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Authors: CRUM, JAMES M., and PRESTWOOD, ANNIE K.

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PREVALENCE AND DISTRIBUTION OF *SARCOCYSTIS* SPP. AMONG WHITE-TAILED DEER OF THE SOUTHEASTERN UNITED STATES[1]

JAMES M. CRUM and ANNIE K. PRESTWOOD, Southeastern Cooperative Wildlife Disease Study, Department of Parasitology, College of Veterinary Medicine, The University of Georgia, Athens, Georgia 30602, USA.

Abstract: Sarcocysts were found by light microscopic examination of muscle in 199 (51%) of 390 white-tailed deer *(Odocoileus virginianus)* from the southeastern United States. *Sarcocystis* infections were detected more frequently in histologic sections of tongue (45%) than of heart (9%). Sarcocysts were significantly more prevalent in adult deer (54%) than fawns (26%) ($P<.01$). Statistically significant differences in prevalence were not found in deer from different physiographic provinces or between sexes. Artificial digestion was more sensitive in detecting *Sarcocystis* infections than examination of histologic sections when both techniques were used to examine tongues of 35 deer. Three different size sporocysts, possibly representing at least two species of *Sarcocystis*, were recovered during feeding trials. Seven dogs *(Canis familiaris)* shed sporocysts 9 to 12 days after eating infected venison. Sporocysts measured 13.4-16.8 × 9.0-12.3μm with an average measurement of 15.2 × 10.9μm (N=195). One of three cats *(Felis catus)* and one of two red foxes *(Vulpes vulpes)* first shed sporocysts of *Sarcocystis* 10 days after eating infected venison. Sporocysts from the cat measured 11.2-13.4×6.72-8.96μm (avg 12.0×8.7μm, N=18), and those from the fox measured 11.2-15.7 × 9.0-11.2μm (avg 13.6 × 10.2μm, N=7).

INTRODUCTION

Sarcocystis spp. are commonly encountered protozoan parasites of wild animals, but the epizootiology of these coccidians is poorly understood. Although the life cycle for a *Sarcocystis* species infecting white-tailed deer *(Odocoileus virginianus)* and the dog *(Canis familiaris)* was recently reported (Crum et al., 1981), knowledge of the prevalence and distribution of *Sarcocystis* in deer from the eastern United States is limited (Prestwood et al., 1976).

Several surveys for *Sarcocystis* infecting white-tailed deer have been conducted in the western and northern portions of the white-tailed deer's range (Karstad and Trainer, 1969; Mahrt and Colwell, 1980; Pond and Speer, 1979). These studies revealed a high prevalence of infection and marked variation in the size of intramuscular cysts.

Information is presented herein on the prevalence and distribution of *Sarcocystis* spp. in white-tailed deer of the southeastern United States.

MATERIALS AND METHODS

Pieces of tongue and heart, and in some cases, portions of diaphragm, esophagus and skeletal muscles were taken from 390 white-tailed deer between 1969 and 1980. One to 20 deer per herd (Table 1) were obtained from 60 counties and 6 parishes of 15 states (Fig. 1). Tissues were fixed in 10% neutral buffered formalin, embedded

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TABLE 1. Histologic examination of white-tailed deer from the southeastern United States for *Sarcocystis* spp.

No. of deer examined from herd (sample size)	No. of herds infected/ no. of herds examined	Total no. of deer infected/ total no. of deer examined
1	2/4	2/4
2	3/4	5/8
3	2/3	3/9
5	46/51	152/255
6	1/1	6/6
7	1/1	2/7
9	1/1	2/9
10	6/6	18/60
12	1/1	6/12
20	1/1	3/20
Total	64/73	199/390

in paraffin, cut at 7 μm and stained with hemotoxylin and eosin. Approximately one square centimeter sections of tissues were examined by light microscopy for intramuscular cysts. Because this survey was a retrospective examination of histologic sections collected over an 11-year period, the anatomical location of the muscle sample varied. To eliminate sampling error, data also were analyzed by comparing only those animals with similar tissue sections (viz., tongue and heart). Analysis of variance was performed using an arcsine transformation of the data.

During 1978 and 1979, samples of diaphragm, esophagus, heart and tongue were collected from five deer in each of seven herds and placed on wet ice. Venison was determined to be infected by finding cysts in frozen sections that were stained with hemotoxylin and eosin. Venison from infected deer (N=30) of each collection was pooled, ground in a food mill and fed daily for 3 to 5 consecutive days to carnivores (Table 2). Mixed breed dogs, cats *(Felis catus)* and red fox *(Vulpes vulpes)* 4 to 5 mo old when fed venison were housed in individual cages and were maintained on a diet of dry dog food or canned pet food. The dogs and cats were born at The University of Georgia Laboratory Animal Care Facility while the red foxes were approximately 6 wk old when placed in the facility. Feces were collected daily and examined by light microscopy after a centrifugal fecal flotation using Sheather's sugar solution. Neither sporocysts nor sporulated oocysts had been found in seven to 38 preinfection fecal samples collected from the carnivores.

In 1979 and 1980, tongues collected from 35 deer were artificially digested and the results compared with those obtained by histologic section. Ten g of coarsely ground tongue were placed in 100ml of digestive fluid (pepsin 0.75% w/v, NaCl 0.86% w/v, HCl 1% v/v) and stirred with a magnetic stirrer for 30 min at 37C. The digest was filtered through gauze and the filtrate centrifuged at 1500 rpm for 10 min. A drop of the pellet was examined microscopically for zoites, and smears were prepared, air dried, fixed in 100% methanol stained with Giemsa and stained zoites measured with a calibrated ocular micrometer.

RESULTS

Cysts were found in 199 of 390 (51%) deer examined by light microscopy. *Sarcocystis* infections were detected in 64 of 73 (Table 1) deer herds examined from all physiographic provinces of the southeastern United States (Fig. 1).

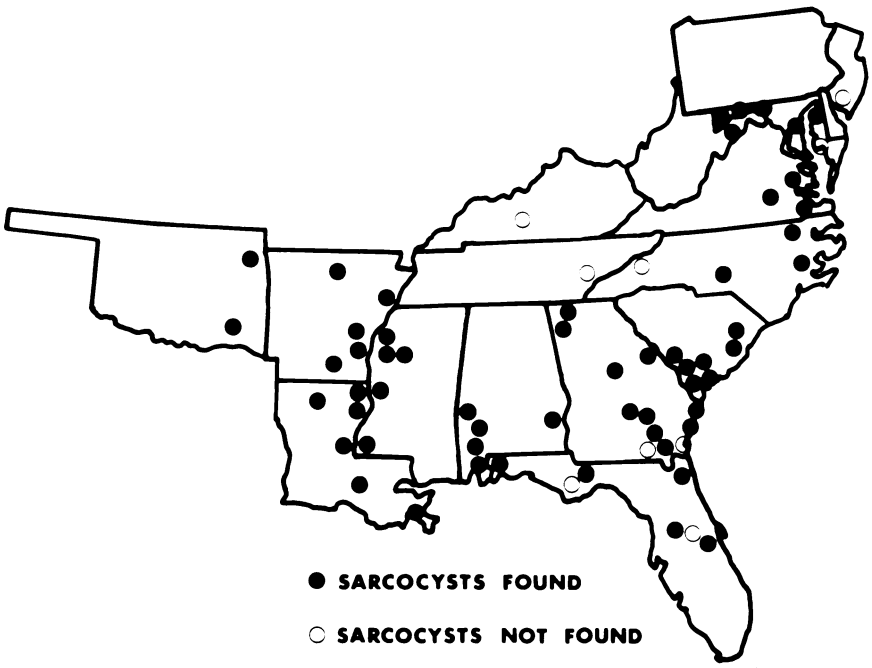

FIGURE 1. Collection sites and distribution of *Sarcocystis* in white-tailed deer of southeastern United States. (STATE, counties or parishes, number deer examined — ALABAMA: Baldwin, 15; Barbour, 1; Clarke, 10; Marengo, 5; Sumter, 5 — ARKANSAS: Arkansas, 5; Bradley, 5; Crittenden, 5; Desha, 5; Stone, 10 — FLORIDA: Brevard, 5; Duval, 9; Escambia, 5; Gadsden, 5; Lake, 10; Liberty, 5; Orange, 1 — GEORGIA: Camden, 5; Charlton, 5; Chatham, 10; Clinch, 5; Floyd, 5; Jeff Davis, 10; McIntosh, 25; Jones, 5; Richmond, 5; Telfair, 5; Ware, 15; White, 5 — KENTUCKY: Edmonson, 3 — LOUISIANA: Concordia, 5; East Carroll, 5; Iberville, 2; Lincoln, 5; Madison, 3; Plaguemines, 5 — MARYLAND: Allegany, 2; Dorchester, 5; Garrett, 2; Kent, 5; Prince George, 12; Washington, 6 — MISSISSIPPI: Coahoma, 5; Issaquena, 5; Leflore, 7; Sunflower, 3; Wilkinson, 5 — NEW JERSEY: Cumberland, 5 — NORTH CAROLINA: Craven, 1; Montgomery, 7; Northhampton, 5; Yancey, 1 — OKLAHOMA: Adair, 5; Pushmataha, 5 — SOUTH CAROLINA: Allendale, 5; Beaufort, 10; Berkeley, 5; Colleton, 5; Hampton, 5; Jasper, 5; Williamsburg, 5 — TENNESSEE: Blount, 2 — VIRGINIA: James City, 3; Nansemond, 5; Nottoway, 5 — WEST VIRGINIA: Hardy, 15).

Table 3 presents results of statistical analyses of the data stratified by sampling techniques and by age, sex and physiographic provinces. Prevalence of *Sarcocystis* spp. among fawns was significantly lower than in adult deer. No significant difference was found in the prevalence between male and female deer ($P > 0.05$).

Results of the feeding trials are presented in Table 2. Dogs began shedding typical *Sarcocystis* sporocysts 9 to 12 days after eating *Sarcocystis*-infected venison. Sporocysts measured 15.2 × 10.9μm (range 13.4-16.8 × 9.0-12.3μm, $N = 195$). A cat in one of three trials and a red fox in one of two trials first shed sporocysts 10 days after eating infected

TABLE 2. Infectivity of *Sarcocystis* spp. of white-tailed deer for carnivores.

Feeding trial	Origin of deer	Carnivore[a]	Infectivity	Prepatent period DPI[b]	Sporocyst size
1	Baldwin County, Alabama	1 Dog	+	9	15.0 × 10.7(13.4-15.7 × 9.0 -11.2)N 60
		1 Cat	-		
2	Telfair County, Georgia	1 Dog	+	12	15.1 × 10.9(13.4-15.7 × 9.0 -11.2)N=30
		1 Red Fox	-		
3	Nansemond County, Virginia	1 Dog	+	9	15.1 × 10.9(13.4-15.7 × 9.0 -11.2)N=30
		1 Cat	+	10	12.0 × 8.7(11.2-13.4 × 6.72- 8.96)N=18
		1 Red Fox	+	10	13.6 × 10.2(11.2-15.7 × 9.0 -11.2)N= 7
4	Jeff Davis and Ware counties, Georgia	1 Dog	+	9	15.3 × 11.0(13.4-15.7 × 10.1 -11.2)N=45
		1 Cat	-		
5	Richmond and Jones counties, Georgia	3 Dogs	+	10,14,21[c]	15.5 × 11.1(13.4-16.8 × 10.1 -12.3)N=30

[a]One control dog and cat were used in feeding trials 1 through 4 and remained uninfected with *Sarcocystis*

[b]DPI = Days Postinfection

[c]Killed dogs for sporocysts

TABLE 3. *Sarcocystis* infections in white-tailed deer stratified by age, sex, and physiographic province and according to histologic section examined.

	Percent Infected		
	All histologic[a] sections (N=390)	Tongue section only (N=365)	Tongue and heart section (N=333)
AGE			
Fawn (< 1 yr)	26[b]	20[b]	14[b]
Adult (> 1 yr)	54	51	51
SEX			
Male	48	44	41
Female	53	49	49
PHYSIOGRAPHIC PROVINCE			
Coastal Plain	47	45	43
Mountain	79	71	71
Piedmont	74	62	57
Interior Highland	35	30	32

[a]Histologic sections varied per deer but included one or more histologic sections containing skeletal and/or cardiac muscle.
[b]Significant ($P<.01$).

venison. Sporocyst production by both the cat and the fox was low. The fox shed sporocysts on days 10-12 and 25 after eating infected venison. The experiment was terminated 30 days postingestion (DPI). The cat shed sporocysts continuously from 10 to 15 DPI, at which time the animal was killed and approximately 2,400 sporocysts were recovered from the intestinal mucosa by artificial digestion. Both infected and control dogs and cats used in the feeding trials sporadically shed unsporulated oocysts. Three different sizes of oocysts were observed in the dogs. The largest unsporulated oocysts measured 37.7 × 30.6μm (range 33.6-40.3 × 26.9-33.6μm, N=12) and were considered to be *Cystoisospora canis*. Oocysts of the two smaller size classes, one measuring 22.0 × 17.5μm (range 15.7-24.6 × 15.7-17.9μm, N=55) and the other measuring 12.48 × 10.56μm (range 11.2-13.4 × 9.0-11.2μm, N=28), were not identified because oocyst size overlapped other coccidia of dogs. Cats shed oocysts measuring 43.0 × 31.9μm (range 39.2-47.0 × 29.1-33.6μm, N=40) which were considered to be *Cystoisospora felis*.

Sarcocystis zoites were detected in 29 of 35 (83%) deer by artificial digestion, but sarcocysts were found histologically in only 15 (43%) of these deer (Table 4). Artificial digestion was more sensitive statistically in detecting *Sarcocystis* infections than was histologic examination when analyzed by the nonparameteric sign test (Ostle and Mensing, 1975) ($P<.01$). Similarly, the prevalence of cysts in histologic sections of tongue was significantly greater ($P<.01$) than the prevalence of sarcocysts in the heart of 333 deer (Table 5).

Measurements of zoites recovered by artificial digestion measured 11.6 × 4.8μm (range 10-18 × 2-11μm, N=133) with no detectable difference in zoite size found among deer from different locales.

Histologically, sarcocyst morphology was similar in all infected deer. Sarcocysts had a striated wall 1 to 2μm thick, although sometimes different planes of sectioning gave the cyst wall a thicker

TABLE 4. Comparison of histologic examination and artificial digestion in detecting *Sarcocystis* in the tongue of white-tailed deer.

Origin of deer	Histological examination No. infected/no. examined	Artificial digestion No. infected/no. examined
Camden County, Georgia	0/5	3/5
Richmond County, Georgia	2/5	5/5
Jones County, Georgia	4/5	5/5
Floyd County, Georgia	3/5	4/5
Plaquemines Parrish, Louisiana	2/5	4/5
McIntosh County, Georgia	1/5	3/5
Arkansas County, Arkansas	3/5	5/5
Total	15/35 (43%)	29/35 (83%)[a]

[a]Significant difference sign test (r=0; $r_{.01}$=1, n=14)

TABLE 5. Comparison of histologic examination of paired tongue and heart tissue sections in detecting *Sarcocystis* in white-tailed deer.

Tissue examined	No. of deer with sarcocysts	No. of deer without sarcocysts	Total no. of deer examined
Tongue	149[a] (45%)	184	333
Heart	29 (9%)	304	333

[a]Significant difference sign test (r=4; $r_{.01}$=49; n=128)

"bottle brush" appearance. Different planes of sectioning limited meaningful cyst measurement to maximum length and diameter of 0.48mm and 0.44mm, respectively.

Usually there were no lesions associated with sarcocysts in deer; however, in seven animals scattered sarcocysts appeared to elicit a local inflammatory response which consisted predominantly of mononuclear cells with scattered eosinophils and macrophages. In five of the seven deer with inflammatory response, the sarcocysts appeared to be ruptured or degenerating.

DISCUSSION

Sarcocystis spp. are prevalent throughout the range of the white-tailed deer, and there are noticeable differences in cyst size and prevalence. Cysts in the present study approached macroscopic size but were not visible grossly as reported for *Sarcocystis* in deer from Montana (Pond and Speer, 1979). As determined by histologic examination in the present study, the prevalence of *Sarcocystis* among white-tailed deer of the southeastern United States is lower than previous reports in white-tailed deer (Karstad and Trainer, 1969). The diges-

tion technique, however, detected a comparable rate of infection. The higher prevalence of cysts in histologic sections of tongue compared with heart in the present study correlates well with previous reports that the tongue is a more consistent source of cysts in both white-tailed deer (Karstad and Trainer, 1969; Mahrt and Colwell, 1980) and mule deer (Sayama, 1952). There appear to be differences in prevalences among herds in the same physiographic provinces of the southeastern United States. Of the nine herds in the present study where *Sarcocystis* was not detected histologically, sample sizes of four herds were not statistically adequate to detect infected deer ($P<.05$) considering the apparent low sensitivity of detection of histologic sections and the average rate of infection (51%) found to exist in infected herds. Statistically, with small sample sizes (less than five deer per herd) there is an appreciable possibility of failing to detect infected deer assuming the real prevalence is as high as 51% in an infinite population. The absence of *Sarcocystis* in five herds with adequate sample sizes may be due to low sensitivity of histologic sections in detecting a low intensity of infection, a prevalence $\leq 50\%$, or the actual absence of the parasite. The low sensitivity of histologic sections to detect infections of *Sarcocystis* is supported by information in Table 4.

The superior sensitivity of the digestion technique in detection of *Sarcocystis* infection has been recognized and demonstrated (Box and Duszynski, 1977; Box and McGuinness, 1978; Jacobs et al., 1960). However, erroneous results could be obtained from the digestion technique without histologic confirmation of cysts. Metrocytes are destroyed by digestive fluids, and thus prevalence of infections may be underestimated. Histologic examination used alone to determine the prevalence of *Sarcocystis* represents a minimal estimate, thus a combination of both histologic and digestive examination of tongue would result in a more accurate estimate of prevalence.

The significant difference in the prevalence of infection between fawns and adults parallels differences shown in previous studies of *Sarcocystis* in a variety of animals (Mahrt and Colwell, 1980; Sayama, 1952). This difference may reflect the time period necessary for cysts to develop to detectable size and/or infection intensity to reach a detectable level.

The feeding trials suggest that more than one species of *Sarcocystis* are present in white-tailed deer. Previous studies with domestic ruminants have also shown multiple species of *Sarcocystis* to occur in a single host (Levine, 1977). The predominant species of *Sarcocystis* in white-tailed deer in the southeastern United States has a deer-dog cycle (Crum et al., 1981). Intramuscular cysts and sporocysts are morphologically similar to *S. odocoileocanis* (Crum et al., 1981). A second less prevalent *Sarcocystis* sp. cycles from deer to cat. The presence of a deer-cat *Sarcocystis* sp. in the present study supplies further evidence to a previous report of *Sarcocystis* sp. which has a deer-cat cycle (Crum et al., 1981).

The passage of sporocysts by a red fox may represent an incidental finding because sporocyst production was very low. The red fox serves as a definitive host for three species of *Sarcocystis* (Ashford, 1977; Erber and Boch, 1976; Fayer et al., 1976; Rommel et al., 1974) which occur in domestic animals. White-tailed deer also may serve as a host for at least one of the monozoic cyst-forming eimeriids of the dog. This possibility is suggested by the fact that the only dog to shed small oocysts (avg $12.4 \times 10.5\mu m$) during the feeding trials did so eight days after eating venison. Further work under more controlled conditions is needed before this can be conclusively determined.

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LITERATURE CITED

ASHFORD, R.W. 1977. The fox, *Vulpes vulpes*, as a final host for *Sarcocystis* of sheep. Ann. Trop. Med. Parasitol. 71: 29-34.

BOX, E.D. and D.W. DUSZYNSKI. 1977. Survey for *Sarcocystis* in the brown-headed cowbird *(Molothrus ater)*: A comparison of macroscopic, microscopic and digestion techniques. J. Wildl. Dis. 13: 356-359.

—— and T.B. McGUINNESS. 1978. *Sarcocystis* in beef from retail outlets demonstrated by digestion technique. J. Parasitol. 64: 161-162.

CRUM, J.M., R. FAYER and A.K. PRESTWOOD. 1981. *Sarcocystis* spp. in white-tailed deer. I. Definitive and intermediate host spectrum with a description of *Sarcocystis odocoileocanis* n.sp. J. Wildl. Dis. 17: 567-579.

ERBER, M. and J. BOCH. 1976. Untersuchungen uber Sarkosporidien des schwarzwildes. Sporozystenausscheidung durch Hund, Fuchs and Wolf. Berl. Münch. Tierärztl. Wschr. 89: 449-450.

FAYER, R., A.J. JOHNSON AND P.K. HILDEBRANDT. 1976. Oral infection of mammals with *Sarcocystis fusiformis* bradyzoites from cattle and sporocysts from dogs and coyotes. J. Parasitol. 62: 10-14.

JACOBS, L., J.S. REMINGTON and M.L. MELTON. 1960. A survey of meat samples from swine, cattle, and sheep for the presence of encysted *Toxoplasma*. J. Parasitol. 46: 23-28.

KARSTAD, L. and D.O. TRAINER. 1969. *Sarcocystis* in white-tailed deer. Bull. Wildl. Dis. Assoc. 5: 25-26.

LEVINE, N.D. 1977. Nomenclature of *Sarcocystis* in the ox and sheep and of fecal coccidia of the dog and cat. J. Parasitol. 63: 36-51.

MAHRT, J.L. and D.D. COLWELL. 1980. *Sarcocystis* in wild ungulates in Alberta. J. Wildl. Dis. 16: 571-576.

OSTLE, B. and R.W. MENSING. 1975. *Statistics in Research*. Iowa State University Press, Ames, Iowa. 596pp.

POND, D.B. and C.A. SPEER. 1979. *Sarcocystis* in free-ranging herbivores on the National Bison Range. J. Wildl. Dis. 15: 51-53.

PRESTWOOD, A.K., S.R. PURSGLOVE and F.A. HAYES. 1976. Parasitism among white-tailed deer and domestic sheep on common range. J. Wildl. Dis. 12: 380-385.

ROMMEL, M., A.O. HEYDORN, B. FISCHLE and R. GESTRICH. 1974. Beitrage zum Lebenszyklus der Sarkosporidien. V. Weitere Endwirte der Sarkosporidien von Rind, Schaf and Schwein und die Bedeutung des Zwischenwirtes für die Verbreitung dieser Parasitose. Berl. Münch. Tierärztl. Wschr. 87: 392-396.

SAYAMA, K. 1952. *Sarcocystis* in deer and elk of California. Calif. Fish and Game J. 38: 99-104.

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