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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 feces 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.

Preliminary histologic examinations conducted on selected tissues from all animals revealed microscopic-sized schizogonous stages 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 tissue 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 cyst was 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 *Sarcocystis 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.[6] Recent research has demonstrated a life cycle similar to that of other coccidial protozoans, but with alternation of the schizogonous and sporogonous stages between herbivores and carnivores, respectively. Specific carnivores fed tissues infected with *Sarcocystis* from sheep (*Ovis aries*), 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.[2,5,9,10] Sporocysts of *S. fusiformis* shed by laboratory-reared dogs (*Canis familiaris*) produced illness and mortality in experimentally infected calves. Various early schizogonous stages and, later, typical cysts were found in the

[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. 4274.

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musculature of the calves. The life cycle of *S. fusiformis* established that carnivores served as the definitive host and herbivores were the intermediate host.[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 *Sarcocystis* cysts in the musculature of fawns collected in the spring of 1974. Further field studies revealed a significant increase in infection levels of *Sarcocystis* which also coincided with the autumnal/winter periods of major fawn mortality. This prompted initiation of experimental studies to investigate cervine *Sarcocystis*.

A preliminary publication reported sporocyst production in coyotes (*Canis latrans*) fed infected mule deer meat.[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 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 *Sarcocystis*. 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 h following the initial feeding.

Starting one day after infected meat was first eaten, daily fecal flotations were prepared with Sheather's sugar solution[7] 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 h 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 h. The sporocyst suspension remaining was finally decanted to a concentration of 1.0×10^4 sporocysts/ml for use as inoculum.

Herbivore Study

Two calves, four lambs, and 14 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 2 to 7 days old from a site in Eastern Oregon (42° 25'N, 120°

[3] W. S. Tyler, Incorporated, Screening Division, Mentor, Ohio 44060, USA.

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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 accordingly into two isolation units.

Seven of the 21 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 x 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 x 10^4, 2.5 x 10^5, and 1.0 x 10^6 sporocysts. Four fawns inoculated with 5.0 x 10^4 sporocysts and two of four inoculated with 2.5 x 10^5 sporocysts were housed together in one isolation unit. The last unit contained the three fawns inoculated with 1.0 x 10^6 sporocysts and the remaining two fawns inoculated with 2.5 x 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, longissmus dorsi, intercostal, masseter and triceps. Tissues were placed in Tissue-Tek II[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 histopathologic findings. Similarly, clinical signs, hematologic 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.

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 x 9.3 μm (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

[4] Lab-Tek Products, Division Miles Laboratories, Inc., Naperville, Illinois 60540, USA.

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63. Mortality rates for the dosage levels 1.0 x 10^6, 2.5 x 10^5 and 5.0 x 10^4 were 100%, 75% and 75%, respectively. The remaining fawn inoculated with 2.5 x 10^5 sporocysts was killed on PID 60 while that inoculated with 5.0 x 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.

Histologic examinations were conducted on esophagus, heart, biceps femoris, semimembranosus, 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 experimental 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.[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 *Sarcocystis* sporocysts through accidental contamination of feed materials or workers' clothing. Transplacental transmission seems improbable since in our and another study,[1] numbers of *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 *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 *Sarcocystis* infections in this group of fawns. The findings of this study demonstrated that the life cycle of at least one microscopic cervine *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]

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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 *Sarcocystis 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.

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