muscles of the pig. This work was reevaluated by Leukart in 1863 who determined what Rainey really described was the
Miescher tubes of the Sarcosporidian of pigs.

The genus Sarcocystis was established in 1882 by Lankester (Scott 1930). From then until the last decade, most of the work on this group consisted of observations on nine or ten of the most common species. These reports covered prevalence and intensity of infections, size and location of cysts and the very divergent views on the pathogenicity and life cycle of the parasite.

Scott (1930) and Eisenstein and Innes (1956) reviewed numerous author's reports of transmission and pathogenicity in considerable detail. These reports described experiments in which sarcocysts resulted from ingestion of infected muscle, feces, or urine from infected animals, or feed contaminated with feces or urine. Other reports described congenital or intrauterine transmission, intramuscular injection of cysts and transmission via an insect vector. Similarly, early reports of the pathogenicity of Sarcocystis varied from harmless to acute. Sarcosporidiosis has also been incriminated historically in such diseases as cattle plague, loco disease of horses, sheep scrapie, "Lamziekte" in either horses or sheep, and eosinophilic myositis.

Historically, the genus Sarcocystis has been included in phylum Protozoa, class Sporozoa, order Sarcosporidia. As recently as 1945, however, Spindler and Zimmerman postulated that sarcosporidiosis was fungal in origin after various transmission studies and culturing of mature sarcocysts yielded a fungus-like growth. Ciesla (1951) and Holz (1954) also reported fungus-like growths from cultures of muscle cysts, while Akün and Holz (1955) reported that histochemical
studies on sarcocysts from sheep indicated a relationship with the fungi. These observations, unverified and divergent from the generally accepted idea that Sarcocystis is a protozoan, remain unexplained. However, these workers failed to conclusively show that the growths reported as occurring in cultures of sarcocysts actually originated from the sarcocyst materials placed in culture. Moreover, Spindler and Zimmerman (1945) were unable to eliminate fortuitous infection as a possibility in their successful transmission trials with sarcocysts.

Levine (1961) reclassified the genus Sarcocystis in class Toxoplasmasida, order Toxoplasmorida. Scholyteck et al. (1970) proposed the order Endodyococcida for them and Levine (1973) retained the term, but reduced the group to suborder Endodyococcidorida, order Eucoccidiorida. Dubey et al. (1970) proposed placing the family Toxoplasmatidae in the suborder Eimeriorina, and Levine (1973) felt this would eventually be done, but only if sexual stages were found to occur in the life cycle. Levine (1973) felt that the life cycle was simple, with no intermediate host involved and no known sexual stages.

Hutchison et al. (1969) discovered that a sexual phase indeed exists in the life cycle of Toxoplasma. From this discovery, a new look was taken at other members of the class Toxoplasmasida, including Sarcocystis, to see if similar stages existed. Ultra-structural studies by Ludvik (1963) on the tissue cysts had previously demonstrated that Sarcocystis is a sporozoan. Fayer (1970, 1972) confirmed this finding and further strengthened the evidence of both sexual
and asexual stages existing in the life cycle when he found gametogony of *Sarcocystis* in tissue culture. He cultivated motile, banana-shaped organisms released from intramuscular cysts of the wild grackle (*Quiscalus quiscula*) in avian and mammalian cell cultures. Mature macro- and microgametocytes and cystlike forms developed following penetration of culture cells.

In the same year, Rommel et al. (1972) first reported the presence of occasionally disporocystid, tetrazoic oocysts in the feces of cats fed macroscopic cysts from sheep muscle. Rommel and Heydorn (1972) and Heydorn and Rommel (1972a) then reported on two experiments further confirming the coccidial nature of *Sarcocystis*. Cats and dogs fed infected beef passed sporocysts in their feces and human volunteers fed infected beef and pork passed sporocysts. The picture became even more complete when Heydorn and Rommel (1972b) were able to demonstrate macrogametocytes and immature and mature oocysts in subepithelial locations in the intestines of cats fed meat infected with *Sarcocystis*.

Other workers were able to confirm this work by various reports of *S. fusiformis* of cattle and *S. tenella* of sheep causing intestinal infections in dogs and cats with subsequent production of sporocysts (Euzéby et al. 1972, Fayer and Leek 1973, Mahrt 1973, Fayer 1974, Ford 1974, Markus et al. 1974a, 1974b, Melhorn et al. 1974, Munday and Corbould 1974).

Fayer and Johnson (1973) provided the first report of asexual multiplication of *Sarcocystis* in tissues of a host orally inoculated with sporocysts passed by an infected carnivore. These schizonts were seen in various tissues of calves infected with sporocysts
collected from dog feces. These authors postulated that when sporocysts are ingested by calves, the sporozoites are released, dissemi-
uate throughout the body, and develop into schizonts. They further hypothesized that the products of these primary stages give rise to
the typical commonly noted muscle cyst stage in the life cycle of
*S. fusiformis.*

The probability of the *Sarcocystis* life cycle being completed in
two vertebrate hosts in a manner similar to that of *Toxoplasma gondii*
(Frenkel et al. 1970, Hutchison et al. 1970, Overdulve 1970, Sheffield and Melton 1970) and other coccidia was therefore established.
Wallace (1973) subsequently reported a cat-mouse-cat cycle in what was presumed to be *S. muris.* Munday and Corbould (1974), Ford (1974), and Markus et al. (1974b) discussed the possibility of a predator-prey transmission role in the life cycle of the Sarcosporidia.

Golubkovan and Kisliakova (1974) reported production of *Sarcocys-
tis* in swine by feeding them cysts passed in the feces of infected cats. Fayer and Johnson (1974) expanded previous studies and again were able to produce sarcocysts in calves inoculated with sporocysts from feces of dogs fed infected beef. They proposed the two host life cycle for *S. fusiformis* based on this work. Rommel et al. (1974) described a two host life cycle for *S. tenella, S. fusiformis,* and *S. miescheriana* (in pigs) with cysts in the muscles of the inter-
mediate host and sexual stages in the gut wall of the definitive host.
Rommel (1975) stated:

"The sarcosporidia have also an obligatory two-host cycle. Carnivores and man are final hosts; herbivores, some omnivores, and rodents serve as intermediate hosts. In con-
trast to all genera so far investigated the bradyzoites of Sarcocystis develop without schizogony directly into gamonts after their oral ingestion by carnivores. Sporulation takes place when the parasites are still within the gut wall leading to the shedding of fully sporulated oocysts or sporocysts."

To date, approximately 70 species have been reported in birds, mammals and reptiles (Kalyakin and Zasukhin 1975). According to Levine (1973), the validity of all species is far from certain as they were delineated primarily on the basis of the host in which they were found, with some importance given to the structure of the cyst wall and the size of the zoites. However, little attempt has been made to complete the life cycle of the majority of the species. Originally a single species was thought to parasitize each host. Recent studies, however, have shown that more than one species may parasitize a single intermediate host and one species may develop in several definitive hosts (Heydorn et al. 1975).

Recent literature has also reported investigations of the effect of the Sarcocystis infections on its hosts. Dubey (1976) felt that the sarcosporidian coccidia are probably non-pathogenic to their definitive hosts, but considered Sarcocystis as an important disease in cattle, swine and sheep; that is, the intermediate hosts of respective species.

Fayer and Johnson (1973) and Johnson et al. (1975) reported acute disease with some fatalities resulting in calves experimentally infected with sporocysts from canine feces. Dalmeny disease in cattle described by Corner et al. (1963) appears synonymous with the acute sarcocystosis experimentally produced in calves. Recently, two field episodes of acute sarcocystosis have been described in
cattle in the United States and Canada (Frelier et al. 1976, Meads 1976). Golubkovan and Kisliakova (1974) reported one species of Sarcocystis in swine to be pathogenic, causing lameness, slow growth and general ill health. Disease, abortions and death were also attributed to sarcocystosis in sheep (Gestrich et al. 1974, Munday et al. 1975, Leek and Fayer 1976).

As has been stated before, most of the work completed to date has been done with Sarcocystis of very few species, primarily those found in domestic and laboratory animals. Literature regarding Sarcocystis infections in wildlife has principally been case reports resulting from incidental findings. Karstad and Trainer (1969) conducted a survey of white-tailed deer (Odocoileus virginianus) from Ontario, Wisconsin and Texas. They found 79 percent of 208 deer harboring sarcocysts in the tongue muscles.
III. MATERIALS AND METHODS

Experimental Design

The experiment was divided into two major phases, preliminary and definitive studies. The preliminary investigation was composed of three small-scale studies designed to provide an opportunity to acquire familiarity with Sarcocystis and research methods used with this parasite. Definitive studies were conducted to fulfill the objectives of the experiment; i.e. to determine 1) if a Sarcocystis life cycle exists between mule deer and coyotes and 2) if Sarcocystis represents a pathogenic agent in experimentally infected mule deer fawns.

Two of the three preliminary studies investigated the carnivore's role in the life cycle of Sarcocystis species. In the first, a dog and a coyote were fed infected beef. Secondly, the same dog and coyote were later fed Sarcocystis infected venison. The third preliminary study was designed to investigate the effect of cervine Sarcocystis sporocysts on two experimentally inoculated mule deer fawns as compared to one uninoculated control fawn.

The definitive studies were conducted in two parts. The first part was to investigate the carnivore host and to obtain sporocyst inoculum for the herbivore segment. Fifteen coyotes and a dog were fed Sarcocystis infected venison and sporocysts passed in their feces were subsequently collected. The second part of the definitive study was to determine the effects of cervine Sarcocystis sporocysts on
various herbivores. Eleven mule deer fawns, two sheep and one calf were all inoculated while three deer, two sheep and one calf were maintained as uninoculated control animals.

As results from this entire research effort were put into sequential publications, it was felt that a deviation from typical thesis format was appropriate. Accordingly, the materials and methods section is a general outline of the work conducted and information on animal rearing. Each segment of the work is, thereafter, given in chapters with detailed materials and methods, results and discussions included.

**Experimental Animals**

Animals used in the experiment had to be obtained from various sources, as the species involved are not available through laboratory animal supply houses. Except for the carnivores used in preliminary studies, all animals were maintained in isolation at the Veterinary Medicine Animal Isolation Laboratory (VMAIL), Corvallis, Oregon.

**Phase I - Preliminary Studies**

The coyote used in the preliminary studies was trapped as an adult for use in experiments in another department. The dog used in the preliminary studies, also acquired as an adult, was purchased from a laboratory animal supply house. After acquisition, the canines were housed in individual modified Horsfall isolation kennels in the School of Veterinary Medicine facilities, Corvallis, Oregon, and maintained on a diet of dry commercial dog feed.

The three fawns used in the preliminary study were hand-reared
on the E. E. Wilson State Game Management Area by Mrs. Donald Kirkpatrick. Fawns were raised in a wire pen adjacent to her house and moved to an enclosed wooden holding pen when weaned. They were transferred, when approximately eight months old, to the medium isolation facilities at VMAIL and were maintained on a diet of alfalfa hay.

Phase II - Definitive Studies

Coyotes used in the definitive study were captured as puppies from dens in Eastern Oregon. The pups, estimated to be between two and three weeks old, were transported from the site of capture to VMAIL. Each coyote was housed in an individual kennel maintained in the medium isolation units. The dog used in the definitive study was born to a bitch maintained in isolation in the School of Veterinary Medicine facilities. This pup was also transferred to a kennel in the medium isolation unit of VMAIL. All puppies were fed a commercial milk-replacer supplemented by liquid multi-vitamins and minerals. Puppies were gradually weaned to a dry puppy chow which was supplemented with bone meal.

The herbivores used in the definitive study were all raised in the maximum isolation facilities at VMAIL. Cattle and sheep were purchased from a local livestock yard when one day old and transported to the isolation facility. They were raised on appropriate milk-replacers. The calves were supplementally fed Calf Manna\(^a\) and the sheep were fed a mixed grain creep feed. When weaned, animals were

\(^a\) Albers Calf Manna, Div. Carnation Company, Los Angeles, CA 98830
fed a pelleted alfalfa and Purina Calf Startena\textsuperscript{b} or Growena\textsuperscript{c}.

Twenty mule deer fawns were captured when approximately two to seven days old from a site in Eastern Oregon (42° 25'N, 120° 25'W). One additional fawn was taken by Caesarean section from a doe shot for another research project in the same area. All fawns were transported to the isolation facility, divided randomly into two groups and placed in two maximum isolation units. Fawns were bottle-fed on a formula of lamb milk-replacer, condensed milk, vitamins and water. When the fawns were approximately five weeks old, alfalfa hay, Purina Calf Startena or Growena, alfalfa pellets, bone meal and mineralized salt were provided free choice. Fawns were gradually weaned onto this diet.

It should be emphasized here, the stringent steps which were taken to preclude accidental infection of these animals. All materials taken in and out of the pens with the herbivores were either steam-sterilized or autoclaved. Personnel entering the pens were required to take skin showers and put on clean coveralls, haircaps, and boots. Raingear was worn over coveralls when entering pens and was showered and scrubbed upon leaving. Face, hands and boots were also scrubbed upon entering and leaving pens. Utmost caution was used in raising these fawns, in order to preclude accidental exposure to \textit{Sarcocystis}.

\textsuperscript{b} Purina Calf Startena, Ralston Purina Co., St. Louis, MO 63188
\textsuperscript{c} Purina Calf Growena, Ralston Purina Co., St. Louis, MO 63188
Experimental Procedures

Phase I - Preliminary studies

**Study 1** - Feces from both the dog and the coyote was examined for six weeks. The animals were considered to be coccidia-free at the time of infection, as neither oocysts nor Sarcocystis sporocysts had been found in the feces. The coyote and dog were each fed 400 g of Sarcocystis infected beef daily for three consecutive days. Starting on post-infection day (PID) 10, microscopic examinations for Sarcocystis sporocysts were conducted on fecal flotations prepared with Sheather's sugar solution (Levine 1973). Feces were examined for four consecutive days each week for a period of 60 days.

**Study 2** - When fecal examinations of the coyote and dog infected in study 1 showed no sporocysts passed for at least 30 consecutive days, the carnivores were fed 600 g of Sarcocystis infected venison from a Steens Mountain deer. Minimal availability of meat precluded follow-up feedings in this trial. Daily fecal flotations were examined microscopically for Sarcocystis sporocysts during PID 10-35. Sporocysts were collected and cleared of fecal debris.

**Study 3** - Sporocysts collected in Study 2 were used as inoculum to infect two mule deer fawns. Fawns were intraruminally infected with 20,000 and 200,000 sporocysts, respectively. A non-infected control fawn was maintained. Weekly blood samples were taken from all fawns for nine weeks post-infection. All fawns were killed nine weeks post-infection. A necropsy was performed on each fawn and tissue samples were collected for histopathological examination.
Tissues were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 5 to 7 μm, and stained with hematoxylin and eosin (HE). Microscopic examination of tissue was conducted for Sarcocystis stages.

Phase II - Definitive studies

Part A - Fecal flotations from all fifteen coyotes and the dog were examined for five consecutive days. No oocysts or sporocysts were found, so animals were considered to be coccidia-free.

On three consecutive days each coyote was fed 700 g ground meat from a Steens Mountain mule deer infected with microscopic-sized Sarcocystis.

Starting on PID one, daily fecal flotations from each animal were prepared as in the preliminary studies and examined microscopically for sporocysts. Daily examinations were discontinued on PID 40. Thereafter, feces were examined every five days until PID 60. Sporocysts were collected and cleaned of fecal debris.

Part B - When nine months of age, the calves were placed in separate isolation units. The four lambs, also nine months of age, were divided between the two isolation units housing the calves. The calf and two lambs in one isolation unit were maintained as uninoculated control animals. The remaining calf and two lambs were each orally inoculated, using an intraruminal tube, with an aqueous suspension of $2.5 \times 10^5$ Sarcocystis sporocysts collected from the coyotes.

Simultaneously, when seven months of age, the 14 fawns which had been successfully reared were placed in three separate isolation
units. The three fawns in one pen were maintained as uninoculated control animals. The remaining 11 fawns were randomized and orally inoculated intraruminally at dosage levels of $5.0 \times 10^4$, $2.5 \times 10^5$, and $1.0 \times 10^6$ sporocysts. Four fawns inoculated with $5.0 \times 10^4$ sporocysts and two of four inoculated with $2.5 \times 10^5$ sporocysts were housed together in one isolation unit. The last unit contained the three fawns inoculated with $1.0 \times 10^6$ sporocysts and the remaining two fawns inoculated with $2.5 \times 10^5$ sporocysts.

All animals were observed at least twice daily throughout the experiment and weighed weekly. Blood samples were taken weekly from each animal, twice prior to infection and for nine weeks post-infection. Animals were manually restrained for sampling prior to infection. Thereafter, each fawn was sedated with 5-10 mg Xylazine\textsuperscript{d} to reduce trauma during weekly blood collections.

The study was terminated 90 days PI. Animals which died or were killed were necropsied and tissue samples were collected for microscopic studies. Tissues were placed in Tissue-Tek II cassettes, individually identified, fixed in 10% neutral buffered formalin and routinely processed as previously described. Tissues were stained with hematoxylin and eosin. In addition, the skeletal and cardiac muscle were stained by Giemsa, PAS and von Kossa methods.

\textsuperscript{d} Xylazine-Rompun, Chemagro, Kansas City, MO 64120
CHAPTER 1. POSSIBLE SPECIES DIFFERENCES BETWEEN SARCOCYSTIS
FROM MULE DEER AND CATTLE

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Abstract:

In preliminary studies with Sarcocystis from beef (Bos taurus)
and mule deer (Odocoileus hemionus hemionus), a coccidia-free labor-
atory dog (Canis familiaris) and captive coyote (Canis latrans) were
fed Sarcocystis-infected local beef and later fed infected mule deer
venison from Eastern Oregon. Sporocysts were passed in the feces of
both canine hosts 10-15 days after ingestion of infected meat. There
was a statistical difference in the size of sporocysts derived from
beef and venison. It was concluded that the Sarcocystis from beef
and mule deer probably constitute distinct species with a life cycle
dependent on the respective ruminant host and a canine host.

The Oregon State Wildlife Commission initiated a study late in
1968 of mule deer on Steens Mountain, Harney County, Oregon to
investigate the causes for declining deer populations. The disease-
related aspects of fawn mortality have constituted a portion of the

Supported by The Oregon State Wildlife Commission and The Oregon
Agricultural Experiment Station. Technical Paper No. 4033.
Overall studies. In the course of these investigations, large numbers of *Sarcocystis* sp. were found in the musculature of most fawns collected in March, 1974 and April, 1975. The significance of this finding is currently under investigation.

Recent research has demonstrated that *Sarcocystis* sp. in some ruminants has a life cycle similar to that of coccidial protozoans, with alternation of hosts.\(^1,2,3,5\) Sexual cycles occur in the intestine of a carnivore, with subsequent production of sporulated sporocysts in the feces. When sporocysts are ingested by a specific ruminant, asexual cycles occur in the endothelial lining of blood vessels; the progeny of these generations subsequently produce the typical cysts in the musculature.\(^2,3,4,5\)

In preparation for work with *Sarcocystis* of deer, *Sarcocystis*-infected local beef was fed to a laboratory dog and captive coyote which were maintained in modified Horsfall isolation kennels. Both animals had been fed dry dog food, and were coccidia free at the time of feeding.

On post-infection days 11 and 15, respectively, individual sporulated sporocysts containing four sporozoites and a granular residuum were first found in fecal flotations prepared with Sheather's sugar solution. As these animals were fed beef for three consecutive days and fecal examinations were not conducted daily, the length of the prepatent and patent periods were not accurately determined. The sporocysts averaged 16.3 x 10.7 \(\mu\)m (N=20) for the dog and coyote. Mean sporocyst size in the dog feces was 16.3 x 10.6 \(\mu\)m (range 15.0 x 9.2 \(\mu\)m to 17.3 x 11.5 \(\mu\)m), while those in the coyote feces were
16.2 x 10.8 µm (range 13.8 x 9.2 µm to 18.4 x 12.7 µm).

Sporocysts were not found in the feces from either animal examined 30, 40, 50, and 60 days post-infection. Sixty days after feeding the infected beef, the same dog and coyote were fed Sarcocystis-infected venison from a Steens Mountain fawn for one day.

Sporocysts were found in their feces on post-infection days 13 and 10, respectively. The length of the patent period again was not determined. The sporocysts resembled those recovered following feeding of infected beef, in that they occurred singly and contained a granular residuum and four sporozoites. The sporocysts differed markedly in size, however, with an average of 14.4 x 9.3 µm (N=20). Mean sporocyst size in the dog feces was 14.5 x 9.2 µm (range 13.8 x 9.2 µm to 16.1 x 9.2 µm), while those in the coyote feces were 14.2 x 9.4 µm (range 13.8 x 9.2 µm to 16.1 x 11.5 µm).

The average sporocyst size from the dog and coyote fed infected beef differed significantly from the average sporocyst size from the same animals fed infected venison (P = .01).

These studies indicate that a species of Sarcocystis from mule deer has a life cycle similar to that of other species of Sarcocystis of ruminants.1,2,3,5 Sporocysts recovered from a dog and coyote fed infected deer muscle, however, were found to differ significantly in size from sporocysts recovered when the same animals were fed infected beef. Since two species of carnivores were used as hosts and measurements agreed between the sporocysts recovered from each when fed either beef or venison, variation in the size of the sporocysts cannot be attributed to modification by the carnivore host, but must
be attributed to inherent differences in the particular *Sarcocystis* sp. infecting cattle and deer. Probably, the parasites represent two distinct species.

**LITERATURE CITED**


CHAPTER 2. CERVINE SARCOCYSTIS:  
A PRELIMINARY STUDY

Abstract: Two mule deer fawns (Odocoileus hemionus hemionus) were intraruminally inoculated with sporocysts collected from feces of a coyote fed Sarcocystis infected venison. An uninoculated control fawn was also maintained. Fawns were observed daily and blood samples collected weekly for nine weeks after inoculation. No changes were seen in any animals. All fawns were killed after nine weeks. Necropsies were performed and tissue samples collected. All fawns were infected with abdominal cyst tapeworms and what appeared to be a rather old Sarcocystis infection. It was concluded the deer had been in contact with canine feces prior to initiation of the trial and the research endeavor was therefore invalidated.

Research with Sarcocystis sp. in some ruminants has demonstrated a life cycle similar to that of coccidial protozoans, with alternation of hosts (Fayer 1973, Munday and Corbould 1974). Epizootologic studies with S. fusiformis have produced mortality in experimentally infected calves (Fayer and Johnson 1974, Johnson et al. 1975).

Hudkins-Vivion et al. (1976) found that coyotes (Canis latrans) or dogs (Canis familiaris) can serve as the definitive host for a species of Sarcocystis found in mule deer (Odocoileus hemionus hemionus). This study was designed to prepare the workers for a large scale investigation to determine whether this Sarcocystis may act as a pathogen in mule deer.
MATERIALS AND METHODS

Three mule deer fawns were hand-reared in a wire pen on E. E. Wilson Game Management Area. When weaned, they were moved to an enclosed wooden corral approximately eight feet high on the same area. They were transferred, when approximately eight months old, to the medium isolation facilities of the Veterinary Medicine Animal Isolation Laboratory (VMAIL).

Two fawns were intraruminally infected with 20,000 and 200,000 sporocysts collected from infected coyote feces (Hudkins-Vivion et al. 1976). Sporocysts had been collected and concentrated by the following procedure. Feces showing useful numbers of sporocysts were soaked in water for 24 hours at 4°C. This suspension was forced through a 325 mesh screen using an apparatus described by Lotze and Leek (1961). The screened material was sedimented in a continuous flow centrifuge. Resulting sediment was mixed with generous amounts of water and decanted for several days at 4°C. The sporocyst suspension remaining after the decanting process was concentrated enough for practical use as inoculum, approximately $1.0 \times 10^4$ sporocysts/ml. This inoculum was administered intraruminally via a stomach tube to the fawns.

All fawns were observed daily and weekly blood samples were taken for nine weeks post-infection. Differential and total white blood cell count, packed cell volume, hematocrit and hemoglobin determinations were performed on each sample.

All fawns were killed nine weeks post-infection and necropsied.
Tissue samples were taken for histopathological examination.

RESULTS AND DISCUSSION

All fawns remained healthy throughout the experiment. Changes were not detected in sequential blood values nor were great deviations observed between animals.

At necropsy, all three fawns were found to have abdominal cyst tapeworms present. Additionally, histopathological examination of muscle samples revealed a well-established Sarcocystis infection present in all three animals.

The probable explanation for these results is that the fawns somehow contacted canine feces prior to the initiation of this study. Hence, the results of this experiment were invalidated. This study did serve to point out, however, the need for stringent isolation procedures for rearing animals to be used in the definitive studies.

LITERATURE CITED


CHAPTER 3. SARCOCYSTIS HEMIONILATRANTIS (SP. N.):
LIFE CYCLE IN MULE DEER AND COYOTES[1][2]

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Abstract: Fifteen coyotes (Canis latrans) shed sporulated sporocysts in their faces after eating freshly ground skeletal muscles from a mule deer (Odocoileus hemionus hemionus) infected with microscopic-sized cysts of Sarcocystis. Sporocysts were shed intermittently from 12 to 36 days after ingestion of the infected meat. Sporocyst size averaged 14.4 x 9.3 μm.

Eleven mule deer fawns orally inoculated with these sporocysts became infected and 9 of 11 died between post-inoculation days (PID) 27 and 63. Clinical signs of anorexia, weight loss, pyrexia and weakness were evident prior to death. A calf (Bos taurus) and two lambs (Ovis aries) orally inoculated with these sporocysts did not become infected and remained healthy throughout the experiment. Similarly, uninoculated control animals consisting of three mule deer fawns, two lambs and one calf remained healthy during the experiment.

[1] This study was supported by the Oregon Department of Fish and Wildlife and the Federal Aid in Wildlife Restoration, Oregon Project W-70-R-5, Subproject F.

Preliminary histological examinations were conducted on selected tissues from all animals. Microscopic-sized schizogonous stages were identified in macrophages, between muscle fibers and near blood vessels in the esophagus, heart, biceps femoris, semimembranosus, diaphragm and tongue from seven of eight fawns which died between PID 27 and 39. Developing or mature muscle cysts were not found in fawn tissues until PID 60. Sarcocysts were found in the three infected fawns examined after this time. Muscle cysts or earlier schizont stages were not found in tissues from the inoculated or uninoculated calves and lambs. A single muscle cysts were found in one control fawn; the other two control fawns were negative for both muscle cysts and other schizogonous stages.

These results established that the life cycle of this species of Sarcocystis can be completed with coyotes as the definitive host and mule deer as the intermediate host. Based on the demonstrated host specificity and earlier findings, the name S. hemionilatrantis is proposed for this parasite of mule deer and coyotes.

INTRODUCTION

Sarcocystis is common in the myocardial and skeletal musculature of many species of birds, reptiles, and mammals. Recent research has demonstrated a life cycle similar to that of other coccidial protozoans, but with alternation of the schizogonous and sporozonous stages between herbivores and carnivores, respectively. Specific carnivores fed tissues infected with Sarcocystis from sheep (Ovis aires), cattle (Bos taurus), swine (Sus scrofa), and mule deer
(Odocoileus hemionus hemionus) developed coccidian sexual stages in the small intestine, with sporulated sporocysts subsequently shed in the feces.\textsuperscript{2,5,9,10} Sporocysts of \textit{S. fusiformis} shed by laboratory-reared dogs (\textit{Canis familiaris}) produced illness and mortality in experimentally infected calves. Various early schizogonous stages and later typical cysts were found in the musculature of the calves. The life cycle of \textit{S. fusiformis} was established in that carnivores served as the definitive host and herbivores were the intermediate host.\textsuperscript{1}

Declining mule deer populations in Oregon prompted initiation of a study in 1968 by the Oregon Department of Fish and Wildlife on Steens Mountain, Harney County, Oregon (42° 30'N, 118° 40'W). One of the findings from the disease-related segment of this study was the presence of large numbers of microscopic-sized \textit{Sarcocytis} cysts in the musculature of fawns collected in the spring of 1974. Further field studies revealed a significant increase in infection levels of \textit{Sarcocystis} which also coincided with the autumnal/winter periods of major fawn mortality. This prompted initiation of experimental studies to investigate cervine \textit{Sarcocystis}.

A preliminary publication reported sporocyst production in coyotes (\textit{Canis latrans}) fed infected mule deer meat.\textsuperscript{5} This report describes completion of the life cycle by determination of the infectivity of these sporocysts for mule deer fawns and demonstration of schizogonous and muscle cyst stages in experimentally infected isolation-reared fawns.
MATERIALS AND METHODS

Carnivore Study

Fifteen coyote pups were used in this study; they were captured from dens in Eastern Oregon when two to three weeks old. The pups were transported to medium isolation facilities at the Veterinary Medicine Animal Isolation Laboratory (VMAIL) in Corvallis, Oregon, and were caged individually within closed concrete buildings. From four until eight months of age, the pups were fed only dry dog feed. After this time, fecal flotations from all coyotes were examined for five consecutive days.

Each coyote was fed ground meat from a Steens Mountain mule deer infected with microscopic-sized Sarocystis. The deer had been shot, skinned and all skeletal muscles collected. The muscles were placed in insulated containers, packed in wet ice and immediately transported to Corvallis. Upon arrival in Corvallis, the meat was ground and weighed into packages containing 700 g each. The coyotes were each fed one package of meat on this day; the remaining meat was refrigerated at 4 C. Each coyote was fed one package of meat 24 and 48 hours following the initial feeding.

Starting one day after infected meat was first eaten, daily fecal flotations were prepared with Sheather's sugar solution and examined microscopically for sporocysts. Daily examinations continued for 40 days; thereafter, feces were examined every five days for an additional 20 days.

Sporocysts were collected by methods learned from instruction at
the Animal Parasitology Institute, Beltsville, Maryland (Fayer 1975, personal communication). Feces showing abundant numbers of sporocysts on qualitative flotations were soaked in water for 24 hours at 4 C. The resultant fecal suspension was washed through a 325 mesh Tyler Analytical Sieve\(^3\) using a rapid screening apparatus previously described.\(^8\) Following the screening procedure, the fecal suspension was sedimented in a continuous flow centrifuge. The resulting clay-like sediment was mixed with generous amounts of water to make a slurry. The slurry solution was kept at 4 C for several days and decanted every 24 hours. The sporocyst suspension remaining was finally decanted to a concentration of \(1.0 \times 10^4\) sporocysts/ml for use as inoculum.

**Herbivore Study**

Two calves, four lambs, and fourteen mule deer fawns were used in this study; they were all raised in the maximum isolation facilities at VMAIL.

Cattle and sheep were purchased from a local livestock yard when one day old and transported to the isolation facility.

Twenty mule deer fawns were captured when approximately two to seven days old from a site in Eastern Oregon (42° 25'N, 120° 25'W). One additional fawn was taken by Caesarean section from a doe shot for another research project in the same area. All fawns were trans-

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\(^3\) W. S. Tyler, Incorporated, Screening Division Mentor, Ohio 44060 U.S.A.
ported to the isolation facility, divided randomly into two groups and placed accordingly into two isolation units.

Seven of the twenty-one fawns died during the first three months of captivity. Muscle samples were taken from the tongue, diaphragm, semimembranosus and heart of six of these fawns. The samples were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin. The slides were then examined microscopically for evidence of Sarcocystis.

When nine months of age, the calves were placed in separate isolation units. The four lambs, also nine months of age, were divided between the two isolation units housing the calves. The calf and two lambs in one isolation unit were maintained as uninoculated control animals. The remaining calf and two lambs were each orally inoculated, using an intraruminal tube, with an aqueous suspension of $2.5 \times 10^5$ Sarcocystis sporocysts collected from the coyotes.

When seven months of age, the 14 fawns were placed in three separate isolation units. The three fawns in one pen were maintained as uninoculated control animals. The remaining 11 fawns were randomized and orally inoculated as described above at dosage levels of $5.0 \times 10^4$, $2.5 \times 10^5$, and $1.0 \times 10^6$ sporocysts. Four fawns inoculated with $5.0 \times 10^4$ sporocysts and two of four inoculated with $2.5 \times 10^5$ sporocysts were housed together in one isolation unit. The last unit contained the three fawns inoculated with $1.0 \times 10^6$ sporocysts and the remaining two fawns inoculated with $2.5 \times 10^5$ sporocysts.

All animals were observed at least twice daily throughout the
experiment. A necropsy was performed on each animal immediately after death or euthanasia and tissue samples were collected for microscopic studies. Tissues collected for microscopic examination included: lung, liver, kidney, spleen, brain, heart, adrenal glands, lymph nodes, pancreas, small and large intestine, stomach, thyroid and salivary glands, reproductive tract, urinary bladder, thymus, esophagus, eye and skeletal muscles, tongue, diaphragm, semimembranosus, semitendinosus, biceps femoris, rectus femoris, longissimus dorsi, intercostal, masseter and triceps. Tissues were placed in Tissue-Tek II\textsuperscript{4} cassettes, individually identified, fixed in 10\% neutral buffered formalin and routinely processed as previously described. Selected tissues were examined microscopically for this report; the remaining tissues will be examined later and the results will be reported in a paper on the histopathological findings. Similarly, clinical signs, hematological and necropsy findings will be reported separately.

RESULTS

Carnivore Study

Oocysts or sporocysts were not found in fecal flotations prepared prior to the feeding of meat. Hence, the coyotes were considered to be free of coccidia.

\textsuperscript{[4]} Lab-Tek Products  
Division Miles Laboratories, Inc.  
Naperville, Illinois 60540 U.S.A.
All coyotes fed Sarcocystis-infected muscle tissue from the mule deer developed infections and passed sporocysts. Sporocysts were shed intermittently between 12 and 36 days after ingestion of infected meat. Average size of the sporocysts was $14.4 \times 9.3 \, \mu m$ (N=80). Sporocysts were individual and contained four sporozoites and a granular residuum.

**Herbivore Study**

Stages of Sarcocystis were not found in tissues from the six fawns which died prior to initiation of the study.

All 11 of the inoculated fawns became clinically ill and nine of the 11 died between PID 27 and 63. Clinical signs of anorexia, weight loss, pyrexia and weakness were evident prior to death. Fawns died on PID 27, 29, 36, 37, 38, 39 and 63. Mortality rates for the dosage levels $1.0 \times 10^6$, $2.5 \times 10^5$ and $5.0 \times 10^4$ were 100%, 75% and 75%, respectively. The remaining fawn inoculated with $2.5 \times 10^5$ sporocysts was killed on PID 60 while that inoculated with $5.0 \times 10^4$ was killed on PID 88.

The three uninoculated fawns remained healthy and continued to gain weight throughout the experiment. One was killed on PID 60 and the other two killed on PID 76. All calves and lambs remained healthy and gained weight throughout the study. The calves and lambs were killed on PID 77 and 74, respectively.

It is appreciated that Sarcocystis forms in herbivores represent various schizogonous stages, including zoites, early schizonts and muscle cysts. For clarification to the reader, the term schizont in this text will refer to earlier stages in various tissues and the
terms muscle cysts or sarcocysts will refer to the later stages found in skeletal musculature.

Histological examinations were conducted on esophagus, heart, beceps femoris, seminembranosus, diaphragm, tongue, kidney, liver, spleen, adrenal glands and lymph nodes from all animals. Microscopic sized schizogonous stages were identified in macrophages, between muscle fibers and near blood vessels in all muscle tissues examined from seven fawns which died between PID 27 and 39, but were not positively identified in one fawn which died on PID 38. A pronounced vasculitis precluded determination of the exact location of schizonts relative to affected blood vessels. Muscle cysts were found in all muscle samples from the fawn which died on PID 63, as well as in samples from the two fawns killed on PID 60 and 88.

Schizonts were not seen in any tissues of the three experimentally uninfected fawns, nor were muscle cysts found in any tissues from two of the uninfected fawns. One small cyst was found in a section of tongue from the remaining control fawn, which was the one delivered by Caesarean section. Early schizonts or muscle cysts were not found in tissues from either the inoculated or uninoculated calves and lambs.

DISCUSSION

This study confirmed the earlier reported finding that after ingestion of Sarcocystis-infected meat from mule deer, coyotes developed an infection with resultant shedding of sporocysts.5 This finding is in agreement, in this respect, with results of trials with
other carnivores.\textsuperscript{2,4,9,10}

Mule deer fawns orally inoculated with sporocysts became infected, developed clinical signs of illness, and schizonts and muscle cysts were found during histopathologic examination of tissues collected at necropsy. All uninoculated fawns remained healthy, as did both inoculated and uninoculated calves and lambs.

The single cyst in the uninoculated fawn may have come from transplacental infection or exposure to a few viable \textit{Sarcocystis} sporocysts through accidental contamination of feed materials or workers' clothing. Transplacental transmission seems improbably since in our and another study,\textsuperscript{1} numbers of \textit{Sarcocystis}-free animals have been reared successfully. The second possibility is more conceivable, despite strict procedures designed to preclude accidental exposure. Although inexplicable at this time, this minimal accidental infection demonstrated the difficulties of rearing \textit{Sarcocystis}-free animals for experimental transmission studies.

It is felt that the implications of this cyst, however, are inconsequential in the overall findings of this study. The findings of schizonts and muscle cysts in fawns infected with sporocysts, the severe disease produced in infected fawns and the absence of parasites and lack of disease in the uninfected fawns indicated that the sporocysts derived from coyote feces were the source of the \textit{Sarcocystis} infections in this group of fawns. The findings of this study demonstrated that the life cycle of at least one microscopic cervine \textit{Sarcocystis} species can be completed through a deer-coyote-deer cycle, which is in agreement with other studies involving two vertebrate
hosts in the transmission of various species of *Sarcocystis* \(^1,3,11\).

As the infection was established in mule deer, but failed to develop in either cattle or sheep similarly inoculated, there appears to be a degree of host specificity involved. Based on this specificity and the earlier reported statistical difference in sporocyst size derived from bovine and cervine source, \(^5\) it is considered appropriate to propose the name *S. hemionilatrantis* for this parasite of mule deer and coyotes. It is appreciated that acceptance of this name may be contingent on taxonomic studies to confirm the specific distinction.

**ACKNOWLEDGMENTS**

We thank the following for their help in various segments of this study: R. Fayer, D. Wyse, B. Schreiber, B. Lindsey and the many persons in the Oregon Department of Fish and Wildlife and the U.S. Fish and Wildlife Service who contributed to successful completion of this study.
LITERATURE CITED


CHAPTER 4. HISTOPATHOLOGY OF EXPERIMENTAL SARCOCYSTIS INFECTION IN FAWNS

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Summary: Mule deer fawns (Odocoileus hemionus hemionus) inoculated with sporocysts of Sarcocystis hemionilatrantis became infected, developed clinical signs of disease, and died due to the infection itself or from intercurrent pneumonia. Clinical signs were first noted 18 days after infection and fawns died from post-infection days (PID) 27 to 63.

Histopathology revealed early lesions in skeletal muscle that consisted of perivascular necrosis with mononuclear and neutrophilic cell infiltration, accompanied by edema, degeneration and focal necrosis of muscle. Subsequently, this reaction subsided and the cellular infiltrate dissipated. An infected macrophage usually remained in the vacuolated muscle space; each macrophage was surrounded by a clear halo. Developing Sarcocystis schizonts were identified in the cytoplasm of the macrophages and the cytoplasmic membrane eventually ruptured releasing zoites. The zoites then developed into typical muscle cysts.

Supported by the Oregon Department of Fish and Wildlife and the Federal Aid in Wildlife Restoration, Oregon Project W-70-R-5, Subproject F.

Technical Paper No. Oregon Agriculture Experiment Station.
The results of this study indicated that *S. hemionilatrantis* is a pathogen of mule deer under experimental conditions. Pathogenicity should be investigated to determine if *S. hemionilatrantis* causes death or debilitation in wild mule deer under natural conditions.

**INTRODUCTION**

*Sarcocystis*, a protozoan parasite, is often identified as an incidental microscopic finding in skeletal and cardiac muscle in a variety of mammals, birds and reptiles (4). Recent work demonstrated that calves (*Bos taurus*) experimentally infected with *S. fusiformis* became anorectic, lost body weight, developed anemia and occasionally died (3). Schizonts were found throughout the body between 26 and 33 days post-infection (PID), while muscle cysts were found between 33 and 54 days PID.

This report describes the histopathologic lesions that developed in mule deer (*Odocoileus hemionus hemionus*) after oral administration of *S. hemionilatrantis* sporulated sporocysts.

**MATERIALS AND METHODS**

Mule deer fawns were captured prior to ten days of age and reared in maximum isolation facilities at Oregon State University until approximately seven months of age at which time they were used in this study. Eleven fawns were infected intraruminally via stomach tube with an aqueous suspension of sporocysts collected from feces of coyotes (*Canis latrans*) that had been fed muscle from a wild mule
deer naturally infected with *S. hemionilatrantis* (2). Four fawns in each of two groups were given $5.0 \times 10^4$ or $2.5 \times 10^5$ sporocysts and three fawns in a third group were given $1.0 \times 10^6$ sporocysts. Three fawns were maintained as uninoculated controls. All animals were observed twice daily PI. The animals were weighed and blood samples were collected weekly, from two weeks prior to infection throughout the experiment. The fawns were tranquilized with 5-10 mg Xylazine[1] immediately prior to handling weekly PI. The clinical and hematological findings will be reported elsewhere.

Seven fawns died during the course of the infection and were necropsied. Deaths occurred on PID 27, 29, 36, 37, 38, 39 and 63 (Table 1). Two fawns were killed when moribund and necropsied on PID 36 and 38. Mortality rates for increasing dosage levels were 75 percent ($5.0 \times 10^4$), 75 percent ($2.5 \times 10^5$) and 100 percent ($1.0 \times 10^6$). The remaining two infected fawns were recovering when they were killed on PID 60 and 88. One control fawn was killed on PID 60 and the other two were killed on PID 76. Brain, bone marrow, eye, gastrointestinal tract, glands - adrenal, salivary and thyroid, kidney, liver, lung, lymph nodes, pancreas, spleen, urogenital tract and muscle samples from the biceps femoris, diaphragm, esophagus, heart, semi-membranosus and tongue were collected at necropsy and fixed in 10 percent buffered formalin. These tissues were processed routinely, sectioned and stained with hematoxylin and

[1] Xylazine - Rompum - Chemagro, Kansas City, Missouri
In addition, skeletal and cardiac muscle sections were stained by Giemsa, periodic acid Schiff, Brown-Hopps, and von Kossa methods.

RESULTS

Clinical Manifestations

Clinical signs of illness were first noted PID 18 and consisted of anorexia, pyrexia, weight loss, weakness, reluctance to rise, and slow, stiff movement. The onset of these clinical signs was sudden, will all fawns in all dosage groups affected simultaneously (Table 1). These clinical signs regressed in most of the fawns between PID 22 and 28; the animals again moved about freely and feed consumption returned to near normal. However, one fawn became severely debilitated, exhibited dyspnea and opisthotonos, and died on PID 27. Clinical signs returned suddenly in the other inoculated fawns on PID 29 and increased in both severity and intensity. Additional signs included increasing dyspraxia, recumbency and death of another seven fawns prior to PID 40. Two of the three remaining fawns slowly began to recover, while the condition of the third fawn continued to deteriorate and it died on PID 63. The condition of the two remaining infected fawns continued to improve until killed on PID 60 and 88. Uninfected control fawns retained normal vigor and continued to gain weight throughout the study.

Macroscopic Observations

The fawns which died or became moribund and were killed were debilitated and body fat was depleted. The sclera was congested and
icteric and the body cavities contained excessive yellow-tinged fluid. The blood was thin and poorly clotted. The skeletal muscles were pale and often yellow-tinged. Petechial and ecchymotic hemorrhages were dispersed throughout the gastro-intestinal tract, urinary bladder, adrenal glands and heart. In some cases, sub-pericardial and subendocardial hemorrhages were markedly suffuse. Visceral lymph nodes were enlarged and edematous. The spleen was generally enlarged and frequently had subcapsular hemorrhages. The kidneys were congested and friable. There was an excess of cerebrospinal fluid and the cerebral vessels were congested.

Six of nine infected animals had a fibrino-purulent pneumonia which contributed to death. Uninfected fawns were free of significant gross lesions.

Histopathology

Significant histopathology lesions associated with sarcocystosis were primarily found in skeletal muscles. The earliest lesions consisted of perivascular necrosis with mononuclear and neutrophilic cell infiltration. Necrosis of blood vessels and adjacent muscle bundles was observed where the response was most intense. These lesions were accompanied by edema, degeneration and focal necrosis of muscle. The necrotic muscle fibers usually exhibited liquefactive necrosis, multiple small granules of mineralization and were infiltrated by large mononuclear cells and occasionally neutrophils. Elsewhere, in the more severely affected muscle, lymphocytes were often dispersed among necrotic muscle fibers. In areas where the acute inflammatory response had subsided, macrophages
that contained developing schizonts were found between muscle fibers in the focal areas of necrosis or were surrounded by a large clear halo in areas where the necrotic debris had dissipated. Infrequently, macrophages that contained schizonts were found in blood vascular spaces.

The earliest schizont stages seen were located intracellularly in macrophages and consisted of a solid basophilic body. As development proceeded, greater intracytoplasmic space was occupied. Mature schizonts consisted of numerous zoites that often filled the entire cytoplasm, displacing the cell nucleus to the periphery of the macrophage. In other cells, the nucleus had disappeared and the zoites were surrounded only by a thin cytoplasmic membrane.

Subsequent rupture of the macrophages released the zoites.

Schizonts and zoites were observed in fawns which died prior to PID 60, while the typical muscle cysts were found in animals examined after PID 60. Tissue reaction was not characteristically found around muscle cysts.

Schizonts and muscle cysts stained blue by Giemsa and red by the Brown-Hopps stain. Schizonts and cysts were generally free of PAS positive material at PID 63, while cysts contained considerable PAS positive material by PID 88.

The lesions in cardiac muscle were similar but milder than those in skeletal muscle; the vascular reaction was less frequent and less intense. Macrophages containing schizonts were uncommon and very few myocardial cysts were found in fawns which lived longer than PID 60.
A few visceral lymph nodes in the infected fawns were very hyperplastic and consisted of a homogeneous mass of lymphocytes; follicles were often obscured. In the fawn examined at PID 88, necrosis of cerebral blood vessels and perivascular hemorrhage were noted.

The adrenal glands in many of the infected fawns contained focal cortical hemorrhages. Acute fibrino-purulent bronchopneumonia occurred in six of the infected fawns and three of these animals had splenitis and lymphadenitis. Portal triads in the livers of a few infected fawns were infiltrated with lymphocytes and less frequently neutrophils.

Lesions attributable to Sarcocystis or other infections were not observed in the three control fawns. One solitary cyst was found in the skeletal muscle of the tongue in an uninoculated control fawn.²

DISCUSSION

This study was designed to determine if S. hemionilatratris was pathogenic for mule deer. Consequently, early histopathology of the disease syndrome was unavailable. It is probable that one or two schizogonous generations preceded death of infected fawns. Perhaps if animals had been examined prior to PI day 27, organisms may have been found in other tissues.

The various stages of lesions described were found in all fawns examined. The earliest lesions noted in these animals was perivascular necrosis with mononuclear and neutrophilic cell in-
filtration in skeletal and cardiac muscles. This was accompanied by edema, degeneration and focal necrosis of muscles in the majority of the fawns. The muscle fibers were liquified, often contained deposits of calcium, and infiltrated by mononuclear cells and neutrophils. As the early histopathologic reaction subsided and the cellular infiltrate dissipated, a macrophage containing schizonts was recognized. A large space occurred around the macrophage leaving the appearance of a macrophage surrounded by a clear halo without inflammatory reaction. The schizonts were first identified in the cytoplasm of macrophages as a solid basophilic body which developed typically and after cytokinesis, mature schizonts contained zoites that filled the entire cytoplasm, displacing the nucleus to the periphery of the cell. Degeneration of the macrophage resulted in the appearance of zoites surrounded by a thin cytoplasmic membrane. Rupture of the membrane released zoites which infected muscle cells and developed into typical cysts in the absence of an inflammatory response.

In calves, schizonts of *S. fusiformis* have been found in the endothelial cells of capillaries, small vessels, glomeruli of the kidney, pulmonary blood vessels, brain, eye, liver and other tissues (3). In deer infected with *S. hemionilatrantis*, however, schizonts were not identified in endothelial cells, but were restricted to tissue spaces and rarely to perivascular spaces in skeletal and cardiac muscle. Furthermore, the schizonts were initially recognized within macrophages and developed within these cells.
Muscles examined in deer that were involved most frequently were those of the tongue and esophagus, less so the diaphragm and semimembranosus and the least affected was cardiac muscle. Calcification was infrequent, but occurred in areas of muscle necrosis. It would appear that if mineralization persisted, the lesions could be confused with those of white muscle disease.

A significant pathologic feature in this study was bilateral adrenal cortical hemorrhage that occurred in the infected fawns. Small hemorrhages in the adrenal cortex may be associated with acute infections as well as other diseases, while massive hemorrhages such as those found in the Waterhouse-Friderichsen syndrome of humans are generally associated with fulminating meningococcal septicemia (1). Similar lesions have been produced in animals by using toxins and toxic compounds (5). Adrenal hemorrhage in the fawns may have resulted from a toxin. In fawns which died from uncomplicated sarcocystosis, clinical signs and the relative paucity of gross lesions suggested release of a potent toxin upon schizont rupture. This premise is substantiated to some degree by necrosis of skeletal muscle and in perivascular areas. Efforts to demonstrate a toxin, however, were inconclusive.

Sarcocystis has not been considered a serious pathogen until recently when it was found that *S. fusiformis* can produce severe disease in calves and therefore result in economic losses to the cattle industry (4). This study established that *S. hemionilatrantis* is pathogenic for mule deer fawns under experimental conditions, but it is improbable that wild fawns would be exposed to sporocyst
dosages used in this study. Investigations are nevertheless warranted to determine if *S. hemionilatrantis* causes direct mortality or debilitates wild mule deer fawns, thereby predisposing them to other mortality factors.
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<tr>
<th>Day Post Infection</th>
<th>Dosage of Isocysts</th>
<th>Occurrence of Clinical Signs and Death in Fawns Fed <em>Sarcocystis merinillosa</em></th>
<th>Status of Isocysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>N</td>
<td>anorexia</td>
<td>anorexia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased water intake</td>
<td>Increased water intake</td>
</tr>
<tr>
<td>21-22</td>
<td>N</td>
<td>anorexia</td>
<td>Increased water intake</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased water intake</td>
<td>Increased water intake</td>
</tr>
<tr>
<td>24-26</td>
<td>N</td>
<td>Feed intake increased</td>
<td>Feed intake increased</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reluctant to rise at times</td>
<td>Reluctant to rise at times</td>
</tr>
<tr>
<td>27-35</td>
<td>N</td>
<td>Anorexia</td>
<td>Anorexia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reluctant to rise at times</td>
<td>Reluctant to rise at times</td>
</tr>
<tr>
<td></td>
<td></td>
<td>One died</td>
<td>One died</td>
</tr>
<tr>
<td>30-42</td>
<td>N</td>
<td>Anorexia</td>
<td>Anorexia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unable to rise</td>
<td>Unable to rise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Two died</td>
<td>Two died</td>
</tr>
<tr>
<td>41-49</td>
<td>N</td>
<td>Feed intake increased</td>
<td>Feed intake increased</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Movement slow</td>
<td>Movement slow</td>
</tr>
<tr>
<td>53-56</td>
<td>N</td>
<td>1 Fawn - down and</td>
<td>Much improved</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reluctant to rise</td>
<td>Reluctant to rise</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Fawn - returning to normal activity</td>
<td>1 Fawn - returning to normal activity</td>
</tr>
<tr>
<td>57-64</td>
<td>N</td>
<td>One died</td>
<td>One killed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Other - normal condition</td>
<td>Other - normal condition</td>
</tr>
<tr>
<td>65-71</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

n = 3 fawns per group; o = 4 fawns per group

N = None
REFERENCES


MACROSCOPIC AND CLINICAL MANIFESTATIONS OF
SARCOCYSTIS HEMIONILATRANTIS IN MULE DEER FAWNS[1][2]

G. Hudkins and T. P. Kistner, School of Veterinary Medicine,
Oregon State University, Corvallis, Oregon 97331, U.S.A.

Abstract: Eleven mule deer fawns (Odocoileus hemionus hemionus) inoculated orally with Sarcozystis hemionilatrantis sporocysts from the feces of coyotes (Canis latrans) developed clinical signs of anorexia, weight loss, pyrexia and weakness. All inoculated animals became severely debilitated, recumbent, and nine of eleven died. Uninfected control fawns remained healthy. Three infected fawns died from uncomplicated sarcocystosis, while the other six also had intercurrent bacterial pneumonia. The most severe clinicopathological changes occurred between 30 and 39 days after infection. At necropsy, all carcasses showed poor physical condition.

Pathognomonic gross lesions of sarcocystosis did not exist, but gross lesions in fawns consisted of pale musculature, icterus, hydrothorax, hydropericardium, ascites, lymphadenopathy and excessive cerebrospinal fluid with edema of the brain and congestion of cerebral blood vessels. Ecchymotic and petechial hemorrhages were evident throughout the gastrointestinal tract, urinary bladder, adrenals, and

[1] This study was supported by the Oregon Department of Fish and Wildlife and the federal Aid in Wildlife Restoration, Oregon Project W-70-R-5, Subproject F.

[2] Oregon Agricultural Experiment Station Technical Publication No.____.
heart. Kidneys and livers were enlarged and friable.

Recent research based on experimental infections of *Sarcocystis hemionilatrantis* in coyotes and mule deer fawns has demonstrated that the life cycle of this parasite can be completed with coyotes as the definitive host and mule deer as the intermediate host (Hudkins and Kistner 1977). This paper describes the clinical signs, gross pathologic lesions and some of the histopathologic lesions which developed during the course of the experimentally induced *Sarcocystis* infections in mule deer fawns.

**MATERIALS AND METHODS**

Mule deer fawns were captured when approximately two to seven days of age and reared in maximum isolation facilities at Oregon State University. When seven months old, 11 fawns were inoculated intraruminally with an aqueous suspension of *Sarcocystis hemionilatrantis* sporocysts (Hudkins and Kistner 1977). Inoculated fawns had been randomly divided into three groups of four, four and three fawns, which were inoculated with $5.0 \times 10^4$, $2.5 \times 10^5$ and $1.0 \times 10^6$ sporocysts, respectively. Three fawns were maintained as uninoculated control animals.

All fawns were observed at least twice daily post-inoculation (PI). The animals were weighed and blood samples were collected weekly from two weeks prior to inoculation and throughout the experiment. Fawns were forcibly restrained for the sample collection the two weeks prior to inoculation. Thereafter, fawns were sedated with
5-10 mg Xylazine\textsuperscript{a} and manually restrained to reduce trauma. Blood was taken from the jugular vein of each fawn. Approximately 10 ml was collected in vacuum tubes containing no anticoagulant, allowed to clot and the serum collected. An additional 3 to 5 ml was collected in a vacuum tube containing EDTA anticoagulant. A few drops of fresh blood were used to make blood smears. The serum, whole blood and smears from each animal were submitted to a commercial diagnostic laboratory\textsuperscript{b} for analysis.

Determinations were made for total white blood cell (WBC) counts, total red blood cell counts (RBC), differential WBC counts, mean corpuscular volume (MCV), hemoglobin (Hb) and packed red cell volume (PCV). Additionally, a profile scan of 18 serochemistry values was performed on each sample. Serochemistry values included alkaline phosphatase (Alk-P), cholesterol, total bilirubin, total protein (TP), albumin, globulin, albumin/globulin ratio (A/G), glutamic oxaloacetic transaminase (SGOT), lactic dehydrogenase (LDH), creatinine, glucose (Glu), blood urea nitrogen (BUN), uric acid, phosphorus, chlorides, sodium, potassium and total calcium.

Following death or euthanasia, animals were subjected to a detailed necropsy examination. Tissue samples for histopathologic studies were collected from all fawns (Hudkins and Kistner 1977, Koller et al. in press).

\textsuperscript{a}Xylazine-Rompun, Chemagro, Kansas City, MO 64120

\textsuperscript{b}ICN United Medical Laboratories, 6060 N.E. 112th, P.O. Box 3932, Portland, OR 97208
RESULTS

Clinicopathologic Manifestations - All fawns remained clinically normal for the first 17 days post-inoculation. On PID 18, signs of clinical illness appeared simultaneously in fawns inoculated at all dosage levels. Early signs consisted of anorexia, pyrexia (40-42 C) rapid weight loss and weakness. Fawns appeared reluctant to rise and moved stiffly and slowly when they did rise.

These clinical signs regressed between PID 22 and 28; the stiffness and soreness disappeared, most animals moved about freely and feed consumption returned to near normal. However, one fawn (inoculated with $1.0 \times 10^6$ sporocysts) became severely debilitated, experienced increasingly labored breathing, opisthotonos, and died on PID 27.

Clinical signs returned suddenly in the other inoculated fawns of PID 29 and increased in both severity and intensity, including increasing dyspraxia, recumbency and seven fawns died or were killed when moribund. Fawns died on PID 29, 36, 37, 38 and 39. Mortality rates for the dosage levels $5.0 \times 10^4$, $2.5 \times 10^5$ and $1.0 \times 10^6$ were 50%, 75% and 100%, respectively.

After PID 40, two of the three remaining inoculated fawns slowly regained normal vigor, while the condition of the third fawn (inoculated with $5.0 \times 10^4$ sporocysts) continued to deteriorate and this fawn died on PID 63. The two remaining infected fawns (inoculated with $2.5 \times 10^5$ and $5.0 \times 10^4$ sporocysts) continued to recover and were killed on PID 60 and 88, respectively. Final mortality rates
were 75%, 75% and 100% respectively.

Clinicopathologic signs were not noted in the uninfected fawns. They retained normal vigor and continued to gain weight throughout the study (Figure 1). One control fawn was necropsied on PID 60 and the other two on PID 76.

**Hematological Observations** - Control and infected fawns had comparable hematologic and serum enzyme levels for three weeks PI. WBC counts of infected fawns became elevated between the third and fourth week PI (Fig. 2), accompanied by an increase in neutrophils and a decrease in lymphocytes. There was a decrease in the RBC counts between three and four weeks PI and Hb levels frequently fell prior to death. Alk-P levels began to drop between the second and third weeks PI and progressively decreased until death (Figure 3). Fawns which recovered showed the same drop as those which died and no increase was seen prior to termination.

Significant changes were not seen in the hematological and serum enzyme values for the control fawns.

**Macroscopic Observations** - General body condition was poor in fawns which died or became moribund and were killed. This was evidenced by rough hair coats, generalized depletion of body fat reserves and wasting of musculature. Animals which died later in the study were noticeably more debilitated than those which died earlier.

Subcutaneous blood vessels were markedly congested at necropsy in the nine fawns which died or became moribund. Six fawns had pneumonic areas in one or both lungs; pneumonia and/or septicemia secondary to pneumonia contributed to death. In some fawns, pneumonia
was accompanied by bronchitis, tracheitis, and/or pharyngitis. A fibrino-purulent pneumonia was present in four fawns.

The remaining three fawns died of uncomplicated acute sarcosporidiosis. Pneumonia was absent, although one fawn had a mild tracheitis and bronchitis and another had subserosal petechial hemorrhages in the lungs. Lesions subsequently described are limited to those found in these three uncomplicated cases.

The sclera was congested and iceric; the pericardial, thoracic and peritoneal cavities contained excessive yellowish fluid. The blood was thin and poorly clotted, while skeletal muscles were pale and often yellowish. Gross lesions attributable to *Sarcocystis* consisted of scattered petechial and ecchymotic hemorrhages throughout the gastrointestinal tract, urinary bladder, adrenals and heart. In several cases, the subpericardial and subendocardial hemorrhages were markedly suffuse.

Visceral lymph nodes were edematous and most were enlarged three to four times normal size. Splenic corpuscles were increased in number and were very prominent within the enlarged spleens; petechial hemorrhages were frequent along the margins of the splenic capsule.

Both kidneys were typically enlarged, friable, congested and the cortex adhered to the renal capsule. Livers were also enlarged, friable and displayed a yellowish discoloration.

Congestion of cerebral vessels was marked; there was an excess of cerebrospinal fluid, as well as edema of the brain.

The fawn killed on PID 60 was found to be in poor condition with lesions similar to, but not as pronounced as those found in the
fawns which died earlier. Areas of atelectasis were present in the lungs, hemorrhagic inflammation was evident in the small intestine, generalized lymphadenopathy was noted and there was an excess of cerebrospinal fluid. The fawn killed on PID 88 was in good physical condition and gross lesions were not evident at necropsy.

Uninfected fawns were unremarkable at necropsy. General body condition was good and all organs and systems appeared normal.

**Microscopic Observations** - Histopathologic results were described in detail in a previous publication (Koller et al., In press). The earliest lesions seen consisted of perivascular necrosis in skeletal muscles with a mononuclear and neutrophilic cell infiltration accompanied by edema, degeneration and focal necrosis of skeletal muscle. Subsequently, this reaction subsided and the cellular infiltrate dissipated. An infected macrophage surrounded by a clear halo usually remained in the vacuolated muscle space. Developing *Sarcocystis* schizonts were identified in the cytoplasm of the macrophages and following schizogony, the cytoplasmic membrane ruptured releasing zoites. The zoites then infected muscle tissue and developed into typical muscle cysts.

**DISCUSSION**

*Sarcocystis* has not generally been considered as a pathogenic disease entity until recently. Johnson, Hildebrandt and Fayer (1975) found that this infection can cause severe pathologic changes in calves and postulated that *Sarcocystis* may be an unrecognized cause of economic losses in the livestock industry.
This study demonstrated that *S. hemionilatrantis* is indeed a pathogen of mule deer. Infected fawns became anorectic, lost body weight, developed fevers, became weak and nine of eleven of the infected fawns died or were killed when moribund. The fact that clinical signs had appeared simultaneously in all infected fawns on PID 18 and returned suddenly on PID 29, is considered relevant in terms of schizogony. We feel that synchronous schizont rupture occurred on these two days PI, with clinical signs resulting from release of a potent toxin from the ruptured schizonts. Efforts to demonstrate a toxin in this study were inconclusive. However, the relative paucity of gross lesions in the uncomplicated cases also suggested release of a potent toxin. Koller et al. stated this was substantiated to some degree by necrosis of skeletal musculature and in perivascular areas and adrenocortical hemorrhage.

Overall, most blood parameters of infected fawns did not exhibit significant changes or deviations from values for control fawns. Values were comparable in all fawns for three weeks PI. Changes detected in infected fawns after this time were of a non-specific nature. Between the third and fourth week PI, WBC counts were elevated, while there was a decrease in RBC counts. Hb levels frequently fell prior to death. It is felt that the animals were much more anemic than the data indicated, as hemoconcentration probably resulted from dehydration. The most notable change was the extreme drop in Alk-P levels. Serum alkaline phosphatase is a non-specific enzyme contained in many tissues of the body. The reason for this decrease in levels is not readily explainable, but perhaps could be
used as a diagnostic aid in the detection of acute sarcocystosis.

We recognize that wild fawns would in all probability not be subjected to the levels of infection administered to fawns in this experiment. It would therefore appear that direct mortality may not be a major decimating factor in wild populations, but that the associated debilitation may be of much greater significance. As demonstrated in this study, debilitation predisposed fawns to pneumonia which contributed to death. The prominent soreness that resulted in recumbency and reluctance to move are of extreme importance when considering the mule deer in its natural environment. It is difficult to extrapolate from an experimental situation to a field problem, but it would seem justifiable to consider that debilitation of similar magnitude or even of a lesser degree would render wild deer undergoing acute *S. hemionilatrantis* infections extremely vulnerable to natural mortality factors, such as predation, starvation and climatic extremes.

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Figure 1. Mean Weight Change per Week

*All animals dead
Figure 2. Weekly Mean White Blood Cell Counts

- All animals dead
- Animals not sedated

Weeks Post-Infection

Control
5.0 x 10^4
2.5 x 10^5
1.0 x 10^6

White Blood Cells thousand/cm
Figure 3. Weekly Mean Alkaline Phosphatase Values

- **All animals dead**
- **Animals not sedated**

- Control
- $5.0 \times 10^4$
- $2.5 \times 10^5$
- $1.0 \times 10^6$
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