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SARCOCYSTIS OF DEER IN SOUTH DAKOTA[1]

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Abstract: The prevalence of *Sarcocystis* in white-tailed deer *(Odocoileus virginianus)* and mule deer *(O. hemionus)* in South Dakota was determined through microscopic examination of tongue samples. The percentage of *Sarcocystis* infection for both species of deer was determined for prairies east of the Missouri River, west of the Missouri River, and the Black Hills of western South Dakota. Sixteen percent (N=62) of the white-tailed deer tongues from East River, 69% (N=42) from West River, and 74% (N=23) from the Black Hills were infected. Prevalence for mule deer was 88% (N=24), 78% (N=63), and 75% (N=12) from East River, West River, and the Black Hills, respectively. Of 50 tongue samples obtained from both species of deer during a special antlerless deer hunt in the Black Hills in 1978, 66% were infected. Coyotes *(Canis latrans)*, dogs *(Canis familiaris)*, red foxes *(Vulpes vulpes)*, a gray fox *(Urocyon cinereoargenteus)*, bobcat *(Felis rufus)*, and raccoon *(Procyon lotor)* were fed muscle from white-tailed deer and mule deer naturally infected with *Sarcocystis* to determine their role as definitive hosts. All coyotes, dogs, and the gray fox shed sporocysts, while none were recovered from the other animals. Sporocysts shed by coyotes were counted and concentrated into an inoculum and administered to a white-tailed deer fawn, which was necropsied 85 days after inoculation. Sections of heart, tongue, esophagus, diaphragm, and skeletal muscle were found to be heavily infected with sarcocysts, while sarcocysts were not detected in a control fawn.

INTRODUCTION

Sarcocystis has a two-host life cycle in which herbivores and carnivores serve as intermediate and definitive hosts, respectively (Dubey et al., 1978; Dubey and Streitel, 1976; Fayer and Johnson, 1975; Fayer et al., 1976; Fayer and Kradel, 1977; Hudkins and Kistner, 1977). Both white-tailed deer *(Odocoileus virginianus)* and mule deer *(O. hemionus)* have been shown to be intermediate hosts of *Sarcocystis* (Mahrt and Colwell, 1980; Pond and Speer, 1979). A preliminary survey for *Sarcocystis* in deer from the Black Hills region of South Dakota in 1976 showed that 93.5% (N=77) of the deer were infected (Hugghins, unpublished, 1977). The present study was undertaken to determine the prevalence of *Sarcocystis* in deer throughout South Dakota and the role of coyotes *(Canis latrans)* and other definitive hosts in the life cycle of *Sarcocystis*.

MATERIALS AND METHODS

Tongues and incisors from hunter and road-killed deer were collected throughout the state during 1977-78. The deer were aged according to the number of annuli present in the incisors. The tongues were frozen and later examined for intramuscular cysts by two methods: (1) A sample from each tongue was removed and sectioned in a cryostat at 5 μm. The tongue sections were then mounted on slides, dehydrated in

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acetone, and refrozen until stained with hematoxylin and eosin. (2) Pieces of tongue were fixed in formalin-acetic acid-alcohol (FAA), dehydrated, cleared, and infiltrated in an Autotechnicon. These samples were then embedded in paraffin, sectioned with a microtome at 12μm, and mounted on slides for staining with hematoxylin and eosin.

The percentage of *Sarcocystis* infection was determined for both species of deer from prairies east of the Missouri River, west of the Missouri River, and the Black Hills (Figs. 1 and 2). A chi-square test was used to determine any significant difference in the frequency of infection from the three regions, along with differences in age and sex.

The carnivores used as experimental definitive hosts (Table 1) were obtained as follows. Five coyote pups and a red fox pup were recovered from dens in Gregory County, South Dakota; by the time they were fed infected deer meat, they were approximately 6 months old. The five dog pups were litter mates born in the dog pens at the University; they were about 5 months old at the time of the feeding experiments. The grey fox was an old fox which had been raised in the Great Plains Zoo at Sioux Falls; he was at least 10 years old. The raccoon was taken from his wild mother in Gregory County and raised on a bottle; he was approximately the same age as the coyotes. The bobcat was borrowed from a zoo in Watertown, South Dakota; he had formerly been a household pet and was about 5 years old. All of the above animals were housed in individual pens with concrete floors and were fed dry-pelleted dog food. When fecal examinations performed each day for a week revealed no sporocysts, the potential definitive hosts were presumed to be *Sarcocystis*-free. Three adult dogs and a pup, plus an adult coyote and a pup were maintained as controls. White-tailed deer fawns which were raised in captivity at South Dakota State University were housed in one outdoor fenced enclosure.

Infected venison for experimental life cycle studies was obtained from road-

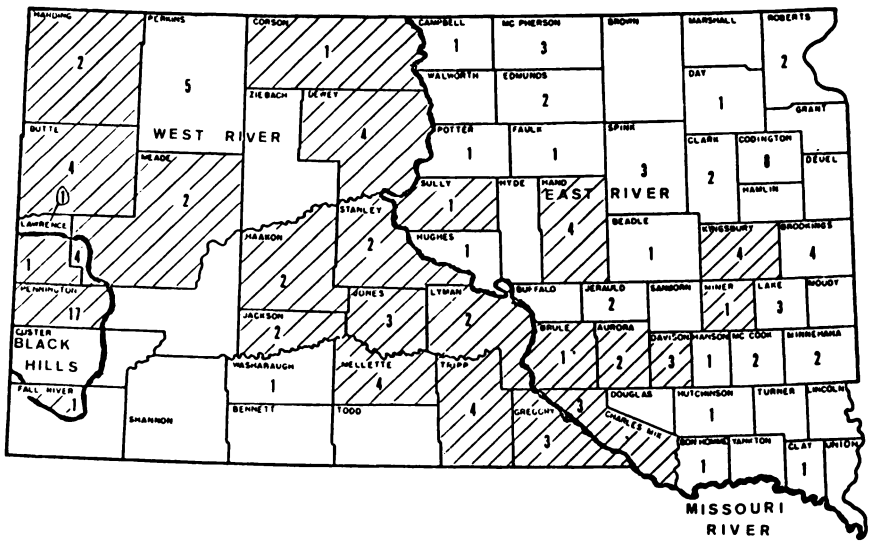


FIGURE 1. Distribution of white-tailed deer tongue samples in South Dakota, 1977-78. Diagonal hash indicates counties containing infected deer.

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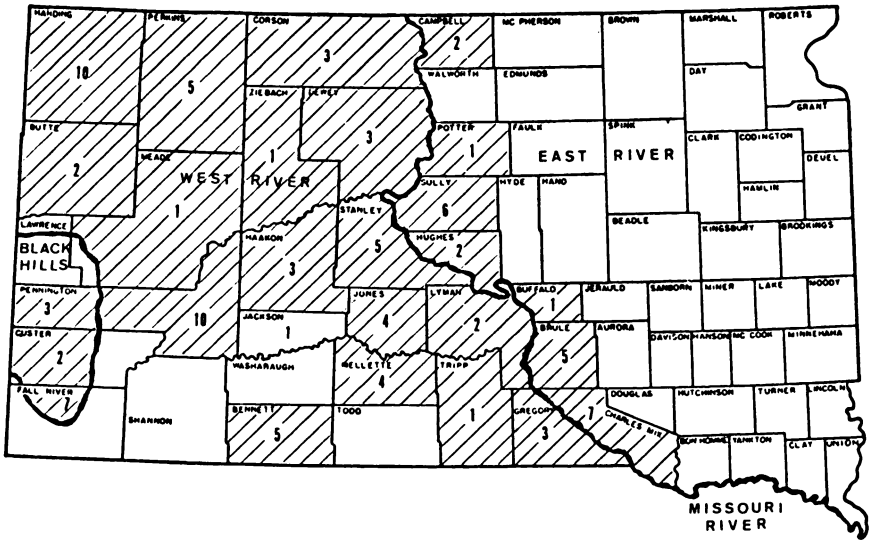


FIGURE 2. Distribution of mule deer tongue samples in South Dakota, 1977-78. Diagonal hash indicates counties containing infected deer.

TABLE 1. The prepatent and patent periods of experimental carnivores fed *Sarcocystis*-infected muscle from white-tailed deer (WT) and mule deer (MD).

Carnivore	Pre-patent (days) WT	Pre-patent (days) MD	Patent (days) WT	Patent (days) MD
Coyote 1	8	-	57[a]	-
Coyote 2	8	-	57[a]	-
Coyote 3	-	8	-	62[a]
Coyote 4	-	7	-	63[a]
Coyote 5	-	8	-	38
Dog 1	9	-	56[a]	-
Dog 2	8	-	57[a]	-
Dog 3	11	-	29[a]	-
Dog 4	-	8	-	62[a]
Dog 5	-	7	-	63[a]
Red fox	-	-	-	-
Gray fox	10	8	50	52[a]
Raccoon	-	-	-	-
Bobcat	-	-	-	-

[a]Fecal collection stopped, but sporocysts still present.

killed white-tailed and mule deer from the Black Hills. The deer skeletal muscle was trimmed of fat and connective tissue and ground in a household-type meat grinder. In order to determine the level of infection, 10 g of ground muscle was placed in a 125 ml flask and combined with 100 ml of pepsin-HCl digestion fluid. The digestion fluid was composed of 2.5 g pepsin, 10 ml HCl, and sufficient 87%

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saline to make one liter. The mixture was placed in an incubator at 36 C for one h and stirred periodically. The fluid was then strained through two layers of cheesecloth and the remaining tissue was squeezed to remove excess fluid. Fifteen ml of the filtrate was centrifuged for 10 min., the supernatent discarded, and the sediment resuspended in 2-5 ml of 87% saline. An aliquot was removed with a pipet, placed in a hemocytometer, and the bradyzoites counted. An approximation of the number of bradyzoites/g of tissue was then calculated from the number of bradyzoites/ml of digestion fluid.

Skeletal muscle that was parasitized with *Sarcocystis* was portioned into 454 g packages, and one package was fed to each prospective final host. Fecal samples were collected daily following the initial feeding of infected meat to determine the prepatent and patent periods. The fecal samples were analyzed for sporocysts by a quantitative sugar flotation technique as described by Fayer (1977).

Feces containing numerous sporocysts were mixed with water and strained several times through metal mesh sieves (series from 1.68 mm to 149 μm mesh). The fecal-water suspension was then placed in flasks and stored at 40 C. Most of the water was decanted and the sediment containing sporocysts was resuspended in the remaining supernatant. The approximate number of sporocysts/ml of feces-water was determined by removing 1 ml of the mixture from the flask and placing it in a centrifuge tube which was then filled with Sheather's sugar solution and capped with a coverslip. After centrifugation, the coverslip was then transferred to a slide in order to count the sporocysts under a microscope. Once the approximate number of sporocysts/ml had been determined from a given flask, the total number of sporocysts for a dose from that flask could be calculated for administration via oral syringe to deer fawns.

RESULTS

Prevalence. A total of 276 white-tailed and mule deer tongues were examined for sarcocysts. The distribution of tongue samples (excluding samples obtained from a special antlerless deer hunt in 1978 from the Black Hills) is illustrated in Figs. 1 and 2. The percentage of infection for both species of deer is shown in Table 2. Of a total of 50 tongue samples from both species of deer obtained from a special Black Hills antlerless deer hunt in 1978, 66% were infected with *Sarcocystis;* analysis of each species showed white-tailed deer 58% (N=36) and mule deer 86% positive (N=14).

The chi-square test revealed a significant difference (28.39) of infection between the two species in comparing all three regions of the state (ldf, .01). The

TABLE 2. The percentage of *Sarcocystis* infection in white-tailed deer (WT) and mule deer (MD) from the East River, West River, and Black hills regions of South Dakota, 1977-78.

	East River		West River		Black Hills[a]	
	% pos.	% neg.	% pos.	% neg.	% pos.	% neg.
WT	16	84	69	31	74	26
MD	88[b]	12	78	22	75	25

[a]Excluding 1978 antlerless season.

[b]These mule deer data were from counties bordering the Missouri River on the east side.

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frequency of infection between mule deer from all three regions was not significant (2df, .01). However, the frequency of infection for white-tailed deer was highly significant (38.57) between East River and the West River-Black Hills areas (2df, .01), while no significant difference was present between the Black Hills and West River prairie regions. No significant difference in the frequency of infection was noted between the sexes of both species (5df, .01), from all three regions in 1977-78.

Age data from white-tailed deer were pooled (young, ½-2½ years, vs. old, 3½-7½ years) for analysis. A significant difference (8.708) was noted in young vs. old white-tailed deer from the East River prairie (ldf, .01), but not in the other regions. Analysis of Black Hills deer from 1978 showed no significant difference of infection according to age (ldf, .01).

Life Cycle Studies. After feeding infected deer meat to experimental canids as described above, daily examination of fecal samples for sporocysts revealed prepatent and minimum patent periods as shown in Table 1. The definitive hosts exhibited peak periods of shedding sporocysts. Fayer (1977) reported similar results. The coyotes and the gray fox showed a fairly constant rise and fall in numbers of sporocysts shed at two-day intervals, as shown in Fig. 3 for two of the coyotes. The dogs' fluctuations in production of sporocysts were less precise, but in late patency settled into a crude 4-day cycle. This appears to be the first mention of this type of cyclic variation, except for sporocysts being shed intermittently (Fayer, 1977; Fayer and Johnson, 1975; Hudkins and Kistner, 1977). By day 40, all of the experimental canids had slowed down considerably in passing sporocysts.

Sporocysts from feces of coyotes fed white-tailed deer meat were concentrated into an inoculum, and approximately 75,000 sporocysts were fed to a 6-mo old white-tailed deer fawn. No clinical signs were observed. Following necropsy on postinoculation day (PID) 85, histological sections of heart, tongue, esophagus, diaphragm, and skeletal muscle were found to be heavily infected with sarcocysts, while a control fawn's tissues were negative for sarcocysts.

DISCUSSION

The prevalence of *Sarcocystis* was significantly higher in the western half of South Dakota than in the East River prairie region. The Black Hills and West River prairies support considerably more coyotes than the eastern region of the state. It is difficult to ignore the apparent positive correlation between prevalence of *Sarcocystis* in deer and abundance of coyotes. Our life cycle studies have indicated that dogs, coyotes, and the gray fox can serve as definitive hosts of *Sarcocystis* in white-tailed and mule deer, with the coyote being implicated as the most likely wild host in South Dakota. However, it should not be overlooked that the domestic dog is reported to be an important predator of deer in South Dakota (Richardson and Peterson, 1974), and this could be an epidemiological factor. The East River region does support red fox populations. Even though the experimental red foxes did not pass sporocysts in the present study, more trials need to be run since only two animals were used, and the red fox has been implicated as a host for *Sarcocystis* spp. elsewhere (Ashford, 1977; Rommel et al., 1974).

The identity of the species of *Sarcocystis* found in this study is difficult to determine. For the species they named *S. hemionilatrantis,* Hudkins and Kistner (1977) reported an average sporocyst size of (9.3μm × 14.4μm) recovered from coyotes fed mule deer meat in Oregon. These figures are similar to our sporocyst measurements of (10.4μm × 14.9μm, N=40) and (10.4μm × 15.8μm,

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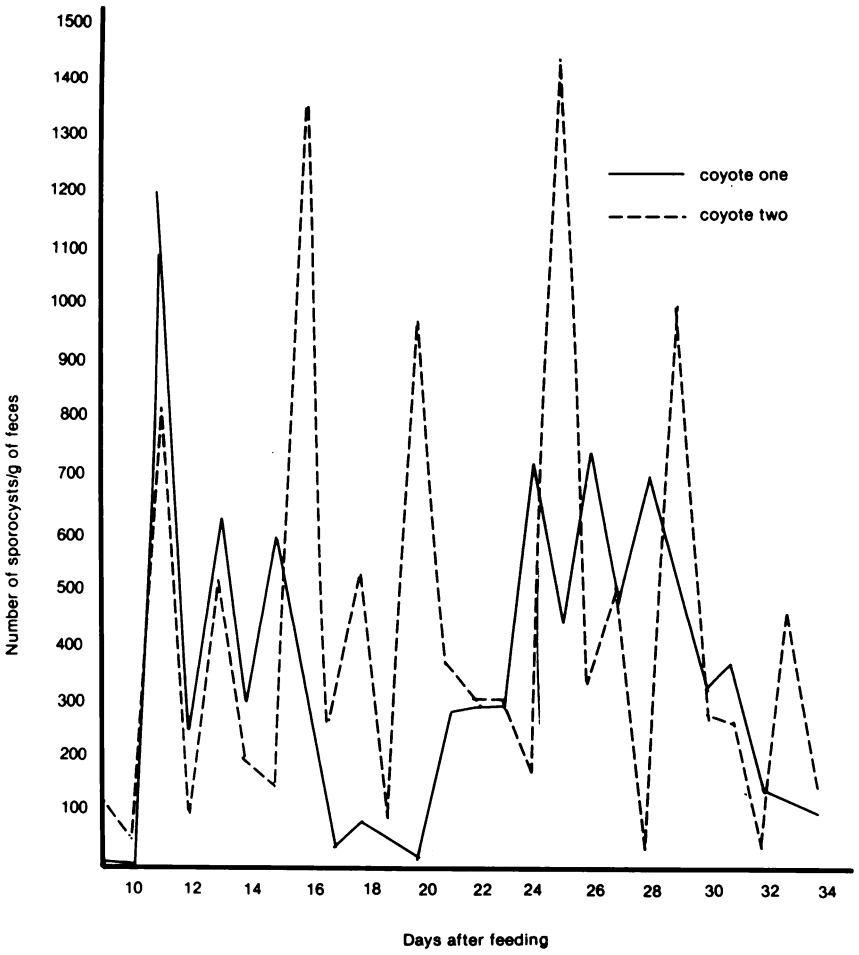


FIGURE 3. Number and pattern of sporocyst shedding by two coyotes fed muscle of white-tailed deer infected with *Sarcocystis*.

N=30) from white-tailed deer and mule deer origins, respectively. A t-test indicated no significant difference in size of sporocysts from our different hosts. The intramuscular sarcocysts from our two species of deer appear to be the same in histological structure. Moreover, they closely resemble sarcocysts we have recovered from other ungulates in western South Dakota.

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