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Endoparasites of Selected Populations of Gray Squirrels (*Sciurus carolinensis*) in the Southeastern United States[1]

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ABSTRACT: Examination of 270 gray squirrels (*Sciurus carolinensis*) from 18 localities in the southeastern United States revealed 29 species of endoparasites, including 5 protozoans, 2 trematodes, 4 cestodes, 1 acanthocephalan, and 17 nematodes. Five of these represented new host records, and one new species of filarial worm was recovered. Data are presented on the prevalence and intensity of infection with each species along with information on geographical distribution. Seven parasites (*Hepatozoon griseisciuri*, *Taenia* sp., *Baylisascaris procyonis*, *Dirofilariaeformia pulmoni*, *Heligmodendrium hassalli*, *Physocephalus sexalatus*, and *Strongyloides robustus*) were observed to produce lesions in the host. Numbers of some intestinal nematodes differed significantly between seasons, age classes, potential natural vegetative types, and localities.

Gray squirrels, *Sciurus carolinensis*, harbor numerous species of endoparasites, but little information is available on the distribution, prevalence, or intensity of infection (Clarke, 1959; Parker, 1971). Similarly, the pathogenicity of most endoparasites and their role as mortality factors in gray squirrel populations remain largely speculative.

Parasitism has been considered a possible factor initiating emigrations of gray squirrels. Although Flyger (1969) did not attribute much significance to parasitism of squirrels emigrating during 1968, Parker and Holliman (1971) emphasized the need for additional parasitologic data during ordinary conditions to serve as a basis for evaluating parasitism during emigrations.

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In an effort to obtain information on endoparasitism in gray squirrels of the southeastern United States, a study was undertaken to (1) identify the endoparasitic fauna; (2) ascertain the prevalence and intensity of infections; (3) evaluate differences attributable to season, age, sex, vegetative type, or collection site; and (4) evaluate the pathogenicity of each species.

Materials and Methods

Three collection sites were sampled in each of the six major potential natural vegetative types (Küchler, 1964) of the southeastern United States: Appalachian oak, oak-hickory, oak-hickory-pine, southern floodplain, southern mixed, and mixed mesophytic (Fig. 1). Five gray squirrels were collected from each site by shooting within the periods of 1–24 May 1972, 15 August–9 September 1972, and 2 January–8 February 1973.

Squirrels were placed in individual plastic bags, kept on ice, and necropsied within 4 hr

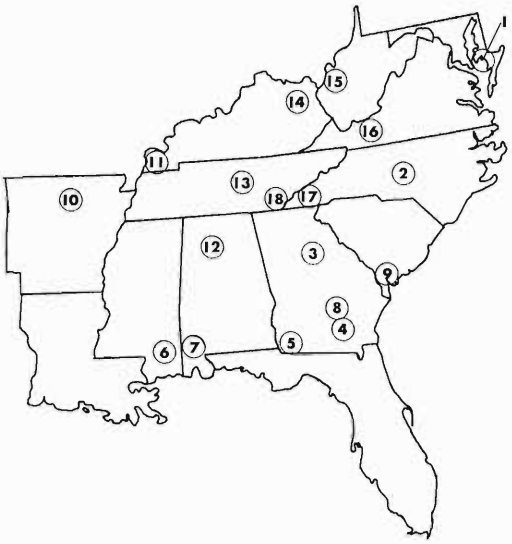

Figure 1. Distribution of collection sites within the southeastern United States. Location of sites within potential natural vegetative types are as follows: (1) Dorchester Co., (2) Montgomery Co., (3) Oglethorpe Co.—Oak–hickory–pine; (4) Jeff Davis Co., (5) Decatur Co., (6) Forrest Co.—Southern mixed; (7) Clarke Co., (8) Jeff Davis Co., (9) Hampton Co.—Southern floodplain; (10) Stone Co., (11) Trigg Co., (12) Lawrence Co.—Oak–hickory; (13) Cumberland Co., (14) Rowan Co., (15) Mason Co.—Mixed mesophytic; and (16) Montgomery Co., (17) Buncombe Co., (18) Monroe Co.—Appalachian oak.

after collection. After skinning, major organs were removed and examined for helminth parasites. The gastrointestinal tract was opened, scraped, the contents washed separately through 100 mesh screens, and the retained material preserved in formalin (5%)-acetic acid (2.5%) solution. Examination for intestinal protozoans was made by direct saline smears. When large numbers of coccidian oocysts were observed, a portion of the intestinal contents was collected in 2.5% potassium dichromate solution, refrigerated, and the oocysts subsequently sporulated at room temperature. Methods of examination for blood parasites and histologic techniques were described elsewhere (Davidson and Calpin, 1975).

Complete parasite counts were made. Parasite numbers and other pertinent data were coded and placed on computer cards. Data on helminth parasites which occurred in more than 25% of the squirrels were analyzed by an analysis of variance test (Service, 1972) for differences related to season, age, sex, vegetative type, or collection site. Prior to analysis, parasite numbers were changed to approximate a normal distribution by the log of n + 1 transformation.

Results and Discussion

Examination of 270 gray squirrels revealed 5 protozoans, 2 trematodes, 4 cestodes, 1 acanthocephalan, and 17 nematodes (Table 1). One species of nematode was previously undescribed, and the gray squirrel was established as a new host for five species. Parasites are discussed in order of prevalence within each phylogenetic group.

Protozoa

Coccidian oocysts similar morphologically to *Eimeria ascotensis*, *E. lancasterensis*, *E. moelleri*, and *E. neosciuri* were observed. Since oocyst morphology often is not adequate for differentiating species, all thin-walled coccidia were considered as *Eimeria* spp. Two species with unique thick-walled oocysts, *E. confusa* and *E. ontarioensis*, were easily distinguished in intestinal smears but were detected rarely. Joseph (1971, 1972) reported that two successive infections with *E. confusa* conferred immunity to subsequent infections. Acquired immunity may account for the low prevalence of infection with this species. Squirrels from the oak-hickory vegetative type had a significantly lower ($P < 0.01$) prevalence of *Eimeria* spp. Joseph (1973, 1975) demonstrated that most eimerians described from gray squirrels occur naturally in or are transmissible to fox squirrels (*S. niger*); thus the epizootiology of these sciurid coccidia apparently involves two hosts.

The pathogenicity of coccidia in gray squirrels apparently varies among species. Webster (1960) noted significant lesions due to *E. neosciuri* infection whereas Joseph (1972) considered *E. confusa* and *E. lancasterensis* as non-pathogenic. Although massive infections with any species probably would be detrimental, the absence of significant lesions due to coccidiosis during this study sug-

Table 1. Endoparasites recovered from 270 gray squirrels collected from the southeastern United States.

Parasite	Percent prevalence	Number per infection		Distribution
		Mean	Range	
PROTOZOA				
Eimeria spp. (1)*	81	–**	–	1–18†
Eimeria confusa (1)	1	–	–	5, 17
Eimeria ontarioensis (1)	1	–	–	8, 16, 17
Hepatozoon griseisciuri (6, 7)	41	–	–	1–12, 14–18
Sarcocystis sp. (5)	8	–	–	1, 2, 4, 5, 7–9, 12, 16, 18
TREMATODA				
Brachylaima sp. (1)	<1	2	1–2	1, 17
Nudacotyle norvica (2)	<1	2	2	7
CESTODA				
Catenotaenia dendritica (1)	<1	2	1–2	11, 18
Hymenolepis diminuta (1)	2	2	1–4	11, 14, 15
Raillietina bakeri (1)	6	4	1–19	5–7, 12
Taenia sp. (7, 8)	3	1	1–3	2, 5, 8, 10, 12
ACANTHOCEPHALA				
Moniliformis clarki (1)	1	1	1	7, 9
NEMATODA				
Ascaris sp. (1)	<1	1	1	18
Baylisascaris procyonis (11)	<1	11	11	1
Bohmiella wilsoni (2)	29	6	1–48	2–10, 12
Capillaria americana (1)	14	3	1–12	1, 4–6, 8–11, 14–16
Citellinema bifurcatum (1)	37	14	1–108	2, 3, 5, 7, 9–18
Dipetalonema interstitium (9)	5	5	1–50	3–5, 9, 13
Dirofilariaeformia pulmoni (10)	2	–	–	1, 11
Enterobius sciuri (3)	26	11	1–152	1–18
Gongylonema pulchrum (4)	5	3	1–29	2, 4, 5, 7–9, 13, 16, 18
Heligmodendrium hassalli (1)	89	124	1–964	1–18
Microfilariae (6)††	31	–	–	1–10, 12–14, 16
Physocephalus sexalatus (2)	<1	41	41	3
Physaloptera sp. (2)	<1	1	1	15
Pterygodermatites parkeri (1)	3	1	1–3	2, 11, 13, 14
Strongyloides robustus (1)	86	51	1–568	1–18
Syphacia thompsoni (3)	5	5	1–34	2, 6, 9, 10, 12, 14, 15, 17
Trichostrongylus affinis (3)	3	2	1–6	3, 5–8
Trichostrongylus calcaratus (3)	16	3	1–36	1–9, 12, 13, 15, 17

* The numbers in parentheses indicate locations in the host: (1) small intestine, (2) stomach, (3) cecum and large intestine, (4) esophagus, (5) muscle, (6) blood, (7) lung, (8) liver, (9) subcutaneous, (10) pulmonary arteries, and (11) thoracic cavity.
** Value not determined.
† Map number.
†† Morphologically similar to *D. interstitium microfilariae.*

gests that coccidiosis is not a major problem in squirrels in the Southeast.

Information on *Hepatozoon griseisciuri* infection in these animals has been presented in detail (Davidson and Calpin, 1976). These authors emphasized that the lungs were a major site of schizogonic development, discussed the probable chronologic epizootiology, and postulated that *H. griseisciuri* infection might be related to mid-winter mortality in gray squirrels.

The prevalence of *Sarcocystis* sp. must be considered minimal since examination of additional muscle sections undoubtedly would have revealed more infected hosts. All sporozoan cysts detected in skeletal muscle tentatively were designated as *Sarcocystis* sp. Recent reports (Frenkel, 1974; Frenkel and Dubey, 1975) suggest that transmission studies are required to differentiate *Sarcocystis* from *Hammondia.* Although the status of the gray squirrel in the life cycle of *Sarcocystis* sp. is unknown, it presumably is similar to that of other intermediate hosts (Frenkel, 1974), with wild carnivores as final hosts. The gray squirrel is established as a new host for *Sarcocystis* sp.

Trematoda

Flukes were encountered rarely, and their sporadic occurrence apparently is related to the dietary habits of squirrels resulting in infrequent ingestion of suitable intermediate hosts.

Brachylaima sp. was recovered from only two squirrels. Although specimens resembled *B. virginiana*, the limited number of specimens precluded identification to species. The gray squirrel is recorded as a new host for *Brachylaima* sp.

Nudacotyle norvica, a common parasite of muskrats, was recovered from a single squirrel. Welborn (1975) recently found *N. norvica* in gray squirrels of western Tennessee, and Olexik et al. (1969) reported *Nudacotyle* sp., probably *N. norvica*, from the same locality.

Cestoda

Raillietina bakeri occurred frequently and with high intensity in several southern sites but did not occur in northern sites. Previous reports of this cestode in other hosts also have been from southern areas (Chandler, 1942; Harkema, 1946; Huggins, 1951), suggesting that the distribution of *R. bakeri* may be related to the distribution of suitable intermediate hosts. The gray squirrel is established as a new host for *R. bakeri*.

Hymenolepis diminuta and *Catenotaenia dendritica* occurred infrequently and in low numbers. Although both species have been reported previously from gray squirrels (Rausch and Tiner, 1948; Parker, 1971; Welborn, 1975), the low prevalence of infection in these reports and the present study suggest that the gray squirrel is an accidental host to both helminths.

Cysticerci of *Taenia* sp. conformed to the morphologic description of *T. pisiformis*; however, three viable cysticerci failed to develop when fed to a young, cestode-free dog. The specific identity of these cestodes therefore is uncertain. Cysticerci were found only in subadult or adult squirrels. Minor lesions associated with *Taenia* sp. infections were focal fibrotic hepatitis.

Acanthocephala

Moniliformis clarki occurred with a prevalence and intensity of infection similar to previous reports in gray squirrels (Parker, 1971; Welborn, 1975). Although none of the squirrels examined had lesions attributable to *M. clarki*, Moore (1946) reported fatal peritonitis in flying squirrels (*Glaucomys volans*) due to heavy *M. clarki* infections. Because of its large size, *M. clarki* also might occlude the intestinal lumen if present in high numbers. The pathogenic potential of *M. clarki* is offset by its infrequent and localized occurrence (Chandler, 1947; Parker, 1971; Welborn, 1975).

Nematoda

Heligmodendrium hassalli was the most frequently encountered parasite and was recovered from each collection site. Harkema (1936), Chandler (1942), and Welborn (1975) also found *H. hassalli* to be the predominant parasite of gray squirrels.

Juvenile squirrels had significantly lower ($P < 0.01$) numbers of *H. hassalli* than subadults, and this difference was attributed to less exposure of the juveniles to infective larvae.

Squirrels from the oak-hickory-pine vegetative type had significantly higher ($P < 0.01$) numbers of *H. hassalli* than squirrels from all other vegetative types. Although lower than in squirrels from oak-hickory areas, squirrels from southern mixed and southern floodplain vegetative types had significantly higher ($P < 0.01$) numbers of *H. hassalli* than did squirrels from the three remaining vegetative types. These differences were attributed to two factors. First, squirrel population densities were generally higher on sites representative of these vegetative types. Welborn (1975) found a positive correlation between nematode infection intensities and squirrel density as estimated by hunter success indices. Second, these sites were in milder climatic regions where survival of eggs and larvae probably would be higher.

Gross and microscopic lesions associated with intense *H. hassalli* infections were mucosal hyperemia and hemorrhagic enteritis. These lesions were found in less than 10% of the squirrels and only in those harboring greater than 300 worms.

Strongyloides robustus had a prevalence of infection and distribution very similar to *H. hassalli*. Although always lower, average numbers of *S. robustus* in squirrels from each site paralleled average number of *H. hassalli* (correlation coefficient = 0.7043). Similar trends also were noted for infections within different age classes and vegetative types. Factors

responsible presumably are the same as postulated for *H. hasalli*.

Strongyloides robustus was the most pathogenic helminth found with substantial frequency. Infections of greater than 150 worms frequently caused a severe hemorrhagic enteritis. Parker (1971) considered *S. robustus* to be the most pathogenic nematode in squirrels of southwestern Virginia. Consistent with the findings of Parker (1971), lesions associated with *S. robustus* were confined to the duodenum.

Citellinema bifurcatum had a prevalence and intensity of infection similar to previous reports (Katz, 1938; Olexik et al. 1969; Parker, 1971; Welborn, 1975). Although *C. bifurcatum* occurred in squirrels from all vegetative types, infections were significantly lower ($P < 0.05$) in squirrels from the southern floodplain and southern mixed vegetative types. In this respect, *C. bifurcatum* apparently is influenced more by environmental conditions than host densities.

Bohmiella wilsoni was recovered frequently from squirrels in all southern collection sites but was absent in northern areas. Although *B. wilsoni* has been reported from more northern areas (Lucker, 1943; Rausch and Tiner, 1948; Parker, 1971; Welborn, 1975), data from this study indicate that *B. wilsoni* is found more frequently in gray squirrels from southern portions of their range.

Enterobius sciuri, which was only recently detected in gray squirrels in North America (Parker, 1971; Parker and Holliman, 1971; Welborn, 1975), had a much higher prevalence than previously reported. This helminth occurred in squirrels from each collection site and was present in significantly higher ($P < 0.01$) numbers during the winter than in other seasons.

Prevalence and intensity of infection by *Capillaria americana* were similar to the findings of Parker (1971) and Welborn (1975). Although this helminth occurred infrequently, it was widely distributed in southeastern gray squirrels.

Dipetalonema interstitium is reported for the second time, having been originally described from gray squirrels in Maryland (Price, 1962). Infection intensities usually were similar to those reported by Price (1962); however, one squirrel harbored a much larger number of worms (50) than had been reported. The high prevalence and wide distribution of microfilariae suggest that *D. interstitium* occurs throughout the non-mountainous areas of the Southeast.

The prevalence and intensity of *Pterygodermatites parkeri* was similar to previous reports (Parker, 1971; Welborn, 1975). According to Parker (1971), worms of the genus *Rictularia* previously reported from gray squirrels (Katz, 1938; Rausch and Tiner, 1948; Parker 1968; Olexik et al. 1969) should be considered *Pterygodermatites*, and in all probability *P. parkeri*.

A description of and information on *Dirofilariaeformia pulmoni* was presented by Davidson (1975). Circulatory lesions associated with *D. pulmoni* were considered severe and conceivably could result in occasional mortality.

Based on the low prevalence of infection and infectivity for other hosts, the gray squirrel probably is an accidental host for *Trichostrongylus calcaratus*, *T. affinis*, *Syphacia thompsoni*, *Gongylonema pulchrum*, *Physocephalus sexulatus*, *Physaloptera* sp., *Ascaris* sp., and *B. procyonis*. The occurrences of *T. affinis* and *P. sexulatus* in the gray squirrel constitute new hosts records.

Although infections apparently were accidental, the pathologic consequences of *P. sexulatus* and *B. procyonis* warrant consideration. Ulcerative gastritis associated with *P. sexulatus*, the swine stomach worm, was the most severe lesion encountered, and such infections would undoubtedly result in occasional mortality. Domestic swine frequented the site from which the squirrel originated, and similar infections could be expected on areas cohabited by squirrels and wild or feral swine.

The ability of *B. procyonis* (= *Ascaris columnaris* of Tiner 1949) larvae to produce fatal neurologic disease in gray squirrels has been demonstrated experimentally (Tiner, 1949). Four of six experimentally-infected squirrels died, and the two survivors had larvae encysted in the myocardium, pericardium, caval veins, lungs, and pulmonary pleura. Larvae found during this study were in similar locations. Considering that infected hosts which develop neurologic disease are more susceptible to predation (Tiner, 1949) and that recently an

epizootic of this disease has occurred within the Southeast (Nettles et al. 1975), the low prevalence of *B. procyonis* may not be indicative of the significance of this helminth for gray squirrel populations.

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Development of *Hammondia hammondi* in Cell Cultures

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ABSTRACT: Artificially excysted sporozoites of *Hammondia hammondi* invade and multiply within primary cell cultures of monkey kidney or mouse embryo or a cell line of human diploid fibroblasts (WI-38). Division was observed at approximately daily intervals through 8 days. Endodyogeny was the only type of division seen. Continuous multiplication was not achieved in long term culture although organisms were present in culture fluids for 4–6 weeks. *H. hammondi* organisms had typical coccidian ultrastructure when observed after penetration and during division. A double membrane pellicle with underlying microtubules, a conoid, micronemes, rhoptries, and a micropore, all resembling those of *Toxoplasma gondii*, were present. A significant difference is that *H. hammondi* organisms contain storage granules which appear to be depleted following cell invasion. Ultrastructure, cultivation, and division of *H. hammondi* are indicative of its close relation to *Toxoplasma gondii*.

Coccidian oocysts resembling those of *Toxoplasma gondii* were recovered from the feces of a cat in Hawaii by Wallace (1973). When these oocysts were fed, after sporulation, to mice toxoplasma-like cysts were found in skeletal muscle and occasionally in the central nervous system. Immunological studies and transmission experiments between cats and between mice indicated that the parasite was not *T. gondii*. Wallace designated the organism WC1170 and presumed it to be *Sarcocystis muris*. Further study allowed differentiation between the latter two parasites (Wallace, 1975). An organism having the same characteristics as WC1170 was isolated from a cat in Kansas by Frenkel (1974) and named *Hammondia hammondi* gen. n. et sp. n.

Hammondia hammondi has similarities to *Toxoplasma*, *Sarcocystis*, and *Besnoitia* (Frenkel, 1974; Wallace, 1975; Wallace and Frenkel, 1975). The oocyst, an isosporan type which morphologically resembles that of *Toxoplasma*, is passed in the unsporulated state and sporulates within 72 hr. When oocysts are fed to mice cysts are produced in the skeletal muscle; bradyzoites within the cysts resemble those of *Toxoplasma*. Schizonts and gametocytes produced in the intestinal epithelium of cats are similar to those of *Toxoplasma*. *H. hammondi* resembles members of the genus *Sarcocystis* by producing cysts in skeletal muscle and, more importantly, by requiring an intermediate host for transmission.

The ultrastructure of the sporozoite of *H. hammondi* closely resembles that of *T. gondii* (Sheffield and Melton, 1974) and the ultrastructural characteristics of *T. gondii* development in cultured cells are known (Sheffield