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Sarcocystis leporum IN COTTONTAIL RABBITS AND ITS TRANSMISSION TO CARNIVORES

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Abstract: Muscle from *Sarcocystis*-infected cottontail rabbits (*Sylvilagus floridanus*) was fed to coccidia-free cats (*Felis domestica*) and dogs (*Canis familiaris*). Only cats became infected and shed sporocysts in their feces. The prepatent period ranged from 10 to 25 days and the patent period from 3 to 46 days. Sporocysts were fully sporulated when shed. They contained 4 sporozoites and a coarse granular residuum and averaged 9.4 by 13.6 μm (N=55). Doses of 200-75,000 sporocysts were orally administered to 5 domestic rabbits (*Oryctolagus cuniculus*). Domestic rabbits did not become infected, suggesting a strict host specificity for the intermediate host *S. floridanus*.

INTRODUCTION

Sarcocystis is commonly found in the cardiac and skeletal muscles of many vertebrates. The life cycle recently has been elucidated for some species of *Sarcocystis* in livestock, wildlife and rodents.[3,4,8,9,10] In general, after infected muscle is ingested by a suitable carnivore, zoites are liberated from the intramuscular cysts and enter cells in the carnivore's intestine. Here they develop into gametes and then oocysts. Oocysts sporulate *in situ*. Fully-developed isosporan sporocysts containing 4 sporozoites are shed in the carnivore's feces. Sporocysts ingested by the appropriate intermediate host initiate an asexual cycle leading to the development of intramuscular cysts.

The life cycle of *Sarcocystis* in cottontail rabbits (*Sylvilagus floridanus*) is unknown. The scant literature on *Sarcocystis* in rabbits, reviewed in great part by Vande Vusse,[11] has dealt with either the morphology of the cyst stage or the prevalence in rabbits based on finding the cyst stage. Although both microscopic and macroscopic cysts have been found in cottontail musculature, Crawley[1] states that cyst size is a function of age and is of no diagnostic value with regard to speciation.

The present study was undertaken to determine (1) the role of carnivores in the life cycle of *Sarcocystis leporum* Crawley, 1914 of cottontail rabbits and (2) the feasibility of establishing a laboratory model with the domestic rabbit (*Oryctolagus cuniculus*) fed sporocysts from a cottontail-carnivore cycle.

MATERIALS AND METHODS

Eighteen cottontail rabbit carcasses acquired at the Veterinary Clinic of the Pennsylvania State University were refrigerated and shipped in insulated containers to the Animal Parasitology Institute (API) at various intervals. Fifteen were used in the following experiments.

Upon arrival at the API, musculature was trimmed from the carcass. Representative pieces of muscle were frozen and sectioned with a cryostat, and other pieces were fixed in 10% neutral buffered formalin and prepared for histologic examination. The remaining muscle

was minced into small pieces and fed to either cats (*Felis domestica*) or dogs (*Canis familiaris*), or both, to determine the infectivity for the carnivore host (Table 1).

Eighteen cats and eight dogs were used in four experiments (Table 1). Each was housed in a separate cage to facilitate fecal collection. All cats and dogs were coccidia-free at the beginning of each experiment. All were fed dry pelleted feed except at the time rabbit muscle was fed. Total feces were collected daily from each animal beginning on or before the day of feeding rabbit muscle and ending at the termination of the experiment.

Feces found to contain *Sarcocystis* sporocysts were refrigerated until many fecal specimens had accumulated. Sporocysts were then cleaned of fecal debris as previously described,[7] resuspended in tap water, and refrigerated until used as inoculum.

Eight 3 to 6-month-old domestic rabbits were used in two experiments. In one experiment, two rabbits were orally inoculated with either 200 or 1000 sporocysts; two others served as uninoculated controls. All were examined daily for signs of disease until they were killed 34 days later. At this time, a thorough postmortem examination was made, and tissues from nearly all organs were fixed in neutral buffered formalin and prepared for histologic examination. In the second experiment, three rabbits were orally inoculated with 25,000, 50,000, or

TABLE 1. **Sarcocystis**-infected cottontail rabbit muscle fed to cats and dogs.

Exp. No.	Carnivore fed cottontail muscle	Carnivore identification No.	Rabbit carcass No.	Sporocysts shed by carnivore (day after carcass fed)	
				Prepatent period	Patent period
1	*Felis domestica*	1	A	20	3
		2	B	15	24
		3-12	None	—	—
	Canis familiaris	1	A	—	—
		2	B	—	—
		3- 6	None	—	—
2	*F. domestica*	1	C,D,E	—	—
		2	F,G	12	5
		3-12	None	—	—
3	*F. domestica*	13	H	10	46
		14	I	—	—
		15	J	11	44
		16	K	12	46
		17,18	None	—	—
4	*F. domestica*	14	L,M,N,O	25	15
		17,18	None	—	—
	C. familiarls	7,8	L,M,N,O	—	—

75,000 sporocysts and examined daily until they were killed 86, 50, or 30 days later, respectively; one rabbit served as an uninoculated control and was killed on day 86. Tissues were taken as in the previous experiment.

RESULTS

Microscopic examination of skeletal muscle from 18 cottontail rabbits submitted to the Veterinary Diagnositc Laboratory revealed that 15 were infected with *Sarcocystis*. Carcasses A-K contained only microscopic cysts. Carcasses L-O contained both macroscopic and microscopic cysts (Table 1). A typical microscopic cyst is shown in Figure 1.

Three cats fed *Sarcocystis*-infected muscle from cottontail rabbits became infected and passed *Sarcocystis* sporocysts in their feces (Exp. 1, two cats; Exp. 4, one cat; Table 1). In contrast, none of four dogs fed parts of the same infected muscle nor any of four unfed dogs and 10 unfed cats passed sporocysts in their feces.

Cats that had passed sporocysts in Experiment 1 were again fed *Sarcocystis*-infected cottontail rabbit muscle and one became reinfected and again passed sporocysts, (Exp. 2, Table 1). None of the unfed cats passed sporocysts.

Three of four additional cats fed *Sarcocystis*-infected cottontail rabbit muscle passed sporocysts, but none of the unfed cats passed sporocysts (Exp. 3, Table 1).

Within the 4 experiments, prepatent periods ranged from 10 to 25 days after feeding. Patent periods ranged from 3 to 46 days. Sporocysts most often were seen as individuals in fecal flotations (Fig. 2) although occasional pairs were observed. Sporocysts were ellipsoid. Each contained four sporozoites and a compact granular residuum. No Stiedae body was present. Fifty-five sporocysts averaged 9.4 by 13.6 μm, with a range of 9.3-11.1 μm by 13.0-16.7 μm.

None of the 5 experimentally inoculated rabbits nor the 3 uninoculated controls showed any signs of illness during the 2 experiments. No lesions were observed at postmortem, nor were any parasites found in histological sections.

DISCUSSION

This is the first report of transmission of *S. leporum* from its intermediate host, the cottontail rabbit, to a definitive host, the domestic cat. In the present study, *F. domestica* was the only felid tested as a possible definitive host. When we consider the recent finding that dogs and

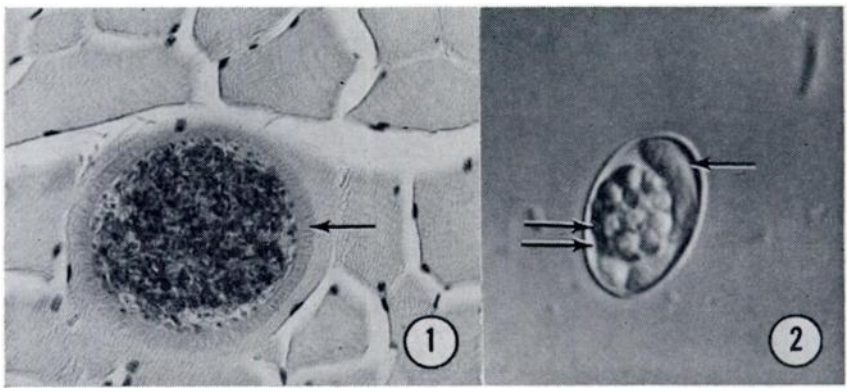

FIGURE 1. Hematoxylin-eosin stained section of cottontail rabbit muscle containing a large cyst.

FIGURE 2. Sporocyst in feces of a cat fed **Sarcocystis leporum**-infected cottontail rabbit muscle.

coyotes serve as final host for a species of *Sarcocystis* in the bovine and that foxes and raccoons also may serve as final host for the same species,[5] we can reasonably speculate that other felids also may serve as the final host for *S. leporum*.

Failure to infect two cats (Exps. 2 and 3) may have been caused by deterioration or death of the parasite. Rabbit carcasses shipped from Pennsylvania to Maryland may have been dead as long as a week before they were fed.

Reinfection of a cat that had stopped shedding sporocysts from a previous infection parallels findings in previous studies.[2] The carnivore final host does not appear to develop immunity to the parasite.[2]

The inability to infect domestic rabbits with *S. leporum* sporocysts from cats may reflect a specificity that parasites of this genus have for the intermediate host. Sporocysts of *S. fusiformis* from dogs fed infected beef were not infectious for sheep nor were *S. tenella* sporocysts from dogs fed infected lamb infectious for calves.[6] Thus, the domestic rabbit apparently will not provide us with a suitable laboratory model with which to study *S. leporum*.

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