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Blood Parasites of Squirrels from Coles and Lake Counties in Illinois

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BLOOD PARASITES OF SQUIRRELS FROM

COLES AND LAKE COUNTIES IN ILLINOIS
(TITLE)

BY

Nicholas I. Obiri

THESIS

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COLES AND LAKE COUNTIES IN ILLINOIS

Presented by

Nicholas I. Obiri

a candidate for the degree of Master of Science
and hereby certify that in their opinion it is acceptable.

23 May 1977

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BLOOD PARASITES OF SQUIRRELS FROM COLES AND LAKE COUNTIES IN ILLINOIS

Nicholas I. Obiri

Abstract: Hepatozoon and microfilariae are described from the blood of fox and gray squirrels captured in Coles and Lake Counties in Illinois during 1976 and 1977. The hepatozoon species is identified as Hepatozoon griseisciuri on the basis of parasite morphology and hosts. A higher rate of infection (35.7% : 11.1%) was observed in gray squirrels than in fox squirrels and more adults were infected than juveniles. No microfilariae were found in gray squirrels but fox squirrels had a 57.1% infection rate with no difference based on age. The observed microfilariae are similar to that described by Davidson from Maryland gray squirrels in 1975. They are presumed to be Dirofilariaeformia pulmoni. Chipmunks and thirteen-lined ground squirrels captured in Coles County, Illinois were negative for blood parasites.

Microfilariae of Dipetalonema interstitium (Price 1962) and Dirofilariaeformia pulmoni (Davidson 1975) have been described from the Eastern gray squirrel (Sciurus carolinensis). Chandler (1942) described two unnamed microfilariae from the fox squirrel (Sciurus niger rufiventer). These are the only reports of microfilariae from North American tree squirrels. Clark (1958) and Davidson (1976) reported the occurrence of Hepatozoon griseisciuri in gray squirrels and Clark described its

life cycle. This species is considered the only Hepatozoon in North American gray squirrels (Davidson and Calpin 1976) and none has been reported from fox squirrels.

Tree squirrels, because of their abundance, importance as game animals (Preno and Labisky 1971), and broad interface with human populations (MacClintock 1970), may be of zoonotic significance to human health. As such, an understanding of fox and gray squirrel parasites is of prime importance. Therefore, this study was undertaken to identify blood parasites of Illinois fox and gray squirrels. Chipmunks (Tamias striatus) and thirteen-lined ground squirrels (Spermophilus tridecemlineatus) captured during the study were also examined.

METHODS

Squirrels were live trapped at four locations in Coles County from February 1976 to January 1977 and from Lake County in February 1976. Sliding door traps (53 x 14 x 17 cm), baited with corn and peanut butter, were used. Traps were checked at least once a day. Animals were etherized and thick and thin films were made from blood obtained from tail or toe snips. A 1 ml blood sample was obtained by heart puncture for use in a blood parasite concentration technique (Knott 1939 as modified by Herman and Price 1954).

Blood films were air dried, fixed in ethanol-ether and stained with Delafield's hematoxylin according to a method described in Department of the Army Technical Manual (1961). Microfilariae observed on stained slides were measured and described.

Hepatozoon and microfilariae concentrated and prepared according to Knott's technique were enumerated by counting the helminths and

gamonts seen while scanning the entire area under a 22 x 22 mm cover slip on a wet-mount slide.

RESULTS

Forty-three animals were captured between February 1976 and January 1977. Four of six gray squirrels were mildly parasitized (1 parasite per 0.04 ml) with Hepatozoon. None of the gray squirrels had microfilarial infections but eight of fourteen fox squirrels were infected. One adult female had a mild infection; the remainder had very heavy (4+ per 0.04 ml) infection. Hepatozoon gamonts were observed in blood from five fox squirrels. Three infections were heavy (2.4 per 0.04 ml) and two were mild (Table 1). The age-sex distribution of infection is summarized in Table 2. Two recaptures were made in the trapping effort but the results of blood examination were in each case consistent with the first results.

Twenty-two chipmunks, Tamias striatus, and two thirteen-lined ground squirrels, Spermophilus tridecemlineatus, were examined but neither microfilariae nor Hepatozoon were seen.

Measurements were obtained from five microfilariae, two of which are shown in Figures 1 and 2. The mean and range of the parameters measured were: Length 243.4 µm (223 - 285). Width 3.62 µm (3.00 - 4.23). Anterior end bluntly rounded. Cephalic space 4.00 µm (3.04 - 5.00), sheath absent. Tail tapering smoothly to a point. Fixed points, actual values followed by percentages of body length are: Cephalic space 4.0 µm (3 - 5), 1.57% (1.35 - 1.75); Nerve ring 46.8 µm (46.6 - 50), 24.7% (17.5 - 22.4); Excretory pore 74.6 µm (71 - 78), 30.9% (24.9 - 33.2); First rectal cell 137.8 µm (121 - 142), 56.7% (46.5 - 63.7); Anal pore midpoint 161.4 µm (129 - 210), 66.2% (51.5 - 76.2).

Table 1. Microfilaria and *Hepatozoon* infection in blood of squirrels examined.

SPECIES	PERIOD OF CAPTURE	SEX	AGE	MICROFILARIA	*HEPATOZOON*
Fox	Feb. 1976*	F	Juv	+++	+
(*Sciurus niger*)		F	Ad	-	-
		F	Juv	+++	+
	March 1976*	M	Ad	+++	++
		M	Ad	+++	++
		M	Ad	+++	-
	July 1976*	M	Ad	-	-
	Oct. 1976*	F	Ad	-	-
		F	Juv	-	-
	Nov. 1976*	F	Ad	-	-
		F	Ad	+++	++
	Jan. 1977*	F	Ad	+	-
		F	Ad	+++	-
		F	Ad	-	-
Gray	Feb. 1976@	F	Juv	-	+
(*Sciurus carolinensis*		M	Ad	-	-
		F	Ad	-	-
		M#	Ad	-	+
		M	Juv	-	+
	March 1976*	F	Ad	-	-

KEY: + 1 parasite per 0.04 ml of wet mount.
++ 2-4 parasites per 0.04 ml of wet mount.
+++ 4 or more parasites per 0.04 ml of wet mount.

* Captured from Coles County.
@ Captured from Lake County.
# Mange observed.

Table 2. Age - sex distribution of squirrels infected with Microfilariae and Hepatozoon. Data expressed as number infected / number examined.

		MICROFILARIA INFECTION				HEPATOZOON INFECTION			
Species	N	Male	Female	Adult	Juv	Male	Female	Adult	Juv
Fox (*Sciurus niger*)	14	3/4	5/10	6/11	2/3	2/4	3/10	3/11	2/3
Gray (*Sciurus carolinensis*)	6	0/3	0/3	0/4	0/2	2/3	2/3	2/4	2/2

Figure 1. Microfilaria recovered from the blood of fox squirrel (*Sciurus niger*). NR = nerve ring, E = excretory pore, G = rectal cell, AP = anal pore.

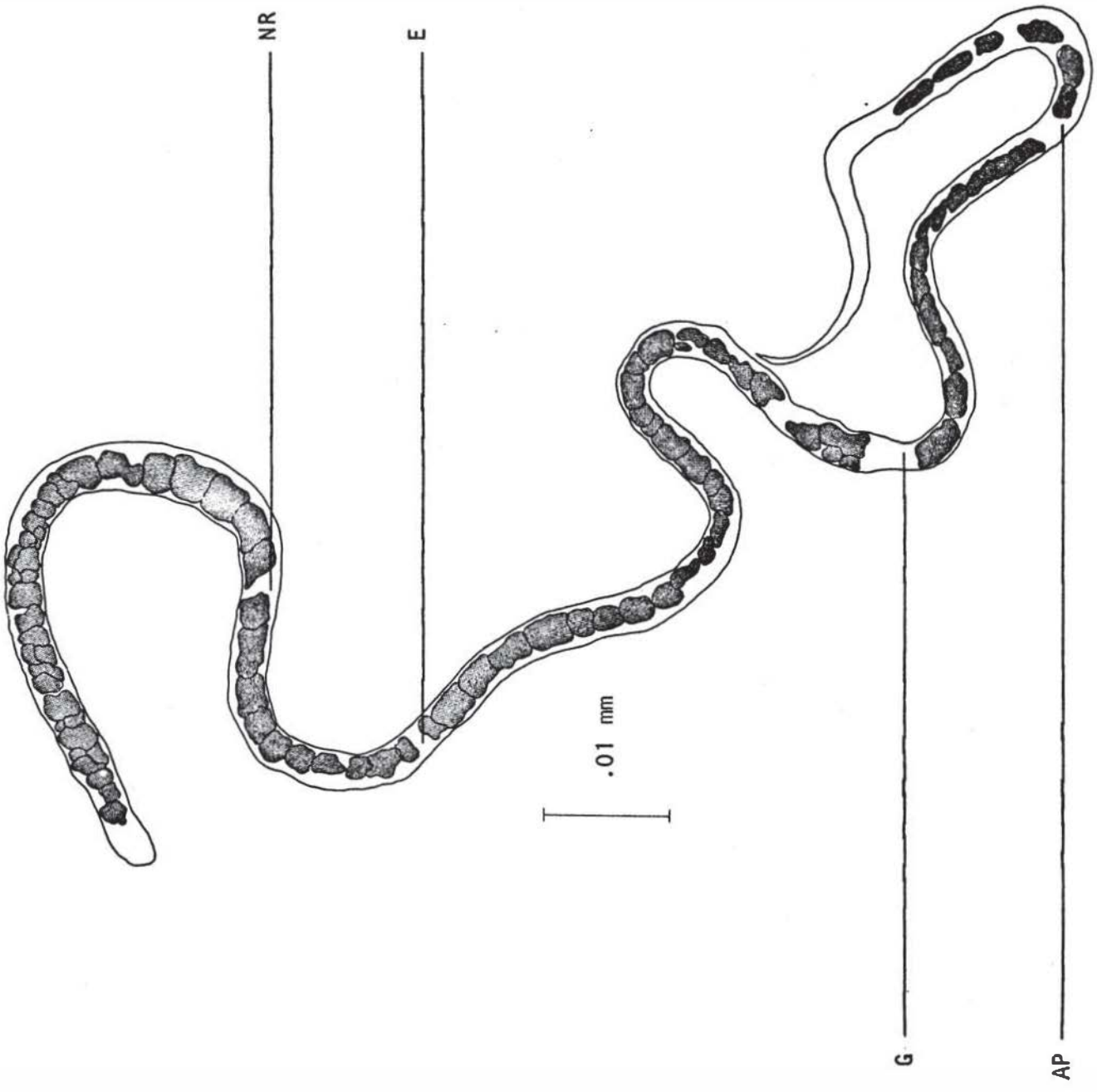

Figure 1

Figure 2. Microfilaria recovered from the blood of fox squirrel (*Sciurus niger*). NR = nerve ring, E = excretory pore, G = rectal cell, AP = anal pore.

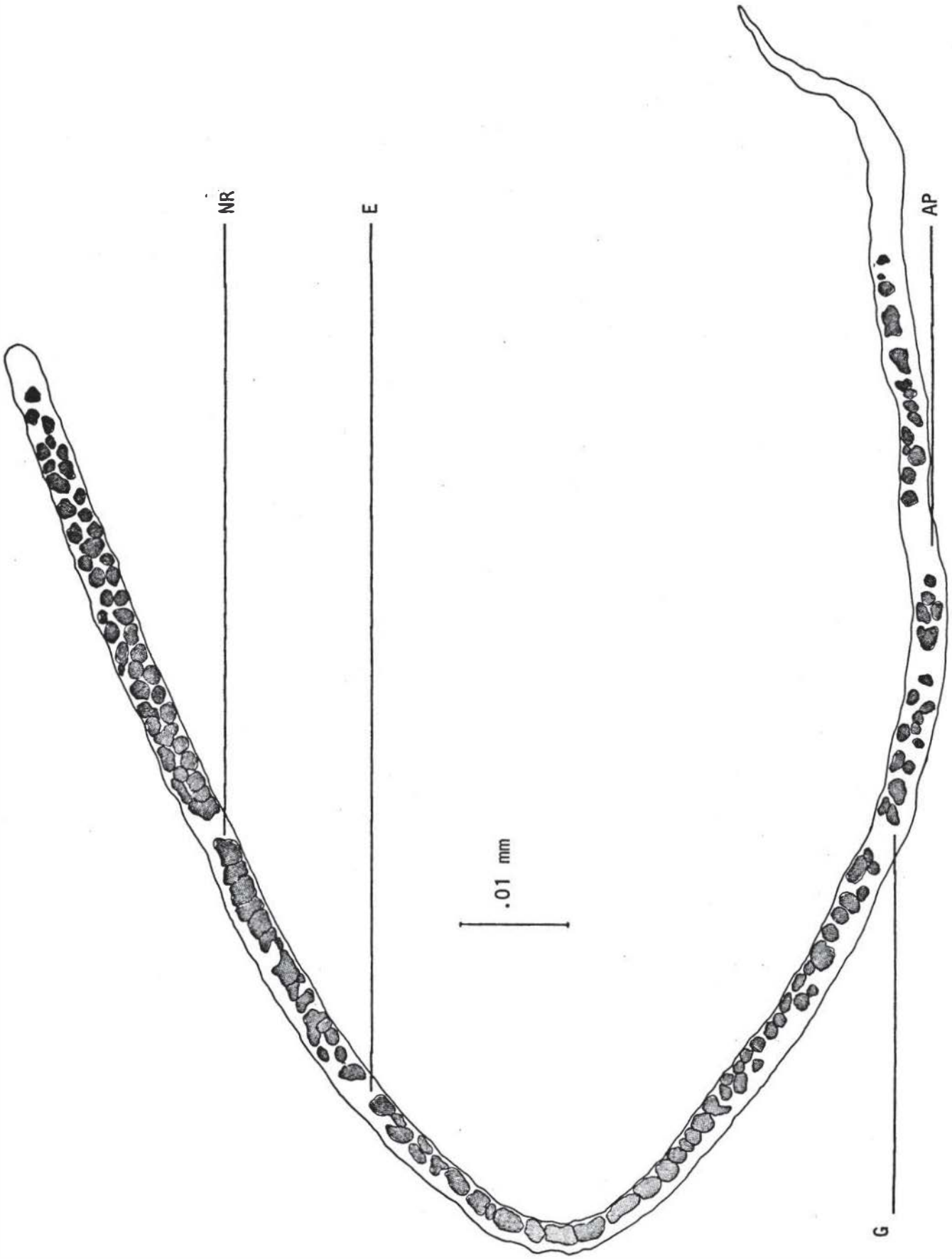

Figure 2

DISCUSSION

Adults accounted for 66.6% of the gray squirrels and 77.7% of the fox squirrels captured suggesting an abundance of adults in this period. This agrees with Brown and Yeager's (1945) data on age classes of squirrels captured in Illinois over a three year period.

Although microfilariae were not observed in the gray squirrels examined, they do host filariid worms (Chandler 1942; Price 1962; Davidson 1975, 1976). However, since the number of gray squirrels examined in this study was small and because five of a total of six animals examined were obtained from the same site, it is reasonable that no worms were found.

The fox squirrels that hosted microfilariae also hosted Hepatozoon except in three instances. Therefore, the presence of one of the parasites does not exclude the other. Possibly two different vectors are responsible for transmission although the mode of transmission of both parasites may be such that if the vectors are available, a host could be infected by both parasites from the same vector. The five gray squirrels from Lake County were not infected with microfilariae. These squirrels were captured in a suburban residential area whereas the Coles County squirrels were captured in a more sylvan setting. Mosquito control measures may have reduced the mosquito population in the suburban area. As such contact between squirrels and mosquito vectors of microfilariae would be fewer in Lake County. This may explain the lower microfilariae infection in the few Lake County squirrels examined.

Literature on filarial infection in Illinois squirrels is scanty. Rausch and Tiner (1948) found Citellium bifurcatum in fox and gray squirrels from McHenry County, Illinois but no mention was made of the occurrence of microfilariae in the peripheral blood. The microfilariae described in this study are morphologically identical to Dirofilariaeformia pulmoni described by Davidson (1975) from a gray squirrel in Dorchester County, Maryland, and it is presumed that both are the same species. However, positive identification demands recovery and description of adult worms, elucidation of preferred vectors and life history studies.

No data on the transmission of Dirofilariaeformia pulmoni were found but there are reports on the transmission of other filaria (Evans 1958; Christensen et al. 1976). Oral ingestion of filarial larvae by jirds was observed under experimental conditions and this mechanism was proposed as a mode of transmission in jirds (Sullivan 1976; Bosworth et al. 1976). Ticks and mosquitoes are intermediate hosts for some filaria worms (Londono 1976; Evans 1958; Christensen et al. 1976; Sullivan 1976; Bosworth et al. 1976). Since both arthropods were available at the sites of capture, either or both could have been the vectors involved.

The data do not suggest a sex-based difference in infection level. More adults than juveniles appear to be infected with microfilariae but when these numbers are compared with the number of adults and juveniles captured (15 adults and 5 juveniles) there was found a 40% infection in both cases. It would therefore seem that there is no age-based preference for Dirofilariaeformia infection in these animals. Price (1962) reported a similar observation in connection with Dipetalonema infection in squirrels.

As regards Hepatozoon infection, the above data indicate a higher infection in gray squirrels than in fox squirrels (11.1% : 35.7%). Clark (1958) demonstrated that the mite Euhaemogamasus ambulans, frequently found in squirrel nests is the natural vector of Hepatozoon griseisciuri but under laboratory conditions, development of H. griseisciuri occurred in a shorter time in Echinolaelaps echidninus. His study also showed that the mosquito Aedes aegypti previously considered a vector of Hepatozoon does not support sporogonic development of the parasite. The higher Hepatozoon infection observed in the Lake County gray squirrels which were actually captured in a suburban residential area may therefore be due to more host-vector interaction, or a difference in host resistance to Hepatozoon infection between the two squirrel species. Whether specimens are differentiated on a habitat or species basis, a higher infection of Hepatozoon griseisciuri is observed in juveniles than in adults. Davidson and Calpin (1976) have postulated that this may be related to midwinter mortality in gray squirrels. Therefore, the acquisition of a natural immunity against these protozoon parasites would be of survival value to these species. A lower infection rate in adult gray squirrels indicates that the animals become less susceptible to Hepatozoon infection as they grow older. Furthermore, Clark (1958) noted that the population of the mite Euhaemogamasus ambulans which he proposed as a natural vector of H. griseisciuri was high in squirrel nests when young were present. This study also indicated prebirth infection in these animals. Both of these observations favor a higher infection rate in juveniles than adults.

There are no accounts of Hepatozoon or microfilarial occurrence in chipmunks and thirteen-lined squirrels. The samples examined in

this study were captured at the same sites as the fox squirrels. The absence of these parasites is therefore probably due to host specificity of the parasites rather than lack of suitable vectors. In addition, since these are ground squirrels with different habits from tree squirrels, exposure to vectors may be reduced to such an extent that infection is prevented. A larger number of thirteen-lined ground squirrels need to be examined to determine whether or not these animals host Hepatozoon and microfilariae.

The similarity of these microfilariae to those described by Davidson (1975) suggests that the filarial worm involved may be Dirofilariaeformia pulmoni. If so, it is the first account of this nematode's occurrence in Illinois. The importance of further studies directed at understanding a broader spectrum of the host-parasite relations between filarial worms and their squirrel hosts in Illinois is appreciated and proposed.

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LOCATION OF TRAPPING SITES

Site 1: 8¼ miles south east of Charleston, Coles County, Illinois. (S.E.¼, S.E.¼, Section 33, T. 12N., R. 10 E.).

Site 2: 13½ miles north east of Charleston, Coles County, Illinois. (S.E.¼, S.E.¼, Section 11, T. 13N., R. 10 E.).

Site 3: Burgner Acres. 7 miles N.W. of Charleston, Coles County, Illinois. (S.W.¼, N.E.¼, Section 1, T. 12N., R. 8 E.).

Site 4: 8 miles south east of Charleston, Coles County, Illinois. (N.E.¼, N.E.¼, Section 4, T. 11N., R. 10 E.).

Site 5: Township of Antioch, Lake County, Illinois.

PARASITES OF NORTH AMERICAN SQUIRRELS

Fox and gray squirrels like most other game animals host a wide range of external and internal parasites (Price 1954, 1962; Eads and Hightower, 1952). Some of these result in hair loss, others cause a wastage of squirrel meat or other more serious pathogenic effects. Diesch et al (1967) reported four cases of leptospirosis in hunters which may have been acquired from squirrels.

PROTOZOON PARASITES

Members of the family Haemogregarinidae (Sporozoa) require 2 hosts to complete their life cycle. They inhabit circulatory vessels of vertebrates and the digestive tract of invertebrate vectors (Cheng 1964). Herman and Price (1954), Clark (1958) and Davidson (1976) have all described the occurrence of Hepatozoon in gray squirrels. Clark (1958) identified the species as Hepatozoon griseisciuri after studying the life cycle and indicated congenital infection across the placenta. His studies showed that the mites Euhaemogamasus ambulans and Echinolaelaps echidninus sustained sporogonic development of the species. Davidson (1976) suggested that this protozoon may have more pathogenic effects than was realized and may in fact be responsible for late winter mortality in the gray squirrels that host them.

In another publication, Davidson (1976) observed Eimeria ascotensis, E. lancasterensis, E. moelleri, E. neosciuri, E. confusa and E. ontarioensis in saline smears of gray squirrels. Webster (1960) noted significant

lesions due to Eimeria neosciuri but Davidson's study did not indicate any significant lesion due to coccidiosis. Davidson's study also established the gray squirrel as a new host for Sarcocystis.

ARTHROPOD PARASITES

Fox and gray squirrels are parasitized by a variety of arthropods. While affecting the squirrels directly, some of these also serve as intermediate hosts of other squirrel parasites. Most of the arthropod parasites belong to two classes -- class Insecta and class Arachnida.

Class Insecta

Members of 3 main orders have been commonly observed on fox and gray squirrels. Sucking lice (order Anoplura) were observed by Harkema (1936) in North Carolina, by Baker (1944) in East Texas, in Virginia by Parker and Morlan (1952) and Parker (1968). Brown and Yeager (1945) observed less than 1% occurrence on Illinois squirrels.

Fleas (order Siphonaptera) were observed by Parker (1968) in Virginia, by Baker (1944) in East Texas, and Harkema (1936) in North Carolina. Freeman (1941) determined that although the flea Orchopeas wickhami was first recognized in Britain, it is more or less specific to the American gray squirrel. Dasgupta and Meedeniya (1958) considered this particular species a vector of Hepatozoon sciuri. Orchopeas howardii was commonly found in overwintering nests of Illinois squirrels (Ross, cited by Brown and Yeager 1945).

Atkeson and Givens (1951) found the larvae of the Cuterebra species of heel fly (order Diptera) in gray squirrels and noted that the resulting sore causes a wastage of squirrel meat. Squirrels are also parasitized by mosquitoes in some of which the development of filarial nematodes have been observed (Evans et al. 1959, Christensen et al. 1976).

Class Arachnida

Most arachnid parasites of squirrels belong to the order Acarina and are essentially mange mites and ticks. Chandler (1942), Graham and Uhrich (1943), Baumgartner (1940), and Allen (1942, 1943) have all noted the occurrence of mange in fox and gray squirrels from Southeast Texas, Southeast Kansas, Ohio, and Michigan respectively. However, Brown and Yeager (1945) reported only 0.94% occurrence of mange in the 1,975 fox and gray squirrels examined over a two year period in Illinois.

NEMATODE PARASITES

Reports of Helminth infection in North American squirrels include that of Chandler (1942) who also found 2 kinds of microfilariae in 17 fox and gray squirrels of Southeastern Texas, Graham and Uhrich (1943) who found Ascaris and Trichonstrongylus along with other helminths. Rausch and Tiner (1948) surveyed the North Central States Sciuridae for helminth parasites. Specimens were examined from McHenry County in Northern Illinois and these were reported to host only the namatode Citellinema bifurcatum. Squirrels from Ohio, Michigan and Minnesota hosted other nematodes in addition. Parker (1968) identified 5 species of nematodes in 16 of 55 Southwest Virginia gray squirrels examined.

In 1962 Price described an adult Dipetalonema interstitium and microfilariae from eastern gray squirrels collected from Maryland. This finding was repeated by Davidson in 1975 who also described a new species, Dirofilariaeformia pulmoni, from the lung tissue. His report included a description of microfilariae found in the animals peripheral blood. He also noted that in one case an adult worm and microfilariae were surrounded by a thrombus containing scattered lymphocytes. The thrombus occluded about two-thirds of the pulmonary artery lumen.

More recently, the taxonomic status of *Dipetalonema fausti* described by Esslinger in 1966 from the liver of a Chinese scaly anteater (*Manis pentadactyla*) was changed to *Chenofilaria filaria*, Kou 1958. The later name having been found to be an older synonym (Esslinger 1976). Meanwhile, Eberhard et al. (1976) described a new genus and species of filaria *Cruorifilaria tuberocauda* from kidney arteries of the Columbian capybara (*Hydrochoerus hydrochaeris*). The report includes a description of an unsheathed microfilariae from the peripheral blood. Prestwood (1976) has also described a new genus and species of filaria, *Didelphostrongylus hayesi*, from the lung of the opposum (*Didelphis marsupialis*) in Georgia. Her report includes a description of the first and third larval forms. The snail *Triodopsis albolabris* is indicated as an intermediate host.

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