PROTOZOAN, HELMINTH AND ARTHROPOD PARASITES OF THE GRAY SQUIRREL IN SOUTHWESTERN VIRGINIA

by

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PROTOZOAN, HELMINTH AND ARTHROPOD PARASITES OF THE GRAY SQUIRREL IN SOUTHWESTERN VIRGINIA

An investigation into the parasites of the gray squirrel, Sciurus carolinensis pennsylvanicus Ord, 1815, in Virginia was begun in September, 1966, at Virginia Polytechnic Institute and State University. The study involved a qualitative and quantitative survey of all parasites of squirrels collected in Montgomery, Craig and Giles counties of southwestern Virginia.

From September, 1966, to July, 1969, a total of 176 squirrels were collected (87 males and 89 females). No fewer than ten squirrels were taken for each month of the year (Table I). Since most specimens were from Montgomery county and only a few from Giles and Craig counties, all will be considered together. It is not feasible to compare host-parasite data on the basis of individual counties due to the large differences in the numbers of hosts collected in each county.

The squirrels were taken by shooting or by live trapping in box traps (Mosby, 1955). Upon collection, cardiac blood samples were taken, blood smears made on glass slides and the carcass placed immediately into a plastic bag to be returned to the laboratory. In the laboratory the animals were examined for protozoa, helminths and arthropods (ectoparasites). Since the literature, materials and methods vary with the several phases of the problem, they are discussed in the appropriate places in the text.

TABLE I. Numbers of gray squirrels collected for study of parasites from the counties of Montgomery, Giles and Craig of southwestern Virginia, September, 1966-July, 1969

Month	Montgomery County		Giles County		Craig County		Total
	males	females	males	females	males	females	
January	3	3	0	0	3	1	10
February	7	9	0	0	0	0	16
March	5	5	0	0	0	0	10
April	8	6	0	0	0	0	14
May	5	6	0	0	0	0	11
June	8	4	0	0	0	0	12
July	9	2	0	0	0	0	11
August	5	7	0	0	0	0	12
September	14	20	0	0	0	0	34
October	9	12	0	1	0	0	22
November	4	4	3	2	0	0	13
December	1	4	1	1	2	2	11
Total	78	82	4	4	5	3	176

The host, Sciurus carolinensis, is a mammal of the order Rodentia, suborder Sciuromorpha and family Sciuridae. It is commonly referred to as the gray squirrel, eastern gray squirrel, common gray squirrel or cat squirrel. In Virginia, Shipley (1941) and Cross (1942) proposed the Appalachian Mountains to be the geographic barrier between the southern subspecies, Sciurus carolinensis carolinensis, on the southeast and the northern subspecies, S. c. pennsylvanicus, on the northwest. It is apparent that gradations between these occur and that there is an intergradation of subspecific characters across this boundary.

According to Hall and Kelson (1959) the measurements of the gray squirrel vary from 430 to 500 mm for total length, 210 to 240 mm for tail length and 60 to 70 mm for hind foot length. Their list of weights varied from 400 to 710 grams. Apparently these data represent a set of averages from samples taken over the range of the species rather than the variation for the total measurements made.

Uhlig (1955) indicated that there was little difference in weight between the sexes of adult squirrels of West Virginia and some other states. Also squirrels at higher elevations appeared to weigh more than those of lower elevations. His weight data on squirrels from various states indicated a north-south cline in average weights, with the heavier squirrels to the north and the lighter ones to the south. Also the weight gradient appeared steeper in areas where the southern and northern subspecies intergrade. This weight cline is in agreement with Bergmann's principle.

In the present study, a number of adult male and female squirrels were measured and weighed (Table II). The averages of these data show

TABLE II. Measurements of adult gray squirrels, Sciurus carolinensis, collected in southwestern Virginia, September, 1966-July, 1969

	Number measured		Size range	
	males	females	males	females
Total length	21	26	402-530 mm Avg. 469 mm	402-550 mm Avg. 469 mm
Tail vertebrae length	20	27	175-255 mm Avg. 209 mm	170-230 mm Avg. 207 mm
Hind foot length	22	28	60-70 mm Avg. 66 mm	56-69 mm Avg. 65 mm
Ear length	22	27	30-35 mm Avg. 32 mm	29-35 mm Avg. 32 mm
Weight	48	53	442-612 g Avg. 518 g	370-646 g Avg. 518 g

no appreciable differences in standard measurements and weights between the sexes and indicates a standard size of adult animals for Montgomery County, Virginia.

The mean weights and measurements of 18 male and 19 female adult gray squirrels from Georgia (Golley, 1962) were consistently less than those included in the present study, but the weights of 28 males and 24 females from Blacksburg, Virginia (Uhlig, 1955) were not significantly different. Average weights given for localities at higher latitudes (Uhlig, 1955) were consistently greater.

The evolutionary history of North American sciurid rodents is poorly known. According to Bryant (1945), there are few fossil sciurids from deposits older than the Upper Miocene. It is Bryant's opinion that the family probably originated during the Eocene. Apparently the family has been derived from the protrogomorphs and appears more closely related to the family Paramyidae (Black, 1963; Wilson, 1949, 1960; Wood, 1955, 1959, 1962) than to any other family of rodents. Also, no other family of primitive rodents possess features which appear to be possibly ancestral to the Sciuridae (Bryant, 1945). Moore (1959) indicates that the ancestors of the northeastern gray squirrel probably evolved in the Old World rather than the New. If this is the case, ancestral tree squirrels must have come across the land bridge where the Bering Strait now separates Siberia and Alaska. It also seems possible that the most recent crossing of the Bering land bridge by tree squirrels could have been as long ago as the Pliocene (Moore, 1959). Black (1963) concluded that squirrels could have moved either of the two directions across the Bering land bridge and leans more toward their having arisen in the

Nearctic region.

The present distribution of the gray squirrel is closely correlated with the distribution of the deciduous forest biome of eastern North America and adjacent areas. They are especially attracted to oak and hickory hardwood stands and, formerly, to chestnuts. According to Bakken (1959), the utilization of foods by gray squirrels varies with the availability. Such foods as acorns, hickory nuts, beech nuts, walnuts, maple samaras, elm seeds, fruits, berries, buds and agricultural crops vary in importance with seasonal availability. Sometimes meat or insects are eaten, but this usually accounts for a small percentage of the diet.

The gray squirrel has been successfully introduced beyond the range of its original habitat. These areas include Colorado and North Dakota (Bakken, 1959), British Columbia (Robinson and Cowan, 1954), Saskatchewan (Nero, 1958), South Africa (Davis, 1950) and England. According to Shorten (1959), major introductions of the gray squirrel in England took place between 1876 and 1929. At least thirty releases took place with the most important center at Woburn Abbey in Bedfordshire. Most of these releases were successful in establishing thriving colonies. Currently, most of England and Wales are populated with gray squirrels which are spreading to Ireland and Scotland. These introduced squirrels appear to have been intermediate between, or a mixture of, the northern and southern subspecies.

Concerning the reproduction of the gray squirrel, the first mating of the year usually takes place in mid-winter, after which one to four young are born following a gestation period of 44 days. A second litter

is commonly produced in late summer. Squirrels nest usually in tree holes, but commonly also in leaf nests. The reproductive biology of this animal has been studied by Deanseley and Parkes (1933), Hibbard (1935), Peery (1948), Flyger (1952), Hoffman and Kirkpatrick (1959) and Brauer and Dusing (1961).

Gray squirrels formerly displayed mass "migrations," or emigrations (since they rarely return to the original location), of many thousands of individuals. Migrations in modern times have been mostly small and localized. The literature concerning migrations include Seton (1920), Fryxell (1926), Shadbolt (1933), Goodwin (1934), Jackson (1935), Hoover (1936), Moore (1942), Schorger (1947) and Fichter (1950). I had the opportunity to witness a gray squirrel "migration" in September, 1968, in North Carolina. This was part of a general "migration" which took place in the east and south-east part of the United States during the month of September, 1968. The observations of this phenomenon have been released by Citron (1968), at the Smithsonian Institution Center for Short-lived Phenomena, and by Flyger (1969). Since high population densities appear to be one possible stimulus for "migrations," it might be anticipated that squirrels from such areas would have a greater burden of parasites than would normally be found. Therefore, a few freshly-killed squirrels were obtained from the afore-mentioned "migration" and examined to determine their parasitic infestations. The findings of this infestigation will be given in the Addendum at the end of this text.

An interest in studying the parasites of the gray squirrel stemmed from an interest in squirrels as game animals. The gray squirrel is a familiar animal to most people in the eastern states and is one of the

most popular small game animals. Millions of squirrels are killed every year and hunters and hunting dogs come in contact with the carcasses. For this reason it would be of clinical importance to have a better understanding of the parasites of the animals and their potential transfer to man or domestic animals. Squirrels have also been known to suffer high mortality rates in some areas, the reasons for which are not understood. Therefore, the present study was proposed to examine in detail a large number of squirrels for parasites, the results of which will be useful to workers in wildlife management and may have public health significance.

The literature concerning such a broad area of study is naturally widely scattered and extensive. Almost 100 parasites of squirrels have been reported in the literature. Many of these reports were published in short notes or from surveys dealing with several species of animal hosts. Also many reports concern only one taxonomic group under study, such as only helminths or only ectoparasites or divisions of these. All of these come from widely scattered areas over the range of the host species and some even from areas to which the gray squirrel has been introduced. The present study is the most comprehensive ever attempted in any one locality.

Among the more intensive studies that have been concerned with gray squirrel parasites were those made by Harkema (1936) who studied 53 gray squirrels in North Carolina and recorded both endo- and ectoparasites; by Katz (1938) who reported endo- and ectoparasites from 72 gray squirrels in Ohio; by Goodrum (1940) who described the occurrence of ectoparasites on this host in Texas; by Chandler (1942) who reported helminths from

tree squirrels in southeastern Texas; by Brown and Yeager (1945) who discussed the ectoparasites of gray squirrels in Illinois; by Rausch and Tiner (1948) who studied the helminths of this host in the North Central states; by Morlan (1952) who reported many ectoparasites from squirrels in Georgia; by Uhlig (1956) who reported both ecto- and endoparasites of gray squirrels in West Virginia; and by Watson (1959) who reported the insect ectoparasites of 267 gray squirrels from Florida. Summaries of the literature dealing with squirrel parasites have been given by Katz (1939) and Clark (1959).

This study will be presented in three major divisions: Protozoa, Helminths and Arthropods. Also included in an Addendum will be notes from studies not directly related to the problem under discussion.

I. PROTOZOA

The blood and intestinal contents of the squirrels were examined to determine if protozoa were present. It was soon evident that three genera of protozoans were present: intestinal coccidia, Eimeria spp.; a haemogregarin in the blood, Hepatozoon sp.; and one intestinal flagellate, Giardia sp.

The literature concerning protozoans from this host is not very extensive and, to the present date, only studies concerning coccidia and Hepatozoon are available. Doran (1954a) provided an excellent comprehensive review of the literature concerning the protozoa reported from rodents in which the gray squirrel representation was very limited. Levine and Ivens (1965) published the single most important work on rodent protozoology. They compiled the descriptions of coccidia and criticized the taxonomic status of species in the genus Eimeria and other genera.

A. Coccidia

There has been much inconsistency in the describing and naming of new species of coccidia. As in many other branches of taxonomy we encounter the so-called "lumpers" and "splitters," and since the number of species of coccidia have been steadily increasing, the problems are steadily getting more complicated. However, over the past few years descriptions have been becoming more standardized and new

techniques are providing more detailed accounts of morphological features. Furthermore, experimental transmissions to other hosts have shed more light on the host specificity of various species.

Since the American gray squirrel has been introduced into various parts of the world, the coccidial studies on this host have been widely scattered. Incidence of coccidiosis has been reported from Sciurus carolinensis in Ohio by Chapman (1938), in Kentucky by Bertram and Gault (1952) and in Virginia by Hanson (1966). Eimeria sp. was reported by Katz (1938) in Ohio. The parasite identified as Eimeria sciurorum Galli-Valerio, 1922 by Möller in 1923 which he found in American gray squirrels in the Berlin Zoological Garden has been redescribed as E. moelleri by Levine and Ivens (1965) because Möller was in error in placing his specimen in that taxon, and Levine and Ivens realized that his organism was a new species. Eimeria neosciuri was described from the American gray squirrel in England by Prasad (1960). Webster (1960) studied coccidia from this host in England simultaneously and independantly from Prasad and considered his species to be the same as that described by Prasad. However, Levine and Ivens (1965) later named the species described by Webster, Eimeria ascotensis. Eimeria sciurorum Galli-Valerio, 1922, was reported in Florida by Bond and Bovee (1958), but Levine and Ivens (1965) considered this to be a different species of Eimeria. Soon and Dorney (1971) described two new species of coccidia from the gray squirrel in Ontario, Canada. These were named Eimeria ontarioensis and Eimeria wongi. They also reported on, but did not name, a species of Eimeria from Ontario gray squirrels which resembled both Eimeria ascotensis and Eimeria neosciuri.

Materials and Methods

From gray squirrels shot and killed in the field or captured alive in box traps, fresh fecal samples or contents of the large intestine were taken and 1 ml of the sample added to 24 ml of 2% potassium dichromate solution to make a 1 to 25 dilution. The potassium dichromate killed bacteria and other organisms but did not harm the resistant coccidial oocysts. The fecal material was crushed and allowed to dissolve until a homogeneous mixture was obtained. The latter was then poured into a petri dish and allowed to stand at room temperature in the covered container for several days or until sufficient time for sporulation. The mixture was stirred every day to insure aeration of the oocysts.

Estimates of coccidial oocyst numbers were made prior to pouring the mixture into the petri dishes for sporulation. In order to make these estimates, the mixture was first stirred until sufficiently mixed and the heavier materials were allowed to settle (about one minute). Then, using a Pasteur pipette, a small amount was drawn from the center of the suspension and transferred into a Petroff-Hausser counting chamber. Under 430 diameters magnification, the coccidial oocysts occurring on the grids were counted (five alternate 0.1 mm squares each from the two grids in the chamber). Since the chamber was 0.1 mm deep and a total of 10 0.1 mm squares were counted, the volume examined amounted to 1 cu mm. This count was then multiplied by the dilution factor and the final figure expressed as the number of oocysts per gram of feces. Since the samples were a 1 to 25 dilution,

oocysts numbering less than 25,000 per gram of feces might not be detected. Such apparently negative counts were recorded as less than 25,000 rather than as negative in the event that the dilution would obscure very low infections.

To separate the oocysts from the potassium dichromate solution, the larger fecal debris was removed by filtration through a number 50 screen, then the filtrate was centrifuged and washed several times using tap water. This concentrated the oocysts into a small volume of fluid and made them readily available for microscopic examination. The concentrated oocysts were retained for study when fresh material was not available. The sporulated oocysts were kept under refrigeration for as long as a year and still appeared to be viable.

The microscopic study of the oocysts was conducted using a Leitz Ortho-Lux microscope equipped with an apochromatic 40/0.95 objective and binocular 20 X eyepieces. Greater contrast for observation was gained by the use of various combinations of colored filters and light intensity. Phase microscopy was used for some observations, but all measurements were made under bright field illumination using a calibrated ocular micrometer. All measurements were recorded in microns. Drawings were made from notes, sketches and average measurements.

Material described in this study was taken from pooled samples from several hosts and also from individual squirrels having less frequent forms. Structures of the oocysts were measured as they were encountered; only a small number of oocysts were measured per slide to avoid duplication and, where possible, similar material was examined

from several different squirrels. These procedures were followed in order to minimize any bias.

In all instances, the material was examined as a wet mount preparation. It was found that after the slide had partially dried around the edges of the coverslip, a small drop of thin lubricating oil could be pulled around the edge of the coverslip and completely seal the slide. Such a preparation was still usable after several days. Oil was found to be more practical in the prevention of slide drying than Vaseline, which was first used.

Portions of the small intestine were fixed in 10% neutral buffered formalin. Histological sections were prepared by embedding in paraffin, sectioning at 10 microns and staining the mounted section with hematoxylin and eosin.

Results and Discussion

Fecal samples from 167 gray squirrels were examined by means of wet mount observations to determine the incidence of coccidia infections. Samples from 152 of these squirrels contained coccidial oocysts i.e., 91% of the squirrels examined were infected. Although the wet mount technique is definitely less efficient than other methods which have been used, e.g., flotation techniques, that may reveal the presence of coccidial oocysts in all cases, its use in the present study expedited the handling of large quantities of material. The time conserved facilitated the detailed observations which follow.

Estimates of coccidial oocysts were made on 48 squirrels of the total number examined in the wet mount observations above. Since these estimates were from individual hosts collected at different times and conditions, it was anticipated that these estimates might provide some insight into the varying intensities of natural infections of coccidia (Table III). From these estimates the average number of coccidia from 28 males was somewhat higher than the average estimated from 20 females. These figures are difficult to compare however, because of the wide range of variation of the individual estimates and the several enormous counts obtained and are, therefore, of no significance.

The one estimate of 3,000,000 oocysts per gram of feces in a male squirrel accounted for the great difference in the averages between the males and females. It is my opinion that probably there are no actual differences in average intensity of infections between the sexes in the whole host population. Since the squirrels I collected were not randomly sampled from the population, similar samples would not necessarily show these same averages. I believe that the extremes of variation in the numbers of coccidial oocysts between individual hosts in the present study indicate that similar extremes are to be expected in the population as a whole.

Although male squirrels appear to have greater home ranges than the females (Bakken, 1952; Robinson and Cowan, 1954; Doebel, 1967), which might influence the incidence of infection in favor of the males, this does not determine intensities of infection. One infective oocyst ingested by a host is sufficient to establish an infection.

TABLE III. Estimates of coccidial oocysts made on 48 gray squirrels from southwest Virginia, August, 1968-July, 1969

Number oocysts per gram of feces	Males	Females
Less than 25,000	2	1
25,000	3	6
50,000	1	3
75,000	6	4
100,000	1	1
150,000	3	1
175,000	1	1
200,000	3	1
250,000	2	0
275,000	1	0
325,000	0	1
350,000	1	0
650,000	1	0
750,000	1	0
1,000,000	1	0
1,875,000	0	1
3,000,000	1	0
Totals	28	20
Estimate averages	324,000	180,000

Therefore the multiplication within the host is apparently due to host-parasite physiological interactions rather than to the initial dosages of the infection. Because of this, the production of oocysts in the course of an infection vary from time to time. Host resistance and susceptibility is little understood.

Only 48 of the hosts examined in the present study were used for estimates of coccidial oocyst numbers because this procedure was begun late in the study and could only be accomplished with precision when the squirrel was actually collected by the author.

No direct correlations between oocyst numbers and host size or age, nor seasonal variations, were indicated by the present observations. The estimates made did show that natural coccidial infections can vary in intensity and on occasions may be very high.

Two morphologically distinct groups of coccidia were encountered in the squirrels of southwest Virginia. The first is characterized by medium-sized, smooth, clear, thin-walled oocysts and the second by large-sized, rough, brownish, thick-walled oocysts. In the first group, I have described what I believe to be three species, tentatively labeled as Eimeria species A, B and C; in the second group, two distinct species have been identified by their specific names. A description and discussion of these follows.

All coccidia observed in this investigation belong to the genus Eimeria, with possibly one exception. Only two oocysts of a coccidian that resemble those of species of Isospora were observed. These are ellipsoidal, clear, thin-walled oocysts that have two subspherical sporocysts that appeared to contain four sporozoites. Enough specimens

are not available for a description. However, this might have been a morphological anomaly of a species of Eimeria since the size of the oocyst is within the range of the thin-walled Eimeria species in the same sample, is basically the same shape and only two were observed.

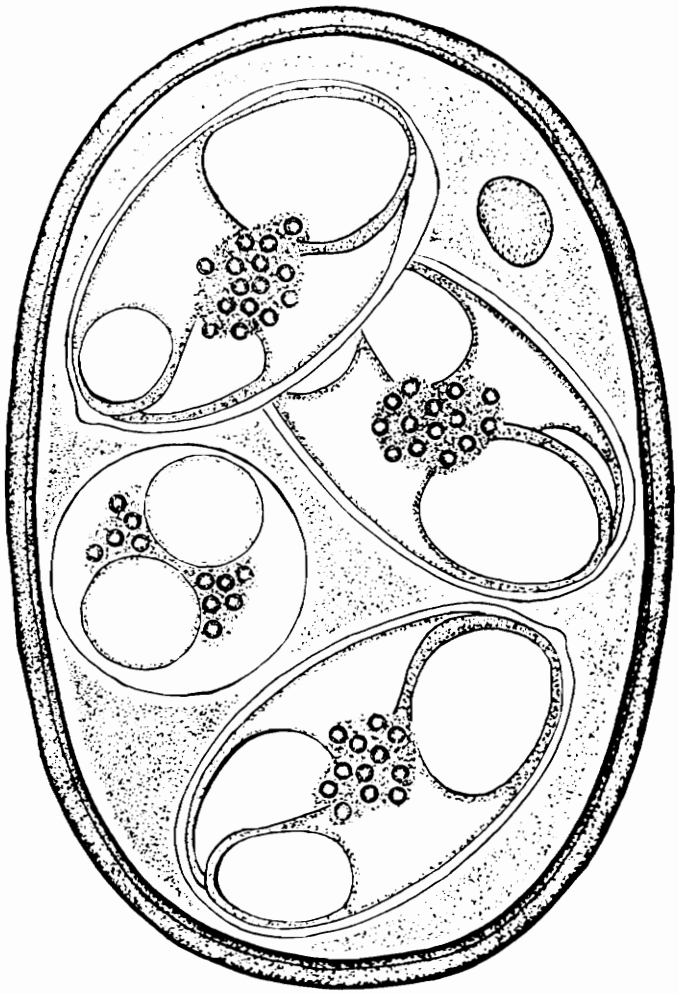
Eimeria sp. A

The oocysts (Figure 1; Plate 1) are ellipsoidal in shape, rarely subspherical. One hundred sporulated oocysts measured 17.0 to 28.0 microns (s.d. 2.7) in length by 10.7 to 18.2 microns (s.d. 1.5) in width, with an average of 22.2 by 14.1 microns. The length to width ratios ranged 1.2 to 2.1 (s.d. 0.2), with an average of 1.6.

The oocyst wall is smooth, composed of two layers and clear and colorless. The outer layer is slightly thicker than the inner layer and the inner layer is not visible until the oocysts are subjected to concentrated hydrochloric acid which separates it from the outer layer. The total wall thickness averaged 1.1 microns. No micropyle is present.

An oocyst residuum is absent, but usually one large or several small refractile granules or polar bodies are present (Plate 1) within the oocyst.

The sporocysts, numbering four per oocyst (tetrasporocystic), are ovoidal, with a small, often inconspicuous, Stieda body. Fifty-four sporocysts measured 7.0 to 11.9 microns (s.d. 1.2) in length by 3.9 to 8.4 microns (s.d. 1.1) in width, with an average of 9.7 by 6.0 microns. The length to width ratios ranged 1.3 to 2.3 (s.d. 0.2), with an



10 μ

FIGURE 1. Eimeria sp. A, mature oocyst

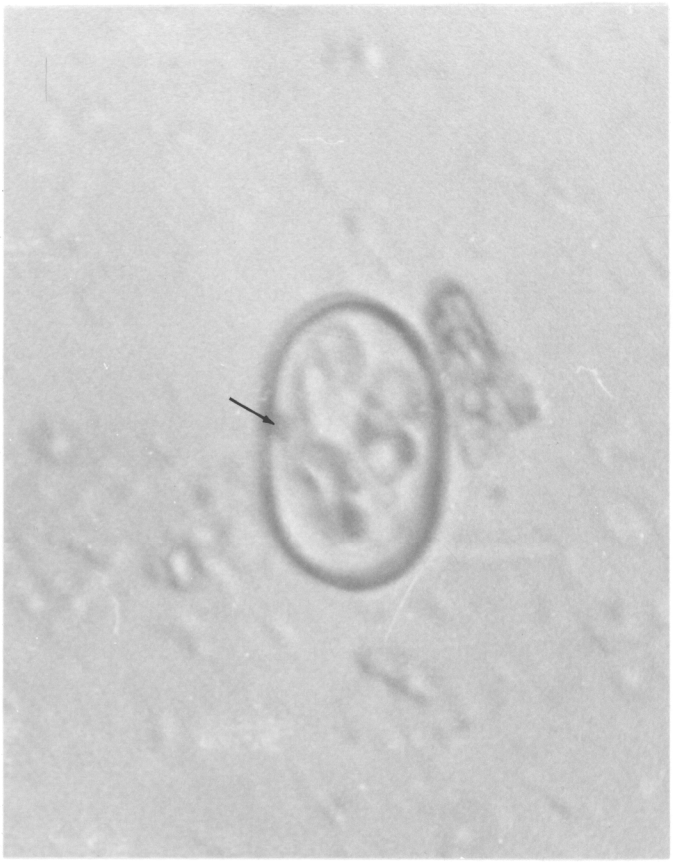


PLATE 1. Eimeria sp. A; photomicrograph of mature oocyst (arrow indicates large oocyst granule); 1800 x

average of 1.6. A sporocyst residuum was present, granular and interspersed between the sporozoites.

There are two sporozoites (dizoic) per sporocyst. The sporozoites are comma-shaped with a large refractile globule near the broad end and usually a smaller one near the narrow end.

This was the most common coccidian found in the gray squirrels in this study and was present in almost all squirrels infected with coccidia. The sporulation time was recorded at 2 to 3 days, with an average of 66 hours.

Histological sections of the ileum revealed the tissue stages of the coccidial parasites. However, due to multiple infections encountered in the naturally infected squirrels, it was impossible to distinguish the species of tissue stages.

Levine and Ivens (1965) cited 12 descriptions of small or medium-sized, thin-walled coccidia from tree squirrels. These include Eimeria serbica Pop-Cenitch and Bordjochki, 1957, Eimeria andrewsi Yakemoff and Gousseff, 1935, Eimeria sciurorum Galli-Valerio, 1922, Eimeria silvana Pellerdy, 1954, and Eimeria sp. Brunelli, 1935, from Sciurus vulgaris Linn., 1758; Eimeria moelleri Levine and Ivens, 1965, Eimeria neosciuri Prasad, 1960, Eimeria ascotensis Levine and Ivens, 1965, and Eimeria sp. Bond and Bovee, 1958, from Sciurus carolinensis Gmelin, 1788; Eimeria parasciurorum Bond and Bovee, 1957, from Glaucomys volans Linn., 1758; Eimeria sp. Henry, 1932, from Sciurus griseus Ord, 1818; and Eimeria kniplingi Levine and Ivens, 1965, from Sciurus niger Linn., 1758. Of these, Eimeria sp. A most closely resembles Eimeria neosciuri. Table IV provides descriptive comparisons of Eimeria sp. A

TABLE IV. Comparison of *Eimeria* sp. A from *Sciurus carolinensis* in southwest Virginia with *Eimeria neosciuri* Prasad, 1960, as described from *Sciurus carolinensis* in England

	Eimeria sp. A	*Eimeria neosciuri*
Oocyst size*	17-28 x 11-18 Avg. 22 x 14	22-28 x 14-18
Oocyst l/w ratio	1.2-2.1 Avg. 1.6	1.55-1.58
Oocyst shape	ellipsoidal, rarely subspherical	ellipsoidal
Oocyst wall	two layers, smooth, colorless	two layers, smooth; outer colorless, inner dark brown
Oocyst granules	+	+
Oocyst residuum	-	-
Micropyle	-	-
Sporocyst size*	7-12 x 4-8 Avg. 10 x 6	11-13 x 5-7
Sporocyst shape	ovoidal	ovoidal
Stieda body	+	+
Sporocyst residuum	+	+
Sporulation time	2-3 days	1½-2 days

*/ Measurements in microns

and E. neosciuri.

The only apparent difference between these descriptions is the size ranges of the oocysts. The smallest oocyst measured in the present study was 4.8 microns shorter and 3.0 microns narrower than that recorded by Prasad. The largest measurements of the oocysts were basically the same in the two studies. This criterion does not constitute a significant difference because size ranges would differ at different times during the infection (Becker, et. al., 1955; Webster, 1960; Soon and Dorney, 1971) and also the number of oocysts measured would greatly influence the range. Other characters were very similar in the two descriptions.

Eimeria sp. A differs from E. moelleri in that the latter has a micropyle and lacks a Stieda body; from E. andrewsi which lacks a sporocyst residuum and Stieda body and by the size of the sporocysts; from E. serbica which lacks a Stieda body; from E. sciurorum and E. ascotensis in their possessing a micropyle (or operculum); from E. parasciurorum by oocyst and sporocyst shape; from E. silvana by oocyst size; from E. kniplingi which has an oocyst residuum and a small micropyle and lacks oocyst granules; and from Eimeria sp. Bond and Bovee, 1958 in that the latter has a micropyle and oocyst residuum and lacks oocyst granules, Stieda body and sporocyst residuum.

Therefore, Eimeria sp. A is considered to be identical to Eimeria neosciuri Prasad, 1960. However, Soon and Dorney (1971) have indicated that they believe E. neosciuri and another described species may be morphological variations of a single species, and they have hesitated to assign them specific names until this question has been

resolved. Because my material appears similar to that studied by Soon and Dorney (1971), I have indicated the species each form resembles, but follow their example by calling them Eimeria species. This form is assigned the tentative name Eimeria sp. A.

Eimeria sp. B

The oocysts (Figure 2; Plate 2) are ellipsoidal to cylindrical. Eighty-one oocysts measured 17.3 to 33.6 microns (s.d. 2.7) in length by 11.9 to 18.2 microns (s.d. 1.4) in width, with an average of 26.3 by 14.7 microns. The length to width ratios ranged from 1.4 to 2.2 (s.d. 0.2) with an average of 1.8.

The oocyst wall is smooth, colorless and composed of two layers. The outer layer is slightly thicker than the inner layer. The inner layer is not clearly visible unless the oocysts are subjected to concentrated hydrochloric acid which separates it from the outer layer. The total wall thickness averaged 1.3 microns. A large micropyle (or operculum) which joined with oval refractile vesicles in the wall is usually prominent near one end and measurements of this structure in 20 oocysts ranged from 2.6 to 8.5 microns, with an average of 5.9 microns.

Usually one large refractile granule or polar body is present, but often hidden among the sporocysts. An oocyst residuum is absent.

Four sporocysts occur in each oocyst. The sporocysts are ellipsoidal and apparently have no Stieda body. Fifty sporocysts measured 8.7 to 15.4 microns (s.d. 1.3) in length by 5.1 to 8.7 microns (s.d.

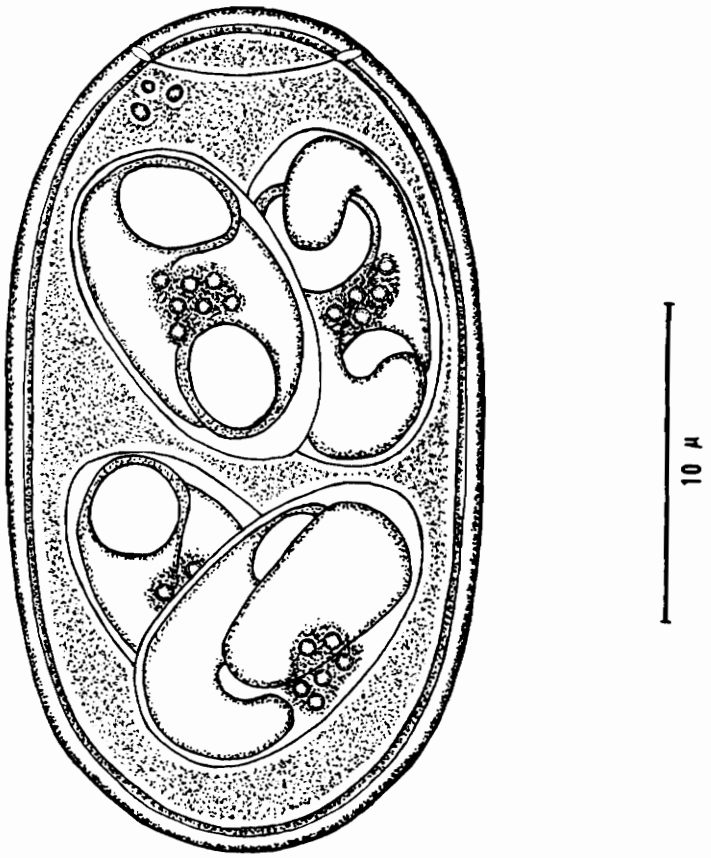


FIGURE 2. Eimeria sp. B, mature oocyst

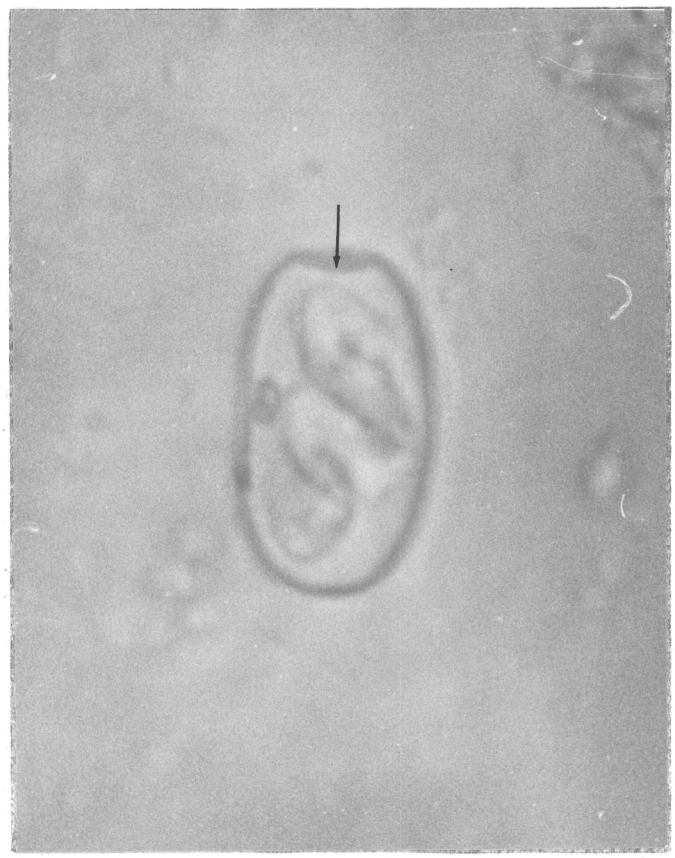


PLATE 2. Eimeria sp. B; photomicrograph of mature oocyst (arrow indicates micropyle); 1800 x

1.0) in width, with an average of 11.0 by 6.3 microns. The length to width ratios ranged 1.3 to 2.2 (s.d. 0.2), with an average of 1.8.

A sporocyst residuum is present and appears as a granular mass located to one side, often interrupting the view of the sporozoites. Two sporozoites occur in each sporocyst. The sporozoites are comma-shaped, with a large refractile globule only at the broad end. The sporozoites were difficult to see in most of the specimens, and thus average measurements were not attempted.

This species is the third most frequently encountered of the five coccidia described in the present study. The sporulation time was 2 days (48 hours).

Of the 12 descriptions presented by Levine and Ivens (1965) that I mentioned in the discussion of Eimeria sp. A, Eimeria moelleri most closely approximates the description of Eimeria sp. B. Table V provides a comparison of the description of Eimeria sp. B of the present study with that of E. moelleri as was described by Möller (1923).

The oocysts studied are, on the average, larger than those of E. moelleri. Since the range overlaps the extremes of the former study in about equal degrees, it appears that more specimens were measured in the present study to give a normal wider range. Möller did not indicate the number of specimens he measured or an average for his measurements. E. moelleri apparently does not have oocystic granules as does my material. This is the most significant difference between the two organisms, however, the oocystic granules are not always a prominent or constant character in my material. Therefore, Möller may have overlooked these in the examination of a few specimens. The final

TABLE V. Comparison of Eimeria sp. B from Sciurus carolinensis in southwest Virginia with Eimeria moelleri Levine and Ivens, 1965 as described from Sciurus carolinensis in the Berlin Zoological Garden by Möller (1923)

	Eimeria sp. B	Eimeria moelleri
Oocyst size*	17-34 x 12-18 Avg. 26 x 15	22-28 x 14-18
Oocyst shape	ellipsoidal to cylindrical	ellipsoidal to cylindrical
Oocyst wall	two layers, smooth, colorless	two layers, smooth colorless
Oocyst granules	+	-
Oocyst residuum	-	-
Micropyle*	+ (3-9)	+ (4-6)
Sporocyst size*	9-15 x 5-9 Avg. 11 x 6	10-14 x 6-8
Sporocyst shape	ellipsoidal	not given
Stieda body	-	(?)
Sporocyst residuum	+	+
Sporulation time	2 days	3 days

*/ Measurements in microns

difference concerns the Stieda body. Möller described the sporocysts as possessing a small "micropyle." Levine and Ivens (1965) proposed that this "micropyle" might actually be a Stieda body. This character is questionable, then, and it is not considered to be a significant difference. The other characters, i.e., oocyst shape, oocyst wall, absence of oocyst residuum, presence of and size of micropyle, sporocyst size and presence of sporocyst residuum, are identical in the two descriptions. The sporocyst shape was not given by Möller, but it is reflected by the dimensions.

Eimeria sp. B significantly differs from E. serbica in that the latter lacks a micropyle and polar granules; from E. andrewsi which lacks a micropyle and sporocyst residuum and has a single-layered wall; from E. silvana which lacks a micropyle, is different in oocyst size and has a single-layered oocyst wall; from E. neosciuri which lacks a micropyle; from E. ascotensis which has two opercula and a Stieda body; from E. parasciurorum which lacks a micropyle and polar granule and has a single-layered wall; and from E. kniplingi which lacks a micropyle and has a single-layered oocyst wall and a Stieda body.

Therefore, Eimeria sp. B resembles Eimeria moelleri more closely than any other coccidia with small or medium-sized, thin-walled oocysts that have been described from tree squirrels, but the present investigation will not assign it to any species and will refer to it as Eimeria sp. B until further study can determine its true taxonomic position. The existing evidence does not warrant the assignment of a new specific name. This conclusion is based on the work by Soon and

Dorney (1971) who raised the possibility that morphological variations may occur within single species of these thin-walled coccidia.

Eimeria sp. C

The oocysts (Figure 3; Plate 3) are ellipsoidal to cylindrical. One hundred sporulated oocysts measured 18.9 to 29.4 microns (s.d. 2.6) in length by 12.2 to 17.0 microns (s.d. 1.0) in width, with an average of 25.2 by 14.3 microns. The length to width ratios ranged from 1.4 to 2.1 (s.d. 0.2), with an average of 1.8.

The oocyst wall is smooth and colorless and composed of two layers. The outer layer appears slightly thicker than the inner one. The latter is not clearly visible until the oocysts have been subjected to concentrated hydrochloric acid which induces the outer wall layer to separate from the inner layer. Two opercula are present, one at each end of the oocyst. Usually both are prominent, one slightly more so than the other, and are connected with oval refractile vesicles in the wall. One operculum is generally a little larger than the other. The average measurements were 7.6 and 6.7 microns, respectively.

An oocyst residuum is absent, but one to three oocyst refractile granules or polar bodies are present.

There are four sporocysts per oocyst. They are ovoidal with a prominent, broadly rounded Stieda body. Fifty sporocysts measured 8.5 to 14.3 microns (s.d. 1.1) in length by 5.1 to 8.5 microns (s.d. 0.7) in width, with an average of 11.0 by 6.0 microns. The length to width ratios ranged from 1.3 to 2.4 (s.d. 0.2) with an average of 1.8.

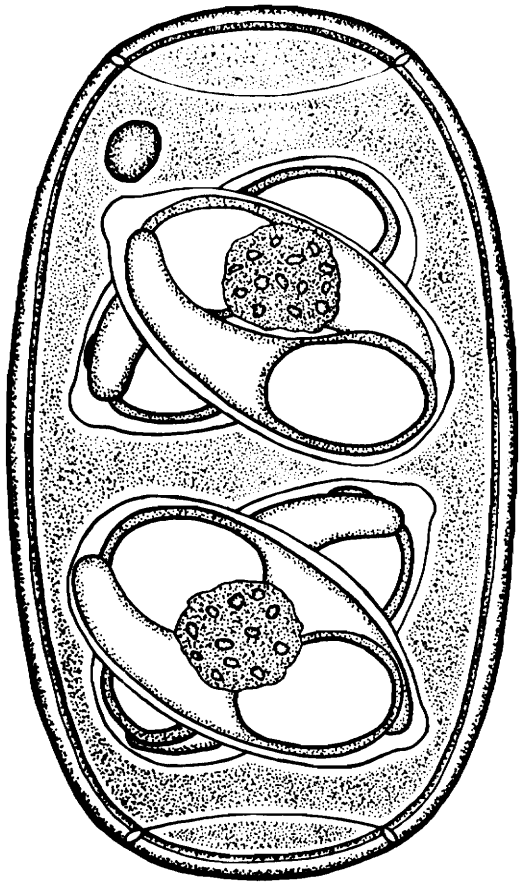


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FIGURE 3. Eimeria sp. C, mature oocyst

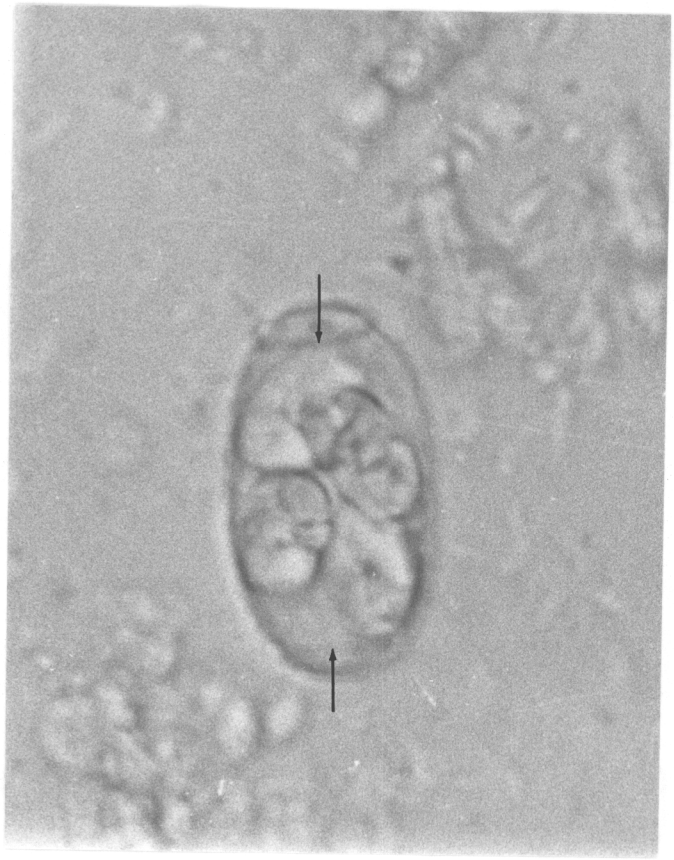

PLATE 3. Eimeria sp. C; photomicrograph of mature oocyst (arrows indicate opercula); 1800 x

A sporocyst residuum is present and appears granular and is concentrated into a small cluster to one side of the sporozoites.

The sporozoites, numbering two per sporocyst, are comma-shaped and have a large refractile globule near the broad end and a second smaller one near the narrow end.

Eimeria sp. C was the second most frequent coccidian parasite encountered in the gray squirrels of southwestern Virginia. The sporulation time was observed as 2 days.

Of the small to medium-sized, thin-walled coccidia which have been described from tree squirrels, only Eimeria ascotensis closely resembles the description of Eimeria sp. C (Table VI). E. ascotensis is the only coccidium that has been described which has an operculum at each end. Webster (1960) described the oocysts as having an operculum at one end and a smaller, less distinct, "terminal cap" at the opposite end. Levine and Ivens (1965) have indicated that this "terminal cap" is probably a second operculum.

The size range given for E. ascotensis by Webster (1960) is somewhat greater than that of the present study. Apparently, this is due to the number of measurements made, since 400 specimens were measured in the former study and 100 in the present study. Eimeria sp. C has an average oocyst size that almost identically conforms with the average size of E. ascotensis as indicated by Webster (1960) from samples taken from four different squirrels. The only significant differences between the two descriptions are numbers of oocyst wall layers and oocyst shape.

TABLE VI. Comparison of *Eimeria* sp. C from *Sciurus carolinensis* in southwest Virginia with *Eimeria ascotensis* Levine and Ivens, 1965, as described from *Sciurus carolinensis* in England by Webster (1960)

	Eimeria sp. C	*Eimeria ascotensis*
Oocyst size*	19-29 x 12-17 Avg. 25 x 14	14-31 x 10-20 Avg. 24 x 15
Oocyst shape	ellipsoidal to cylindrical	ovoidal (drawn as ellipsoidal)
Oocyst wall	two layers, smooth, colorless	three layers, smooth
Oocyst granules	+	+
Oocyst residuum	-	-
Operculum	++	++
Sporocyst size*	9-14 x 5-9 Avg. 11 x 6	9-10 x 6-7
Sporocyst shape	ovoidal	ovoidal
Stieda body	+	+
Sporocyst residuum	+	+
Sporulation time	2 days (48 hours)	3 days (76 hours)

*/ Measurements in microns

Webster (1960) described E. ascotensis as having 3 wall layers. However, Levine and Ivens (1965) and Soon and Dorney (1971) appear convinced that the third layer observed by Webster was actually an optical illusion since they had seen it many times in microscopic studies of coccidia. This optical illusion was also observed in the present study. Therefore, much emphasis cannot be placed on this character.

The oocysts of Eimeria ascotensis were described as being ovoidal (Webster, 1960). Actually, the illustrations given by Webster showed them to be ellipsoidal, the same as those of Eimeria sp. C.

Therefore, since Eimeria sp. C has two opercula and the description agrees with E. ascotensis in oocyst size, presence of oocyst granules, absence of oocyst residuum, in sporocyst shape and size, presence of a Stieda body and sporocyst residuum and no other descriptions of coccidia from tree squirrels have been cited with two opercula, the present material is tentatively identified as Eimeria ascotensis. However, as mentioned earlier, Soon and Dorney (1971) raised the possibility that morphological variations may occur within single species of coccidia which are encountered in single infections in squirrels in Canada. Therefore, the present material is designated as Eimeria sp. C until this question is resolved.

Eimeria wongi Soon and Dorney, 1971

The oocysts (Figure 4; Plate 4) are subspherical to ellipsoidal. One hundred sporulated oocysts measured 31.9 to 44.2 microns (s.d. 2.5) in length by 25.1 to 35.3 microns (s.d. 1.8) in width, with an average of 38.5 by 31.0 microns. The length to width ratios ranged from 1.1 to 1.4 (s.d. 0.1), with an average of 1.2.

The oocyst wall is composed of two layers. The outer layer is 1.5 microns thick, very rough (Figure 5) and brownish-yellow in color, while the inner layer is 1.0 microns thick, clear and smooth. The total wall thickness averages 2.5 microns. A small micropyle is present at one end. This small micropyle was not observed until the technique employed by Soon and Dorney (1971) was used. By subjecting the oocysts to concentrated acid, the small micropyle becomes visible when the outer layer of the wall of the oocysts separates from the inner layer. It appears to be a true micropyle and measured 2.9 microns across its greatest width. It is present on the inner layer of the wall, rather than the outer layer.

Usually one to four refractile granules or polar bodies are present within the oocyst. An oocyst residuum is absent.

Four sporocysts occur in each oocyst. They are ovoidal and each has a prominent Stieda body (Plate 5). One hundred sporocysts measured 14.0 to 22.4 microns (s.d. 1.6) in length by 9.7 to 13.1 microns (s.d. 0.7) in width, with an average of 18.1 by 11.6 microns. The length to width ratios ranged from 1.2 to 2.0 (s.d. 0.1). with an average of 1.6. Sporocyst residual material is granular and generally dispersed among the sporozoites. Details of the sporocyst are more readily

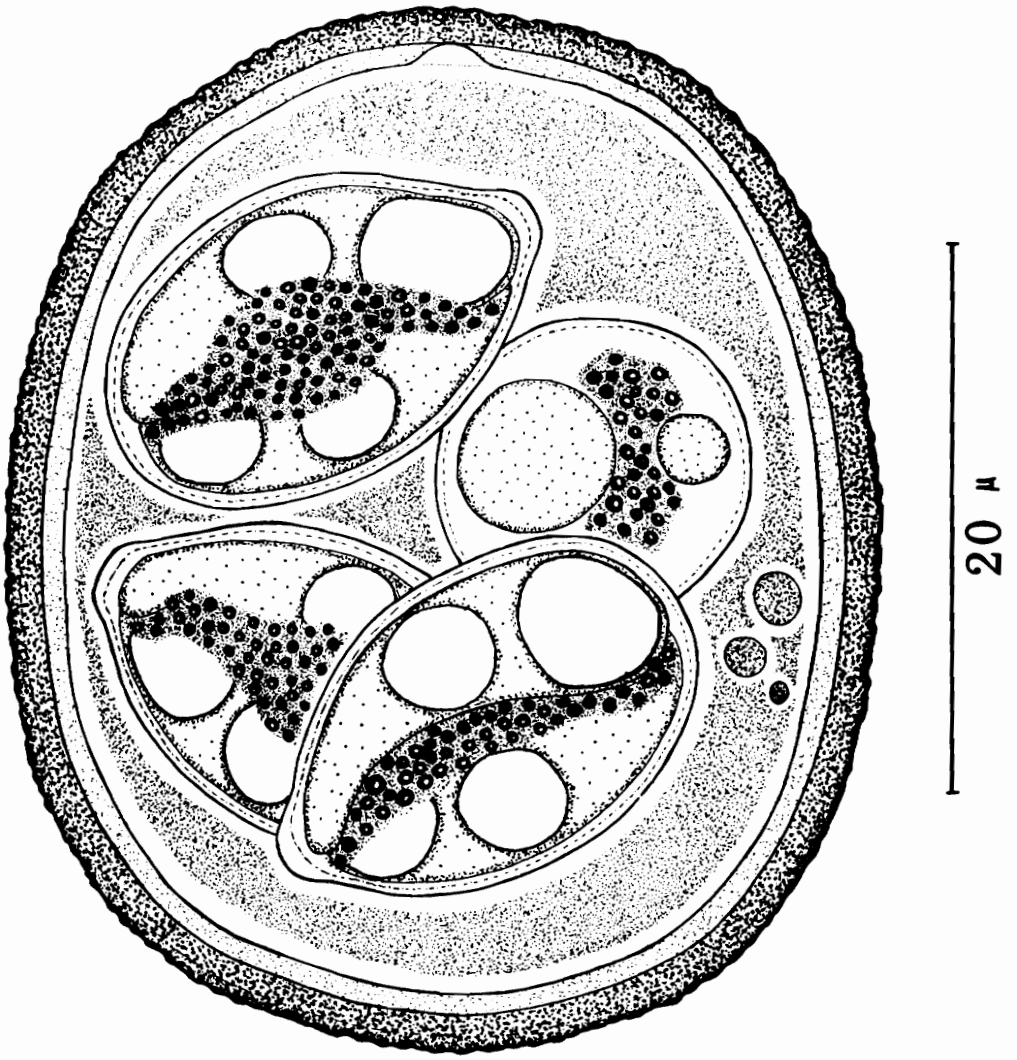

FIGURE 4. Eimeria wongi, mature oocyst

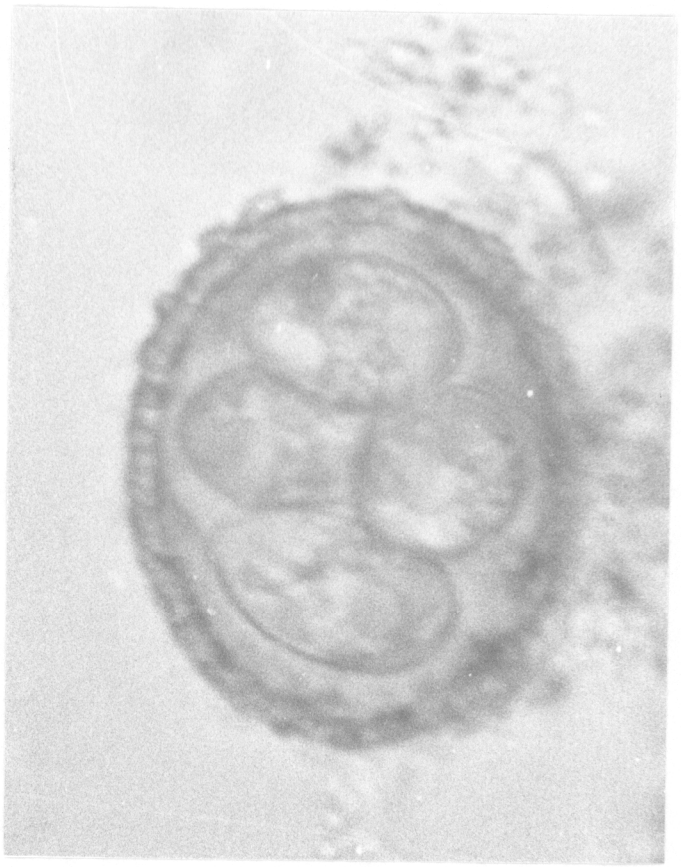

PLATE 4. Eimeria wongi Soon and Dorney, 1971; photomicrograph of mature oocyst; 1800 x

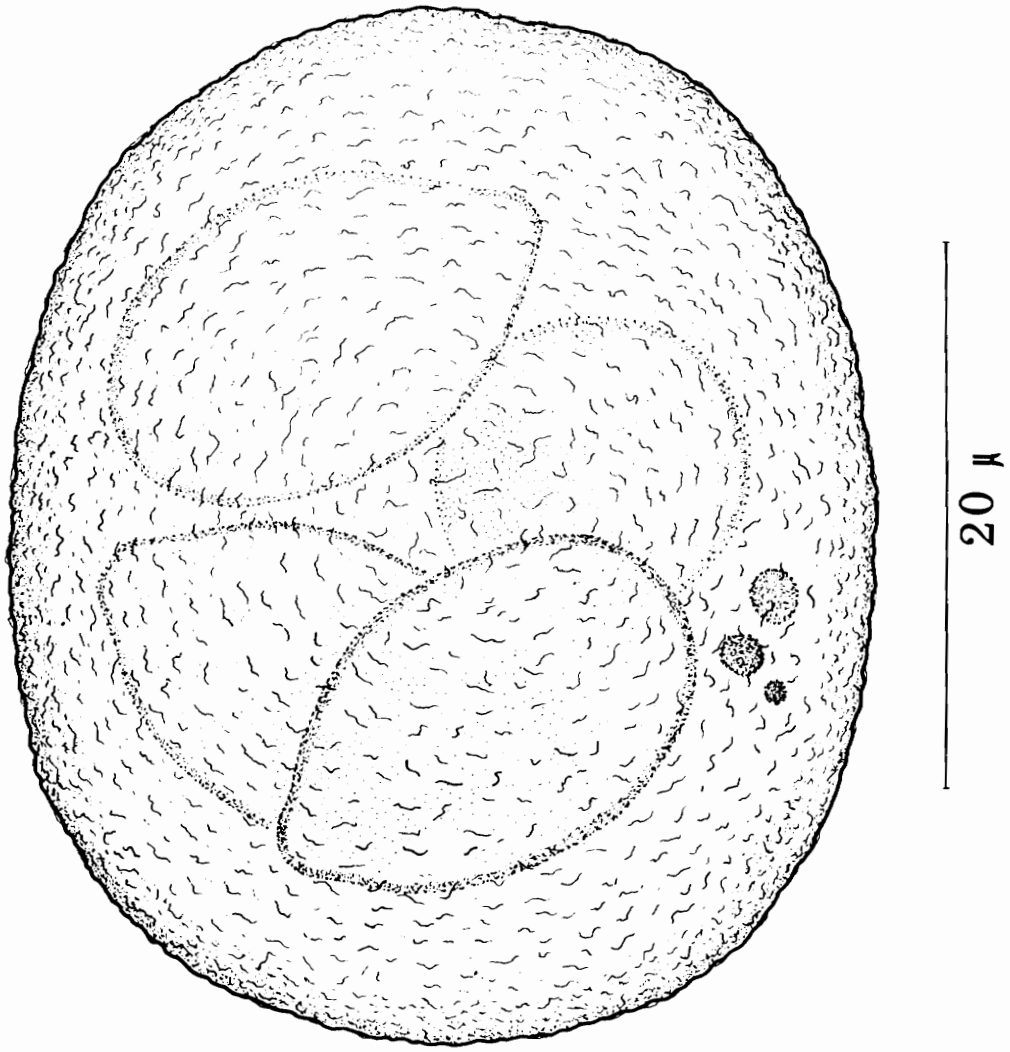

FIGURE 5. Eimeria wongi, rough oocyst wall

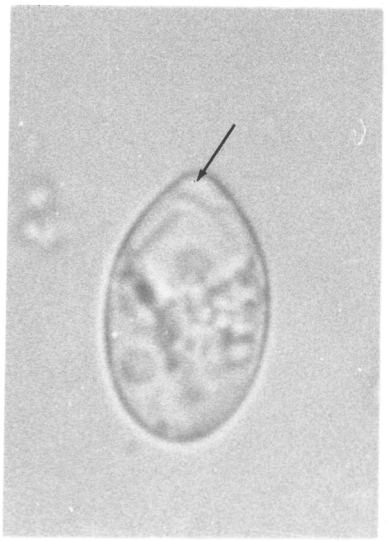

PLATE 5. Eimeria wongi Soon and Dorney, 1971; freed sporocyst liberated from oocyst by crushing (arrow indicates the Stieda body); 1800 x

observed by crushing the oocysts between the slide and coverslip in order to free them because the thick oocyst wall obscures the view.

There are two sporozoites per sporocyst. The sporozoites are comma-shaped and have a large clear refractile globule located at the broad end and usually a second smaller one centrally located or slightly toward the small end of the sporozoite. Sixteen encysted sporozoites measured 9.0 to 18.2 microns (s.d. 3.4) in length by 3.4 to 8.0 microns (s.d. 1.4) in width, with an average of 13.1 by 4.6 microns.

Only one host was infected with this species and a single culture required 45 days in order for 52% of the oocysts to sporulate. A higher percentage was never reached.

A review of the literature dealing with coccidia of the host family Sciuridae reveals several coccidian species that are morphologically similar to that of the present description. These include Eimeria toddi Dorney, 1962, from Tamiasciurus hudsonicus Erxleben, 1777; E. wisconsinensis Dorney, 1962, from Tamias striatus Linn., 1758; E. larimerensis Vetterling, 1964, from several species of Spermophilus and Cynomys; E. tuscarorensis Dorney, 1965, from Marmota monax Linn., 1758; and E. bilamellata Henry, 1932, from several species of Spermophilus. Of these, the material I examined closely resembles both Eimeria wongi and E. toddi. Since E. wongi is parasitic in gray squirrels and E. toddi is parasitic in red squirrels, and because Soon and Dorney failed to transmit E. wongi to red squirrels in their study in Canada, I identify my material with E. wongi. Also Dorney (personal comments) examined specimens from my southwestern Virginia material and he also considered it to be identical to E. wongi.

Table VII provides a comparison of E. wongi in Canada (Soon and Dorney, 1971) with that of southwestern Virginia. The only notable differences in these descriptions are the size ranges of the oocysts and they are not significant.

The smallest oocyst measured by Soon and Dorney (1971) was 5.3 microns smaller than the smallest recorded herein; the largest measured was 1.3 microns larger than the largest of those among my material. These differences appear to be normal differences in range due to a larger sample, since Soon and Dorney (1971) measured 287 specimens as compared with 100 in the present description. Further, it has been shown (Becker, Zimmerman and Patillo, 1955; Webster, 1960; Soon and Dorney, 1971) that oocyst size ranges may vary at different times during an infection and from one host to another.

Further comparisons show the remaining data to be almost identical in the two descriptions.

This account constitutes the first report of Eimeria wongi in Virginia and the United States of America.

Eimeria ontarioensis Soon and Dorney, 1971

The oocysts (Figure 6; Plate 6) were pyriform, with a short bottleneck. One hundred sporulated oocysts measured 25.0 to 39.9 microns (s.d. 3.1) in length by 17.1 to 27.4 microns (s.d. 2.0) in width, with an average of 31.5 by 22.9 microns. The length to width ratios ranged from 1.2 to 1.7 (s.d. 0.1), with an average of 1.4.

The oocyst wall is composed of two layers. The outer layer is thick, rough, brownish-yellow and is 1.0 microns whereas the inner

TABLE VII. Comparison of *Eimeria wongi* Soon and Dorney, 1971, from *Sciurus carolinensis* in Canada with that of southwestern Virginia

	Present study	Soon & Dorney, 1971
Geographic distribution	U.S.A. (S W Virginia)	Canada (Ontario)
Oocyst size*	32-44 x 25-35 Avg. 39 x 31	27-46 x 23-36 Avg. 36 x 28
Oocyst l/w ratio	1.1-1.4 Avg. 1.2	Range not given Avg. 1.3
Oocyst shape	subspherical to ellipsoidal	subspherical, rarely ellipsoidal
Oocyst wall*	two layers; outer thick & rough, inner clear & smooth (2.5)	two layers; outer thick & rough, inner clear & smooth (2.9)
Oocyst granules	+	+
Oocyst residuum	-	-
Micropyle*	+ (2.9)	+ (2.0)
Sporocyst size*	14-22 x 10-13 Avg. 18 x 12	16-20 x 9-13 Avg. 18 x 11
Sporocyst shape	ovoidal	ovoidal
Stieda body	+	+
Sporocyst residuum	+	+
Sporulation time	45 days (52%)	9-11 days (one culture required 43 days)

*/ Measurements in microns

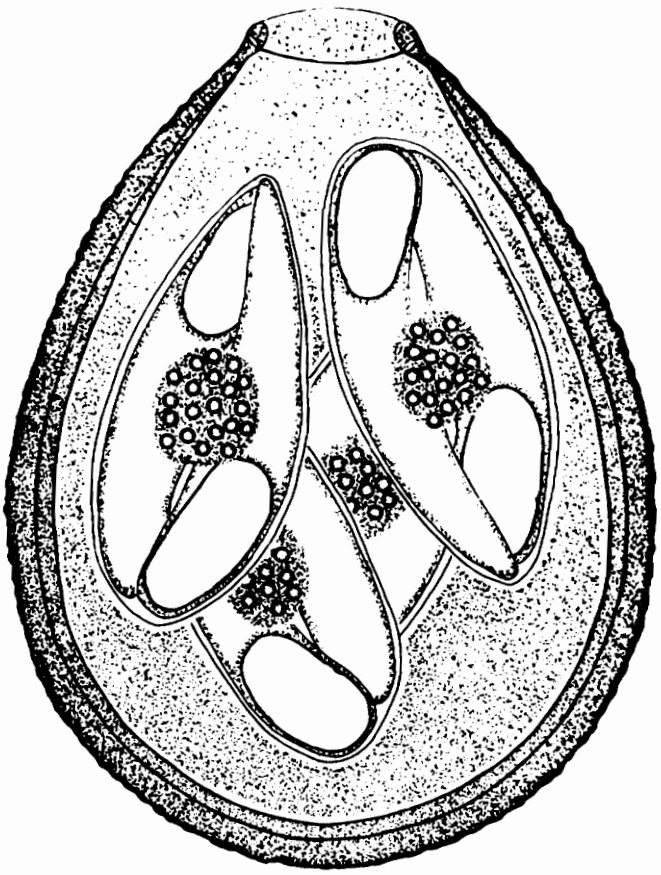

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FIGURE 6. Eimeria ontarioensis, mature oocyst

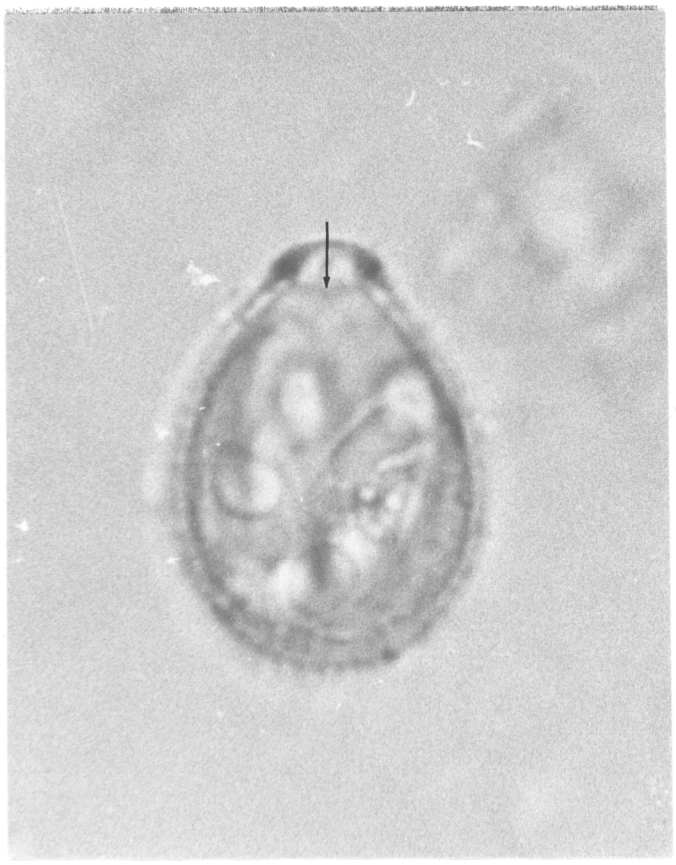


PLATE 6. Eimeria ontarioensis Soon and Dorney, 1971; photomicrograph of mature oocyst (arrow indicates the micropyle); 1800 x

layer is thin, smooth and colorless and measures 0.6 microns. The total wall averages 1.6 microns thick. In specimens that were crushed under the coverslip the outer wall layer ruptured and broke in several places leaving the oocyst contents bound by the very thin rigid inner wall.

A large micropyle is located at the narrow end, the greatest diameter of which ranged 3.4 to 9.4 microns, with an average of 6.1 microns (average of 25 oocysts). An extension of the outer wall appears to form a cap or vesicle over the micropyle. The micropyle was not visible on the inner membrane of the crushed specimens described above.

No refractile granules or oocyst residuum were observed in any of the specimens examined.

The sporocysts (Plate 7), numbering four per oocyst, are lanceolate with one end slightly rounded and the opposite somewhat pointed, forming a Stieda body. Fifty sporocysts measured 11.4 to 22.1 microns (s.d. 2.2) in length by 5.6 to 9.1 microns (s.d. 0.8) in width, with an average of 17.3 by 7.2 microns. The length to width ratios ranged from 1.6 to 3.3 (s.d. 0.3), with an average of 2.4. The sporocyst residual material is somewhat granular and clumped together to form a rosette-shaped mass which often partially obscures the sporozoites.

There are two sporozoites within each sporocyst which are elongate-comma-shaped. Each sporozoite has a very large refractile globule only at the broad end. Twenty encysted sporozoites measured 12.1 to 18.2 microns (s.d. 1.8) in length by 3.6 to 6.3 microns (s.d. 0.8) in width, with an average of 15.5 by 4.6 microns.

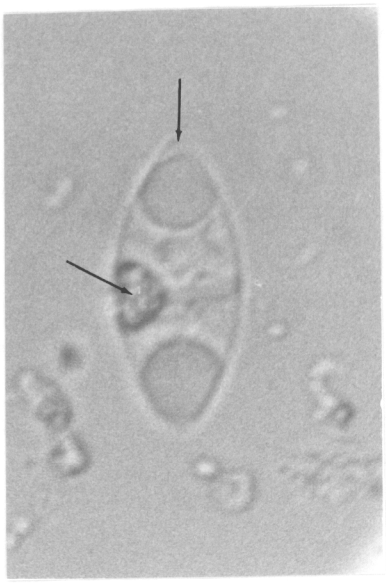


PLATE 7. Eimeria ontarioensis Soon and Dorney, 1971; freed sporocyst liberated from the oocyst by crushing (arrow at top indicates the Stieda body; arrow at side indicates the sporocyst residuum); 1800 x

With the exception of the single infection of Eimeria wongi encountered, E. ontarioensis was the second least abundant coccidium found in this study. Of two cultures observed for sporulation time, one revealed mature sporocysts in 4 days while the other required 13 days.

Soon and Dorney (1971) recently described Eimeria ontarioensis from Sciurus carolinensis in Ontario, Canada. The above description deviates from theirs only in a few minor respects (Table VIII). These include oocyst size, absence of oocyst granules, oocyst wall thickness and sporocyst size. These do not appear to be significant differences as will be explained as follows.

The oocyst size range differs only to a small extent and the average measurements are similar. The size range of the oocysts in the present study overlaps that of the Ontario specimens. The smallest oocyst length measured in the Virginia material differed from that of the Ontario material by only 1.3 microns; the largest oocyst length measured was 11.1 microns smaller than the largest recorded in the Ontario material. The average values for oocyst size differ by 5.3 microns in length and 0.6 microns in width in the two studies. These differences appear to be normal variations, since Soon and Dorney (1971) provided evidence that an increase in the average oocyst size occurred in this species during the first few days of the patent period in experimentally infected gray squirrels. The Virginia material was taken from naturally infected gray squirrels, and thus the time of infection was unknown.

TABLE VIII. Comparison of *Eimeria ontarioensis* Soon and Dorney, 1971, from *Sciurus carolinensis* in Canada with that of southwestern Virginia

	Present study	Soon & Dorney, 1971
Geographic distribution	U.S.A. (S W Virginia)	Canada (Ontario)
Oocyst size*	24-40 x 17-27 Avg. 32 x 23	26-51 x 18-28 Avg. 37 x 24
Oocyst l/w ratio	1.2-1.7 Avg. 1.4	not given
Oocyst shape	piriform, with short bottleneck	piriform, with short bottleneck
Oocyst wall*	two layers, outer thick & rough, inner thin & smooth (1.6)	two layers, outer thick & rough, inner thin & smooth (3.4)
Oocyst granules	+	+ (45%)
Oocyst residuum	-	-
Micropyle*	+ (3.4-9.4) Avg. 6.1	+ Avg. 8.9
Sporocyst size*	11-22 x 6-9 Avg. 17 x 7	18-27 x 6-11 Avg. 22 x 8
Sporocyst l/w ratio	1.6-3.3 Avg. 2.4	1.8-3.6 Avg. 2.7
Sporocyst shape	lanceolate	cigar shaped
Stieda body	+	+
Sporocyst residuum	+	+
Sporulation time	4-13 days	4-8 days

*/ Measurements in microns

Oocyst granules were not observed in the present study, but were observed in 45% of those oocysts from the Ontario squirrels. Soon and Dorney (1971) found these oocystic granules difficult to see unless the outer wall was removed by crushing or subjection to sulphuric acid. Since the outer wall layer was not regularly removed in the present study, this difference cannot be emphasized.

Both studies show the oocyst wall to be composed of two layers; the outer layer thick, rough and brownish in color and the inner layer thin, smooth and colorless. However, the average total wall thickness in my material is 1.6 microns (outer layer 1.0 microns, inner layer 0.6 0.6 microns) while that of the Ontario material is 3.4 microns (outer layer 2.3 microns, inner layer 1.1 microns). Also there are size differences among the sporocysts. The average sporocyst length in my material is about 4.5 microns shorter than that of the Ontario specimens while the average width differed by only 1.0 microns. The reason for wall and sporocyst differences is not understood. They may be due to differences in degree of infection or to age of infection in the host. Also, it is not known what natural variations may occur in these parasites over the extent of the host species range or what effects slight differences in physiological conditions in the host at different times may have upon the parasites.

None of the above discrepencies warrant the assignment of a new species name to the Virginia specimens. A review of the taxonomic, morphological and clinical properties of similar species of coccidia from the host genus Sciurus as outlined by Soon and Dorney (1971),

shows that the Virginia material most closely resembles that of Eimeria ontarioensis and it is hereby assigned to that species.

This is the first report of E. ontarioensis from Virginia and the United States of America. No other species of coccidia of the host family Sciuridae which have pyriform oocysts with a short bottle-neck have been reported from the continent of North America. Similar species have been described from Sciurus species in Europe and South America. Soon and Dorney (1971) discussed the zoogeographic significance of E. ontarioensis in this respect.

Pathology

Squirrels infected with coccidia appeared to be alert and healthy animals, and upon autopsy showed little signs of alimentary tract pathology. No hemorrhages were apparent in the gut wall and well-formed feces was a constant character. The only macroscopic indication of diseased areas were observed along most of the length of the ileum. This region of the small intestine appeared very thin and transparent as compared with the other areas of the digestive tract, and gas bubbles in the lumen could readily be seen through the intestinal wall. Histological preparations (10 micron sections stained with hematoxylin and eosin) revealed destruction of columnar epithelial cells on many of the villi (Plate 8) and occasionally cellular destruction of the submucosa was evident and the tissue stages of the parasites (Plate 9) could often be observed. Some of these spaces were filled with blood corpuscles.

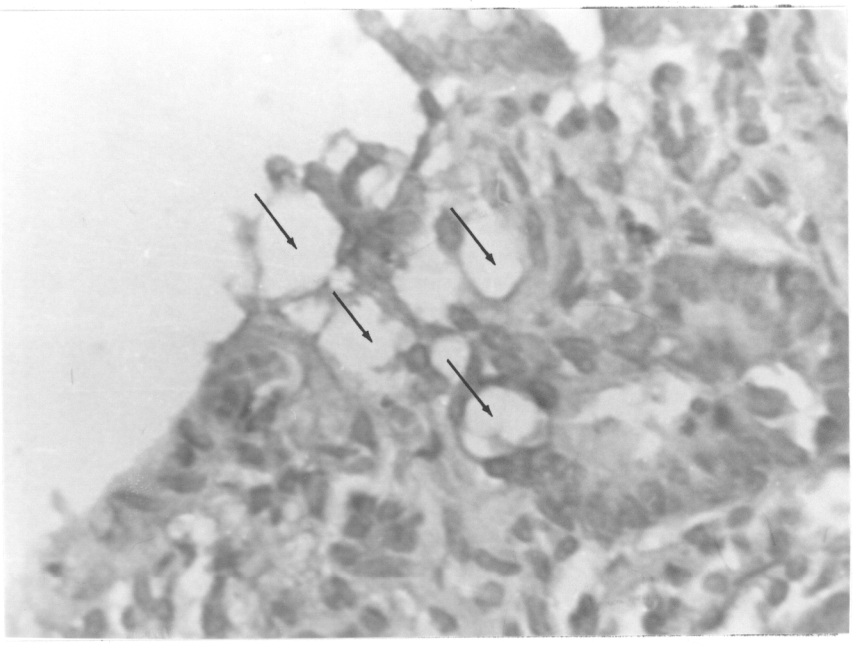


PLATE 8. Tissue section of ileum; vacuolated areas indicated by arrows represent cellular destruction by coccidial organisms (10 microns, hematoxylin and eosin); 800 x

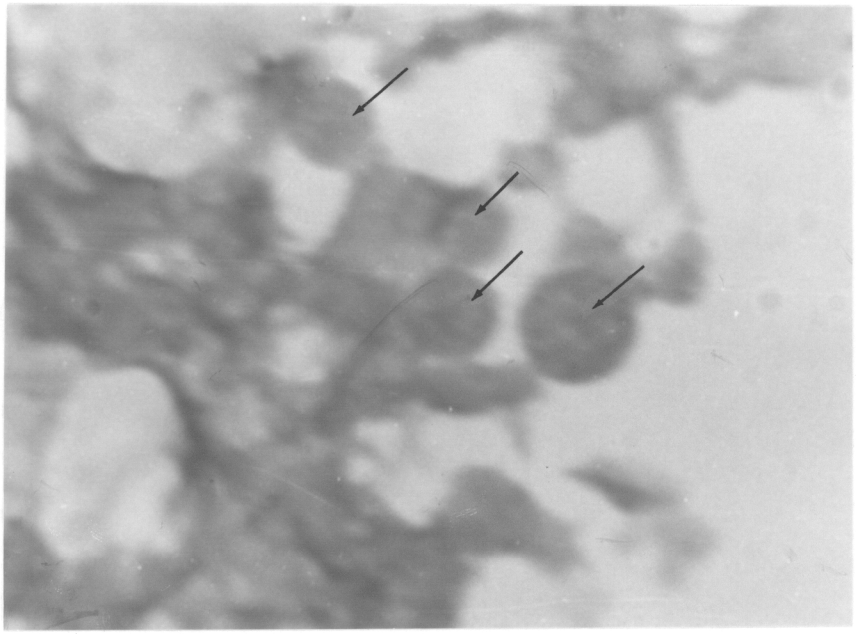


PLATE 9. Tissue section of ileum; schizonts containing merozoites are indicated by the arrows (10 microns, hematoxylin and eosin); 1400 x

Estimates of the numbers of coccidial oocysts passed in the feces were made on one squirrel daily for a period of 40 days, and single estimates were made on 48 animals as already mentioned. Fluctuations in the numbers of oocysts passed varied a great deal from day to day and from animal to animal, but the averages indicate that a tremendous number of intestinal cells were being destroyed. Taking into consideration that the single squirrel mentioned was a confined animal and that these estimates were made on only one individual over a period of time or were single counts from 48 individuals at an unknown period of infection, the evidence indicates that squirrels must have a high rate of intestinal cell regeneration and must be capable of maintaining a physiological equilibrium with the parasite by repair and immunological mechanisms. This conclusion is also based on the fact that significant squirrel "die offs" are rarely reported and that most infected squirrels observed appeared to be healthy animals. What effects these parasites may have during periods of high population densities, food shortages and bad weather conditions or what influences other organisms such as viruses, bacteria, intestinal protozoa, nematodes and tapeworms may have in conjunction with coccidial infections is left largely to speculation.

Summary and Conclusions

This study has provided evidence that the majority of squirrels within the area harbor coccidia in their intestines and the oocysts can be demonstrated in their feces. Numbers of coccidial oocysts

being passed in the feces of naturally infected squirrels are quite variable and may at times be very high.

Coccidia of five types have been described. Three of these are coccidia with small-sized, thin-walled oocysts, while two have large, thick-walled ones. Within the small-sized, thin-walled group were described Eimeria spp. A, B and C, which resemble Eimeria neosciuri, Eimeria moelleri and Eimeria ascotensis, respectively. Within the large-sized, thick-walled group were described two species that are identical with Eimeria wongi and Eimeria ontarioensis. The order of frequency of occurrence of the parasites were, first, Eimeria sp. A; second, Eimeria sp. C; third, Eimeria sp. B; fourth, E. ontarioensis; and fifth, E. wongi. This is the first report of any of these species from gray squirrels in the state of Virginia, and of E. wongi and E. ontarioensis from the United States of America. Table IX provides a summary and comparison of the coccidia in this study.

Soon and Dorney (1971) studied the small, thin-walled coccidia of the gray squirrel in Canada, which they called Eimeria sp. They considered all of these to belong to a single species in which, of 854 oocysts examined, 92.3% had no micropyle, but 7.7% had an operculum. Of those having an operculum 1.8% had "terminal caps" (or a second operculum) at the opposite end. In other words, they believed that their material showed characteristics of both E. neosciuri and E. ascotensis, and raised the possibility that these may be morphological variations within a single species. They declined to assign specific names for these forms by calling them Eimeria sp. Therefore, due to the occurrence of morphological forms in the present study which are

TABLE IX. Comparison of the eimerian parasites observed in Sciurus carolinensis from southwestern Virginia, September, 1966-July, 1969

	Eimeria sp. A	Eimeria sp. B	Eimeria sp. C	Eimeria wongi	Eimeria ontarioensis
Oocyst size*	17-28 x 11-18 Avg. 22 x 14	17-34 x 12-18 Avg. 26 x 15	19-29 x 12-17 Avg. 25 x 14	32-44 x 25-35 Avg. 39 x 31	25-40 x 17-27 Avg. 32 x 23
Oocyst l/w ratio	1.2-2.1 Avg. 1.6	1.4-2.2 Avg. 1.8	1.4-2.1 Avg. 1.8	1.1-1.4 Avg. 1.2	1.2-1.7 Avg. 1.4
Oocyst shape	ellipsoidal, rarely subspherical	ellipsoidal to cylindrical	ellipsoidal to cylindrical	subspherical to ellipsoidal	piriform, with short bottleneck
Oocyst wall	two layers, smooth, colorless	two layers, smooth, colorless	two layers, smooth, colorless	two layers; outer thick & rough, inner thin & smooth	two layers; outer thick & rough, inner thin & smooth
Oocyst granules	+	+	+	+	-
Micropyle	-	+	++	+	+
Sporocyst size*	7-12 x 4-8 Avg. 10 x 6	9-15 x 5-9 Avg. 11 x 6	9-14 x 5-9 Avg. 11 x 6	14-22 x 10-13 Avg. 18 x 12	11-22 x 6-9 Avg. 17 x 7
Sporocyst l/w ratio	1.3-2.3 Avg. 1.6	1.3-2.2 Avg. 1.8	1.3-2.4 Avg. 1.8	1.2-2.0 Avg. 1.6	1.6-3.3 Avg. 2.4
Sporocyst shape	ovoidal	ellipsoidal	ovoidal	ovoidal	lanceolate
Stieda body	+	-	+	+	+
Sporulation time	2-3 days	2 days	2 days	45 days (52%)	4-13 days

*/ Measurements in microns

similar to the Eimeria sp. of Soon and Dorney (1971), I have preferred to refer to them as Eimeria sp. A, B and C, rather than assigning them to the described forms which they resemble.

Observations into the pathology of these parasites revealed that, although the hosts appeared alert and healthy, there was considerable tissue damage to the ileum in these animals. However, the hosts appear to be able to tolerate these parasites; and thus, they do not appear to be extremely pathogenic under the conditions in which the squirrels I examined were collected.

Further studies on these coccidia should include experimental infections on parasite-free hosts using a drug such as Amprolium to eliminate natural infections in the experimental host. According to the technique of Remmler and McGregor (1964) single species of coccidia may be isolated and administered to the hosts. Using these procedures, it would be possible to determine the daily oocyst production during the patent period with varying initial dosages; to determine what effects pregnancy would have on coccidial numbers; to study the tissue stages of the life cycle of each species; and to determine if those morphological types are actually different or variations of the same species. Also, further studies on experimental infections of other sciurid rodents with these coccidia would provide information on the host specificity of these parasites. Finally, a study of the immunological mechanisms of this host and serological analysis may provide clues to species identification of coccidia.

B. Haemogregarins

A survey of protozoan organisms occurring in the blood of gray squirrels was conducted and one species, Hepatozoon griseisciuri Clark, 1958, was discovered. Hepatozoon is a genus of sporozoan parasites, which, according to Hall (1953), is classified in the sub-phylum Sporozoa, class Telosporidea, sub-class Coccidia, Order Adeleida, because the gametocytes are associated in isogamy during differentiation. It is in the sub-order Haemogregarina because the life cycle involves two hosts and the zygote is a motile ookinete and in the family Hepatozoidae because the large oocysts contain sporocysts, each with numerous sporozonts. Hepatozoon is the type genus.

The genus Hepatozoon was first described by Miller (1908) who observed and recorded H. perniciosum (now H. muris) in white laboratory rats from Washington, D. C. Wellman and Wherry (1910) were the first to report the occurrence of Hepatozoon in a mammal indiginous to the neartic region: H. citellicola from the California ground squirrel, Citellus beecheyi (Richardson, 1829). Another observation of Hepatozoon was made in 1953, by Herman and Price (1954), who observed an abundance of gametocytes of the parasite in the blood of gray squirrels on the Patuxent Research Refuge in Laurel, Maryland. These findings have stimulated an interest in the study of the life histories of these organisms and a search for them in other localities and in other hosts.

Reviews on the species of Hepatozoon have been given by Brumpt (1946) and by Clark (1956). Clark (1956) indicates that there are about 59 names in the literature that have been assigned to the genus

Hepatozoon, many he believed to be synonymous. Added to the above number, about 16 forms have been reported without names. The taxonomic validity of many of these is doubtful as determined by their accompanying descriptions. Species of Hepatozoon are known to occur in a wide range of mammals and even in some birds and lizards. The distribution of the genus is cosmopolitan, however, it may be more common in the tropics. I have found 14 references to 6 species of Hepatozoon that are parasites of rodents in the family Sciuridae.

The species of Hepatozoon that have been reported from the family Sciuridae include the following: Hepatozoon sciuri (Coles, 1914) from Sciurus vulgaris Linnaeus, 1758, in England by Coles (1914) and in Italy by Franchini (1932), and tentatively from Sciurus carolinensis in England by Dasgupta and Meedeniya (1958), in Washington, D. C. and Maryland by Herman and Price (1954, 1955) and in Rhode Island by Weidanz and Hyland (1958); H. griseisciuri Clark, 1958, from Sciurus carolinensis in Maryland and Washington, D. C. by Clark (1958), in Wisconsin by Dorney and Todd (1959) and in Virginia by Parker (1968); H. citellicola (Wellman and Wherry, 1910) from Citellus beecheyi in California by Wellman and Wherry, 1910); H. mereschkowski (Tartakowskii, 1913) from Citellus suslicus guttatus (Pallas, 1770) in Russia by Tartakowskii (1913); H. gaetulum (Sergent, 1921) from Atlantoxerus getulus (Linn., 1758) in North Africa by Sergent (1921); H. funambuli (Patton, 1906) from Funambulus pennanti Wroughton, 1905 in India by Patton (1906) and from Spermophilus musicus=Citellus pygmaeus musicus Menetries, 1832 in Russia by Tarkatowskii (1913); several others without specific names have also been reported.

Hepatozoon has also been reported in the rat, Rattus norvegicus Berkenhout, 1769, in the eastern United States by Miller (1908), Price and Chitwood (1931), Andrews and White (1936), Herman (1939) and Eyles (1952).

Herman and Price (1955) have postulated that some organisms assigned to the genera Leucocytozoon, Haemogregarina and Leucocytogregarina may be synonymous with Hepatozoon. Wenyon (1910) and Brumpt (1946) also considered that some of these organisms may be Hepatozoon.

Clark (1956) summarized the characters considered in distinguishing between the various species of Hepatozoon as follows: preferred intermediate and definitive hosts, time required for sporogonic development, size of oocysts, number of sporocysts developing within each oocyst, number of sporozoites developing within each sporocyst, location of schizogony in the host, size of the schizonts (segmenters), number of merozoites produced by each schizont, the time required for the gametocytes to appear in the peripheral circulation and the production of immunity.

Clark (1958) elucidated the life cycle of H. griseisciuri that parasitizes gray squirrels. At present, it is believed that this is the only species found in this host in the United States and that those reports from this host by Dorney and Todd (1959), Weidanz and Hyland (1958), Herman and Price (1954, 1955) and Parker (1968) are actually only one species.

Materials and Methods

Two methods of diagnosis were used to determine the incidence of Hepatozoon griseisciuri among gray squirrels in southwestern Virginia. Herman and Price (1955) first began using both these methods for comparison, and they both have subsequently been used by other investigators.

Herman and Price (1955) prepared Giemsa stained blood smears which were examined at 450X with a compound microscope for 5 minutes or until 100 leukocytes were observed. This technique was utilized in the present study except Wright stain was substituted on the blood smears. The second method was a modification (by Herman and Price, 1955) of Knott's (1939) concentration technique for diagnosis of microfilariae. The procedure is summarized as follows: A sample of blood is taken by cardiac puncture from freshly killed or anesthetized squirrels using a needle (#20) and syringe (5 or 10 cc capacity). One to 2 cc of blood is added to 10 cc of 2% solution of formalin in a 15 cc conical centrifuge tube. The blood and formalin are mixed by inverting the tube several times. Within the next 48 hours, the tube is centrifuged for 5 minutes at about 1750 rpm. The supernatant fluid is then poured off and the tube inverted onto a paper towel to drain a few minutes, the leukocytes remaining in the apex of the tube. Finally, within 5-10 minutes, the tube is set upright and a few drops of a 1 to 1000 aqueous solution of methylene blue is added to improve microscopic contrast. Upon examining the sediment under 100X, the parasites appear as refractile bodies with bluish nuclei within the

leukocytes or sometimes free, the latter especially when the blood has been forced from the syringe through the needle thus disrupting cells and freeing the parasites.

Histological sections (10 microns) from several hosts were stained with Ehrlich's hematoxylin and eosin to be examined for the schizogonic stages of the parasite. The following material was prepared for examination: brain, heart, lungs, liver, spleen, lymph nodes, muscle, kidney, small intestine, cecum, large intestine, gall bladder, esophagus, stomach, pancreas and skin. Also femural bone marrow smears were stained and examined.

In vitro cultivation of the blood forms of the parasites was attempted. In the first culture attempts, the following DIFCO culture media were employed: T-soy broth, Albimi's Brucella broth and Brain-Heart infusion media. These media were prepared according to directions, and then each was divided into two equal quantities in cotton-stoppered flasks. To one-half of each of the divided media was added 0.1% agar. The material was autoclaved at 15 pounds pressure for 15 minutes. Then 10 cc of each medium was placed into sterile, screw-cap test tubes. Fresh cardiac blood was taken under sterile conditions from an etherized squirrel known to be infected with Hepatozoon. A 10 cc syringe and # 20 needle was used, and heparin was flushed through the syringe to moisten the inner surfaces to prevent coagulation of the blood. When 10 cc of blood filled the syringe it was then transferred into a 15 cc, sterile, conical centrifuge tube, stoppered and placed in a tube rack inside an incubator at 37-38° C, the approximate body temperature of the gray squirrel. After 2-3 hours the leukocytes formed a layer at

the top of the contents of the tube. This layer of leukocytes was extracted with a Pasteur pipette and 3-6 drops were inoculated into 3 tubes each of the 6 media preparations. One additional tube containing the medium was not inoculated and served as the control. The tubes were then incubated at 37-38° C and samples were taken from each tube daily and examined by phase microscopy.

Another culture procedure was also attempted using products manufactured by Microbiological Associates, Inc. This method involved the following preparation: 10 cc of Medium 199 (10 X conc.) was diluted with 76.9 cc distilled, pyrogen free water; a solution of 1% agar in water was prepared by heating until dissolved then 13.1 cc of this 1% agar was added to the diluted Medium 199 to give a final agar concentration of .15%; next 10 cc of bovine serum was added to the medium, then .46 cc of a 7.5% solution of sodium bicarbonate was added to produce a concentration of approximately .03%; then 1 cc of L-Glutamine (200 mM) was added and finally 2 cc of penicillin/streptomycin mixture (25,000 units) was added to conclude the preparation. The medium was dispensed into 4 test tubes with screw caps. Three tubes were inoculated with 1-2 drops of leukocytes obtained as described in the procedures above and incubated at 37-38° C, while the fourth tube served as a control. Samples from each tube were examined twice a day under phase microscopy for 5 days.

Results and Discussion

Table X shows the incidence of Hepatozoon griseisciuri in the squirrels of southwestern Virginia. The concentration technique revealed an incidence of 79%, while the stained smears revealed a less efficient estimate of only 58%. The incidence of infection between the sexes and between different age classes showed no differences and thus the parasite appears to be generally distributed in our sample of the squirrel population. Also there were no marked differences of incidence during different seasons of the year.

The only stage of the parasite that was observed in this survey was the gametocyte encysted within monocytes. Plate 10 illustrates several gametocytes that have been freed from the monocytes in the blood concentrates. When the cardiac blood was forced from the syringe through the needle under pressure, it was found that many of the leukocytes would be ruptured and subsequently free the gametocytes from the cell. Plate 11 illustrates encysted gametocytes in a Wright's stained blood smear as they usually appear encysted.

The encysted gametocytes in the Wright's stained material measured 10.3 to 12.8 microns long (s.d. 0.68) by 2.4 to 5.1 microns wide (s.d. 0.83) with an average (10 specimens) of 11.3 by 3.6 microns. The nucleus measured about 5.3 by 3.0 microns. Encysted gametocytes in the fresh blood concentrates measured from 9.1 to 13.7 microns long (s.d. 1.07) by 3.0 to 4.6 microns wide (s.d. 0.48) with an average (18 specimens) of 11.4 by 3.6 microns. The nucleus measured about 4.0 by 2.5 microns. These measurements are comparable with those given by Clark

TABLE X. Incidence of Hepatozoon griseisciuri Clark, 1958 in the gray squirrel, Sciurus carolinensis Gmelin, 1788, in southwestern Virginia as indicated by two diagnostic procedures

	Modified Knott's concentration technique		Wright's stained blood smears	
	No. examined	% infected	No. examined	% infected
Male squirrels	41	80	42	60
Female squirrels	39	77	42	57
Totals and Averages	80	79	84	58

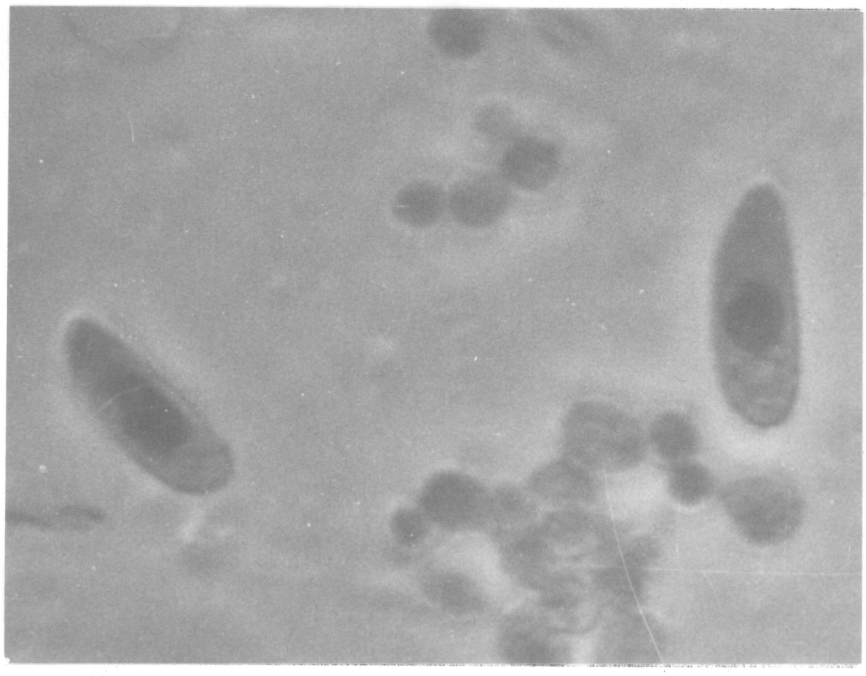


PLATE 10. Freed gametocytes of Hepatozoon griseisciuri Clark, 1958, as seen in blood concentrates (4000 x)

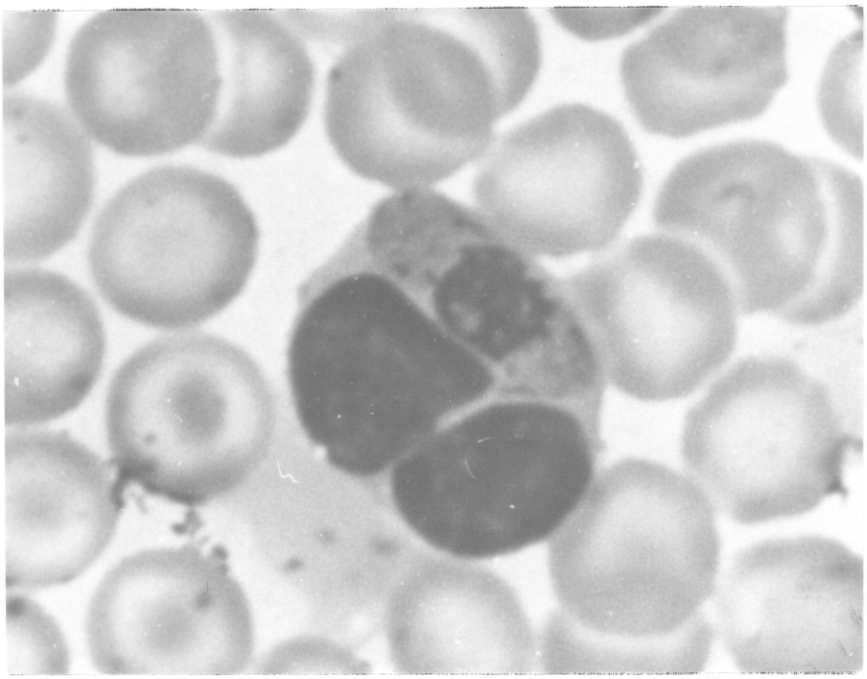


PLATE 11. Gametocyte of Hepatozoon griseisciuri Clark, 1958, encysted within leukocyte as photographed from a Wright's stained smear (4000 x)

(1958) who gave a range of 9.9-11.8 microns (average 10.9 microns) for length and 3.3-4.0 microns (average 3.4 microns) for width for encysted specimens in blood smears and an average of 12 microns long by 4 microns wide for encysted gametocytes in fresh blood concentrates.

In addition to the gray squirrels herein reported to be infected with Hepatozoon in southwestern Virginia, I have had occasion to examine materials acquired from squirrels in North Carolina, Georgia and Florida. Table XI provides a comparison of results of previous studies with that of the present one. These data extend the presently known distribution of Hepatozoon griseisciuri in Washington, D. C., Maryland, Rhode Island and Wisconsin to southwest Virginia, western North Carolina to northwest Georgia. The Florida material that I examined was negative.

I examined histological sections of various tissues and femoral bone marrow smears from juvenile to adult squirrels which revealed no evidence of the schizogonic stages of the parasite. However, one squirrel was born in captivity of an infected parent and died the same day. I prepared histological sections of the liver, spleen and kidney and upon examination, I saw numerous schizonts (Plate 12) in various stages of development in the liver and spleen. Since there were apparently no mites in the cage or on the parent squirrel and there was not sufficient time for the extent of development of the parasites herein observed from the time of birth to death, transmission apparently occurred across the placenta. These results help to substantiate the findings of Clark (1958) who was unable to locate these stages in any squirrels older than 36 hours. The hypothesis put forth by Clark

TABLE XI. Present records on the distribution of Hepatozoon griseisciuri Clark, 1958, in the gray squirrel, Sciurus carolinensis Gmelin, 1788

Locality	Stained smears		Concentrates		References
	No. examined	% infected	No. examined	% infected	
Patuxent Refuge, Maryland	86	48	64	100	Herman and Price (1955)
Takoma Park, Maryland	10	50	12	100	ibid.
Baltimore Co., Maryland	45	71	--	--	ibid.
Natl. Zool. Park, Wash., D.C.	19	16	21	100	ibid.
Rhode Island	11	27	--	--	Weidanz and Hyland (1958)
Wisconsin	21	5	4	50	Dorney and Todd (1959)
Virginia (southwestern)	84	58	80	79	present study
North Carolina (western)	9	55	9	66	present study
Georgia (northwestern)	5	60	5	100	present study
Florida (north central)	4	0	--	--	present study

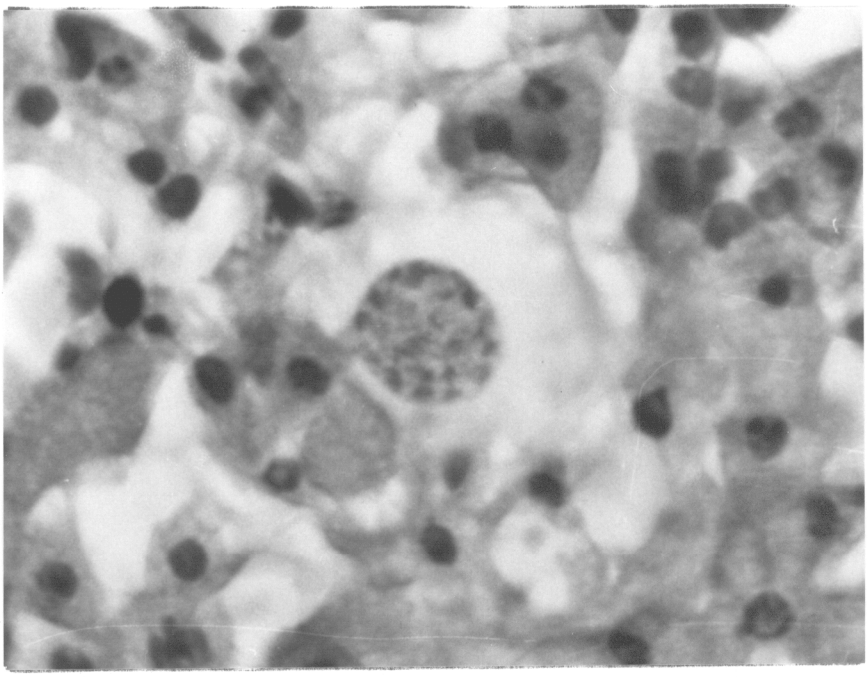


PLATE 12. Photomicrograph of schizont of Hepatozoon griseisciuri Clark, 1958, from 10 micron section of liver of a one-day-old gray squirrel (643 x)

(1958) for their apparent absence in the older squirrels was that possibly schizogony occurred ". . . throughout the blood spaces of the reticuloendothelial system without special emphasis on a particular organ." I was not able to confirm his hypothesis. If, indeed, there are no tissue stages in older squirrels, the perpetuation of the parasite in the blood remains mysterious since I found squirrels known to be at least 4 years old (from retrap tags) to be relatively heavily infected.

The in vitro cultivation attempts using leukocyte inoculum in various media proved unsuccessful in all but one case (Table XII). Only in the Brain-Heart infusion medium plus 0.1% agar were any Hepatozoon organisms observed. On the day following inoculation vermicular organisms were seen moving in this medium upon examination by phase contrast microscopy. These organisms were broad at one end, revealed a granular cytoplasm, and the smaller end appeared to serve as a holdfast structure (Figure 7). The movement appeared to consist of whip-like or thrashing movements into C- or U-shapes with the small end usually remaining stationary against the slide or coverslip. They did not appear motile or show any phototaxic or photophobic responses.

These organisms were observed each day for four days after which no movement could be detected, and they were assumed to be dead. The procedure using the Brain-Heart infusion medium plus 0.1% agar was repeated as initially outlined using blood inoculum from a different infected squirrel. The results from this second attempt were identical to the first. Subsequent repeats were unsuccessful.

TABLE XII. Culture procedures used in an attempt to maintain Hepatozoon griseisciuri Clark, 1958, using leukocyte inoculum from infected gray squirrels, Sciurus carolinensis Gmelin, 1788

Culture medium	Leukocyte inoculum	Tubes inoculated	Results	Controls
T-soy broth[a]	3-6 drops	3	Negative	1 (negative)
T-soy broth + 0.1% agar	3-6 drops	3	Negative	1 (negative)
Albimi's Brucella broth[a]	3-6 drops	3	Negative	1 (negative)
Albimi's Brucella broth + 0.1% agar	3-6 drops	3	Negative	1 (negative)
Brain-Heart infusion[a]	3-6 drops	3	Negative	1 (negative)
Brain-Heart infusion + 0.1% agar	3-6 drops	3	Positive[c]	1 (negative)
Medium 199[b]	1-2 drops	3	Negative	1 (negative)

a/ DIFCO products
b/ Microbiological Associates, Inc. product
c/ Maintained alive in vitro for four days

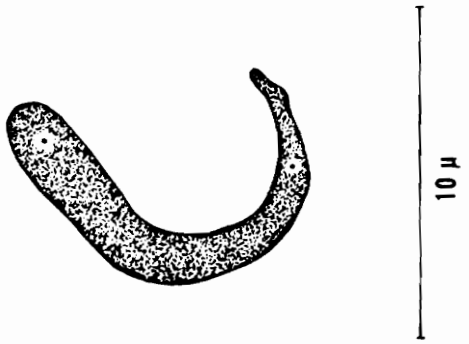


FIGURE 7. Hepatozoon griseisciuri, motile gamete from Brain-Heart infusion culture + .1% agar

From these observations, it is reasonably certain that the stage of the organism that survived in the culture medium was the motile gametes which had become excysted. These are apparently the same stages (gametes) observed by Clark (1958) who liberated the gametocytes from the leukocytes by osmotic disturbance (dropping several cc of blood into distilled water), washing several times to remove the cellular debris and examining in saline. He did not mention whether or not he was able to maintain them alive outside the host for any notable length of time.

No multiplication, growth or union of these gametes was observed. However, these experiments do show that the parasite may be kept alive for at least 4 days outside the host. Modifications of the procedure and/or serial transfers using fresh media might be ways to maintain them for longer periods in future studies. Also, if the schizogonic stages could be recovered and cultured, possibly some in vitro multiplication would be possible. In vitro cultivation of these organisms would be very helpful in order to gain more information concerning physiological, nutritional, behavioral and other aspects of these parasites.

Pathology

In general, pathogenic effects are not observed in animals infected with the various species of Hepatozoon, and there is little evidence in this study of pathological conditions precipitated by this parasite in squirrels, except that many of the squirrels examined had very large spleens and the one-day-old squirrel exhibited cell

destruction in the liver and spleen as the result of schizogonic development.

I can briefly summarize the pathological evidence which exist to date. Richards (1961) reported no pathogenic effects of Hepatozoon procyonis Richards, 1961 in raccoons. However, Furman (1966), working with Hepatozoon balfouri (Laveran, 1905) in jerboas (Jaculus jaculus jaculus), reported massive involvement of liver tissue with parencymal cell destruction by the growing trophozoites and the spleen enlarged three to four times. However, there was no other evidence of host reaction. Brumpt (1946) has shown that intense infections of H. muris in rats reveal hemorrhagic spots and small lesions on the liver at autopsy, and Miller (1908) indicated that the liver is distinctly enlarged and dull yellow in chronic cases. The lungs also showed small surface hemorrhages (Miller, 1908) and the spleen was dark in color and enlarged (Miller, 1908; Brumpt, 1946). The blood may show a great increase in the number of leukocytes (Brumpt, 1946) and also may be thin and watery (Miller, 1908). Very heavy infections often resulted in the death of the rat (Miller, 1908; Brumpt, 1946).

Conclusion and Summary

Hepatozoon griseisciuri appears to be prevalent (79% incidence, Table XI) in the squirrels of southwest Virginia. These figures are less than those reported by Herman and Price (1955) in Maryland and Washington, D. C. (100%) and in the present study from Georgia (100%), but greater than the figures given by Weidanz and Hyland (1958) in

Rhode Island, by Dorney and Todd (1959) in Wisconsin and by the present data from North Carolina. As more data become available, it may be possible to determine some correlations of incidence of infection with geographical conditions such as altitude and climate. Also correlations between the distribution of Hepatozoon and that of the mite vector should be studied. This could determine the importance of prenatal infection across the placenta (as proposed by Clark, 1958).

Specimens, in addition to those from southwest Virginia, were examined by me and served to extend the known distribution of this parasite into North Carolina and Georgia.

A search for schizogonic stages in histological preparations failed to demonstrate the parasites in the juvenile to adult squirrels examined. However, these stages were seen in sections of liver and spleen which was taken from a one-day-old squirrel born in captivity. Their apparent absence in the tissues of older squirrels and high prevalence of gametocytes in the circulating blood requires more study in order to clarify this situation.

Several attempts were made to maintain living parasites in vitro by using several culture media. The moving, excysted gametes were kept alive in vitro for four days without any apparent multiplication or union of gametes. Further studies along similar avenues may produce a method for maintaining them for longer periods and make available new techniques for study.

No pathological disturbances could be ascertained except that splenomegaly was observed in many of the hosts and in a one-day-old squirrel examined there was evidence of cell destruction by the

schizogonic development of the parasite.

C. Flagellates

In the examinations of fecal material from gray squirrels collected in southwestern Virginia, I discovered the cysts of the intestinal flagellate, Giardia sp. This is apparently the first observation of the parasite in this host. I also found one squirrel of nine examined from North Carolina to be infected with this parasite (Parker and Holliman, 1971; also included in the Addendum).

Giardia sp.

Giardia has been found in a wide variety of animals, including mammals, birds, reptiles, amphibians, fish and even nematodes. I found two reports of Giardia in rodents of the family Sciuridae: Giardia sp. in Citellus richardsoni Sabine, 1822, (Mackinnon and Debb, 1938) and Giardia beckeri Hegner, 1926, in Citellus tridecemlineatus Mitchill, 1821, in Iowa (Hegner, 1926). Ansari (1952) has maintained that Giardia is strictly host-specific and that reports of the same species in different hosts have been due to false identifications of either parasite or host or both. Therefore, it has traditionally been the case to label almost all giardias occurring in different hosts by different specific names. Early cross-transmission attempts from one host to another appeared negative. Lavier (1924) failed to infect laboratory rats with Giardia lamblia from man; and Armaghan (1937) inoculated rats with trophozoites from the muskrat and cysts from rabbits and mice with negative results. Other evidence seems to point toward a less rigid

host specificity. Felice (1952) did not report any significant morphological differences between the giardias of various wild rodents and those of laboratory rats. A number of successful cross-transmissions have been reported but as yet have not initiated the stimulus necessary to begin a taxonomic evaluation of the genus. Hegner (1927), Armaghan (1937) and Halba (1956) have succeeded in infecting laboratory rats with Giardia from man; and Bonestell (1935) experimentally infected 4 wood rats, Neotoma fuscipes, with Giardia from man and concluded that rats may possibly serve to spread human giardiasis. I suggest that a concentrated effort is needed to study the host specificity of this organism before it can be determined if the many described species are indeed valid species or if all are actually one or several species, some of which may show host induced morphological variations.

The in vitro cultivation of Giardia had been unsuccessful until Karapetyan (1960) succeeded in culturing giardias from humans in mixed cultures with human intestinal yeasts and chicken fibroblasts, a trypsin digested protein extract, human serum and a balanced salt solution. Karapetyan (1962) also similarly cultured giardias from rabbits, but, instead of using chicken fibroblasts, he substituted chicken embryo extract and instead of intestinal yeasts, he used ordinary baker's yeast. Substitutions of different blood serums were also equally successful when transitions from one to the other were gradual. Meyer and Pope (1965) are apparently the only other investigators who have attempted to duplicate the culture procedures of Karapetyan. They found it necessary to modify the procedure somewhat in their cultivation of giardias from rabbits and chinchillas. Their modification involves

the daily reinoculation of yeast to the cultures instead of only one initial inoculation, also they adjusted the pH daily using a 0.1 N solution of sodium bicarbonate.

The present study sought to determine the prevalence of Giardia in our sample of gray squirrels; to perform cross-transmissions in order to learn something about the host specificity of Giardia from the gray squirrel; to attempt in vitro cultivation of the organism and to present a brief description of Giardia from the gray squirrel.

Materials and Methods

To determine the prevalence of the intestinal flagellate, Giardia, in the squirrels I collected, examinations of the wet mount preparations of fecal material from each squirrel, in conjunction with the study on coccidia already discussed, were made to determine if the cysts of this organism were present. In addition, trapping operations were initiated in a campus woodlot using box traps (Mosby, 1955) to obtain squirrel hosts infected with Giardia. This woodlot had yielded infected squirrels in the initial survey. These additional squirrels were collected for use only in the study of this parasite and are not included in the numbers collected for any other survey. Trapped squirrels were brought to the laboratory in the traps, their fecal material was examined, and if the animal proved to be negative, it was taken back to the place of capture and released. After 23 animals had been trapped and examined, I finally succeeded in acquiring one infected host. This animal was initially recorded as being negative, but after

retaining it in the laboratory for several days and feeding it on cracked corn, subsequent examinations demonstrated a high number of Giardia cysts in the feces. Fecal samples were taken from this animal and dissolved in tap water. The mixture was stirred and poured through several layers of cheese cloth, then centrifuged and washed several times. This procedure yielded a high number of giardial cysts suspended in a small volume of water which I then used to experimentally infect other hosts. This material was added to the drinking water of the animals to be infected. For cross-transmission experiments, 3 parasite-free gray squirrels were exposed to the drinking water containing the cysts to insure a source of cysts for experimental work. Then, 6 hamsters, 6 laboratory white mice and one chipmunk, Tamias striatus, were fed cysts in a similar manner. These animals had been checked for Giardia infections before being exposed to the parasite.

Experimentally infected gray squirrels, laboratory white mice and hamsters were sacrificed and the trophozoites of the parasites were recovered and used for culture studies. Attempts to maintain Giardia in cultures were made by following both the procedures of Karapetyan (1962) and the modification by Meyer and Pope (1965). The culture medium was prepared using the following constituents:

25% inactivated bovine serum
10% Hottinger's digest (tryptic meat digest)
5% Chick embryo extract (purchased from Microbiological Associates, Inc.)
60% Hank's balanced salt solution with phenol red as a pH indicator

The medium was adjusted to pH 7.3 and a penicillin/streptomycin mixture added to make a final concentration of 500 and 250 units per ml respectively.

For preparations of the inoculum, the infected animal was killed with chloroform or ether, the body cavity opened and about 15-25 cm of the duodenum removed and placed in a sterile petri dish. The segment of the intestine was slit open lengthwise and the inside scraped with a scalpel. The remaining shell of the intestine was discarded and the tissue and intestinal contents retained. To this I added 15-20 ml of Hank's balanced salt solution and mixed. Then I filtered the material through several layers of washed cheese cloth and the filtrate centrifuged at 1,000 rpm. for 10 minutes. The supernatant fluid was drawn off using a Pasteur pipette and the sediment containing the giardial trophozoites inoculated into small culture bottles containing 1 to 3 ml of media. These bottles were about 46 mm long and 14 mm in diameter with a smaller neck fitted with rubber stoppers. Following inoculation of Giardia, a loopful of yeast (Saccharomyces cerevisiae) from a 48 hr old culture was also inoculated into the medium and the culture bottles were placed in an incubator at 36-38 degrees Centrigrade, the bottles being slanted at a 5-7 degree angle. The cultures were examined daily and two-thirds of the medium was removed and replaced with fresh medium daily. Some cultures were reinoculated with the yeast daily (modification by Meyer and Pope, 1965), while others had only the one initial inoculation (after Karapetyan, 1960, 1962). Stock medium was maintained in a stoppered flask and was examined periodically, serving as my control.

Results and Discussion

In conjunction with the coccidia study, fecal samples from 167 gray squirrels were examined by the wet mount method for the presence of Giardia cysts. These examinations demonstrated 8 (4.79%) squirrels to be infected with the intestinal flagellates (6 males and 2 females). I suspect that this is an infection rate that is much lower than that which actually occurs, since one additional squirrel, not included in this survey, was trapped and maintained for a short time in the laboratory and was negative on initial examination of the feces, but after being fed cracked corn for several days, numerous cysts appeared in the feces. It is likely that the rough food material in some way promoted the movement of Giardia trophozoites down the alimentary tract where they encysted before passing in the feces. I have not been able to support this hypothesis with further evidence to date. Using a hemocytometer, estimates were made of the numbers of Giardia cysts occurring in the feces of 5 naturally infected hosts. These estimates yielded 336,000, 504,000, 600,000, 840,000 and 1,920,000 cysts per gram of feces in each of these hosts. The occurrence of Giardia in the gray squirrel constitutes a new host record.

As noted earlier, the assignment of different specific names for giardias occurring in different hosts has been a common practice, many without reference to cross-transmission studies to substantiate their apparent host-specificity. Possibly many of these so-called species are synonymous. Attempts to infect other rodents with the Giardia I found in squirrels were undertaken. Table XIII outlines

TABLE XIII. Animals given drinking water contaminated with Giardia sp. cysts obtained from a naturally infected gray squirrel

Experimental host	No. animals exposed	No. animals infected	Relative abundance of trophozoites at autopsy
Gray squirrel	3	3 (100%)	Very high
Hamster	6	6 (100%)	Moderate to high
White mice	6	6 (100%)	Low to moderate
Chipmunk	1	1 (100%)	Very low

the results of those attempts. My results show that all of the animals exposed to the cysts in the drinking water became infected with Giardia from the naturally infected gray squirrel. The average prepatent period was about 5 to 7 days. Cysts could be demonstrated in the feces of the gray squirrels, hamsters and most of the white mice. However, no cysts were ever observed in the feces of the chipmunk and, when this animal was sacrificed, trophozoites were relatively scarce when compared with the numbers occurring in the other experimental hosts. The magnitude of infection of the chipmunk was expected to be similar to that of the gray squirrel since they are more closely related, taxonomically, than the squirrel is to hamsters and mice. Possibly the low infection of the chipmunk was due to its drinking habits in that it appeared to drink less water than the other animals, thus receiving fewer cysts. The most important fact is, however, that Giardia obtained from a gray squirrel can be transmitted to other hosts. Our work shows no host specificity for the parasite. The naturally infected gray squirrel has been retained in captivity for 8 months in the laboratory, during which time it has continually passed a high number of Giardia cysts in the feces.

In my attempts of in vitro cultivation of Giardia taken initially from one naturally infected gray squirrel and experimentally transferred to other gray squirrels, hamsters and laboratory white mice, I have only been partially successful. Trophozoites taken from experimentally infected gray squirrels were inoculated into cultures as previously outlined. Following the procedures of Karapetyan (1962) in which one initial yeast inoculum was used, I kept the trophozoites

alive in cultures for 14 days. Signs of multiplication were evident about the fifth day, but from the tenth to the fourteenth day, they began dying until none were alive in the cultures. Using the same stock medium, other culturing was done in which I followed the modification of Meyer and Pope (1965) by reinoculating with yeast daily. By using this modification, I was able to keep the trophozoites alive in culture for 21 days. Multiplication was apparent on the sixth day and continued up to the nineteenth day after which the numbers rapidly decreased until all were dead at the end of the twenty-first day. Two other attempts, using experimentally infected gray squirrels as a source for the trophozoites, did not yield any better results. Several attempts to culture Giardia trophozoites recovered from experimentally infected hamsters and white mice were even less successful. These trophozoites only survived from one to five days in the culture without any apparent signs of multiplication.

Several reasons may be given for our inability to maintain Giardia in culture for extended periods of time. Foremost was the problem of pH change which occurred continually, both in the stock media and in the culture tube. More pronounced shifts occurred in the latter. The changes in pH, which tended toward the acid, were partially alleviated by the use of a 0.1 Normal solution of sodium bicarbonate to return to pH 7.3. However, after use of this to adjust the pH, undoubtedly there was some change in the balance of the salt ions. There was also the problem of mold contaminating the media. Other factors may have been the strain of yeast and the chicken embryo extract used which could

not have been identical to those used in the studies by Karapetyan (1962) and Meyer and Pope (1965).

The following is a brief description of the Giardia sp. from the gray squirrel: The trophozoites (Plate 13; Figure 8a) are somewhat pyriform in shape. Twenty-five trophozoites measured 13.68 to 20.52 microns in length by 8.55 to 11.97 microns in width, with an average of 16.90 by 10.50 microns. There are two nuclei, two sucking discs and 8 flagella present. The shapes of the median bodies are highly variable. Some appear rounded while others appear as straight and curved bars. The latter were noted particularly in actively dividing forms. The cysts (Figure 8b) are usually ellipsoidal and contain four nuclei near one end. Several thickened flagella are normally visible within the cyst. Twenty-five cysts measured 13.16 to 15.96 microns in length by 8.40 to 10.64 microns in width, with an average of 14.12 by 8.91 microns. The trophozoites generally occur throughout the upper small intestine, becoming less numerous in the ilium and absent in the cecum and large intestine. The cysts are usually absent in the duodenum, a few are to be found in the ilium, but are mainly seen in the cecum and large intestine before passing with the feces.

Pathology

The squirrels observed infected with giardias in the general survey did not exhibit any gross pathology or clinical symptoms of distress. However, one squirrel, experimentally infected with giardia in the laboratory, regurgitated frequently and also had diarrhetic stools.

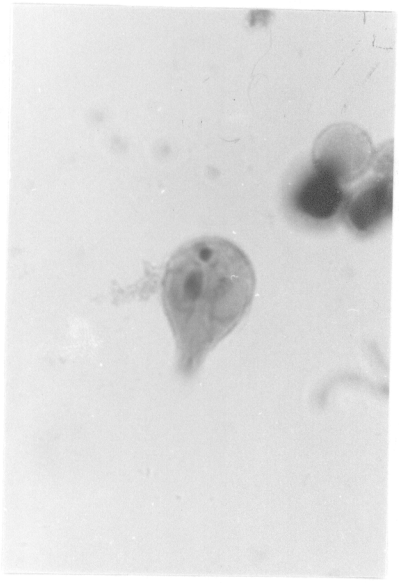


PLATE 13. Photomicrograph of a trophozoite of Giardia sp. from the gray squirrel; stained with iron-alum hematoxylin; 1360 x

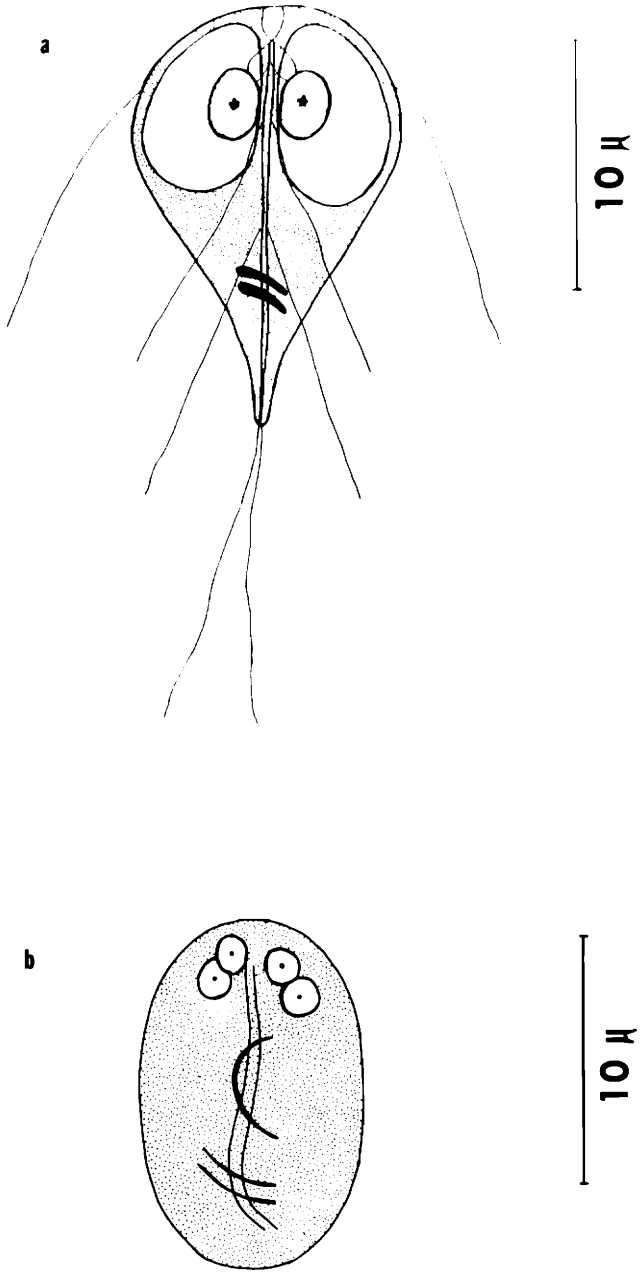


FIGURE 8. Giardia sp. a. trophozoite b. cyst

The animal was rather sickly in appearance and had a dull, brittle coat. Upon sacrifice of the animal no gross pathological lesions were evident in the small intestine.

Conclusions and Summary

In my survey of squirrel feces for the occurrence of the intestinal flagellate, *Giardia* sp., I found a relatively low incidence of cysts (about 5%) in the squirrels examined. I believe that the actual occurrence is probably higher than this in which there may be many subclinical infections in which the cysts are not demonstrable in the feces.

From cross-transmission studies it is concluded that there is little host specificity shown by the *Giardia* sp. from the gray squirrel. I was able to establish infections in 6 hamsters, 6 white mice and one chipmunk (100% of the attempts), however, the degree of infection was not observed to reach the intensity seen in 3 experimentally infected gray squirrels. The prepatent period was about 5-6 days, while the patent period appears to last indefinitely. All experimentally infected animals were sacrificed while infected.

I have attempted to culture this organism *in vitro* using the procedures of Karapetyan (1962) and also the modification by Meyer and Pope (1965). I succeeded in maintaining them in culture, in which multiplication was apparent, for a maximum period of 21 days using the modification by Meyer and Pope (1965). The procedure of Karapetyan (1962) allowed survival in cultures for a maximum period of only 14

days. Attempts to culture the Giardia from experimentally infected hamsters and white mice using both procedures demonstrated survival of the organisms only up to 5 days, usually 1 to 3 days, with no apparent multiplication. These differences in the reaction to the culture medium between the Giardia from its natural host (squirrel) and an experimental host (hamster and mouse) suggest that there may have been some induced changes in the parasite. It is hypothesized that possibly some change in the organism had occurred as a result of host influence. If so, these were physiological changes since no morphological differences were noted.

A new host record is established by my finding Giardia in the gray squirrel. This is the third host species of the rodent family Sciuridae known to harbor giardias. This discovery has potential medical significance because hunters come in contact with squirrel carcasses and thus may be exposed to infection. The lack of parasite host specificity shown by this organism indicates that it may be of clinical importance and the array of species named from various hosts may contain much synonomy.

II. HELMINTHS

Bibliographies which include the literature on helminths from gray squirrels have been given by Katz (1939), Doran (1954a, 1954b, 1955) and Clark (1959). These cited references to the occurrence of one species of acanthocephala, one trematode, at least nine cestodes and at least nineteen nematode parasites. In view of the numbers of squirrels examined, the more extensive studies related to helminths of this host have been Harkema (1936), Katz (1938), Rausch and Tiner (1948) and Parker (1968). Further discussion of the literature will follow when each group of organisms is considered.

Materials and Methods

The viscera and body cavities of 175 gray squirrels were examined for helminths. Visceral organs were either injected with and preserved in 10% buffered formalin for later examination or examined in physiological saline while fresh. The larger portions of the alimentary tract (stomch, small intestine, cecum and large intestine) were slit open longitudinally and washed in running tap water over a #325 screen. The residue left on the screen was collected in a minimal amount of water and, a little at a time, was examined at 15X under a dissection microscope. The inner surfaces of these hollow organs were also examined under the dissection microscope. The duodenum and esophagus

were pinned to a wax-bottomed petri dish for careful observation. The other visceral organs were dissected and examined under 7x. These included liver, spleen, gall bladder, lungs, diaphragm, kidney, heart, mesenteries and urinary bladder. The helminths were collected, counted, identified and stored in 70% ethanol.

Examinations for microfilariae were made in conjunction with the Hepatozoon survey already discussed (modification by Herman and Price, 1955, of Knott's, 1939, concentration technique) and helminth eggs occurring in the feces were recorded in conjunction with the coccidia study discussed earlier.

A. Phylum Platyhelminthes

Class Trematoda

Trematodes have seldom been reported in this host. Chapman (1938) recorded a "fluke" from a gray squirrel in Ohio and Olexik, Perry and Wilhelm (1969) recently reported the trematode, Nudacotyle sp., from the small intestine of three gray squirrels in Shelby County, Tennessee. I did not encounter any trematodes in this study.

Class Cestoda

Hall (1911) reported larval Multiceps serialis (Gervais, 1847) Stiles and Stevenson, 1905, from the gray squirrel in the United States. In 1928, Schwartz noted the occurrence of "larval tapeworms" in various organs of gray squirrels in Falls Church, Virginia, and Bowie, Maryland. Harkema (1936) reported larval Taenia taeniaeformis (Batsch,

1786) Wolffhugel, 1911, from the gray squirrel in North Carolina. Katz (1938) listed Catenotaenia sp. and Hymenolepis sp. in gray squirrels in southern Ohio. In 1946, Rankin reported Cittotaenia pectinata americana (Goeze, 1782) Stiles and Hassel, 1896, in western Massachusetts. Rausch and Tiner (1948) reported Catenotaenia pusilla (Goeze, 1782) Janicki, 1904, and Hymenolepis diminuta (Rudolphi, 1819) Blanchard, 1891, in the North Central states, and Freeman (1954) successfully infected gray squirrels with the eggs of Taenia crassiceps (Zeder, 1800) Rudolphi, 1810, and obtained mature cysticerci. Packard, in 1956, reported Catenotaenia sp. in Kansas. Oldham (1961) recovered a single Cysticercus fasciolaris from one squirrel and some other unidentified larval cestodes from two other squirrels out of 100 he examined in southeastern England. Parker (1968) reported Catenotaenia sp. from southwestern Virginia. Recently Olexik, Perry and Wilhelm (1969) reported the occurrence of Hymenolepis nana (von Siebold, 1852) and an unidentified species of pseudophyllidian cestode from gray squirrels of southwest Tennessee. These last two are questionable since the H. nana "adults" were reportedly "encysted" in the liver and the pseudophyllidian was identified only on the basis of gravid proglottid anatomy and operculated eggs. No scolex was recovered. Other cestodes of tree squirrels have been mentioned in the literature, but the identification of the host species is not very clear in these.

Results and Discussion

Five hosts (3.00%) harbored adult cestodes in the small intestine from which two species were recovered. No larval cestodes were observed in any of the squirrels examined, however, one squirrel used in experimental studies of the Giardia sp. was found to be parasitized by larval cestodes. Dr. J. A. McLeod of Brandon University, Brandon, Manitoba, Canada, tentatively identified these as sparganid-type larvae of one of the spirometrids of the cestode order Pseudophyllidea. Spirometra mansonoides (Mueller, 1935) appears to be the only known species in North America. Specific identification could not be made from the larval forms which to date have been poorly defined. A total of 18 plerocercoids were recovered from this one squirrel in which 15 were encysted and 3 were free in the body cavities. Of those encysted, one was located on the liver and 14 were attached to the mesenteries. There was a double cyst wall composed of an outer host generated wall and an inner parasite generated wall. The larvae occurring free in the coelom numbered one in the thoracic cavity between the lobes of the lungs and two in the abdominal cavity among the viscera. These larvae appear to be of the non-budding type with a single holdfast at one end.

Order Cyclophyllidea

Family Catenotaeniidae Spassky, 1950

Catenotaenia dendritica (Goeze, 1782)

Four hosts harbored Catenotaenia dendritica and the number of worms per infection ranged from 1 to 3 (average 2.00). The hosts of these worms were 2 subadult females, one adult female and one subadult

male. Three hosts were collected from the fringes of forested areas and one from a remote forest area. Two hosts were captured in the months of August and two in September.

This tapeworm utilizes an intermediate host which is a free-living tyroglyphid mite in which the larval stage is a merocercoid. The definitive host, in this case the gray squirrel, becomes infected upon accidentally ingesting the mites which contain the merocercoid larvae.

Apparently the gray squirrel is not the usual definitive host for this worm due to the low percentages of infestation. This appears to be the first report of this species from the gray squirrel. Other hosts from which it has been recorded that inhabit the areas from which the infested squirrels were collected include Clethrionomys gapperi (Vigors) and Peromyscus maniculatus (Wagner). Perhaps these are the usual definitive hosts.

The particular intermediate host involved in the transmission to squirrels is not known at the present time.

Family Hymenolepididae Railliet et Henry, 1909

Hymenolepis diminuta (Rudolphi, 1819) Blanchard, 1891

One host harbored one adult Hymenolepis diminuta. This worm was recovered from an adult male host captured on a farm woodlot in the month of June. The worm measured 160 mm in length and 3 mm at its greatest width. The eggs were about .068 by .063 mm.

This species is a common parasite of many kinds of rodents including the genera Rattus, Mus and Microtus which occur in

southwestern Virginia. It has also been recorded in man. It requires an intermediate host in which the larva is a cysticercoid. The intermediate hosts recorded for this species are numerous and varied, but according to Chandler and Read (1962) are predominantly grain-infesting insects such as meal months, earwigs and grain beetles and also dung beetles, flea larvae and myriapods.

This appears to be the third report of H. diminuta in gray squirrels and the first in Virginia. Previous reports of the genus occurring in squirrels were in Ohio (Katz, 1938) and the North Central states (Rausch and Tiner, 1948).

This worm could constitute some pathological importance to squirrels if it became frequent in numbers and in occurrence. Squirrels appear to be accidental hosts, but in such cases possibly serve as reservoirs and aid in the dissemination of the parasites. In southwestern Virginia the apparent rarity of this species in squirrels does not constitute a significant problem.

B. Phylum Acanthocephala

The only acanthocephalan reported from gray squirrels has been Moniliformis clarki (Ward, 1917) Van Cleave, 1924, by Chandler (1947) from squirrels in Florida. No acanthocephala have been encountered in the present study, however, in the addendum I will report on the occurrence of Moniliformis clarki in squirrels from Florida.

C. Phylum Aschelminthes

Class Nematoda

Price (1928) described Heligmodendrium hassalli (Price, 1928) Travassos, 1937, from the gray squirrel in Maryland. Cameron (1932) described Enterobius sciuri from the introduced American gray squirrel in Scotland. In 1936, Harkema reported Longistriata hassalli=Heligmodendrium hassalli from this host in North Carolina. Katz (1938) listed larval Ascaris lumbricoides Linn., 1758, Citellinema bifurcatum Hall, 1916, Heligmodendrium hassalli and Rictularia sp. in Ohio. "Nematoda" sp. and "pinworms" were given by Goodrum (1940) in Texas. Chitwood and Graham (1940) noted the occurrence of Strongyloides sp. in Texas. Reiber and Byrd (1942) listed Citellinema bifurcatum, Citellinema sleggsi Manter, 1930 and Strongyloides papillosus (Wedl, 1856) Ransom, 1911. Citellinema bifurcatum and C. sleggsi were shown to be synonymous by Dikmans (1938). Also in 1942, Chandler reported Heligmodendrium hassalli and described Strongyloides robustus from the gray squirrel in southeastern Texas. In 1943, Lucker described the nematode, Böhmiella wilsoni, from the stomachs of gray squirrels in West Virginia, Virginia and Georgia. Impalaia sp. and Mecistocirrus sp. were reported by Brown and Yeager (1945) in Illinois. Rausch and Tiner (1948) listed a number of nematodes found in gray squirrels in some of the North Central states. These included Böhmiella wilsoni, Heligmodendrium hassalli, immature Physaloptera sp., Rictularia sp., Rictularia halli Sandground, 1935, and Trichostrongylus calcaratus Ransom, 1911, from Wisconsin; Capillaria sp. from Ohio and Wisconsin;

Strongyloides sp. from Michigan and Wisconsin and Citellinema bifurcatum and Trichostrongylus sp. from Minnesota. Capillaria americana was described by Read (1949) from gray squirrels in Illinois, Ohio and Wisconsin. Also, in 1949, Tiner reported the experimental infection of a gray squirrel in Illinois with larval Ascaris columnaris Leidy, 1856. Packard (1956) reported the occurrence of Strongyloides robustus in gray squirrels in Kansas. Microfilariae were reported from gray squirrels in the Washington, D. C.-Baltimore, Maryland area by Price (1954) and in Georgia by Robinson (1954). Evans, Phillips and Bickley (1959) reported the occurrence of the microfilaria, Dipetalonema sp., in Maryland. In 1962, Price described a microfilaria from the gray squirrel in Maryland, giving it the name Dipetalonema interstitium. Parker (1968) reported five species of nematodes from gray squirrels in southwestern Virginia. They were Citellinema bifurcatum, Böhmiella wilsoni, Contracaecum sp., Rictularia coloradensis Hall, 1916 and Sypahcia thompsoni Price, 1928. Olexik, Perry and Wilhelm (1969) listed Citellinema sleggsi (=C. bifurcatum), Rictularia sp., Heligmosomum sp. (probably the genus Heligmodendrium), Strongyloides sp., Capillaria sp. and a large stomach worm of the Trichostrongylidae (probably Bohmiella) from gray squirrels in southwestern Tennessee. Lichtenfels (1970) recently described the nematode, Pterygodermatites parkeri, from this host in southwestern Virginia. Table XIV provides a cross-reference to this literature for easy reference and locality distribution comparisons. It can readily be seen that the data from many localities within the gray squirrel's distribution are widely scattered or lacking.

TABLE XIV. Cross reference to the literature of nematode parasites of the gray squirrel, Sciurus carolinensis, for reported localities

Nematode species	Localities															References
	Georgia	Illinois	Kansas	Maryland	Michigan	Minnesota	North Carolina	Ohio	Tennessee	Texas	Virginia	Washington, D. C.	West Virginia	Wisconsin	Scotland[1]	England[1]	
Ascaris columnaris[2] (larval)		X															Tiner (1949)
Ascaris lumbricoides (larval)								X									Katz (1938)
																X	Oldham (1961)
Bohmiella wilsoni	X										X		X				Lucker (1943)
														X			Rausch and Tiner (1948)
											X						Parker (1968)
Capillaria sp.														X			Rausch and Tiner (1948)
											X						Hanson (1966)
									X								Olexik, et. al. (1969)
Capillaria americana		X						X						X			Read (1949)
								X						X			Rausch and Tiner (1948)
Citellinema bifurcatum						X											Rausch and Tiner (1948)
									X								Reiber and Byrd (1942)
								X									Katz (1938)
											X						Parker (1968)
Citellinema sleggsi=C. bifurcatum									X								Reiber and Byrd (1942)
			X														Packard (1956)
Contracaecum sp. (larval)											X						Parker (1968)
Dipetalonema sp.				X													Evans, et. al. (1959)
Dipetalonema interstitium				X													Price (1962)
Enterobius sciuri															X		Cameron (1932)
Heligmodendrium hassalli														X			Rausch and Tiner (1948)
										X							Chandler (1942)
				X													Price (1928)
								X									Katz (1938)
Impalaia sp.		X															Brown and Yeager (1945)
Longistriata hassalli							X										Harkema (1936)
Mecistocirrus sp.		X															Brown and Yeager (1945)
microfilariae				X								X					Price (1954)
	X																Robinson (1954)
nematoda species										X							Goodrum (1940)
Physaloptera sp. (larval)														X			Rausch and Tiner (1948)
pinworms										X							Goodrum (1940)
Pterygodermatites parkeri											X						Lichtenfels (1970)
Rictularia sp.														X			Rausch and Tiner (1948)
								X									Katz (1938)
									X								Olexik, et. al. (1969)
Rictularia coloradensis											X						Parker (1968)
Rictularia halli														X			Rausch and Tiner (1948)
Strongyloides sp.					X												Rausch and Tiner (1948)
										X							Chitwood and Graham (1940)
									X								Olexik, et. al. (1969)
Strongyloides papillosus									X								Reiber and Byrd (1942)
Strongyloides robustus										X							Chandler (1942)
			X														Packard (1956)
Syphacia thompsoni											X						Parker (1968)
Trichostrongylus sp.						X											Rausch and Tiner (1948)
Trichostrongylus calcaratus														X			Rausch and Tiner (1948)
Trichostrongylus retortaeformis															X		Cameron and Parnell (1933)

1/ Introduced American gray squirrel host
2/ Experimental infection

Results and Discussion

A total of 12 species of nematodes, representing 8 families and 6 orders, were recovered from 175 gray squirrels. Table XV lists these species and gives the percent of squirrels infected, numbers recovered, range of intensity and average per infected squirrel. A total of 109 (61.71%) squirrels harbored nematodes. The number of nematode species per host ranged 1 to 6 (average 1.87) species per host infection, and the number of worms per infection ranged 1 to 170 (average 20.76) worms per infected host. The most common species was Citellinema bifurcatum (45.23%), with Strongyloides robustus and Böhmiella wilsoni second and third, respectively.

The nematodes I observed may be divided into four categories on the basis of their modes of transmission and development: those which exhibit free-living stages in the soil and reach the host by penetrating the skin or by ingestion (Strongyloides, Trichostrongylus, Citellinema, Böhmiella and Heligmodendrium); those which have a direct life cycle and the larvae usually develop and remain in the egg to be eaten by the host (Capillaria, Ascaris, Syphacia and Enterobius); those which require an intermediate host to complete their development (Gongylonema); and those in which the life history is improperly known at the present (Pterygodermatites and Contracaecum).

In examining the data of all hosts harboring nematodes, considerations were made to determine what correlations might exist between incidence and degree of nematode infection and sex and age classes of the hosts, localities in which hosts were collected and seasonal variations.

TABLE XV. Nematode parasites recovered from 175 gray squirrels, Sciurus carolinensis Gmelin, 1788, in southwestern Virginia, September, 1966-July, 1969

Species	No. squirrels infected	% infected[1]	Total no. recovered	Range	Avg./inf. squirrel
Citellinema bifurcatum	76	45.23	1146	1-158	15.08
Strongyloides robustus	47	28.14	528	1-92	11.23
Böhmiella wilsoni	18	13.85	123	1-31	6.83
Capillaria americana	12	7.14	22	1-3	1.83
Heligmodendrium hassalli	11	6.58	228	1-65	20.73
Syphacia thompsoni	9	5.35	151	1-135	16.78
Pterygodermatites parkeri	7	4.16	18	1-10	2.57
Trichostrongylus calcaratus	7	4.16	10	1-4	1.43
Enterobius sciuri	4	2.38	14	1-11	3.50
Gongylonema pulchrum	4	2.38	13	1-6	3.25
Ascaris lumbricoides (larval)	1	0.59	1	--	1.00
Contracaecum sp. (larval)	1	0.59	1	--	1.00

1/ From host to host the visceral organs sometimes differed slightly because other investigators removed them before I could examine them. Therefore, refer to the text for the exact numbers of hosts examined for each species according to the organs where they occur.

Table XVI illustrates the percent of squirrels infected in relation to the sex and age classes of the hosts. In examining the percentages of the age classes for the total examined, it appears that the juveniles and adults have higher infection rates than the subadults. However, this may not be the real condition in the population. It is expected that infection rates should increase with the age of the host. Since I did not get these results, I believe it is due to the sample size in each age class. The juvenile sample totaled 21 hosts; the subadult sample totaled 49 hosts; and the adult sample totaled 105 hosts. Therefore, the smallness of the juvenile sample in comparison with the subadult and adult samples apparently influenced these results. In examining the average percentages according to host sex, the numbers in each sample size are similar and indicate a higher incidence of nematode infection in the males.

Data regarding squirrels collected from different localities were considered. Since the entire collecting area was basically a continuum of the eastern deciduous forest biome and vegetational types did not appear to differ sharply from one collection site to another, it was decided that it might be possible to study squirrel nematode parasites on the basis of the nearness of the squirrels collected to human environmental influence. Therefore, four such areas were selected and are herein defined:

TOWN - squirrels that were collected within the town limits of Blacksburg or from the V. P. I. campus proper where there is ready access to lawns, and people and domestic animals are present;

TABLE XVI. Incidence of nematode infection according to the sex and age classes of the gray squirrel hosts

Age classes	Males		Females		Totals	
	Examined	% inf.	Examined	% inf.	Examined	% inf.
Juveniles	14	64.28	7	71.42	21	66.66
Subadults	24	66.66	25	40.00	49	53.06
Adults	48	70.83	57	61.40	105	65.71
Totals and averages	86	68.60	89	56.06	175	61.71

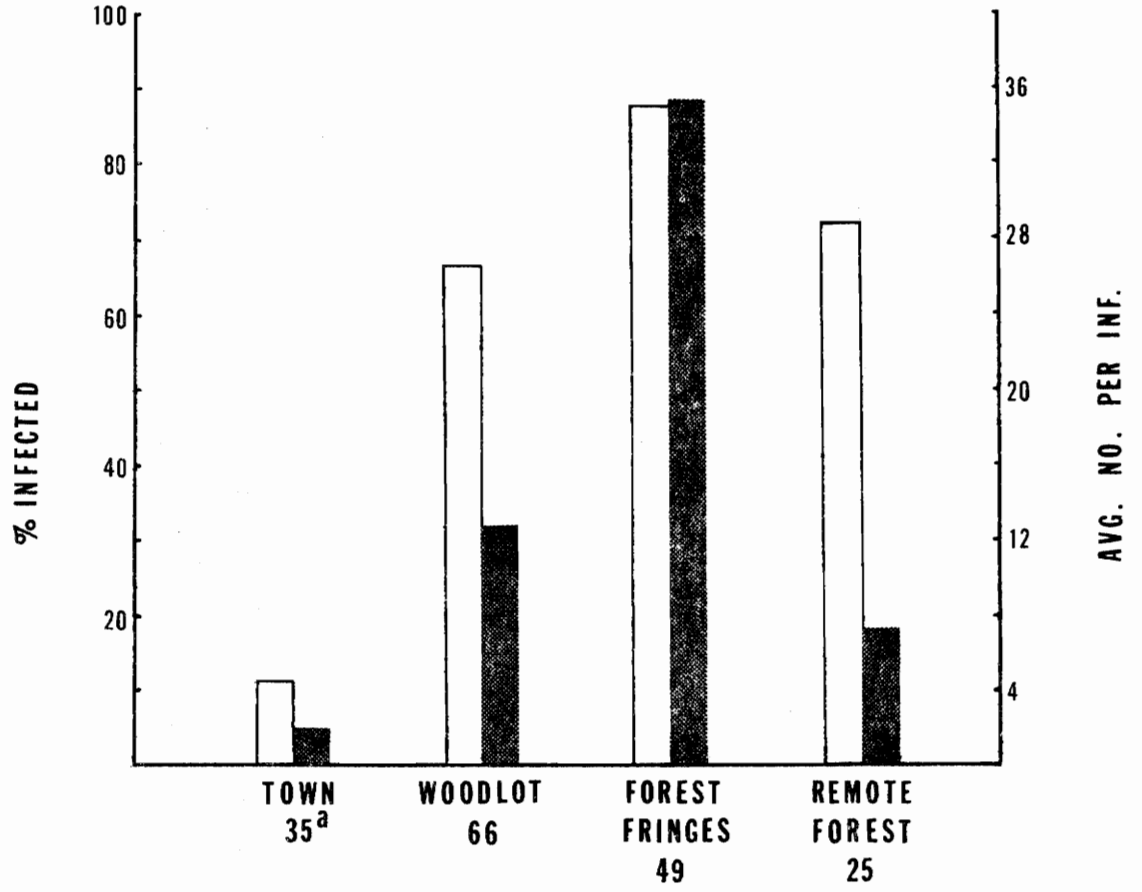
WOODLOT - squirrels collected from farm woodlots which are, for the most part, isolated stands of trees, which are usually separated from other such areas by pastureland or fields and where there may be occasional visits by man or domesticated animals;

FOREST FRINGES - squirrels collected from the extremities of extensively forested areas with moderate to heavy understory, that may be adjoining farmlands and roads, but regular intrusions by man is not as pronounced as in the first two areas;

REMOTE FOREST - squirrels collected deep in the interior of heavily forested areas, considered to be remote from human habitation as compared with the other areas.

Figure 9 shows a comparison of percentages of infection and average numbers of nematodes per infection between these designated areas. The town sample yielded the lowest percentage (11.10%), the woodlot and remote forest samples were higher, each with similar rates, 66.66% and 72.00%, respectively. The highest percentages occurred in the forest fringes sample (87.75%). The forest fringes area also showed a greater nematode species diversity and averaged 2.21 species per infection. The town, woodlot and remote forest samples averaged 1.00, 1.54 and 1.55 species per infected host, respectively. The average number of worms per infected host was 1.75 in the town sample, 12.72 in the woodlot sample, 35.53 in the forest fringes sample and 7.22 in the remote forest sample. Katz (1938) observed in Ohio that squirrels collected from forested areas did not show a significantly greater susceptibility to parasitism than those taken from small woodlots, however, higher numbers of parasites per infection occurred in the forests than in the woodlots. It is therefore evident that certain characteristics of the forest fringes environment exist which better fosters the transmission of nematode parasites than the other areas.

FIGURE 9. Percent infected and average number nematodes per infection according to habitat of the hosts

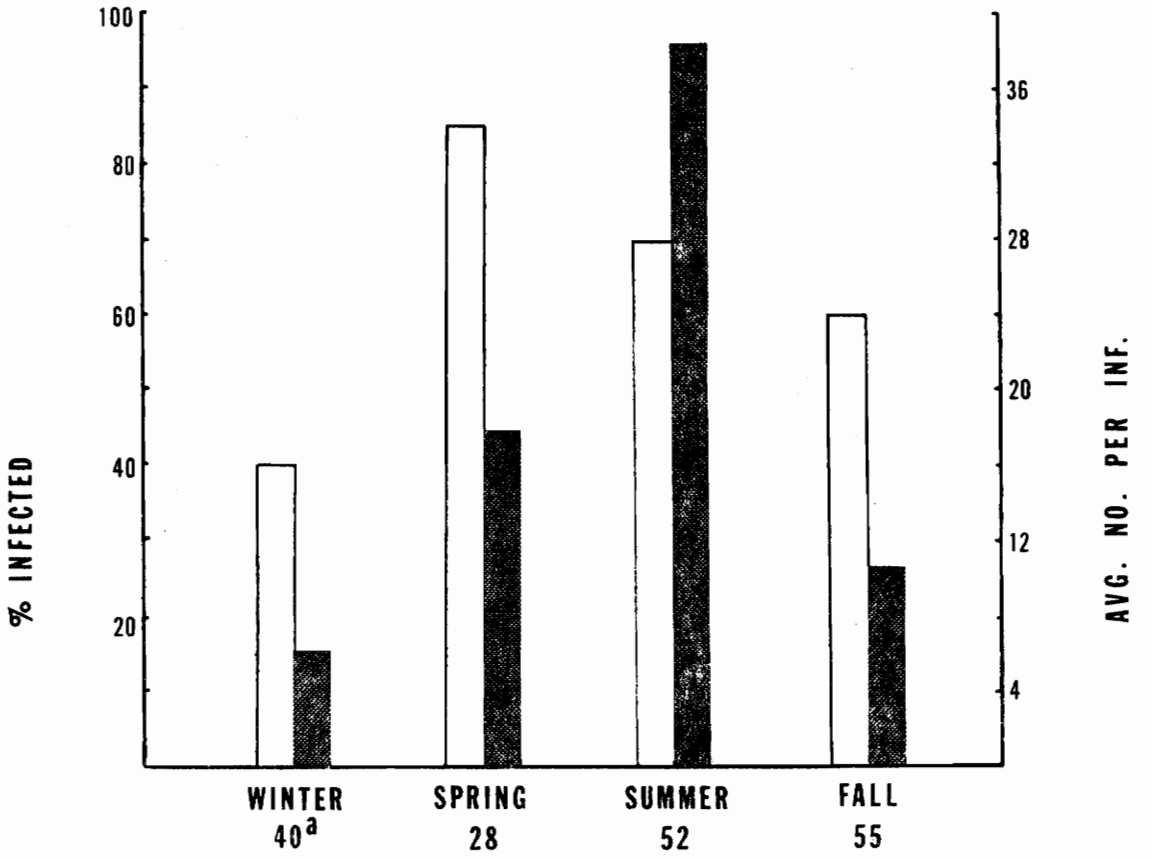


a/ number examined

One can only surmise what these may be. Perhaps there is greater diversity of habitat due to a transitional zonation. Possibly shade, moisture and soil conditions are more favorable here than in the other areas yielding greater survival of the parasites to infect squirrels.

The data were examined to see what seasonal variations might occur for the total number of nematodes harbored by the squirrels. The calendar dates December 21-March 20 were the collection dates for the winter sample; March 21-June 20 the spring sample; June 21 to September 20 the summer sample; and September 21 to December 20 the fall sample. Figure 10 shows, by means of a bar graph, the seasonal percentages of infection and average number of nematodes harbored per host. The lowest percent infected occurred in winter (40.00%), the highest in spring (85.71%), with a steady decline into summer (69.23%) and fall (60.00%). If the spread of nematodes among squirrels was directly related to the density of the squirrel population, the results obtained herein should closely follow that assumption because the population, being at a low point in winter, reaches its high point in spring and decreases through summer and fall although there is a second litter produced in late summer or early fall which retards the decline. Looking at the average number of nematodes per infected squirrel, there is a steady increase from a minimum in winter to a maximum in summer. A considerable decrease is noted in the fall. Perhaps there is some sort of lag phenomenom involved. Possibly, due to the maximum number of squirrels infected in spring, the spring squirrels contaminate the soil, from which the summer squirrels become infected. Since the results showed a somewhat lower percentage infected in summer, perhaps

FIGURE 10. Seasonal incidence and intensities of nematodes in 175 gray squirrels, 1966-1969



a/ number examined

squirrels may spend more time in the trees than on the ground at this time of year than at other times, whereby the ones that do frequently visit the ground become more heavily infected. These aspects will be given more consideration in the discussion of the three most common species of parasites in this study.

On the next few pages will be given a brief discussion of each nematode species encountered in this study.

Subclass Aphasmidia
Order Trichuridea
Family Trichuridae Ralliet, 1915

Capillaria americana Read, 1949

This nematode was found in the small intestine of 12 (7.14%) squirrels. The worms were usually embedded in the mucosa of the gut wall and were always located from 5 cm below the pyloric sphincter to the lower end of the duodenum. None were recovered from the jejunum or ileum. All infections were light. The numbers of worms ranged from 1 to 3 with an average of 1.83 per infected host. All the worms recovered were females. The incidence shown by this study is somewhat lower than that of Olexik (1968) who recorded Capillaria sp. in 7 (17.5%) of 40 gray squirrels examined in southwest Tennessee.

The type host for this worm is Glaucomys volans volans (Linn., 1758). Other hosts given by Read (1949) are Sciurus carolinensis leucotis Gapper, 1830, Peromyscus maniculatus bairdii Hoy and Kennicott, 1857, and Peromyscus leucopus noveboracensis (Fischer, 1829). The type locality is Illinois, with another locality in Wisconsin. The worms recovered from gray squirrels and reported in the literature under the

name of Capillaria sp. by Rausch and Tiner (1948), Hanson (1966), Olexik (1968) and Olexik, Perry and Wilhelm (1969) probably belong to this species.

These small worms have a simple life cycle as do most of those in the family Trichuridae. The eggs (Plate 22), which exhibit the characteristic barrel-shape, with a plug on each end, reach the soil by way of fecal contamination and, with sufficient moisture, become infective after about 3 to 6 weeks of embryonation (Chandler and Read, 1962). Infection results from ingesting material containing embryonated eggs or hatched larvae.

Capillaria americana does not appear to pose any serious pathogenic problems to squirrels in southwestern Virginia as indicated by the low incidence and light worm burdens. The tunneling of these worms in the gut mucosa does not appear to cause any significant pathology. It is not known what pathology heavy infections would induce.

Subclass Phasmidia
Order Rhaddiasidea
Family Strongyloididae Chitwood and McIntosh, 1934

Strongyloides robustus Chandler, 1942

This species occurred in the duodenum of 47 (28.14) of 167 hosts examined. Numbers ranged from 1 to 92 (average 11.2), and all the worms recovered were parasitic females; the males being free-living in soil. Olexik (1968) reported Strongyloides sp. in 19 (47.5%).

Generally, these worms are restricted to the upper portion of the duodenum near the pyloric sphincter. They are usually embedded in the mucosa, and often bore through it and become tangled in the intestinal

villi. Occasionally the worms are found in the tissue surrounding the opening of the bile duct. They are rarely encountered more than 15 cm below the pyloric sphincter.

This species was most difficult to remove without breaking. A #1 dental pulp file with the first few millimeters of the tip bent at a right angle to the shaft was used to recover the worms. With this, the duodenum, which had been pinned to a wax-bottomed petri dish, was examined for worms by using raking strokes with the tip passing between and through the villi. This method tended to dislodge worms that were not readily visible on the mucosal surface. Digestion techniques for the recovery of these worms were not used because many of the host viscera had been preserved in formalin.

This species was first described from the gray squirrel by Chandler (1942). All those organisms from this host listed in the literature as Strongyloides sp., as well as the name S. papillosus by Reiber and Byrd (1942), probably belong to the same species, S. robustus (see Table XIV). Apparently, no redescriptions of S. robustus have been presented since the original one by Chandler (1942). Since his description was based upon only 13 specimens, data from additional specimens from other localities, such as those reported on herein, should be beneficial to future students. Table XVII presents the data as gleaned from 20 specimens and provides a comparison with those given in the original description by Chandler (1942). The measurements recorded herein show slightly greater ranges which are to be expected in a larger sample size. None of the characters appear to differ significantly.

TABLE XVII. Characteristics of Strongyloides robustus Chandler, 1942, from the gray squirrel in southwestern Virginia as compared with those described from the gray squirrel in Texas

	Present study	Chandler (1942)
Locality	Virginia	Texas
Parasitic females		
No. specimens measured	20	13
Total length (mm)	3.90-7.84 (6.01)[a]	4.5-6.8 (6.1)
Maximum diameter (mm)	.050-.069 (.055)	.040-.075
Esophagus length (mm)	.98-1.30 (1.13)	.86-1.26 (1.14)
Esophagus % total length	14.80-26.79 (19.37)	17.7-19.0
Vulval distance from posterior end (mm)	1.37-3.56 (2.41)	1.60-2.88
Post-vulvar length % of total length	29.4-48.4 (39.8)	35-43
Shape of tail	Clavate	Not given
Eggs (mm)	.040-.071 x .023-.035 (.054 x .028)	.055-.060 x .027-.030
Incidence	28% of 167 hosts	50% of 4 hosts
Degree of parasitism	1-92 worms (11.2)	Moderate numbers

a/ Average

In examining my data concerning this species, attempts were made to determine what correlations might exist between the occurrence of this worm and sex and age classes of the host, localities in which hosts were collected and variations between seasons.

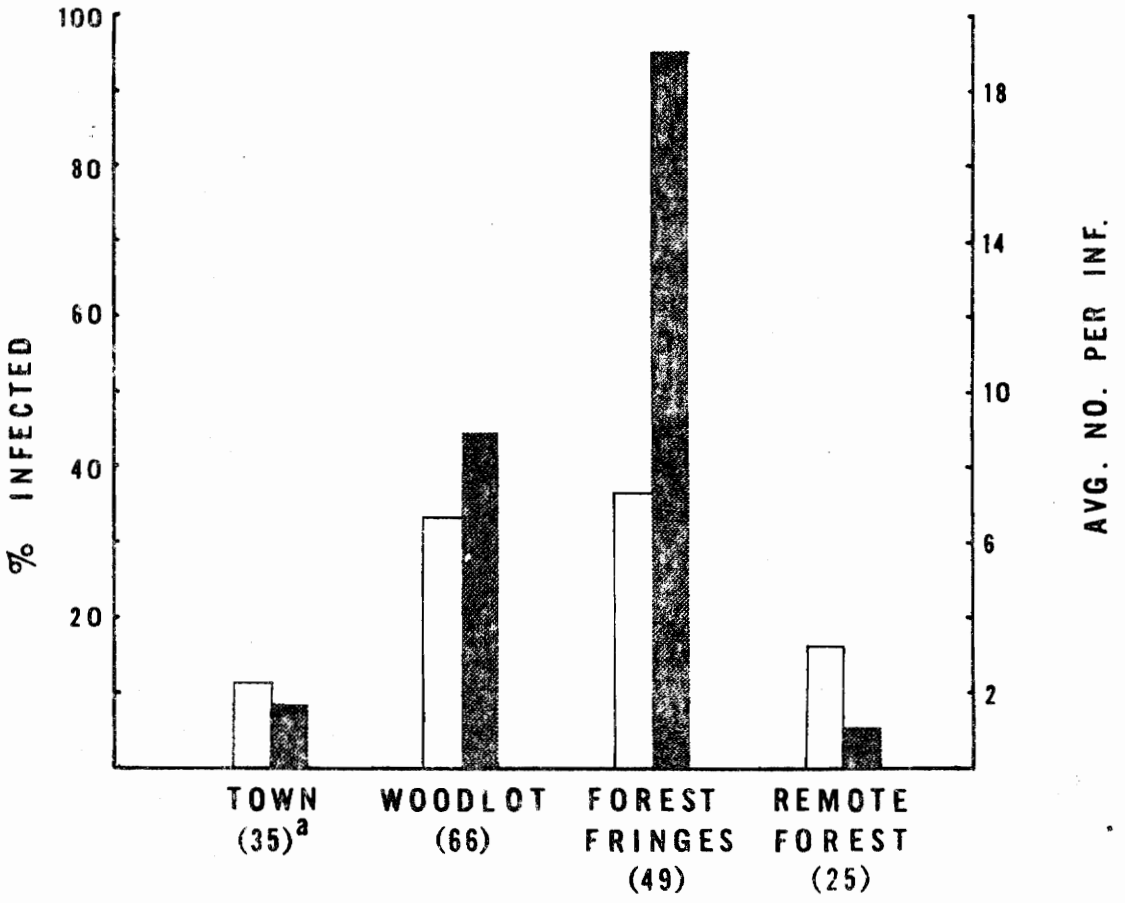
Table XVIII shows the percent of squirrels infected, subdivided into the sex and age classes of the hosts. The juvenile and subadult males and females had a lower frequency of infection than the adults of either sex. When considering all the males of all ages as one group and all the females as another, the infection appears to be slightly higher in the males. In speculating over the reasons for this, one idea I might suggest is that perhaps this is due to males having a greater home range than females (Bakken, 1952; Robinson and Cowan, 1954; Doebel, 1967), thus facilitating more frequent opportunities to encounter infective stage larvae in the soil. These data, however, differ from that of Olexik (1968) who recorded Strongyloides sp. in 3 (21.4%) of 14 male gray squirrels and in 16 (61.5%) of 26 female gray squirrels he examined in southwest Tennessee. When considering the total of both sexes as a separate age classes, the juveniles show a slightly lower percentage infected than the subadults; and similarly, the subadults show a lower percentage than the adults. This is to be expected since I assume that the older the animal becomes, the chances of encountering infective larvae become greater.

Figure 11 shows relative differencies in percent infected and average number of worms per infection when hosts from different localities are compared. The group collected from town yielded low percentage and numbers, those from woodlots a little higher and those from

TABLE XVIII. Incidence of infection with *Strongyloides robustus* Chandler, 1942, related to sex and age classes of gray squirrel hosts

Age classes	Males		Females		Totals	
	Examined	% inf.	Examined	% inf.	Examined	% inf.
Juveniles	14	14.29	6	0.00	20	10.00
Subadults	21	9.52	24	12.50	45	11.11
Adults	46	47.83	56	32.14	102	39.22
Totals and averages	81	32.10	86	23.72	167	28.14

FIGURE 11. Percent infected and average number per infection of Strongyloides robustus Chandler, 1942, correlated with habitat



a/ Number examined

forest fringes highest, while those from remote forest areas were within a range comparable with the town group. These differences are difficult to explain. Perhaps one explanation for the higher figures in the forest fringe group may be due to a greater access of the hosts to more diverse habitat conditions in which some factor or factors would enable greater survival rates of the larvae in the soil. Possibly these may include soil humidity, soil type, humus thickness and leaf litter cover, pH, temperature, etc. Chandler and Read (1962) state that "The larvae of Strongyloides are easily destroyed by cold, desiccation, or direct sunlight, and are rather short-lived even under the most favorable conditions. This probably accounts for the infrequence of Strongyloides infections outside warm, moist climates."

It is my opinion that these percentage differences may be due, in part at least, to altitudinal differences, or rather to environmental differences which exist as a result of differences in elevation. This conjecture was proposed after a reexamination of the data in which estimates of the altitude at which each squirrel was collected were considered. The group of squirrels collected from town came from an average elevation of 2100 ft., those from woodlots also 2100 ft., those from forest fringe areas 1900 ft. and those from remote forest areas 2680 ft. Regarding Figure 11, the highest percent infected and the greatest numbers of worms was in the forest fringes group, and this group was also collected from the lowest average elevation. To further pursue this point, Table XIX shows percent infection and average number of worms per infection according to elevation. Although there were marked differences in the sample sizes at various elevations and thus the data

TABLE XIX. Incidence of infection and average numbers of Strongyloides robustus Chandler, 1942, harbored at various elevations at which the gray squirrel hosts were collected

Elevation (ft.)	Number examined	Per cent infected	Average no. worms/ inf.
1700	14	50.00	17.86
1800	9	77.77	25.29
1900	2	50.00	12.00
2100	120	24.16	7.38
2500	14	21.42	1.67
2700	2	0.00	0.00
3300	2	0.00	0.00
3500	4	0.00	0.00

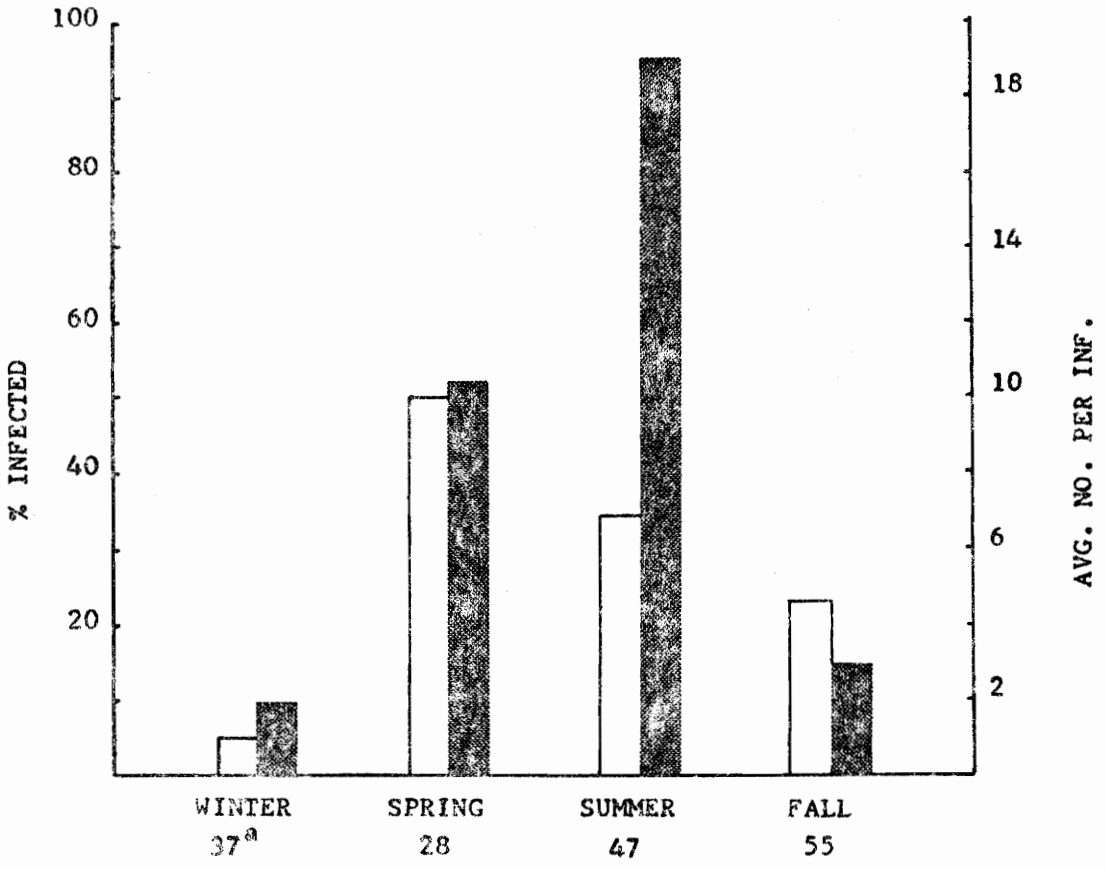
are not conclusive, apparently there is a decrease in the percent of squirrels infected with Strongyloides, and also an apparent decrease in the number of worms per infection, as altitude increases.

Since the town sample and woodlot sample were both taken at an average altitude of 2100 ft., the lower percentage infected in the former may be due to desiccation and sunlight reducing the numbers of larvae in soil whereas more shade and moisture fostered a higher percentage of infection in the latter habitat.

Squirrel population densities may possibly be higher in town and in woodlots than in the other areas, due to availability of food and nesting sites, and reduced predation. If this be the case, the present data do not show any correlation between host density and percent infected with this species in the habitats designated. Therefore, for the present, I must assume that certain environmental conditions are involved which limit rates of infection.

Further, percent infected and average numbers of worms per squirrel were examined to determine what variation might occur between seasons (Figure 12). It was observed that the lowest numbers of squirrels infected occurred in winter (5.41%), the highest in spring (50.00%), with a decrease through summer (38.29%) and fall (23.63%). The average number of worms per squirrel, however, increased from winter to a peak in summer, then showed a considerable drop in the fall. The percent infected was greatest in the spring when the host population density is high, probably accounting for a greater dissimination of parasites. However, the height of the bar for the spring sample must be accepted cautiously because this may have been influenced by the relatively

FIGURE 12. Percent infected and average number per infection of Strongyloides robustus Chandler, 1942, correlated with seasons



a/ Number examined

smaller sample size. It is difficult to explain why the summer sample showed about 12% lower rate of infection than the spring sample but averaged about twice as many worms per infection. It is my opinion that the movements of squirrels on the ground at this time of year is minimal and possibly the squirrels that do visit the ground become more heavily infected due to more infective larvae being in the soil at that time. These hypotheses demand further attention in future studies.

Order Ascaridida
Family Heterocheilidae Railliet and Henry, 1915

Contracaecum sp. (larval)

One larval Contracaecum sp. occurred in the small intestine of one squirrel (0.59%). It appears that this occurrence is entirely accidental. Members of the genus Contracaecum are worldwide in distribution and are parasites of fish, birds and fish-eating mammals. Yamaguti (1961) listed no mammals in the southeastern United States as hosts of any species of the genus. However, he does list several birds and fish that are native to the area as hosts for certain species of Contracaecum. At present no further explanations for this occurrence in squirrels can be given.

Family Ascarididae Blanchard, 1849

Ascaris lumbricoides Linn., 1758

One specimen of an immature Ascaris lumbricoides was recovered from the small intestine of a single (0.59%) gray squirrel. The specimen was a female worm about 112 mm in length. There was no

evidence of egg production.

Ascaris lumbricoides has been reported in several hosts including man, mice, monkeys, bears, muskrats and squirrels. The worm has been reported in the fox squirrel, Sciurus niger rufiventer, by Brown and Yeager (1945) and Tiner (1951) and in the gray squirrel by Katz (1938) and Oldham (1961). Katz (1938) recorded one immature female Ascaris lumbricoides which measured 72 mm in length. Only one host was infected of the 72 (1.4%) that he examined.

According to the results of the present study, in which this species showed a low percentage of occurrence and the worm was immature, it appears that this occurrence is accidental. To bear out this assumption, the reports from tree squirrels in the literature appear to have been of larval stages also.

Order Oxyuridea
Family Oxyuridae Cobbold, 1864

Syphacia thompsoni Price, 1928

This nematode was harbored in the cecum and large intestine of 9 (5.35%) gray squirrels. Numbers of worms harbored ranged from 1 to 135 with an average of 16.78 per infection. These figures may be somewhat misleading because the infections were, for the most part, very light. Six hosts harbored only one worm each; one host harbored two worms; one harbored eight worms; and one exceptional individual harbored 135 worms.

Syphacia thompsoni is a new host record for the gray squirrel and was so designated in my preliminary research report (Parker, 1968).

Other hosts of this species in the United States include the flying squirrel (type), Glaucomys volans volans, in Virginia by Price (1928) and Glaucomys sabrinus macrotus in Michigan and Wisconsin by Rausch and Tiner (1948), and the red squirrel, Tamiasciurus hudsonicus in Wisconsin by Tiner and Rausch (1949).

The life cycle of this nematode is simple and direct. Upon infection the larvae become sexually differentiated in about 48 hours. Further details into the life cycle are given by Chan (1951; 1952).

Enterobius sciuri Cameron, 1932

This nematode occurred in the cecum and large intestine of four (2.38%) hosts. Infections were light, ranging from 1 to 11 worms per host with an average of 3.50 worms per infected squirrel.

Enterobius sciuri was first described by Cameron (1932) from the introduced American gray squirrel in Scotland. Subsequently, this species has only been reported in the United States from the flying squirrel, Glaucomys volans volans, and fox squirrel, Sciurus niger rufiventer, in Ohio, Michigan and Wisconsin by Rausch and Tiner (1948). Therefore, this is the first report of its occurrence in the gray squirrel in North America. I have also observed the species in North Carolina gray squirrels in which infections were very heavy (see Addendum).

The eggs of Enterobius (Plate 16) are voided with the feces and the partially developed embryo matures in several hours. Infection results from swallowing the eggs containing fully-developed larvae. In

the gut, the larvae hatch and temporarily burrow into the mucosal wall, usually in the region of the cecum, where they remain for a while before growing to maturity in the lumen (Chandler and Read, 1962).

Order Strongylidea
Family Trichostrongylidae Leiper, 1912

Trichostrongylus calcaratus Ransom, 1911

This nematode was found in the small intestine and cecum of 7 (4.16%) squirrels. Numbers ranged from one to four with an average of 1.43 worms per infected host. These worms are very small and have a narrow cephalic and esophageal region and the body increases in diameter posteriorly. They are difficult to observe even when the gut is pinned out and viewed at 15X under the dissection microscope. Usually the worms have their narrow head portion embedded in the mucosa of the gut and are not readily dislodged by the washing technique.

In addition to the gray squirrel, T. calcaratus occurs in rabbits (Sylvilagus sp.) and hares (Lepus sylvaticus and Oryctolagus sp.), muskrats (Ondatra zibethica), woodchucks (Marmota monax) and fox squirrels (Sciurus niger rufiventer) in the United States.

The eggs of the nematode are passed with the feces of the host and, under favorable conditions, the larvae hatch and develop to the infective stage in about 4-6 days. For some species it has been shown that the larvae migrate on vegetation to where they are vulnerable to the grazing activities of herbivores (Levine, 1963; Soulsby, 1965). These movements have been shown to be influenced by temperature, light intensity and moisture. The morphological features of the

developmental stages of Trichostrongylus is given by Douvres (1957).

The pathogenesis and immunology of Trichostrongylus spp. have been given for domestic animals. The effects on gray squirrels is unknown, and, among those examined, there were only light infections and no indication of significant pathology.

Citellinema bifurcatum Hall, 1916

This nematode was the most common helminth harbored by the squirrels studied. A total of 1146 worms were recovered from the duodenum and stomach of 76 (43.43%) hosts. Most of the worms were found in the first few centimeters below the pyloric sphincter. The head portions were often embedded in the duodenal mucosa; and in heavy infections many worms were entangled together only to be separated with much difficulty. Numbers of worms ranged from 1 to 158 with an average of 15.08 worms per infected host. Apparently the few worms recovered from the stomach were the result of post mortum migrations since none were found to be attached to the stomach mucosa. These data may be compared with that of Katz (1938) who found this nematode in 25 (34.7%) gray squirrels out of 72 examined with an average number of worms per infestation of 11.7, while the maximum number recovered from an individual host was 122. Olexik (1968) recovered this worm in 17 (42.5%) of 40 gray squirrels examined in southwest Tennessee. The highest degree of infestation recorded was 78 worms. The infection rates of my animals most closely corresponds to that reported in the latter study.

Citellinema bifurcatum has been reported from a number of species of tree and ground squirrels in the United States and a few in Canada. A list of the reports of this species in gray squirrels is given in Table XIV. Most of the other host species are to be found in Hall (1916), Katz (1938), Dikmans (1938), Reiber and Byrd (1942), Rausch and Tiner (1948) and others which are catalogued by Doran (1955a).

Apparently the details of the life cycle of this worm have not been elucidated. Probably the eggs mature in the soil and either hatched larvae or infective larvae remaining in the eggs penetrate the skin or are swallowed by the host to ultimately reach the small intestine. The worms encountered in the present study were either adults or immatures completing their final stages of maturation. All were distinguishable as males and females, however, the sizes were greatly variable. The small sizes and absence of egg production in some of the worms indicated that development was not complete.

As with Strongyloides robustus discussed earlier, attempts were made to determine what correlations might exist between the incidence of Citellinema bifurcatum in relation to the sex and age classes of the host, localities in which the hosts were collected and variations between seasons.

Table XX shows the percent of squirrels infected, subdivided into the sex and age classes of the hosts. These data show a slightly higher percentage of infected squirrels in the males (47%) than in the females (40%). References were cited in the discussion of Strongyloides indicating that males have greater home ranges than females, in which I speculated that it may be one factor accounting

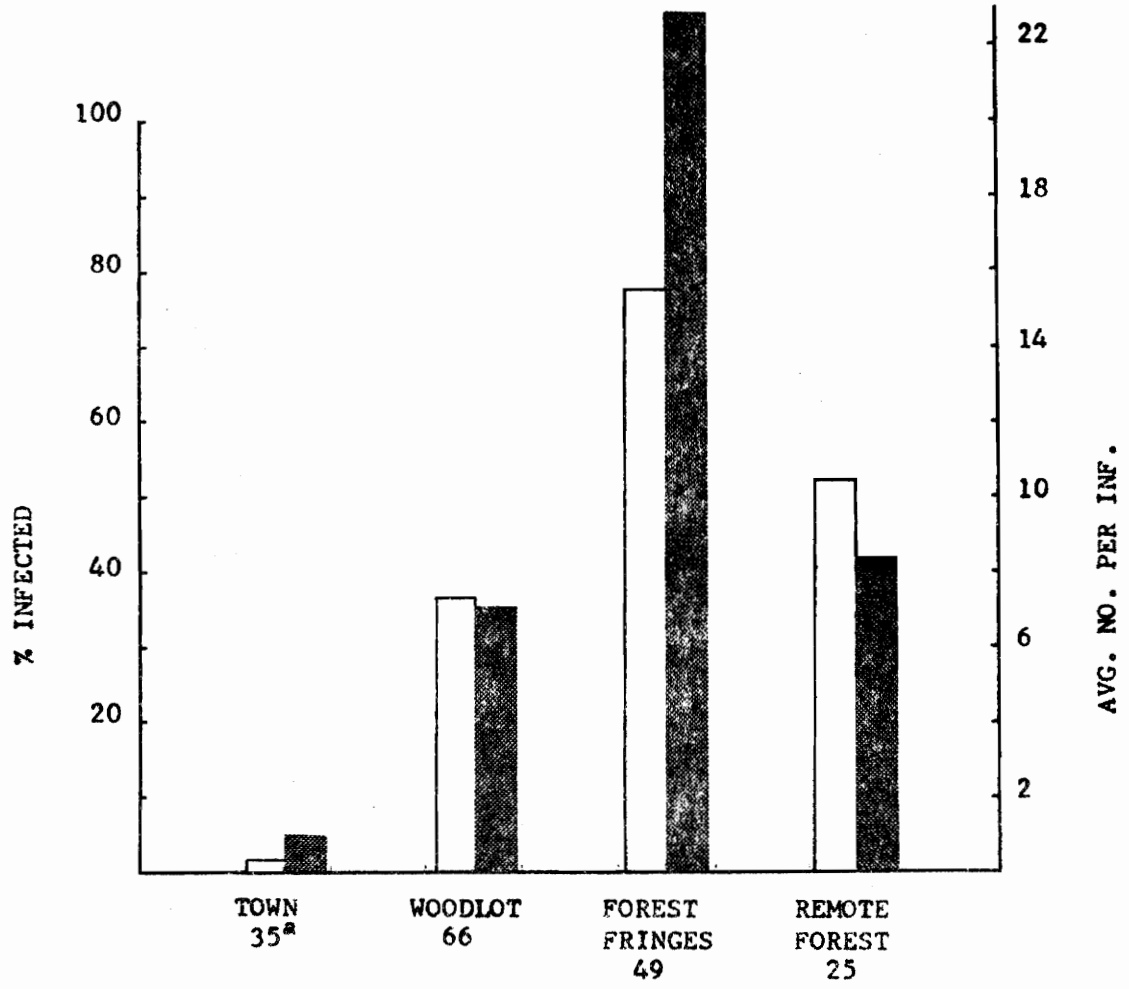
TABLE XX. Incidence of infection with Citellinema bifurcatum Hall, 1916, related to sex and age classes of the gray squirrel hosts

Age classes	Males		Females		Totals	
	Examined	% inf.	Examined	% inf.	Examined	% inf.
Juveniles	14	42.86	7	71.43	21	52.38
Subadults	24	37.50	25	16.00	49	26.53
Adults	48	52.08	57	47.37	105	49.52
Totals and averages	86	46.51	89	40.45	175	43.43

for the higher rates in the males. The higher percentages shown by the juvenile females over subadult and adult females, and similarly, the juvenile males over the subadult males, cannot be explained by the data presented herein. Higher percentages in the older age classes would be expected but my data do not support this assumption. The main factor to consider on this point is the smallness of our juvenile sample. These data are opposite from that of Olexik (1968) who recorded a higher incidence in the females in which this worm occurred in 5 (35.7%) of 14 male gray squirrels examined as compared with 12 (46.1%) of 26 female gray squirrels examined.

Figure 13 shows relative differences in percent infected and average number of worms when hosts from different localities are compared. The town sample shows the smallest percent infected (2.85%), the woodlot sample is considerably higher (36.64%), while the highest was recorded in the forest fringes sample (77.55%). The sample from remote forest areas showed a lower percentage (52.00%), but constituted the second highest area for infection with this worm. The average number of worms per host infection was closely correlated with the percentages of infection in each area. These differences between the localities are similar to those explained in the discussion of Strongyloides. Also these data closely reflect the observations of Katz (1938) who noted that C. bifurcatum occurred more frequently in forest inhabiting squirrels as compared with those from small woodlots. He showed that 34.7% of 72 squirrels examined from heavy forest were infected whereas only 25.0% of 16 animals examined from small woodlots were infected.

FIGURE 13. Percent infected and average number per infection of Citellinema bifurcatum Hall, 1916 correlated with habitat



a/ Number examined

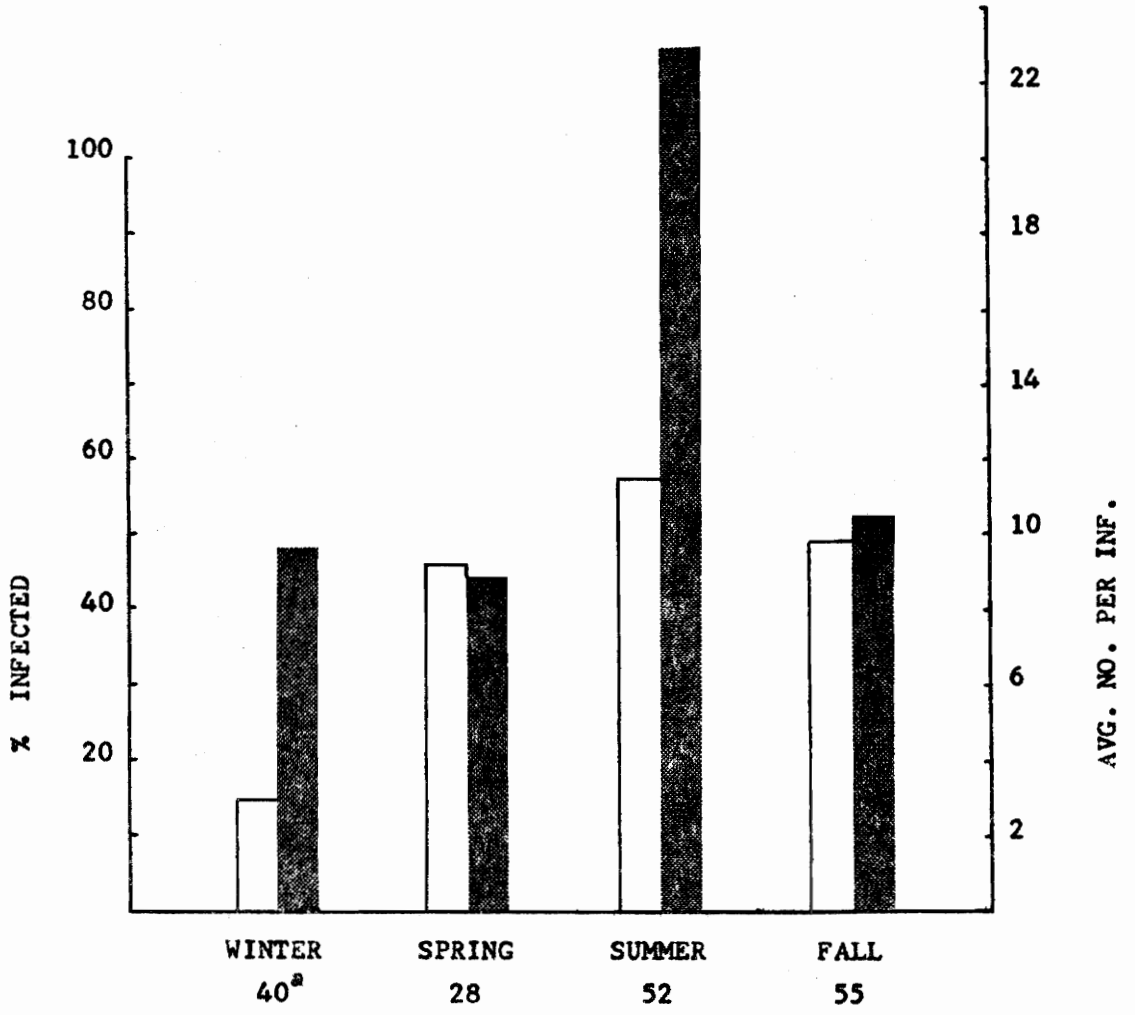
Table XXI shows the percent infected and average number worms per infection according to the altitudes at which the hosts were collected. Unlike the results for Strongyloides, Citellinema bifurcatum does not show any particular correlation with elevation, except that higher worm burdens were encountered at the lower altitudes. In future studies, larger sample sizes collected at each altitude may provide enough information to reach some conclusions in this area of study.

Finally, the percentage of infected squirrels and the average number of worms per animal were examined to determine what variations might occur between the seasons of the year (Figure 14). These data showed the lowest percentage of infections in winter (15.00%), a considerably higher percentage in spring (46.43%) and the highest in summer (57.69%), while the percentage in the fall dropped off again (49.09%). The average number of worms per squirrel were very similar in the winter (9.67), spring (8.85) and fall (10.48); however, the summer average was more than twice that of any other season (23.00). These seasonal percentages differ somewhat from that of Strongyloides, which had the highest percentage of infection in spring. However, the average number of worms per host were closely correlated in that highest worm burdens occurred in summer. Apparently, environmental conditions are optimal for the survival of the larval stages and squirrel activities and densities are adequate so that higher rates of infection and degrees of infection result with this species in summer.

TABLE XXI. Incidence of infection and average numbers of Citellinema bifurcatum Hall, 1916, harbored at various elevations at which the gray squirrel hosts were collected

Elevation (ft.)	Number examined	Per cent infected	Average no. worms/ inf.
1700	14	85.71	33.75
1800	14	71.43	31.40
1900	2	100.00	50.00
2100	123	34.15	6.17
2500	14	42.86	6.50
2700	2	0.00	0.00
3300	2	100.00	11.50
3500	4	50.00	12.50

FIGURE 14. Percent infected and average number per infection of Citellinema bifurcatum correlated with seasons



a/ Number examined

Böhmiella wilsoni Lucker, 1943

This large nematode was found in the stomach of 18 (13.85%) squirrels out of 130 stomachs examined and constituted the third most common nematode encountered (see Table XV). Post mortem migrations into the esophagus and duodenum were observed. Usually the worms were localized in the pyloric region of the stomach but were never observed to be attached to the stomach mucosa. The numbers of worms ranged from 1 to 31 with an average of 6.83 per infected host. Counts to determine the sex ratio of this worm were made from 17 infected hosts which yielded 70 (60%) males and 48 (40%) females.

The details of the life history of this nematode have not been well studied. Some development probably occurs in the soil from which the host becomes infected. Future studies should include the development of the worm and methods of infection of the host.

In addition to the gray squirrel, B. wilsoni has also been reported in the fox squirrel, Sciurus niger niger, by Lucker (1943) and S. n. rufiventer by Rausch and Tiner (1948) and in the rat, Neotoma floridana osagensis, by Murphy (1952).

In examining my data concerning B. wilsoni attempts were made to determine what correlations might exist between the incidence of this worm and the sex and age classes of the host, localities in which the hosts were collected and variations between seasons.

Table XXII shows the percentage of squirrels infected, subdivided into the sex and age classes of the hosts. The average incidences in the juvenile and subadult male and female age classes are lower than the averages for the adults of both the males and females. This confirms

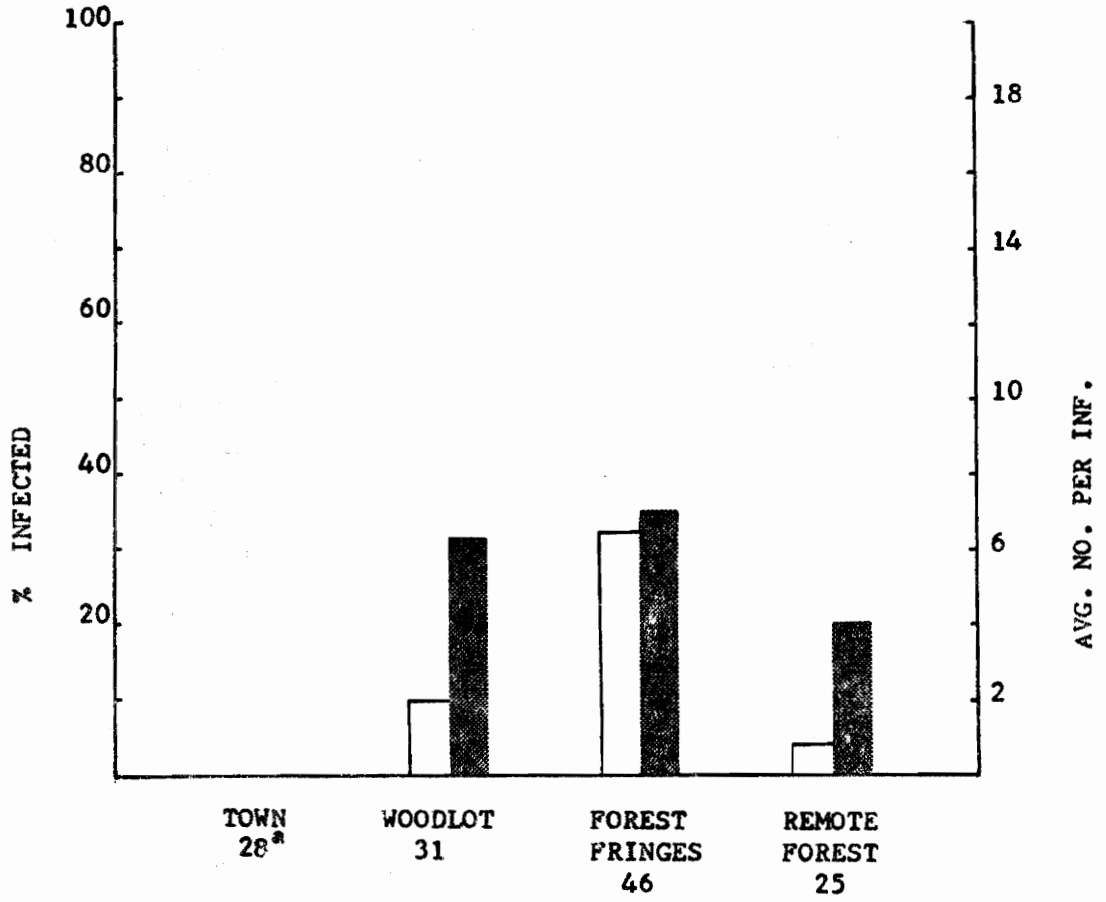
TABLE XXII. Incidence of infection with Böhmiella wilsoni Lucker, 1943, related to the sex and age classes of the gray squirrel hosts

Age classes	Males		Females		Totals	
	Examined	% inf.	Examined	% inf.	Examined	% inf.
Juveniles	12	0.00	5	20.00	17	5.88
Subadults	20	15.00	13	7.69	33	12.12
Adults	39	12.82	41	19.51	80	16.25
Totals and averages	71	11.27	59	16.95	130	13.85

the assumption that the older animals would have greater opportunity to become infected. The average for all ages of females is somewhat higher than the average for all ages of males. Since it has been shown that males have a greater home range than females (Bakken, 1952; Robinson and Cowan, 1954; Doebel, 1967), I speculated that it may be a factor which influences a higher incidence in males. The higher incidence of this worm in females cannot be explained at this time. Sufficient data for this species were not available for making any conclusions concerning individual age classes within each sex.

Figure 15 shows relative differences in percent infected and average numbers of nematodes per infection when hosts from different localities are compared. No squirrels from the town sample were found to harbor this species. Of the hosts from woodlots 9.68% were infected, whereas the highest rate of infection was recorded from the forest fringes areas at 30.43%. Squirrels from remote forest areas showed a low percentage of only 4.00%. The average number of worms per infection was not very dissimilar between localities with 6.33 in woodlots, 7.14 in forest fringes and 4.00 in remote forest. It is my opinion that possibly greater diversity of habitat and certain microhabitat factors could be instrumental in producing higher rates of infection in the forest fringes. Also, it was pointed out in the discussion of Strongyloides that the hosts from forest fringes areas were collected at an average elevation of 1900 feet, whereas the town and woodlot samples averaged 2100 feet and remote forest areas averaged 2680 feet in elevation. In summary, Table XXIII shows the incidences of infection at various altitudes. The sample sizes were considerably different at each altitude,

FIGURE 15. Percent infected and average number per infection of Böhmiella wilsoni Lucker, 1943 correlated with habitat



a/ Number examined

TABLE XXIII. Incidence of infection and average numbers of Böhmiella wilsoni Lucker, 1943, harbored at various elevations at which the gray squirrel hosts were collected

Elevation (ft.)	Number examined	Per cent infected	Average no. worms/ inf.
1700	11	27.27	4.00
1800	14	71.43	8.30
1900	2	100.00	4.50
2100	81	3.70	6.33
2500	14	0.00	0.00
2700	2	0.00	0.00
3300	2	0.00	0.00
3500	4	0.00	0.00

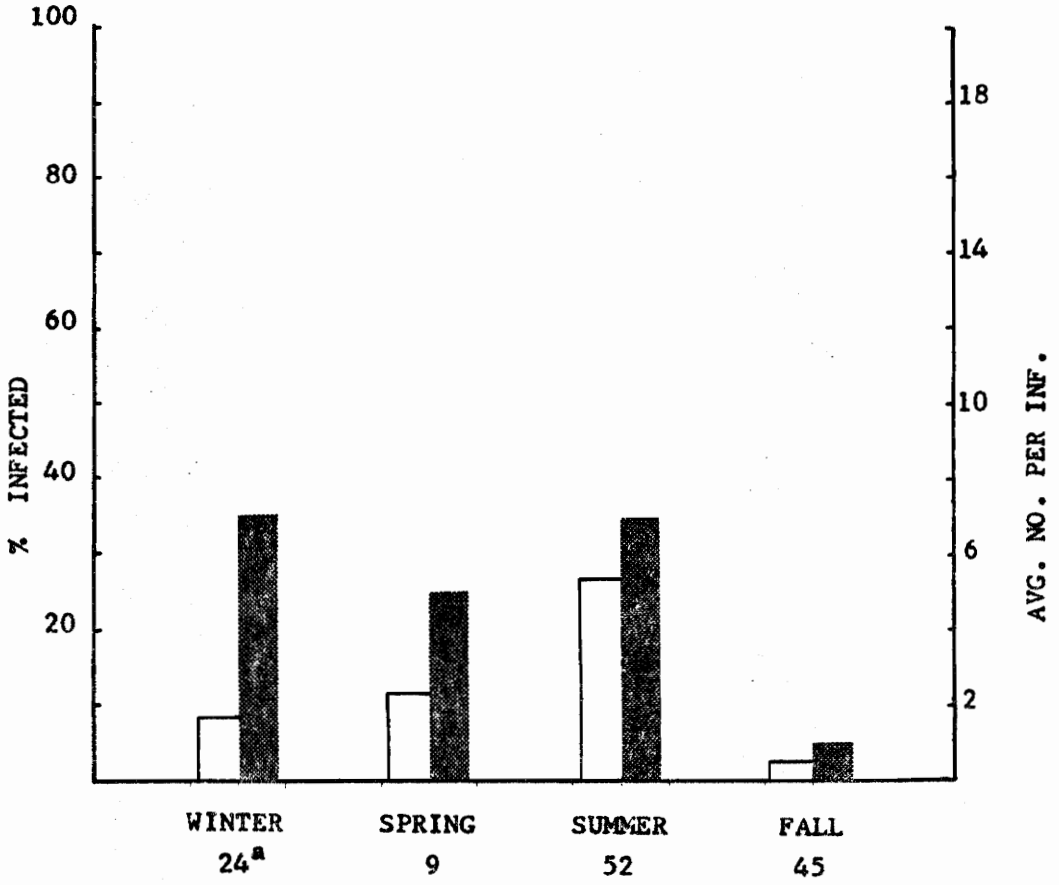
however, in view of the total squirrels (18) found to be infected with this worm, 15 (83%) were collected from altitudes below 2100 feet and 3 (17%) at 2100 feet elevation. No squirrels were found to harbor B. wilsoni at elevations above 2100 feet. Since the majority of hosts were collected at 2100 feet and similar numbers of hosts were collected below and above this altitude (27 and 22, respectively), it is possible that certain environmental factors which differ with altitude, at least in part, limit the distribution of this worm.

My data were examined further to determine what variation in incidence of this worm occurred between seasons of the year (Figure 16). It appears that rates of infection increase from winter to spring to a peak in summer, with a low point in the fall. The size of the spring sample was considerably lower than the others and, therefore, must be accepted with caution when comparing it to the other seasons. The average number of worms per infection are comparatively similar during winter, spring and summer, but they are reduced in the fall.

Heligmodendrium hassalli (Price, 1928)

This nematode was found in the small intestine (usually duodenum) of 11 (6.58%) gray squirrels. The numbers of worms per infection ranged from 1 to 65, with an average of 20.73 per infected host. Katz (1938) found this species to be the most common nematode occurring in gray squirrels in Ohio. He reported a higher incidence in 50 (69.4%) hosts of 72 examined, with an average of 74.6 worms per host and a maximum of 443 worms in one host. Counts to determine the sex ratio of this worm in my study were made from 10 infected hosts which yielded 70 (45%)

FIGURE 16. Percent infected and average number per infection of Böhmiella wilsoni Lucker, 1943 correlated with seasons



a/ Number examined

males to 95 (55%) females.

All squirrels infected with H. hassalli were adult or subadult squirrels: ten infected hosts were collected during the summer months and one during the fall months and infected squirrels for the most part came from lower altitudes. Three infected squirrels were collected at 1700 feet elevation, six at 1800 feet, one at 1900 feet and one at a high elevation of 3300 feet.

In addition to the gray squirrel, H. hassalli has been reported in fox squirrels, Sciurus niger bryanti, in Maryland by Dozier and Hall (1944) and Sciurus niger rufiventer in Ohio by Katz (1938), in Wisconsin by Rausch and Tiner (1948), in Kansas by Ulhrich and Graham (1941), and in Texas by Chandler (1942), and in the red squirrel, Tamiasciurus hudsonicus, in Wisconsin by Rausch and Tiner (1948).

Order Spiruridea
Family Rictulariidae Railliet, 1916

Pterygodermatites parkeri Lichtenfels, 1970

This species was found in the small intestine of 7 (4.2%) squirrels. The numbers per infection ranged from 1 to 10 (average 2.6). A total of 18 worms (16 female, 2 male) were recovered. Katz (1938) reported Rictularia sp. in 7 (9.7%) of 72 hosts examined, with an average number of 2.6 per host and a maximum of 6 worms in one host.

The genus Rictularia Froelich, 1802, (Rictulariidae) has now been separated into two genera by Quentin (1969). These are Rictularia Froelich, 1802, and Pterygodermatites Wedl, 1861. Some of the material recovered in the present study was examined by Dr. J. Ralph Lichtenfels

at the Beltsville Parasitological Laboratory in Beltsville, Maryland, the result of which led to the description of Pterygodermatites parkeri. The species referred to as Rictularia coloradensis by Parker (1968), Rictularia halli by Rausch and Tiner (1948) and Rictularia sp. by Katz (1938), Rausch and Tiner (1948) and Olexik, Perry and Wilhelm (1969) from gray squirrels probably all belong to this same species, Pterygodermatites parkeri. Specimens collected during the course of the present study have been deposited in the USNM Helminthological Collection as follows: No. 63207 (holotype: female) and No. 63208 (allotype: male).

With the permission of the author, the description of P. parkeri Lichtenfels, 1970, is quoted as follows:

> Buccal cavity contains three equally large buccal teeth that extend anteriorly from its floor about one-third its depth. Buccal teeth consist of three main portions with middle portion of each tooth shorter than lateral portions, giving anterior edge of each tooth a concave appearance.
>
> FEMALE (Based on 12 complete specimens and some fragments): Length 19.9-34.4 mm. Diameter at vulva 315-536. Buccal cavity round in en face 65-100 deep by 65-105 wide. Mouth roughly oval, slightly elongated laterally, bordered by 14-19 perioral denticles up to 13-16 long. Esophagus length 2.70-4.14 mm; anterior muscular portion 610-810 long. Nerve ring 315-449 and excretory pore 518-555 from anterior end. Cervical papillae 415-700 from anterior end at level of 6th or 7th pr. of combs. Vulva posterior to end of esophagus (except specimens from Napeozapus in which it is anterior), between pairs of cuticular processes numbers 30 and 32, 2.25-4.27 mm from anterior end. Eggs embryonated, length 40-50; width 30-33. Total number pairs cuticular processes 61.67 including 32-34 pairs of combs. Largest combs 147-240 by 60-120 high. Maximum spine length 970167. Tail length 255-442.
>
> MALE (allotype): Length 6.18 mm. Diameter: maximum 268; at base of buccal capsule 100. Buccal cavity 43 by 43. Mouth bordered by 13 perioral denticles up to 11 long. Esophagus length 1.40 mm; anterior muscular portion 400. (Excretory pore not seen.) Nerve ring 175 from anterior end between 3rd and 4th combs. Cervical

papillae between 5th and 8th pairs of combs 300 from anterior end. Total of 42 pairs of combs; largest 162 long by 59 high; last one 500 from tail end. Ventral cuticular fans absent. Tail length 243. Spicules nearly equal, curved ventrally, left 270, right 256, long. (Neither gubernaculum nor accessory piece discernible.) Ventral surface of tail and pericloacal region with 10 paired and one unpaired genital papillae, and a finely rugose papillation in the pericloacal region (Figure 14).

Figure 17 (a-d) is after Lichtenfels (1970) and in his paper were indicated as figures 11 to 14, respectively.

The life cycle of this species is unknown at present. It is possible that larval development requires some intermediate host and insects are suspected.

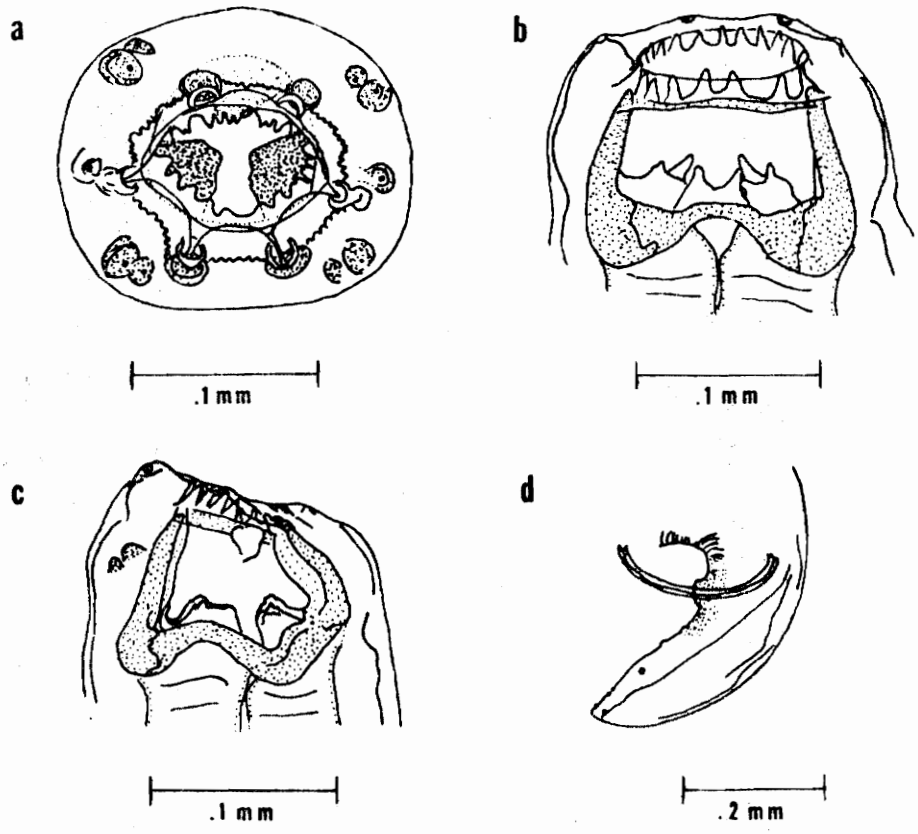


FIGURE 17. Pterygodermatites parkeri Lichtenfels, 1970 a. en face, female b. dorsal view of head, female c. left lateral view of head, female d. male tail

Order Spiruridea
Family Spiruridae Oerley, 1885

Gongylonema pulchrum Molin, 1857

In examinations of the esophagus of 168 gray squirrels, I found Gongylonema pulchrum in four (2.38%) hosts. All of the worms except one were embedded, according to their characteristic wavy pattern, in the mucosa of the esophagus. One worm, however, was recovered free in the stomach and obviously was the result of post mortum migration. I did not regularly examine the tongue, buccal cavity and pharynx in this study and it is possible the incidence reported herein might be somewhat conservative. The number of worms per infected host ranged from one to six (average 3.25) and a total of 13 worms were recovered (4 males, 9 females). These findings constitute a new host record for this worm in the gray squirrel. Apparently this is only the fourth species of the order Rodentia found to harbor these worms and is the first from the family Sciuridae.

The esophagus was removed from the squirrel, split lengthwise and pinned to a wax-bottomed petri dish for examination under a dissection microscope at 15X. Considerable care and patience was required for the removal of the worms without fragmentation. This was minimized by the use of a number 1 dental pulp file, the tip of which had been bent at a right angle. Damage incurred to worms by the initial incision could not be prevented.

Gongylonema pulchrum appears to be relatively non-specific with reference to choice of host and is worldwide in its distribution. A review of the literature for reported hosts of G. pulchrum yielded

mammals from seven orders: Insectivora, in the hedgehog; Rodentia, in the Guinea pig, nutria and rat; Lagomorpha, in the rabbit and hare; Perissodactyla, in the horse and donkey; Artiodactyla, in the European wild boar, domestic swine, goat, sheep, cattle, camel, water buffalo, roe deer, red deer and white-tailed deer; Carnivora, in the black bear, fox and dog; and Primates, in the Rhesus macaque, monkey and man. Young chicks and turkeys have also been experimentally infected with G. pulchrum. Human infections have been reported from the United States (Georgia and Arkansas), Russia, China, Morocco and Panama. Ward (1916) provides an interesting case account of the pathology of this species in man.

The intermediate hosts of G. pulchrum are also numerous and little specificity is shown. The more extensive studies on intermediate hosts for this worm have been conducted in Russia. Popova (1959) found infective larvae in 27 species of beetles; Iwashkin and Khromova (1961) found them in at least 5 species; and Chebotareu and Polishchuk (1961) found them in 28 species of Scarabaeidae and Tenebrionidae. Threlkeld (1958) gave three intermediate hosts for this worm in southwestern Virginia: the dung beetle, Aphodius lividus (Olivier); the scavenger beetle, Dermestes vulpinus Fabr.; and the cockroach Parcoblatta sp. Perhaps one of these insects was involved in the transmission to the squirrels in this study.

Gongylonema pulchrum produced only minor pathological effects in the gray squirrel in which the tunneling under the esophageal mucosa was most evident. There was no evidence of inflammation, edema or

blockage caused by these worms. The pathology in domestic animals has been reported by several investigators.

Morgan and Hawkins (1949) reported no significant pathology in swine, cattle and sheep infected with G. pulchrum, but they added that the worms rendered the esophagus of these animals unfit for use as food. More specific pathological descriptions in similar animals have been given by Popova (1960, 1962, 1964) and Baunann (1961) who recorded tissue destruction and lesions, inflammation, edema, hyperemia and resultant chronic conditions in the organs affected.

Since this is a new host record and morphological dimensions of this species differ between various hosts, a brief description appears to be in order. The following measurements are in millimeters:

Five female worms measured 27.8-37.9 (31.3) in length; greatest width .170-.225 (.203). Buccal capsule .039-.051 x .008-.013 (.045 x .010). Anterior portion of esophagus .353-.454 (.409); posterior portion of esophagus 3.622-4.258 (3.625); total esophagus 3.512-4.708 (4.033) and 12.4-14.6% (13.4%) of the total length. Distance from anterior end to nerve ring .176-.245 (.219). Vulva located 1.984-3.100 (2.284) from posterior end. Tail length 165-.240 (.200). Origin of cervical alae from anterior end .137-.231 (.184). Origin of cuticular bosses from anterior end .011-.028 (.017) and terminate at .604-1.186 (.851). Ten eggs measured .044-.052 x .023-.024 (.048 x .023).

Four males measured 17.2-19.1 (18.0) in length; greatest width .136-.163 (.147). Buccal capsule .032-.046 x .007-.009 (.040 x .008). Anterior portion of esophagus .365-.463 (.416); posterior portion of esophagus 2.975-3.164 (3.038); total esophagus 3.438-3.529 (3.454) and

17.8-20.0% (19.0%) of the total length. Left spicule 6.940-9.208 x .027-.029 (8.054 x .028). Right spicule .093-.119 x .011-.023 (.108 x .017). Gubernaculum .074-.086 x .024-.029 (.080 x .027). Length of right caudal alae .245-.465 (.368); length of left caudal alae .325-.535 (.463). Origin of cervical alae from anterior end .131-.171 (.153).

The occurrence of G. pulchrum in squirrels is of particular interest since the possibility exists that squirrels may serve as reservoir hosts and aid in the dissemination of these worms to domestic animals and man. Prestwood, Smith and Mahon (1970) have recently shown G. pulchrum to be widely distributed in deer in 13 southeastern states. Therefore, owing to the apparent non-specificity for host, I suggest that future studies of wild mammals include examinations of the upper alimentary tract for this worm. Perhaps there are other animals which serve as reservoir hosts and serve to disseminate this worm to domestic animals and, occasionally, to man. The importance of G. pulchrum in the gray squirrel demands further attention.

Examination for Microfilariae

In conjunction with the study of Hepatozoon, which was discussed earlier, Knott's (1939) concentration technique (modification by Herman and Price, 1955) was employed in the examination of cardiac blood from 80 squirrels (41 males, 39 females). The results yielded no positive cases of microfilariae in the blood concentrates from squirrels of southwestern Virginia.

Nematodes referred to in the literature as "microfilariae" in the gray squirrel have been reported by Price (1954) and Robinson (1954);

microfilariae under the name Dipetalonema sp. was discussed by Evans, Phillips and Bickley (1959); and Price (1962) described Dipetalonema interstitium from gray squirrels in Maryland. Various species of mosquitoes have been implicated in the transmission of microfilariae to squirrels (Evans, et. al., 1959).

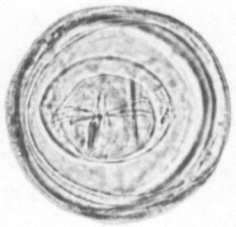
Fecal Analysis for Helminth Ova

In conjunction with my survey of the coccidia of gray squirrels using the "wet-mount" preparation, fecal samples were examined for the presence of helminth ova to determine the reliability of fecal analysis in the diagnosis of helminth infections. Samples from 167 squirrels were examined of which 61 (36.53%) were positive. Many squirrels found to harbor helminths at autopsy did not exhibit ova in the feces by this technique, especially when only a few worms were harbored. Greater accuracy might have been gained in the use of floatation techniques, but this was not done in this study because of the amount of time involved. Plates 14-25 provide a comparative index to the helminth ova which might be encountered in the feces of gray squirrels, but for practical purposes I find it more reliable to dissect the alimentary tract and search for the worms. The ova were removed from the adult worms in order to photograph them for these plates.

Pathology

For most of the helminths encountered in this survey, few produced signs of pathology in the host upon gross examination. The majority of

14. Hymenolepis diminuta

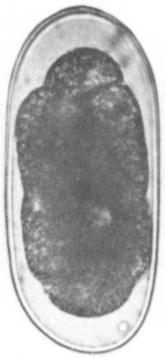


15. Catenotaenia dendritica

16. Enterobius sciuri

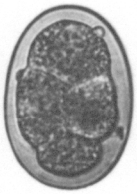
17. Syphacia thompsoni

18. Bohmiella wilsoni



19. Heligmodendrium hassalli

20. Citellinema bifurcatum



21. Trichostrongylus calcaratus

22. Capillaria americana

23. Gongylonema pulchrum

24. Pterygodermatites parkeri

25. Strongyloides robustus

PLATES 14-25. Comparative index to the helminth ova likely to be encountered during fecal analysis of Sciurus carolinensis (400X)

the helminths were found in the small intestine, while some were found in the esophagus, stomach, caecum and large intestine. The following helminths were often attached to the mucosa of the organs in which they occurred: Catenotaenia dendritica, Hymenolepis diminuta, Citellinema bifurcatum, Trichostrongylus calcaratus, Heligmodendrium hassalli and Pterygodermatites parkeri. Except for only slight local disruption of tissues, no other pathology caused by these worms was observed.

More extensive pathological damage was caused by the worms, Gongylonema pulchrum, Strongyloides robustus and Capillaria americana. These worms produced tissue damage by their tunneling movements in the mucosa. Gongylonema pulchrum exhibited its typical zig-zag tunneling pattern in the mucosa of the esophagus, yet there was no evidence of inflammation in this area resulting from it. Strongyloides robustus appeared to be responsible for the most severe nematode pathology. These worms, although very small, perforated the duodenal mucosa (Plate 26); in certain heavy infections many of the intestinal villi were badly eroded. The worms did not appear to be randomly distributed, but were localized in patches or areas where many minute holes could be seen in the villi. In some infections, worms were observed to inhabit the tissue surrounding the opening of the bile duct, and several patches of these worms could usually be found in the duodenal enlargement near the opening of the pyloric sphincter. Capillaria americana tunnels just under the duodenal mucosa in a fashion similar to Gongylonema. Due to the few numbers encountered the pathology was not significant in any infections that I observed.

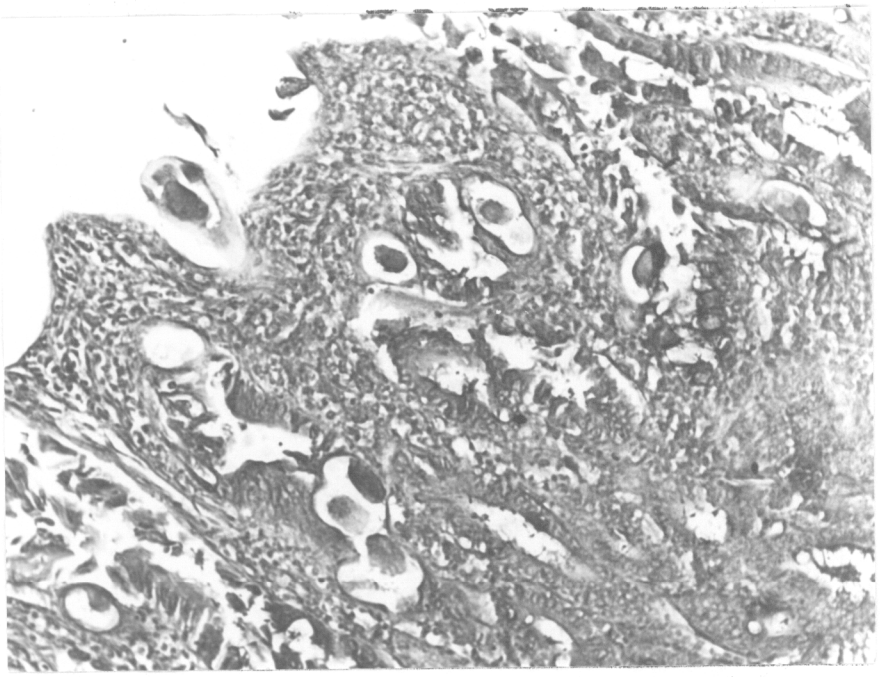


PLATE 26. Photomicrograph showing the pathology caused by Strongyloides robustus Chandler, 1942, in the duodenum of the gray squirrel (164 x)

Summary and Conclusions

Two species of adult cestodes were observed in the squirrels in this study: Catenotaenia dendritica and Hymenolepis diminuta. The first species is a new host record. No larval cestodes occurred in the squirrels of my survey, but one squirrel collected subsequent to the survey to be used for experimental work was found to be infected with sparganid larvae of a pseudophyllidian tapeworm. No representatives of Trematoda or Acanthocephala were found in this study. In the Aschelminthes, 12 species of nematodes were harbored by the squirrels examined. Citellinema bifurcatum, Strongyloides robustus and Böhmiella wilsoni were the most frequently encountered species and occurred in 45.23%, 28.14% and 13.85% of the hosts, respectively. Capillaria americana, Heligmodendrium hassalli, Trichostrongylus calcaratus, Citellinema bifurcatum, Strongyloides robustus and Ascaris lumbricoides are new distributional records for this host in Virginia, and Syphacia thompsoni, Gongylonema pulchrum and Contracaecum sp. are new host records. Enterobius sciuri is herein reported for the first time in the gray squirrel in North America. One nematode, Pterygodermatites parkeri, that I recovered in this study was described as a new species by Lichtenfels (1970).

According to the examination of data concerning the three most prevalent nematodes, it appears, for the most part, that male squirrels show higher incidences of nematode infections than females and usually incidence also increases with the age of the host. Collection localities designated as town, woodlot, forest fringes and remote forest were

defined. The forest fringes yielded the highest rates of infection. It was speculated that perhaps this was due, in part, to diversity of habitat in this area and to environmental differencies resulting from differences in elevation. Seasonal variations in three species showed peaks of incidence to occur in the spring for Strongyloides robustus and in the summer for Citellinema bifurcatum and Böhmiella wilsoni.

No microfilariae were observed in the examinations of blood concentrates from 80 hosts.

Fecal analysis showed 36.53% of 167 squirrels to have helminth eggs in the feces but was concluded to be a poor diagnostic method. A comparative index to helminth ova of squirrels is included.

The pathology of helminth infections in this study for the most part was insignificant. Strongyloides robustus appeared to be the most important species for its pathogenesis.

III. ARTHROPODS

The final section of this study of gray squirrel parasites concerns the arthropods present on the skin and in the fur of these animals and also these occurring in gray squirrel nests. The following groups of organisms will be considered: Insecta, order Mallophaga (biting lice), order Anoplura (sucking lice), order Diptera (files) and order Siphonaptera (fleas); and Acarina (ticks and mites). The literature dealing with these forms on gray squirrels is rather extensive and widely scattered. The larger works come from Harkema (1936) who reported species of lice, mites and ticks in North Carolina; from Katz (1938) who reported botfly larvae and fleas in Ohio; from Bishopp and Trembly (1945) who reported ticks from various areas of the United States; from Brown and Yeager (1945) who reported species of fleas, lice, mites and ticks from Illinois; from Morlan (1952) who reported fleas, lice, mites and ticks in Georgia; from Allison (1953) who reported fleas, botfly larvae and mites from North Carolina; from Burbutis (1956) who reported fleas on this host in New Jersey; and from Watson (1959) who reported fleas and lice in Florida. A more specific summary of the literature with reference to the individual groups and species is given below.

Class Insecta
Order Mallophaga

No species of biting lice were reported on gray squirrels until Bruelia rotundata (Osborn, 1896) was reported by Parker (1968) in

Virginia. This species was found in the early phases of my study, the details of which will be given in the results.

Order Anoplura

Among the Anoplura, or sucking lice, Ferris (1916) reported Enderleinellus longiceps Kellogg and Ferris, 1915, on the gray squirrel from Nebraska and in 1919 from Mississippi and Nebraska. Ferris (1921) described Hoplopleura sciuricola from this host in Mississippi and Florida. In 1936, Neohaematopinus sciurinus (Mjoberg, 1910) and Hoplopleura sciuricola were listed by Harkema in North Carolina. Keegan (1943) reported Neohaematopinus antennatus from this host in Iowa. Baker (1944) reported Neohaematopinus sp. in eastern Texas. In 1945, Brown and Yeager listed Hoplopleura sciuricola in Illinois. Morlan (1952) listed Hoplepleura sciuricola, Polyplax spinulosa (Burmeister), Neohaematopinus sp. and Neohaematopinus sciurinus from this host in Georgia. Scanlon (1959) reported Neohaematopinus sciuri Jancke, 1932, in New York. Watson (1959) listed Hoplopleura sciuricola and Neohaematopinus sciuri from this host in Florida. Parker (1968) listed Enderleinellus longiceps, Hoplopleura sciuricola and Neohaematopinus sciuri in Virginia. Johnson (1959) showed N. sciurinus to occur on Sciurus niger in North America and N. sciuri to occur both on the European Sciurus vulgaris and the North American Sciurus carolinensis. He separated these species on differences in antennae morphology. Therefore, reports of N. sciurinus on the gray squirrel are likely misidentifications of N. sciuri.

Order Diptera

Cuterebra sp., the botfly or warblefly larva, has been reported on gray squirrels in Ohio by Katz (1938) and Chapman (1938), in Virginia by Cross (1942), in Alabama by Atkeson and Heflin (1948) and Atkeson and Givens (1951), in North Carolina by Allison (1953), in West Virginia by Uhlig (1956) and in eastern Tennessee by Dunaway, Payne, Lewis and Story (1967). It was also reported by Parker (1968) in Virginia. Cuterebra emasculator Fitsch was reported in this host in North America by von Bau (1906).

Order Siphonaptera

A number of fleas have been reported on gray squirrels. In 1904, Baker reported Ceratophyllus wickhami (Baker) Wagner from New York, Michigan, Iowa, Nebraska and Georgia. Shaftesburg (1934) reported Pulex irritans Linnaeus, 1758, in North Carolina. Shipley (1941) and Cross (1942) listed Orchopeas sp. from squirrels in Virginia. In 1945, Brown and Yeager reported Orchopeas nepos Rothschild and Orchopeas howardii (Baker, 1895) in Illinois. In 1947, Hubbard showed O. wickhami to be synonymous with O. howardii, the latter taking precedence. Morlan (1952) listed Xenopsyllus cheopis Rothschild, 1903, Orchopeas howardii and Echidnophaga gallinacea (Westwood, 1875) from this host in Georgia. Allison (1953) reported Orchopeas wickhami=O. howardii in North Carolina. Also, in the same year, Dean (1953) reported O. howardii from Alabama, and in 1959, Watson also reported it from this host in Florida. Burbutis (1956) listed Orchopeas sp., Nosophyllus fasciatus (Bosc d'Antic, 1801), Ctenocephalides felis (Curtis, 1820), Cediopsylla simplex Baker, 1904,

and Ceratophyllus gallinae (Schrank, 1803) from New Jersey. Goodrum (1961) reported "fleas" on this host in Texas. Olexik (1968) and Olexik, Perry and Wilhelm (1969) reported Orchopeas howardii from the gray squirrel in southwest Tennessee, and Parker (1968) reported the same species in Virginia. Other literature reports of fleas on squirrels are not clear as to the identity of the host species.

Class Arachnida
Order Acari

Ticks

At least eight species of ticks are reported in the literature as parasitic upon gray squirrels. Harkema (1936) listed Ixodes cookei in North Carolina. In 1940, Hixon reported Amblyomma maculatum Koch in Georgia, and Shipley (1941) recorded "ticks" in Virginia. Dermacenter variabilis (Say, 1821) was reported by Cross (1942) in Virginia, by Brown and Yeager (1945) in Illinois, by Bishopp and Trembly (1945) in the United States, by Anastos (1947) in New York and by Morlan (1952) in Georgia. Ixodes marxi Banks, 1908, was listed by Katz (1941) from Ohio and by Clifford, Anastos and Elbl (1961) from Maryland, Alabama, Delaware, Maine, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, South Carolina, Vermont and the District of Columbia. Amblyomma americanum (Linn.) has been reported by Baker (1944) in eastern Texas, by Bishopp and Trembly (1945) in the United States and by Morlan (1952) in Georgia. Bishopp and Trembly (1945) also reported Ixodes cookei Packard, 1869, and Haemophysalis leporis-palustris (Packard) from the gray squirrel in the United States. Anastos (1947) has recorded the

species, Ixodes muris Bishopp and Smith, 1937, from this host in New York.

Mites

Harkema (1936) reported the mite, Haemolaelaps glasgowi Ewing, 1925, from the gray squirrel in North Carolina. This species was again reported in Georgia by Morlan (1952). Sarcoptes sp., the mite often associated with mange in wild animals, has been reported by Chapman (1938) and Martin (1938) in Ohio, by Brown and Yeager (1945) in Illinois, by Uhlig (1956) in West Virginia, by Goodrum (1961) in Texas and by Madson (1964) in Michigan. Trombicula (Eutrombicula) tropica-alfreddugesi has been reported from squirrels in east Texas by Goodrum (1940). Trombicula (Neotrombicula) whartoni Ewing, 1929, has been reported from this host by Brennan and Wharton (1950) in North Carolina and by Morlan (1952) in Georgia. Morlan (1952) also reported Trombicula (Eutrombicula) splendens Ewing, 1913, Trombicula (Eutrombicula) alfreddugesi (Oudemans, 1910), Euschongastia sp., Ornithonyssus bacoti (Hirst, 1913), Parasitus americanus Berlese and Haemolaelaps megaventralis from gray squirrels in Georgia. Allison (1953) recorded Trombicula sp. from this host in North Carolina. Clark (1958) reported Haemogamasus ambulans (Thorell, 1872) from this host in Maryland, and he (Clark, 1960) also described a new species of nasal mite, Speleognathopsis sciuri. Goodrum (1961) reported "chiggers" in Texas, and Shoemaker and Joy (1966) noted the occurrence of Trombicula fitchi Loomis, 1954, in West Virginia.

Materials and Methods

Upon bringing collected squirrels to the laboratory, the animals were carefully skinned over a white enamel pan and the skin rolled and frozen in the same plastic bag used. The skinning was accomplished by the following process: Midway on the body, the skin was cut with scissors all around so as to divide it into anterior and posterior halves. Then each half was carefully removed from the carcass toward its appropriate end. The anterior portion was peeled to the tip of the nose and cut away from the head, whereas the posterior portion was peeled to the tail and the tail pulled out of the skin covering it. The skin on the legs was peeled to the tip of the toes and pulled away from them. Thus the skin of each squirrel was recovered in two halves, resembling a shirt and pants. The enamel pan over which the skinning was done was washed with 70% ethanol and the fluid retained to be inspected later.

To determine the incidence of ectoparasite species on the squirrels, the squirrel skins were thawed and brushed over a white enamel pan. Next, the skin was placed into a gallon-size jar containing 70% ethanol and vigorously shaken for several minutes. The skin was removed from the jar, the enamel pan and plastic bag washed and all of the fluid was collected and examined, fractions at a time, in a petri dish under a dissection microscope (7x). The arthropods were removed from the fluid using a #1 or #2 dental pulp file, the end of which had been shaped in the form of a loop, and were stored in screw-cap vials containing 70% ethanol until identification could be made. Some of the arthropods had to be mounted on microscope slides and observed under a compound

microscope before identification was possible. These were transferred from the 70% ethanol directly into a drop of Hoyer's medium on a slide and the coverslip mounted. This is a rapid method involving only the one step which clears the speciman and makes a permanent mount.

It was decided that attempts should be made to determine the total numbers of ectoparasites which occur on some of the individual squirrels and also the densities of these ectoparasites in relation to the surface area available for them on the body of the host. It was anticipated that these data might provide information concerning preferences of various species for certain body regions. Therefore, 24 adult squirrels (12 males and 12 females) were selected to be processed by the following method: The skin was thawed and cut into the various body regions over a white enamel pan. These regions were designated as head, back, underparts, legs and tail. The head region was separated by cutting the skin about one-fourth inch behind the base of the ears, separating it from the other parts. The back was composed of the entire dorsal area and the sides down to where the white belly hair began. The underparts were designated as the ventral area covered by the white belly hair, except that on the underside of the head and legs. The legs were cut away from the body proper on a straight line where they joined the body. All four legs were considered together as one body region. The tail was cut where it joined the body. Thus the skin covering each body region as designated was removed and manipulated separately from each of the other regions. Figure 18 shows a diagrammatic scheme of the body regions as they appeared when separated and ready for tracing.

The skin of each body region was spread onto a piece of cardboard

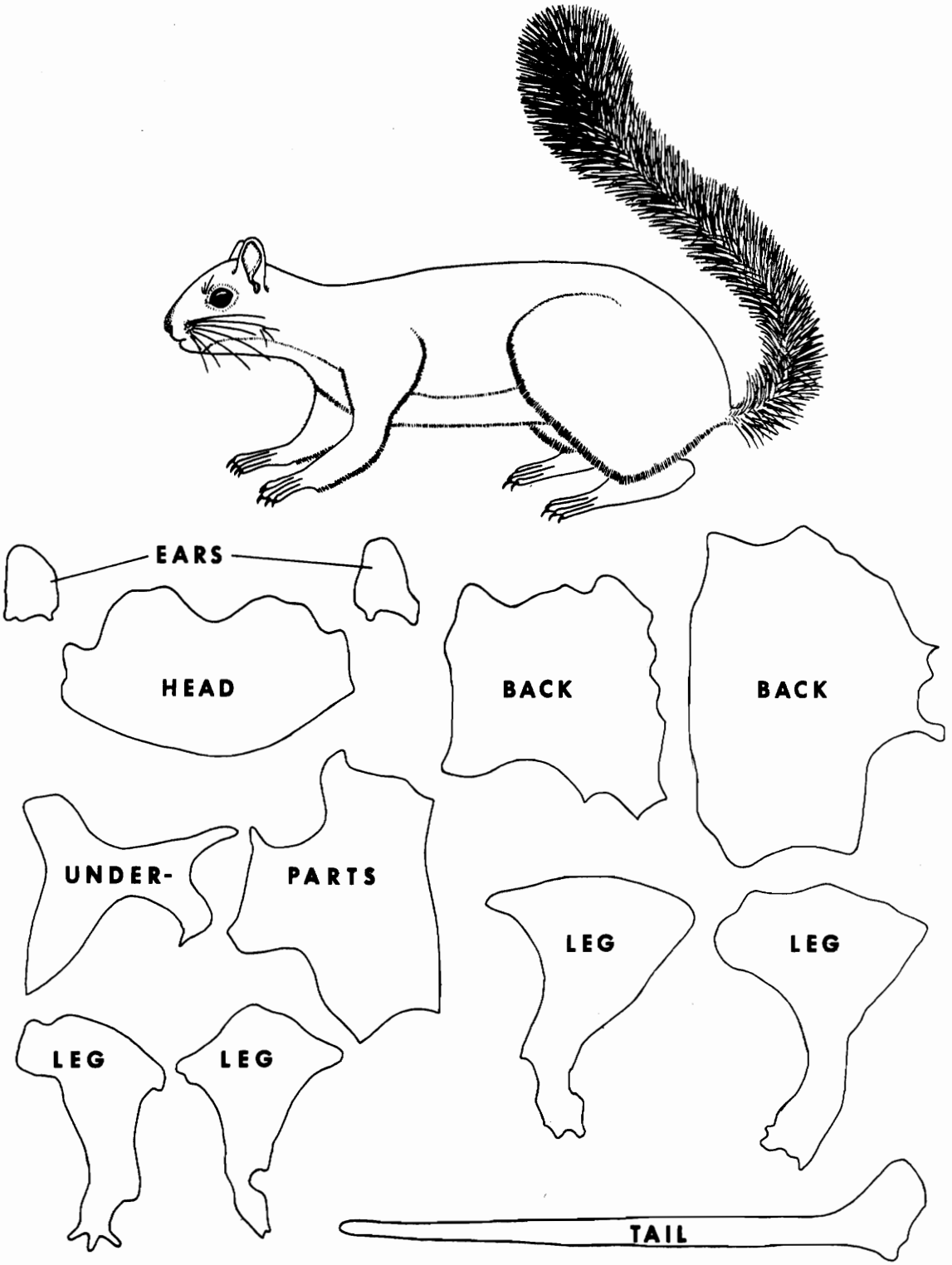


FIGURE 18. LAYOUT OF TRACINGS OF BODY REGIONS FOR AREA DETERMINATIONS

in a manner to avoid distortion and stretching. Then each was traced around its margin with a pencil. Afterwards, the traced area of each body region was traced again using a Keuffel and Esser Co. compensating polar planimeter which was calibrated to read directly in square centimeters. These procedures yielded the approximate surface area of the skin of each body region. By adding the surface area readings from the planimeter of all of the regions of the skin, the approximate total skin surface area of each animal was determined. Next, the skin from each body region was placed into separate beakers and a 5% solution of solium hydroxide was poured into the beaker to cover the skin. This phase of the procedure is the Hopkins (1949) hair dissolving technique for the removal of ectoparasites. The skin was allowed to remain in the 5% sodium hydroxide solution overnight by which time the hair had dissolved. The remaining hairless skin was examined for arthropods still clinging to it and then discarded. All of the fluid in the beakers, which by this time was a dark brown color due to the hair pigments, was examined under a dissection microscope (7x) in small quantities poured into a petri dish. The petri dish was methodically examined and the arthropods removed and counted. All the arthropods washed from the plastic bag and those in the enamel pan had become detached from the skin were also identified and counted. Because I was not certain from which body region these came, they were classed under an additional heading entitled "uncertain location". With the surface areas of each body region determined and the ectoparasites counted, I was able to calculate the parasite densities. Density is expressed as the number

of organisms per square centimeter.

The methods I have described made it possible to determine, as accurately as possible, the total numbers of ectoparasites on the host, the densities of the parasites on each body region and the regions of the body on which various species occurred. Only 24 squirrels were examined by this technique due to its excessive time consumption. Only adult squirrels were examined because I did not consider it feasible to compare adult surface areas with those of immature squirrels.

Another project was undertaken in this study which involved the survey of arthropod parasites occurring in the nests of gray squirrels, in which I anticipated that I might find some species not regularly found on the squirrels. Rather than collect the nests for a single sample, I thought it more desirable to produce a device for monitoring the same nests for considerable lengths of time. Therefore, I modified the construction of the "nest-funnel" device proposed by Drummond (1955; 1957), who used this device to survey acarine populations in the nests of Peromyscus leucopus and I have utilized the approximate shape and dimensions given by Barkalow and Soots (1965) for gray squirrel nest boxes. Figure 19 shows my modified nest-funnel design. Mason-size jar lids for the attachment of pint-sized canning jars were attached to the funnels under the nest boxes. Fourteen nest-funnels were constructed and placed in two woodlots on the campus of Virginia Polytechnic Institute and State University, 7 in Crumpacker woodlot and 7 in Prices Fork woodlot. One nest-funnel in each woodlot had the entrance hole covered with one-fourth inch mesh hardware cloth to keep squirrels out but to allow small arthropods to enter. These served

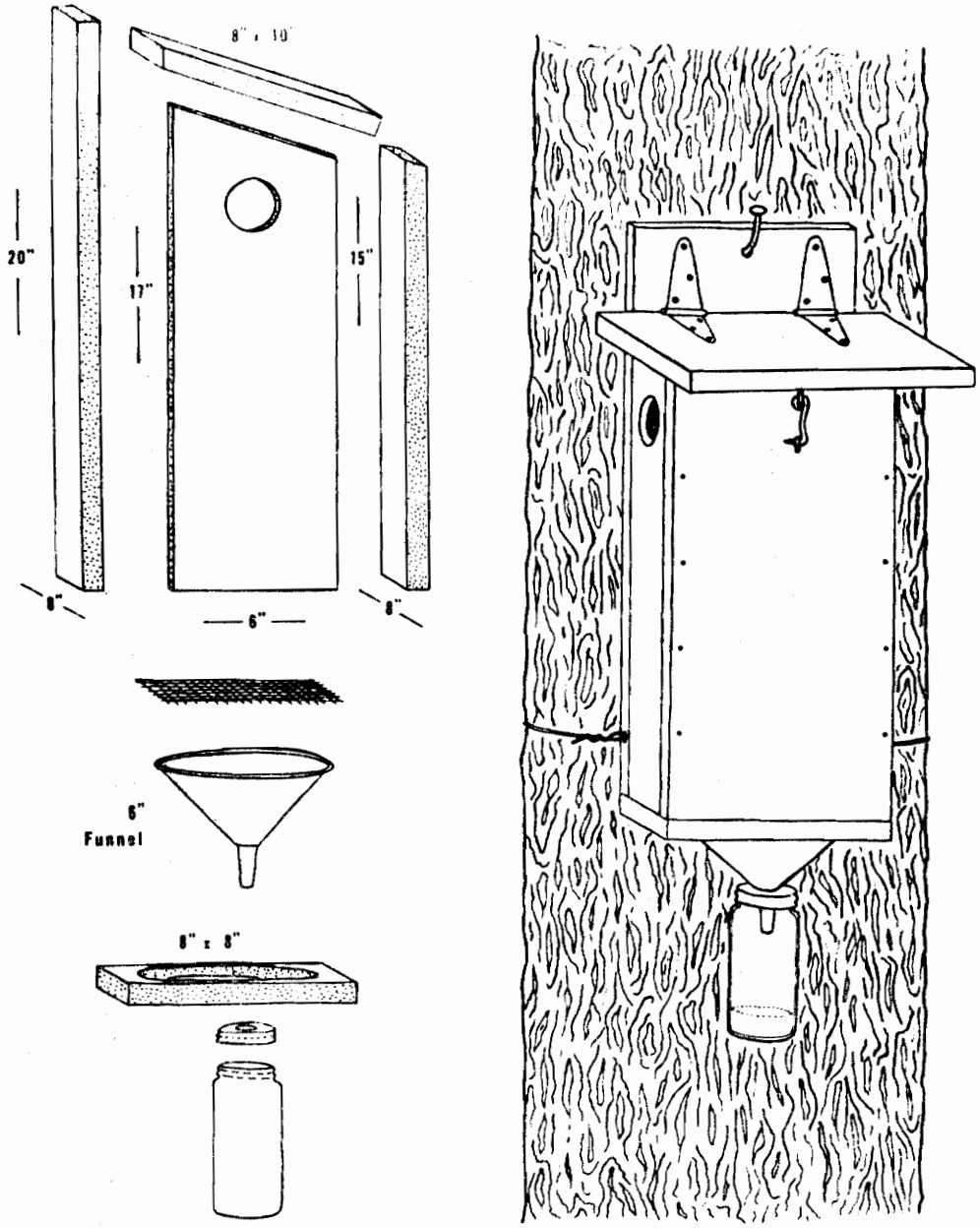


FIGURE 19. Modified nest-funnel design

as controls. The nest-funnels were attached to large trees about 20 feet above the ground. After installation, I allowed four months for the squirrels to become accustomed to the nest-funnels and, hopefully, to construct nests in them. After the four months, fresh jars were positioned under the funnels. About one inch of 35% ethanol with 10% glycerol and several thymol crystals to retard mold was placed into each jar. The jars were changed once each month for a year, during which time the nest-funnels were also inspected for occupation by squirrels and the presence of leaf litter. The jars I collected each month were returned to the laboratory and inspected for parasitic arthropods. A 24 foot, aluminum, extension ladder was easy to transport and facilitated the close inspection of the nest-funnels and the changing of the jars.

Results and Discussion

This portion is presented in four sections. First are the results of the general survey in which incidence and species were determined on the 106 squirrels examined; second are the results of the density studies which were made on 24 adult squirrels; third is the discussion of the species found in these studies, with comments and discussion relating to both the general survey and the density studies; and last are the results of the nest-funnel survey.

1. General Survey

Of the 176 gray squirrels collected for parasite investigations, 106 of these were examined for ectoparasitic arthropods occurring on the skin and in the fur. These examinations revealed 103 (97%) to be infested with parasitic arthropods. These arthropods included four species of lice (one Mallophaga; three Anoplura), two species of flies (Diptera) and one flea (Siphonaptera) among the insects and one species of tick (Ixodidae) and at least six species of mites among the acarines. Table XXIV shows the data concerning the number of squirrels observed to be infested with each species and the percent of squirrels infested. The louse, Neohaematopinus sciuri, was the most frequently observed species, being found on 81% of the squirrels examined. The second most common species was the flea, Orchopeas howardii, being present on 74% of the hosts. Other species in decreasing order of prevalence were the lice, Enderleinellus longiceps 68% and Hoplopleura sciuricola 55%; the mite, Haemogamasus ambulans 30%; the tick, Ixodes marxi 23%; chiggers (Trombiculidae) 14%; and the mite, Androlaelaps casalis 12%. Other species were less common and each was present on fewer than 10% of the hosts. These included the warble fly, Cuterebra sp. 8%; some very small unidentified mites (Rodentopididae) 2%; the louse, Bruelia rotundata 2%; the black fly, Simulium sp. 2%; and the mites, Echinolaelaps sp. 1%, Haemogamasus sp. (probably pontiger) 1% and Hirstionyssus sp. 1%.

2. Density Studies

All of the 24 adult male and female gray squirrels examined for total numbers of ectoparasites had at least a few parasites on them.

TABLE XXIV. Ectoparasitic arthropods recovered in the examinations of 106 gray squirrels in southwestern Virginia, September, 1966-July, 1969

Ectoparasites	No. squirrels infested	Percent infested
Insecta (insects)		
Mallophaga (biting lice)		
Bruelia rotundata	2	1.89
Anoplura (sucking lice)		
Neohaematopinus sciuri	86	81.13
Hoplopleura sciuricola	58	54.71
Enderleinellus longiceps	72	67.92
Diptera (flies)		
Cuterebra sp.	8	7.55
Simulium sp.	2	1.89
Siphonaptera (fleas)		
Orchopeas howardii	78	73.58
Acari (ticks and mites)		
Ixodidae		
Ixodes marxi	24	22.64
Laelaptidae		
Androlaelaps casalis	13	12.26
Haemogamasus sp. (probably *pontiger*)	1	0.94
Haemogamasus ambulans	32	30.19
Echinolaelaps sp.	1	0.94
Macronyssidae		
Hirstionyssus sp.	1	0.94
Rodentopididae?		
genus?	2	1.89
Trombiculidae		
chiggers	15	14.15

The twelve males yielded a total of 6,156 ectoparasites (average 513) and the twelve females yielded a total of 3,686 ectoparasites (average 307) for a total of 9,842 ectoparasites (average 410) on the 24 squirrels. The total number of individual species on the 24 squirrels reflect slightly higher rates of occurrence than those of the general survey of the total 106 squirrels. This indicates the hair-digest method to be the more efficient. Neohaematopinus sciuri was found on 22 (91.6%) hosts, Orchopeas howardii was found on 20 (83.3%) hosts, Enderleinellus longiceps was found on 20 (83.3%) hosts, Hoplopleura sciuricola was found on 18 (75.0%) hosts and Ixodes marxi was found on 9 (37.5%) hosts. Other species were found less frequently, which included Haemogamasus ambulans that was on 8 (33.3%) hosts, Androlaelaps casalis that was on 3 (12.5%) hosts and chiggers (Trombiculidae) that were on 3 (12.5%) hosts. These incidences of occurrence do not necessarily reflect the density of individual species. A total of 3,799 Neohaematopinus sciuri comprised 38.6% of the total ectoparasite fauna on the 24 squirrels; 137 Orchopeas howardii comprised only 1.4% of the ectoparasite fauna; 2,845 Enderleinellus longiceps comprised 28.9% of the ectoparasite fauna; 2,564 Hoplopleura sciuricola comprised 26.0% of the ectoparasite fauna; 203 Ixodes marxi comprised 2.1%; 192 Haemogamasus ambulans comprised 2.0%; 12 Androlaelaps casalis comprised 0.1% and 88 chiggers (Trombiculidae) comprised 9.9% of the total ectoparasite fauna.

The averaged calculations of the surface areas of the bodies of the 24 squirrels are shown in Table XXV. The average weights of the 12 males compared with the 12 females were remarkably similar, the males

TABLE XXV. Averaged body surface areas of 24 gray squirrels (12 males, 12 females) calculated from tracings of skin of various body regions using a compensating polar planimeter, with the averaged weights

Body region	Male surface areas[1]	Female surface areas	Total surface areas
Back	161.64	154.33	157.99
Legs	117.56	107.11	112.33
Underparts	86.73	86.89	86.81
Head	66.80	63.59	65.19
Tail	33.27	30.00	31.63
Entire body averages	465.99	441.92	453.95
Average weights[2]	508.30	507.70	508.00

1/ Numbers represent square centimeters
2/ Average weights are in grams

averaging 508.3 grams and the females 507.7 grams. Although the actual measurements given in Table XXV show very close similarities between the males and females, Table XXVI shows that when the area of each body region is expressed as a percentage of the total body surface, the measurements for both males and females are almost identical. With this in mind, I feel that it is proper to compare the male and female parasite densities. The largest body region is the back (34.80%), followed by legs (24.75%), underparts (19.12%), head (14.36%) and tail (6.97%).

Since the surface area was measured in square centimeters, parasite densities are expressed as the number of ectoparasites per square centimeter. Four-digit numbers after the decimal were used because of convenience in calculations and because some densities were extremely low so that they could not be expressed in any other manner. Therefore, these numbers are not the result of extreme accuracy, but to the calculations from my data.

In the paragraphs which follow, I will summarize my data concerning the numbers and densities of all ectoparasites as a group according to body regions and host sex. Individual species numbers and densities will be discussed later.

On the body region I designated as the back, ectoparasite numbers on male squirrels ranged from 5 to 446 (average 194.7), whereas the densities ranged from 0.0296 to 2.8875 per square centimeter (average 1.1211). On the back region of females, numbers ranged from 2 to 544 (average 112.8), whereas the densities ranged from 0.0164 to 3.6899 per square centimeter (average 0.7543). The averages for the back region of all those examined (12 males and 12 females) were 153.7 ectoparasites

TABLE XXVI. Average surface areas of body regions expressed as percentages of the total body surfaces of 24 adult gray squirrels (12 males, 12 females)

Body region	Males %	Females %	Average of total %
Back	34.69	34.92	34.80
Legs	25.23	24.24	24.75
Underparts	18.61	19.66	19.12
Head	14.33	14.39	14.36
Tail	7.14	6.79	6.97
Totals	100.00	100.00	100.00

per host back and 0.9377 per square centimeter. The highest average number and the greatest average density occurred on this body region.

On the legs, parasite numbers on males ranged from 1 to 452 (average 147.6), whereas the densities ranged from 0.0081 to 4.0082 per square centimeter (average 1.2978). Numbers on females ranged from 0 to 262 (average 50.6), whereas the densities ranged from 0.0000 to 2.3196 per square centimeter (average 0.4755). Averages for the total examined were 99.1 ectoparasites per host legs region and 0.8866 per square centimeter.

On the underparts, parasite numbers on the males ranged from 4 to 215 (average 65.6), whereas the densities ranged from 0.0468 to 2.2194 per square centimeter (average 0.7634). On the female underparts, ectoparasite numbers ranged from 1 to 436 (average 52.4), whereas the densities ranged from 0.0106 to 4.5060 per square centimeter (average 0.6102). Averages for the 24 squirrels were 59.0 ectoparasites per host underparts and a density of 0.6868 per square centimeter.

On the head region, parasite numbers on male hosts ranged from 0 to 146 (average 53.3), whereas the densities ranged from 0.0000 to 2.0977 per square centimeter (average 0.7832). On the head region of females, parasite numbers ranged from 0 to 330 (average 53.9), whereas the densities ranged from 0.0000 to 5.1969 per square centimeter (average 0.8454). Averages for the 24 squirrels examined were 53.6 ectoparasites per host head region and a density of 0.8143 per square centimeter.

Ectoparasites on the tail region were found in fewest numbers and least densities. On male hosts, parasite numbers ranged from 2 to 58 (average 17.5), whereas the densities ranged from 0.0500 to 1.6667 per

square centimeter (average 0.5402). On the females, the numbers ranged from 0 to 54 (average 11.8), whereas the densities ranged from 0.0000 to 1.9636 per square centimeter (average 0.4139). Averages for the 24 squirrels were 14.7 ectoparasites per host tail and a density of 0.4771 per square centimeter.

To summarize the results given above for entire body averages, ectoparasite numbers on male hosts ranged from 17 to 1167 (average 513), whereas the densities ranged from 0.0353 to 2.6742 per square centimeter (average 1.1136). On female hosts, ectoparasite numbers ranged from 7 to 1685 (average 307) and the densities ranged from 0.0161 to 3.7617 per square centimeter (average 0.7026). The averages for all 24 hosts were 410 ectoparasites per host and 0.9081 per square centimeter. Table XXVII provides the total numbers of ectoparasites recovered from each body region of the males and females and summarizes the average densities. On the back region, 3,689 ectoparasites constituted 37.5% of the total recovered; 2,378 ectoparasites on the legs constituted 24.1% of the parasites recovered; 1,416 ectoparasites on the underparts constituted 14.4% of the parasites recovered; 1,287 ectoparasites on the head constituted 13.1% of the parasites; and 352 ectoparasites were recovered from the tail which accounted for 3.6% of the total. During handling processes, 720 ectoparasites became separated from the host and could not, with certainty, be assigned to any body region. These were catagorized under the title "uncertain location", and they constituted 7.3% of the total parasites recovered.

Therefore, my data on ectoparasite densities indicate the highest densities to occur on the back region, with decreasing densities on

TABLE XXVII. Ectoparasite numbers and average densities according to body regions on 24 adult gray squirrels (12 males and 12 females)

Body regions	Males		Females		Total	
	No.	Avg. density[1]	No.	Avg. density	No.	Avg. density
Back	2,336	1.1211	1,353	0.7543	3,689	0.9377
Legs	1,771	1.2978	607	0.4755	2,378	0.8866
Head	640	0.7832	647	0.8454	1,287	0.8143
Underparts	787	0.7634	629	0.6102	1,416	0.6868
Tail	210	0.5402	142	0.4139	352	0.4771
Uncertain location	412	---	308	---	720	---
Body totals and averages	6,156	1.1136	3,686	0.7026	9,842	0.9018

1/ Number of ectoparasites per square centimeter of body surface

the legs, head, underparts and tail. Watson (1959), in his study of the insect ectoparasites of gray squirrels in Florida, observed that ectoparasites appeared to be more numerous on the back between the shoulders. His conclusions were based on observation, whereas my conclusions are based on calculations and actual numbers.

I believe this study is the first in which ectoparasite densities have been calculated for the total surface area of any host. Apparently, this is also the first time that surface areas have been determined for the gray squirrel. I suggest that expressions of densities of parasites on hosts are more valuable and more meaningful than mere total counts which have long been used. Total count figures do not reflect the effects of the parasites on the host if the size of the host is unknown. In my study I have relied on the calculation of the surface areas of the host, which, after the ectoparasites had been counted, yielded the densities of the parasites in numbers per unit area. I believe this study introduces a more meaningful method of ectoparasite research and can be modified to conform to future studies. The principle disadvantage of the technique was its great time consumption which limited the number of specimens that could be examined. It would not be practical to use my method on animals much larger than squirrels unless sample areas are studied rather than the entire host.

3. Species Accounts

A discussion of the ectoparasite species I found in this study follows and includes data and observations from both the general survey and the density study.

Class Insecta
Order Mallophaga

Bruelia rotundata (Osborn, 1896)

As a rule, lice of the order Mallophaga, which are the so-called biting lice, are generally found on birds. Therefore, due to the apparent host specificities of most lice, few mallophagan parasites have been reported on mammals. In my study of ectoparasites of gray squirrels, two squirrels were killed by a wildlife student and brought to me for examination. Both squirrels were infested with Bruelia rotundata. The lice were still clinging to the squirrel carcass when inspected. No other squirrels I examined harbored this species and thus it occurred on 2 (1.89%) of the 106 examined. This constitutes the first report of a mallophagan parasite on squirrels. Since the usual host is the crow, Corvus corone brachyrhynchus Brehm., I cannot explain the presence of this species on squirrels, unless the hunter who collected the squirrels had previously carried dead crows in his game bag. In any event, these lice were actively feeding or attempting to feed on the carcasses of the dead squirrels.

Order Anoplura

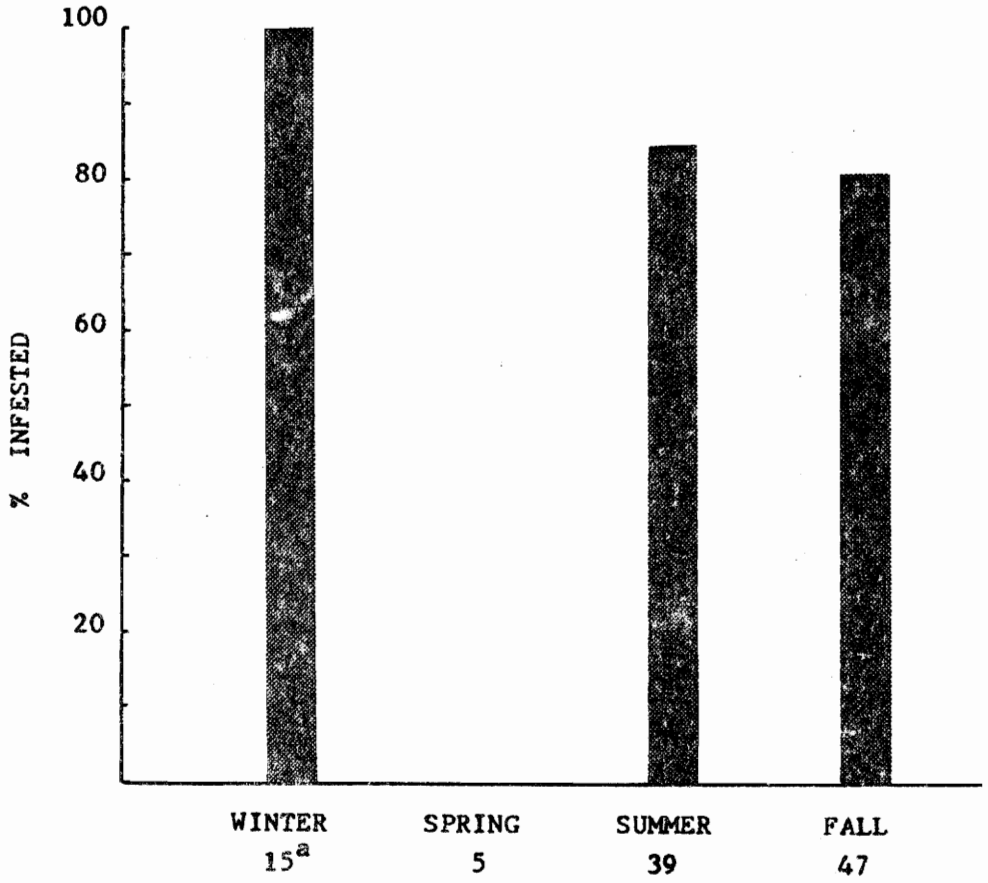
Neohaematopinus sciuri Jancke, 1932

In the general survey of 106 gray squirrels, N. sciuri was present on 86 (81%) of the hosts. Table XXVIII shows the incidence of infestation in relation to the sex and age categories of the hosts. The averaged data indicate no significant differences in incidence between the sexes or age classes. Figure 20 illustrates the seasonal incidence

TABLE XXVIII. Incidence of Neohaematopinus sciuri Jancke, 1932, on the gray squirrel in southwestern Virginia related to the sex and age categories of the hosts

Host age	Males		Females		Total	
	Examined	% infested	Examined	% infested	Examined	% infested
Juvenile	12	75.00	3	100.00	15	80.00
Subadult	9	77.78	12	83.33	21	80.95
Adult	31	93.55	39	71.79	70	81.43
Totals and averages	52	86.53	54	85.04	106	81.13

FIGURE 20. Percent squirrels infested with Neohaematopinus sciuri Jancke, 1932 correlated with seasons



a/ Number examined

of this species. The data show that a peak in incidence occurs in winter (100%); the lowest occurs in spring (0%); another high peak occurs in summer (85%), followed by a slight decline in fall (81%). Harkema (1936) in North Carolina noted that the highest incidence of this louse occurred in December (100%). There was a little decline in January (90%), but a sharp decline was noted in February (17%). Animals he examined during March, April and May were reported negative. He had no June, July or August samples, but beginning in September (14%), there was an increase in incidence by October (50%) and November (88%). Also, Watson (1959) indicated that N. sciuri populations on Florida gray squirrels increased to a peak in early winter, declined to a low in February, then showed a moderate increase in March, reaching another perk in May. He also suggested that these population changes were correlated with seasonal conditions. Apparently lower temperatures and less rainfall favored increases in the lice populations in Florida.

In the density determinations undertaken on 24 adult squirrels, N. sciuri comprised about 39% of the total ectoparasite fauna and yielded the highest numbers and greatest average density of any single species I recovered. Twenty-two hosts (91.6%) were infested with this species, 12 males (100%) and 10 females (83.3%). Parasite numbers on infested males ranged from 12 to 826 (average 228.2), whereas the numbers on infested females ranged from 3 to 317 (average 106.1). The average number for all infested squirrels in the density studies was 172.7 N. sciuri per host.

The data relating this species to the various body regions will be summarized in the paragraphs that follow.

On the body region I designated as the back, N. sciuri occurring on the male hosts ranged in numbers from 3 to 292 (average 92.8), whereas the densities ranged from 0.0178 to 1.8905 per square centimeter (average 0.5785). On infested females the numbers ranged from 1 to 167 (average 45.6), whereas the densities ranged from 0.0067 to 1.1327 per square centimeter (average 0.3059). The averages for the total of the infested males and females were 71.4 parasites per host back and 0.4546 per square centimeter. This species occurred in highest numbers and densities on the back region.

On the legs, N. sciuri numbered from 1 to 209 (average 53.1) parasites per host legs on infested males, whereas the densities ranged from 0.0081 to 1.8866 per square centimeter (average 0.4597). On the legs of infested females, parasite numbers ranged from 1 to 81 (average 24.3) parasites per host legs, whereas the densities ranged from 0.0084 to 0.7034 per square centimeter (average 0.2283). The averages for the total (males and females) were 40.0 parasites per host legs and 0.3545 per square centimeter.

On the underparts region, N. sciuri numbered from 2 to 136 (average 40.6) among infested males, whereas the densities ranged from 0.0234 to 1.8865 per square centimeter (average 0.4780). Among infested females, N. sciuri numbers on the underparts ranged from 0 to 43 (average 12.5), whereas the densities ranged from 0.0000 to 0.7264 per square centimeter (average 0.1590). The averages for the total infested squirrels were 27.8 parasites per host underparts and 0.3330 per square centimeter.

On the tail region of infested males, N. sciuri numbers ranged from 1 to 45 (average 13.0), whereas the densities ranged from 0.0250 to

1.6304 per square centimeter (average 0.4198). Among infested females, numbers ranged from 0 to 14 (average 6.3), whereas the densities ranged from 0.0000 to 0.4521 per square centimeter (average 0.2260). The tail averages for the total infested squirrels were 9.9 parasites per host tail and 0.3317 per square centimeter.

The least numbers and densities were found on the head region. Parasite numbers among infested males ranged from 0 to 57 (average 10.3), whereas the densities ranged from 0.0000 to 0.8282 per square centimeter (average 0.1514). Among infested females, numbers ranged from 0 to 20 (average 6.6) parasites per host head region, whereas the densities ranged from 0.0000 to 0.2801 per square centimeter (average 0.1006). For the total infested squirrels, the averages were 8.6 parasites per host head and 0.1283 per square centimeter.

Table XXIX summarizes the results of the density determinations and total numbers for the louse, N. sciuri, according to host sex and provides the averages for each body region.

Out of 3,799 N. sciuri which occurred on 22 squirrels of 24 examined for numbers and densities, 41.3% occurred on the back, 23.2% occurred on the legs, 16.1% occurred on the underparts, 5.7% occurred on the tail and 5.0% occurred on the head. Due to some handling losses, 8.7% of the total were included under the heading "uncertain location", because I was not certain of their exact location on the host. Correspondingly, the greatest densities occurred on the back, followed by decreasing densities on the legs, underparts, tail and head. I also observed that adult lice were more numerous on the back, head and tail

TABLE XXIX. Numbers and average densities of Neohaematopinus sciuri Jancke, 1932, related to body locations on adult gray squirrels

Host body region	12 males		10 females		Total	
	Total number	Avg. densities[1]	Total number	Avg. densities	Total number	Avg. densities
Back	1114	0.5785	456	0.3059	1570	0.4546
Legs	637	0.4597	243	0.2283	880	0.3545
Underparts	487	0.4780	125	0.1590	612	0.3330
Tail	155	0.4198	63	0.2260	218	0.3317
Head	124	0.1514	66	0.1006	190	0.1283
Uncertain location	221	---	108	---	329	---
Entire body totals and averages	2738	0.4175	1061	0.2040	3799	0.3204

1/ Number per square centimeter of skin surface

with the majority of immatures occurring on the legs and underparts.

Enderleinellus longiceps Kellogg and Ferris, 1915

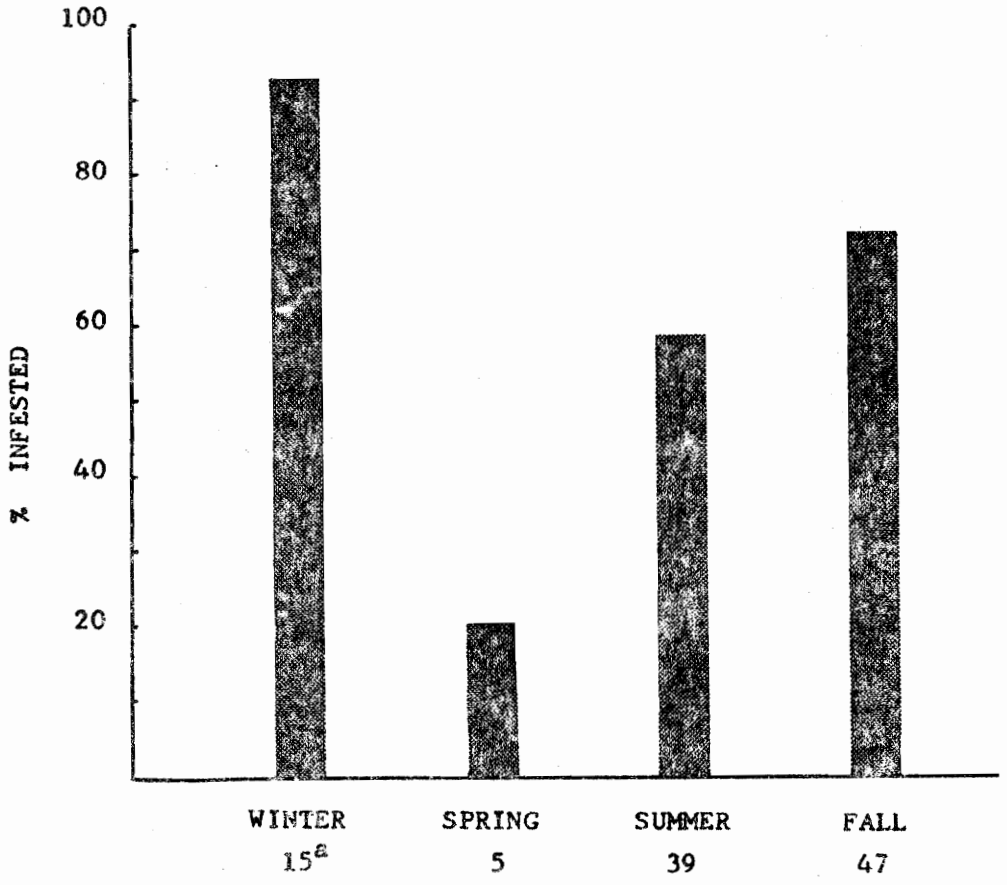
From the general survey of the 106 gray squirrels examined, E. longiceps was found on 72 (68%) of the hosts. Table XXX shows the incidences of infection related to the sex and age categories of the hosts. The incidence appears to be the highest among the male squirrels and the totals for the age groups of juvenile and subadult show a slightly higher percentage than the adults. Since the sample sizes in these two age categories were less than those of the adult category, a proper comparison cannot be made. However, this is a very small louse which must cling to the smaller diamter hair shafts and, therefore, I cannot completely rule out the possibility that it shows preference for younger animals.

Figure 21 illustrates the seasonal incidence of this species. The highest percentage of squirrels infested were collected during winter, with 14 (93.3%) animals infested of 15 examined. The lowest percentage of infestation occurred in the spring, with 1 (20.0%) animal infested of 5 examined. This low was followed by an increase in summer, with 23 (58.9%) animals infested of 39 examined, and fall, with 34 (72.3%) animals infested of 47 examined. Therefore, there appears to be a definite seasonal relationship in percentages of infection with E. longiceps. This trend shows a steady increase in the percentage of squirrels infested from a low in spring to a high in winter. Watson (1959) showed that the frequencies of certain species of lice increase during cold,

TABLE XXX. Incidence of Enderleinellus longiceps Kellogg and Ferris, 1915, on the gray squirrel in southwestern Virginia related to the sex and age categories of the hosts

Host age	Males		Females		Total	
	Examined	% infested	Examined	% infested	Examined	% infested
Juvenile	12	66.67	3	66.67	15	66.67
Subadult	9	77.78	12	75.00	21	76.19
Adult	31	83.87	39	51.37	70	65.71
Totals and averages	52	78.85	54	57.41	106	67.92

FIGURE 21. Percent squirrels infested with Enderleinellus longiceps Kellogg and Ferris, 1915 correlated with seasons



a/ Number examined

dry climatic conditions and decrease in warm, moist conditions.

The incidence of E. longiceps on the 24 squirrels in the density studies was somewhat higher than was shown by the general survey. Twenty squirrels (83.3%) were infested, 11 males (91.7%) and 9 females (75.0%). The numbers occurring on individual infested hosts ranged from 13 to 314 on male squirrels and from 3 to 1,264 on female squirrels. The average number for all infested squirrels was 142.3.

On the body region I designated as the head, the numbers of E. longiceps on the region in infested males ranged from 1 to 133 (average 39.6), whereas the densities ranged from 0.0151 to 1.8839 per square centimeter (average 0.7039). The numbers found on the head of infested females ranged from 0 to 296 (average 58.9), whereas the densities ranged from 0.0000 to 4.6614 per square centimeter (average 0.7580). The average on the head of the total (11 males, 9 females) were 48.3 parasites per host head and 0.7337 per square centimeter. Enderleinellus longiceps occurred in the highest numbers and densities on this body region.

The underparts region yielded an average density that was the second highest over other body regions. Numbers ranged from 2 to 75 (average 18.8) among infested males, whereas the densities ranged from 0.0245 to 0.7478 per square centimeter (average 0.2640). Among infested females, parasite numbers on the underparts ranged 0 to 401 (average 54.2), whereas the densities ranged from 0.0000 to 4.1443 per square centimeter (average 0.5059). The underparts averages for the total (11 males and 9 females) were 34.8 parasites per host underparts and 0.3971 per square centimeter.

On the back region, E. longiceps numbers ranged from 0 to 91 (average 27.6) among infested males, whereas the densities ranged from 0.0000 to 0.6399 per square centimeter (average 0.2125). Among infested females the numbers ranged from 0 to 353 (average 50.3), whereas the densities ranged from 0.0000 to 2.3944 per square centimeter (average 0.2806). The averages for the total were 37.9 parasites per host back region and 0.2500 per square centimeter.

On the legs, parasite numbers ranged from 0 to 48 (average 10.9) on infested males, whereas the densities ranged from 0.0000 to 0.4256 per square centimeter (average 0.1187). On infested females the numbers ranged from 0 to 173 (average 25.6), whereas the densities ranged from 0.0000 to 1.5317 per square centimeter (average 0.1900). The averages on the legs of the total were 17.5 per infested host and 0.1580 per square centimeter.

This species occurred in fewest numbers and densities on the tail region. On infested males, the numbers ranged from 0 to 1 (average 0.2), whereas the densities ranged from 0.0000 to 0.0362 per square centimeter (average 0.0075). None of the females harbored this species on the tail. The total averages were 0.1 parasites per host tail and 0.0034 per square centimeter.

Therefore, of 2,845 Enderleinellus longiceps collected from 20 adult gray squirrels, 34.0% of these occurred on the head region; 26.6% occurred on the back region; 24.4% occurred on the underparts region; 12.3% occurred on the legs; and 0.1% occurred on the tail. During handling manipulations, 2.6% of the total became separated from the

host and could not be assigned to any certain body region. These were classified as "uncertain location". These percentages do not relate exactly to the densities which occur on the body regions. The head region does show the highest parasite numbers and the greatest average density. However, the underparts which shows the second highest density actually had fewer parasites than the back region which had the third highest density. I suggest that these densities (which are number per unit area), rather than actual numbers of parasites, indicate parasite preferences for location on the host. Therefore, from the density study, I show that E. longiceps prefers the ecological conditions of the head pelage, with decreasing preferences for underparts, back, legs and tail. Table XXXI summarizes these data according to sex and body regions and provides the total parasite numbers and average densities of all hosts examined.

Enderleinellus longiceps is restricted for the most part to gray squirrels and fox squirrels. Sciurus carolinensis is the type host. Kim (1966) has published a revision of the genus Enderleinellus including a key to the species.

Hoplopleura sciuricola Ferris, 1921

In my general survey, the louse, Hoplopleura sciuricola, was found on 59 (55.7%) squirrels of 106 examined. Table XXXII shows the percentages of squirrels infested related to the sex and age categories of the hosts. The males demonstrated a higher incidence of this species in which 35 (67.3%) were infested of 52 examined as compared with 24(44.4%) females infested of 54 examined. The data seems to indicate the highest

TABLE XXXI. Numbers and average densities of Enderleinellus longiceps related to body locations on adult gray squirrels

Host body region	11 males		9 females		Total	
	Total number	Avg. densities[1]	Total number	Avg. densities	Total number	Avg. densities
Head	436	0.7039	530	0.7580	966	0.7337
Underparts	207	0.2640	488	0.5059	685	0.3971
Back	304	0.2125	453	0.2806	757	0.2500
Legs	120	0.1187	230	0.1900	350	0.1580
Tail	2	0.0075	0	0.0000	2	0.0034
Uncertain location	30	---	45	---	75	---
Entire body totals and averages	1099	0.2613	1746	0.3469	2845	0.3084

1/ Number per square centimeter of skin surface

TABLE XXXII. Incidence of Hoplopleura sciuricola Ferris, 1921, on the gray squirrel in southwestern Virginia related to the sex and age categories of the hosts

Host age	Males		Females		Total	
	Examined	% infested	Examined	% infested	Examined	% infested
Juvenile	12	25.00	3	0.00	15	20.00
Subadult	9	88.89	12	50.00	21	66.66
Adult	31	77.42	39	46.15	70	60.00
Totals and averages	52	67.31	54	44.44	106	55.66

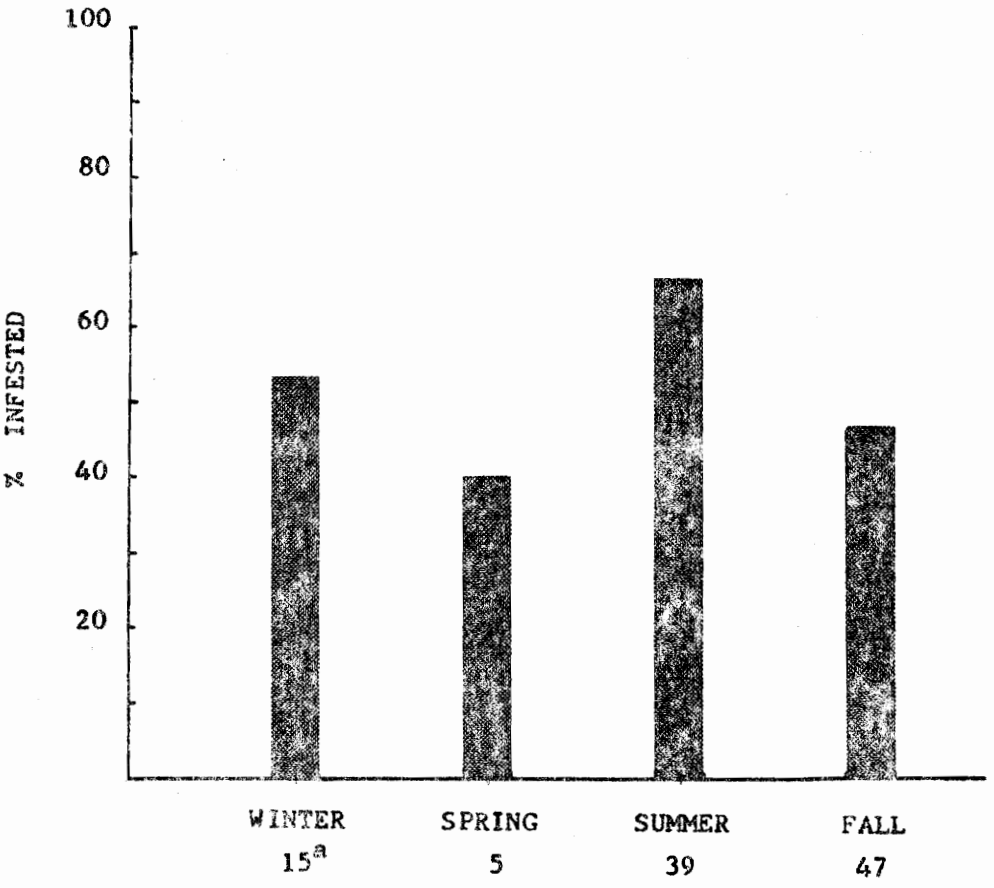
percentage of infestation in the subadult age category, with the adults showing a slightly lower percentage. The juvenile squirrels had significantly lower percentages than the other age categories. It must be considered, however, that the juvenile and subadult categories included fewer numbers of squirrels examined and do not provide good comparison with the adults.

By examining the incidence of H. sciuricola in relation to the seasons of the year (Figure 22), the highest percentage of squirrels are infested in winter (53.3%) and summer (66.7%) and the lowest in spring (40.0%) and fall (46.8%).

The incidence of this species on the 24 squirrels in the density studies was somewhat higher than that shown by the general survey. Eighteen squirrels (75.0%) were infested with H. sciuricola, 11 males (91.7%) and 7 females (58.3%). Numbers on infested hosts ranged from 2 to 637 (average 185.6) among the male squirrels and from 1 to 273 (average 74.6) among the females, with an average for the total of 142.4.

On the body region I designated as the legs, the numbers of H. sciuricola on infested males ranged from 0 to 370 (average 90.5), whereas the densities ranged from 0.0000 to 3.2810 per square centimeter (average 0.7354). The numbers of this species on the legs of infested females ranged from 0 to 43 (average 13.4), whereas the densities ranged from 0.0000 to 0.4220 per square centimeter (average 0.1392). The leg averages for the total infested were 60.5 parasites per host and 0.5157 per square centimeter. This louse species occurred in higher densities on the legs than on any other body region.

FIGURE 22. Percent squirrels infested with Hoplopleura sciuricola Ferris, 1921 correlated with seasons



a/ Number examined

On the back region, the numbers of H. sciuricola on infested males ranged from 1 to 228 (average 79.7), whereas the densities ranged from 0.0054 to 1.5557 per square centimeter (average 0.4756). The numbers on the back of infested females ranged from 0 to 220 (average 55.9) parasites per host, whereas the densities ranged from 0.0000 to 1.4701 per square centimeter (average 0.3650). The back averages for the total infested were 70.4 parasites per host and 0.4348 per square centimeter.

The numbers of H. sciuricola occurring on the head region of infested males ranged from 0 to 15 (average 5.1), whereas the densities ranged from 0.0000 to 0.2155 per square centimeter (average 0.0750). The numbers found on infested females ranged from 0 to 8 (average 1.7), whereas the densities ranged from 0.0000 to 0.1220 per square centimeter (average 0.0268). The averages of the head region of the total infested were 3.8 parasites per host and 0.0572 per square centimeter.

On the body region I designated as the underparts, H. sciuricola numbers ranged from 0 to 28 (average 6.3) on infested males, whereas the densities ranged from 0.0000 to 0.3097 per square centimeter (average 0.0644). On the underparts of infested females, the total numbers ranged from 0 to 4 (average 1.0), whereas the densities ranged from 0.0000 to 0.0392 per square centimeter (average 0.0104). The averages for the underparts of the total squirrels infested were 4.2 lice per host underparts and 0.0445 per square centimeter.

Hoplopleura sciuricola occurred in fewest numbers and densities on the tail. Parasite numbers on infested males ranged from 0 to 2 (average 0.3), whereas the densities ranged from 0.0000 to 0.0661 per square centimeter (average 0.0081). On infested females, the numbers ranged

from 0 to 1 (average 0.1), whereas the densities ranged from 0.0000 to 0.0334 per square centimeter (average 0.0048). The averages for the tail region of the total squirrels infested were 0.2 parasites per host tail region and 0.0069 per square centimeter.

Therefore, of 2,563 H. sciuricola occurring on the infested squirrels in the density study, 49.5% occurred on the back region and 42.5% on the leg region (together comprising 92.0%). On the underparts, head and tail occurred only 2.9%, 2.6% and 0.2% of the total, respectively. Due to the fact that some lice became separated from the host during the handling processes, I collected an additional 2.3% under the heading "uncertain location". Although the greatest numbers of H. sciuricola occurred on the back, my data indicates that this species occurred in the highest densities on the legs. During the processes of gathering these data, I observed that most of the H. sciuricola on the legs were immatures, whereas on the back there was also a majority of immatures, but the numbers of adults were greater. Perhaps it is more advantageous for the adults to remain on the back because these large adult lice are probably more subject to the scratching activities of the host on other areas. I conclude, therefore, that H. sciuricola generally prefers the habitat of the legs and back over the other body regions since the parasite numbers and densities appear to be significantly greater there.

Table XXXIII summarizes these data according to sex and body regions and gives the total parasite numbers and average densities.

TABLE XXXIII. Numbers and average densities of Hoplopleura sciuricola Ferris, 1921, related to body locations on adult gray squirrels

Host body regions	11 males		7 females		Total	
	Total number	Avg. densities[1]	Total number	Avg. densities	Total number	Avg. densities
Legs	995	0.7354	94	0.1392	1089	0.5157
Back	877	0.4756	391	0.3650	1268	0.4348
Head	56	0.0750	12	0.0268	68	0.0572
Underparts	69	0.0644	7	0.0104	76	0.0445
Tail	3	0.0081	1	0.0048	4	0.0069
Uncertain location	42	---	17	---	59	---
Entire body totals and averages	2042	0.2717	522	0.1092	2568	0.2118

1/ Number per square centimeter of skin surface

Order Diptera

Cuterebra sp.

The botfly or warblefly maggot, Cuterebra, is one of the most important parasite species of the gray squirrel with regard to game management. Due to the numbers of squirrels harboring these large maggots under the skin during the early hunting seasons in certain localities, many of these squirrels killed by hunters are discarded due to the idea that they render the animal unfit as a food item. For this reason, and others, several states have postponed the opening of their squirrel hunting seasons until most of the larvae have emerged.

I have found relatively few squirrels infested with Cuterebra sp. in the present study. Eight hosts (7.5%) of 106 examined harbored them. All infested hosts were collected between August 31 and October 4. One host (10.0%) of 10 examined in the month of August was infested; three hosts (10.7%) of 28 examined in September were infested; and 4 out of 18 (22.2%) examined in October were infested. Numbers per infestation ranged from 1 to 2 (average 1.1). All infested hosts were collected at an elevation of approximately 2100 feet. Two hosts came from farm woodlots, whereas six were from forest areas. Among the eight infested hosts four were males (1 juvenile; 2 subadults; 1 adult) and four were females (1 juvenile; 2 subadults; 1 adult). The parasites were located on the throat, on the head behind the ear and on the anterior thoracic area. No warblefly was found on any of the 24 squirrels examined in the density study.

Chapman (1938) showed that 44 (12.2%) of 326 gray squirrels examined were infested with Cuterebra sp. in southeastern Ohio. In Virginia,

Cross (1942) revelaed that 29 (28.2%) of 103 squirrels examined were infested. Parasite numbers ranged from 1 to 4 (average 2). The peak of infestation apparently occurred between September 1 and 15. Atkeson (1948) listed 151 (7.1%) of 2,128 gray squirrels in Alabama to be infested. There the peak of infestation apparently occurred between October 1 and 15. The most intensive investigation of this parasite has been conducted by Allison (1953) in North Carolina. He noted the seasonal and geographical distributions of Cuterebra sp. as the result of hunter bag checks and questionnaires sent to license holders. The bag checks showed that peaks of infestation in the Mountain Region occurred between September 1 and October 1; in the Piedmont Region between September 15 and October 15; and in the Upper and Lower Coastal Regions between October 1 and November 1. Percentages of infestation averaged 7.9% in the Mountain Region, 30.5% in the Piedmont Region, 28.0% in the Upper Coastal Region and 0.6% in the Lower Coastal Region. Questionnaire responses provided similar rates. The results of the present study in which 7.5% of the hosts were infested are comparable with the 7.9% for the Mountain Region percentages in the North Carolina study. According to the findings of Cross (1942), it appears that other localities in Virginia have higher rates of infestation than was shown in my study.

Although larval Cuterebra have been successfully reared to adults by several investigators (Johnson, 1930; Greene, 1935; Beamer, Penner and Hibbard, 1943; Allison, 1953), it appears that no one has identified or described the adults of larvae recovered from gray squirrels.

Apparently the larvae do not have significant morphological differences to facilitate specific identification. Therefore, most investigators have relied on the use of only the generic name. I unsuccessfully attempted to rear these to adults in a jar with soil and damp Sphagnum moss. I did manage to secure pupated larve, but the adults never emerged.

Payne and Cosgrove (1965) have described the pathology and healing processes associated with Cuterebra infestation in gray squirrels. They observed some muscular involvement and slower healing of the wounds in the gray squirrel than in other animals they observed infested with similar species.

Simulium sp.

Two specimens of the black fly (adults), Simulium sp., were found on 2 (1.89%) gray squirrels of the 106 examined in my general survey. One occurred on an adult male host collected on September 18; and the other occurred on a subadult female collected on September 30. The occurrance of Simulium on gray squirrels suggest that they are also parasitic on them. It is well known that these flies feed by sucking the blood of animal victims. Some have been shown to transmit certain protozoan and nematode diseases of domestic animals and man, which is facilitated by their feeding activities. Even if these flies feed frequently on gray squirrels, actual recoveries of them on hosts killed by shooting would be rare. The specimens collected in this study were obviously flies that had inadvertently become trapped in the fur of the squirrel.

One specimen of Simulium sp. was tentatively identified as Simulium sp. (jenningsi group).

I also recovered Simulium sp. from gray squirrel nests.

Order Siphonaptera

Orchopeas howardii (Baker, 1895)

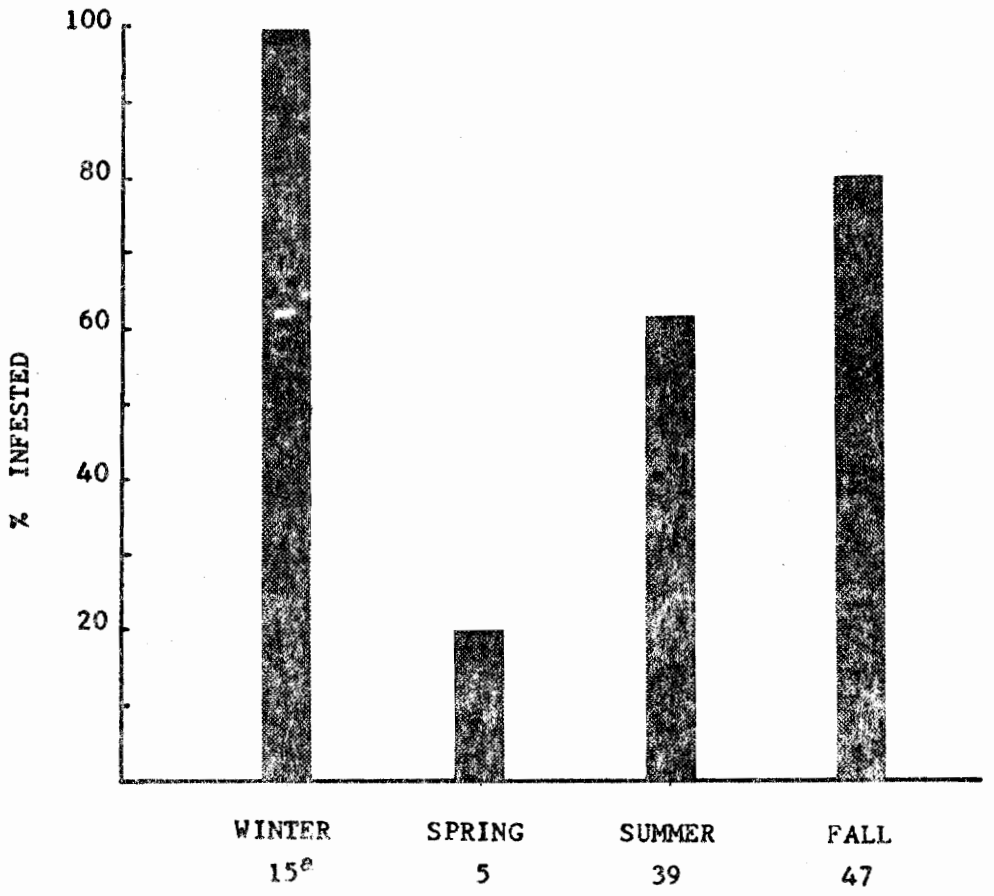
In my general survey the flea, Orchopeas howardii, occurred on 78 (73.6%) of the 106 gray squirrels examined. This was the second most frequently encountered species. Table XXXIV records the incidence as related to the sex and age categories of the hosts. Accepting the data at face value, it appears that the highest percentages of squirrels infested with O. howardii are the juveniles, with decreasing frequencies on the subadults and adults. It also appears that a greater percentage of males are infested than are the females. However, I am cognizant that these data are not conclusive, due to the difference in sample sizes between the age categories.

I examined my data to see what correlations exist between the incidence of O. howardii and the seasons of the year. Figure 23 comparatively illustrates my results. The winter sample of 15 squirrels showed 100% to be infested with O. howardii; the spring sample of 5 yielded 1 (20.0%); the summer sample of 39 yielded 24 (61.5%); and the fall sample of 47 squirrels yielded 38 (80.9%). Therefore, it appears that fleas increase in frequency on gray squirrels, from a low in spring, with increases in summer through fall, to a high in winter. These observations may be compared to those of Watson (1959) who made ectoparasite counts on squirrels in Florida. He noted the highest number

TABLE XXXIV. Percent gray squirrels infested with Orchopeas howardii related to the sex and age categories of the hosts

Host age	Males		Females		Total	
	Examined	% infested	Examined	% infested	Examined	% infested
Juvenile	12	91.66	3	100.00	15	93.33
Subadult	9	88.88	12	75.00	21	80.95
Adult	31	74.19	39	61.53	70	67.14
Totals and averages	52	80.76	54	66.66	106	73.58

FIGURE 23. Percent squirrels infested with Orchopeas howardii (Baker, 1895) correlated with seasons



a/ Number examined

of fleas occurred on squirrels in November, with a decline to very low numbers in January, February and March. A slow increase began in April to May, with higher numbers in June, July and October. He had no August and September samples.

The density studies of the 24 adult squirrels yielded a slightly higher percentage infested than did the general survey. Twenty (83.3%) hosts were infested, 10 males (83.3%) and 10 females (83.3%). The numbers occurring on individual hosts ranged from 1 to 28 (average 9.1) among the male squirrels and from 1 to 14 (average 4.4) among the females, for an average of the total of 6.8.

On the body region designated as tail, O. howardii numbers ranged from 0 to 4 (average 0.8) on infested males, whereas the densities ranged from 0.0000 to 0.0984 per square centimeter (average 0.0240). On infested females, parasite numbers ranged from 0 to 4 (average 0.9), whereas the densities ranged from 0.0000 to 0.1633 per square centimeter (average 0.0318). The tail averages for the total infested were 0.8 fleas per host tail region and 0.0281 per square centimeter. The highest densities were encountered on this body region.

On the legs, numbers ranged from 0 to 6 (average 1.1) on infested males, whereas the densities ranged from 0.0000 to 0.0522 per square centimeter (average 0.0103). On infested females, the numbers ranged from 0 to 3 (average 0.5), whereas the densities ranged from 0.0000 to 0.0261 per square centimeter (average 0.0047). The averages for the total infested were 0.8 fleas per host legs and 0.0074 per square centimeter.

On the back region, flea numbers on infested males ranged from 0 to 6 (average 1.4), whereas the densities ranged from 0.0000 to 0.0325 per square centimeter (average 0.0091). On infested females, flea numbers on the back region ranged from 0 to 3 (average 0.7), whereas the densities ranged from 0.0000 to 0.0183 per square centimeter (average 0.0045). The averages for the total infested were 1.1 fleas per host back region and 0.0067 per square centimeter.

On the underparts region, flea numbers on infested males ranged from 0 to 2 (average 0.3), whereas the densities ranged from 0.0000 to 0.0235 per square centimeter (average0.0037). The numbers on infested females ranged from 0 to 1 (average 0.1), whereas the densities ranged from 0.0000 to 0.0107 per square centimeter (average 0.0011). The averages for the total infested were 0.2 fleas per host underparts and 0.0023 per square centimeter.

Fleas occurred in least numbers and densities on the head region. On infested males, the numbers ranged from 0 to 1 (average 0.2), whereas the densities ranged from 0.0000 to 0.0151 per square centimeter (average 0.0033). On infested females, no fleas occurred on the head region of any examined. The head averages for the total infested were 0.1 fleas per host head region and 0.0015 per square centimeter.

Of the 135 fleas, O. howardii, which occurred on 20 of the squirrels in the density survey, 12.6% occurred on the tail region, 11.8% on the legs, 15.6% on the back, 3.0% on the underparts and 1.5% on the head. Of all the species I collected in the density studies, O. howardii showed the greatest tendency of becoming detached from the host during

handling procedures even though they had been killed with ether upon immediate collection of the host. From "uncertain location", 55.5% of the fleas recovered were placed in this category. Therefore, the results of the density study which showed decreasing densities from tail to legs, back, underparts and head, cannot be conclusive, since the numbers on these areas amounted to less than half of the total population collected. Table XXXV summarizes the data of the density study as related to O. howardii and the body regions and sex of the host.

Class Arachnida
Order Acari
Family Ixodidae

Ixodes marxi Banks, 1908

In my general survey the tick, Ixodes marxi, was found on 24 (22.6%) of the 106 hosts examined, 11 (21.2%) males and 13 (24.1%) females. Table XXXVI provides the percent infested related to the sex and age categories of the hosts. There appear to be no definite patterns between the age categories, in which the highest percentage was shown by the juveniles (33.3%), the second highest the adults (24.3%), and the sub-adults least (9.5%). These results may have been influenced by differences in sample size. Otherwise the higher incidence in the juveniles cannot be explained. The averages for the total of the males and females do not appear to be significantly different.

In examining my data concerning the seasonal incidence of I. marxi, I discovered that no squirrels collected during winter and spring months harbored them and that 6 (15.4%) of 39 hosts examined in summer and 18

TABLE XXXV. Numbers and average densities of Orchopeas howardii (Baker, 1895) related to body locations on adult gray squirrels

Host body region	10 males		10 females		Total	
	Total number	Avg. densities[1]	Total number	Avg. densities	Total number	Avg. densities
Tail	8	0.0240	9	0.0318	17	0.0281
Legs	11	0.0103	5	0.0047	16	0.0074
Back	14	0.0091	7	0.0045	21	0.0067
Underparts	3	0.0037	1	0.0011	4	0.0023
Head	2	0.0033	0	0.0000	2	0.0015
Uncertain location	53	---	22	---	75	---
Entire body totals and averages	91	0.0189	44	0.0101	135	0.0145

1/ Number per square centimeter of skin surface

TABLE XXXVI. Percent squirrels infested with Ixodes marxi Banks, 1908, related to the sex and age categories of the hosts

Host age	Males		Females		Total	
	Examined	% infested	Examined	% infested	Examined	% infested
Juvenile	12	16.67	3	100.00	15	33.33
Subadult	9	11.11	12	8.33	21	9.52
Adult	31	38.10	39	23.08	70	24.29
Totals and averages	52	21.15	54	24.07	106	22.64

(38.3%) of 47 examined in fall harbored them. Figure 24 illustrates these seasonal differences for visual comparison.

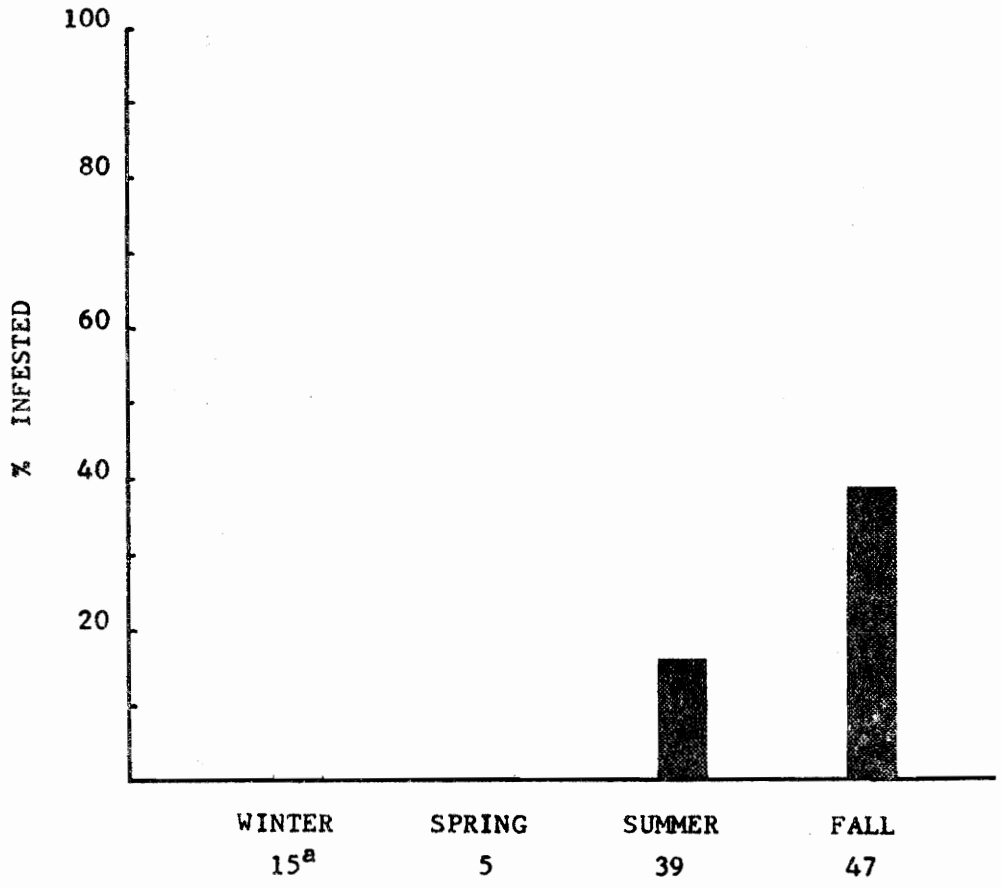
The incidence of this species on the 24 squirrels of the density study was somewhat higher (37.5%) than was shown by our general survey (22.6%), demonstrating a more efficient recovery technique in the use of the Hopkin's hair dissolving method. Numbers on individual infested hosts ranged from 2 to 138 (average 36.2) among the males and from 1 to 26 (average 11.6) among the females, with an average of 22.5 on all infested hosts.

On the body region designated as the tail, I. marxi numbered from 0 to 26 (average 6.5), whereas the densities ranged from 0.0000 to 0.6399 per square centimeter (average 0.1599). No ticks occurred on the tail region of infested females. The averages for the total infested were 2.9 ticks per host tail region and 0.0711 per square centimeter.

On the head region, I. marxi numbers ranged from 0 to 14 (average 4.0) on infested males, whereas the densities ranged from 0.0000 to 0.2003 per square centimeter (average 0.0579). On infested females, the numbers ranged from 0 to 11 (average 4.8), whereas the densities ranged from 0.0000 to 0.1678 per square centimeter (average 0.0746). The average for the total infested was 4.4 ticks per host head region and 0.0672 per square centimeter.

On the body region designated as the back, I. marxi numbers ranged from 0 to 24 (average 6.7) on infested males, whereas the densities ranged from 0.0000 to 0.1507 per square centimeter (average 0.0430). On infested females, tick numbers on the back ranged from 0 to 14 (average 5.2), whereas the densities ranged from 0.0000 to 0.0936 per square

FIGURE 24. Percent squirrels infested with Ixodes marxi Banks, 1908, correlated with seasons



a/ Number examined

centimeter (average 0.0343). The averages for the total infested were 5.9 ticks per host back region and 0.0382 per square centimeter.

On the underparts region, the numbers on infested males ranged from 0 to 17 (average 4.7), whereas the densities ranged from 0.0000 to 0.1995 per square centimeter (average 0.0557). On infested females, tick numbers ranged from 0 to 1 (average 0.2), whereas the densities ranged from 0.0000 to 0.0116 per square centimeter (average 0.0023). The averages for the total infested hosts were 2.2 ticks per host underparts and a density of 0.0260 per square centimeter.

Ixodes marxi occurred on the legs in the least numbers and densities. On the legs of infested males, the numbers ranged from 0 to 7 (average 1.7), whereas the densities ranged from 0.0000 to 0.0486 per square centimeter (average 0.0121). On infested females, tick numbers on the legs ranged from 0 to 2 (average 1.0), whereas the densities ranged from 0.0000 to 0.0196 per square centimeter (average 0.0092). The averages for the total infested were 1.3 ticks per host legs and a density of 0.0105 per square centimeter.

The summary of our data given above and in Table XXXVII was provided for future reference and should be considered cautiously, since one host had an unusually high number of ticks which accounted for 67.9% of the total collected. From my observations in this study, it is my opinion that I. marxi usually prefers the environs of the head and back over other positions on the body.

Of the 9 hosts which harbored I. marxi in the density studies, 6 hosts had these ticks located on the back region, accounting for 26.1%

TABLE XXXVII. Numbers and average densities of Ixodes marxi Banks, 1908, related to body locations on adult gray squirrels

Host body region	4 males		5 females		Total	
	Total number	Avg. densities[1]	Total number	Avg. densities	Total number	Avg. densities
Tail	26	0.1599	0	0.0000	26	0.0711
Head	16	0.0579	24	0.0746	40	0.0672
Back	27	0.0430	26	0.0343	53	0.0382
Underparts	19	0.0557	1	0.0023	20	0.0260
Legs	7	0.0121	5	0.0092	12	0.0105
Uncertain location	50	---	2	---	52	---
Entire body totals and averages	145	0.0731	58	0.0261	203	0.0470

1/ Number per square centimeter of skin surface

of the total recovered; 7 had them on the head region, accounting for 19.7%; 1 had them on the tail, accounting for 12.8%; 3 had them on the underparts, accounting for 9.9%; and 4 had them on the legs, accounting for 5.9%. An additional 25.6% was listed as "uncertain location."

The majority of the specimens of I. marxi occurring on the squirrels in this study were 6- and 8-legged larval stages. Very few adults were collected.

Clark (1956) reported finding this species in the nests of gray squirrels in Maryland and Washington, D. C. I also recovered this species from nests in the present study in southwestern Virginia.

Family Laelapidae

Androlaelaps casalis (Berlese, 1887)

In the general survey for ectoparasites on 106 gray squirrels the mite, Androlaelaps casalis, was found on 13 (12.3%) hosts, 6 males (11.5%) and 7 females (13.0%). Table XXXVIII provides the percent incidence related to the sex and age categories of the hosts. Due to the few numbers of hosts infested, a proper appraisal of the differences cannot be made. My data showed that subadults had the highest percentage (19%), whereas the juveniles were second (13%) and the adults least (10%). There were no significant differences between the average percentages on each sex.

In examining my data in relation to the seasonal incidence of this species, I discovered that all infested squirrels were collected during the months of July, August, September and October, with percentages of

TABLE XXXVIII. Percent squirrels infested with Androlaelaps casalis (Berlese, 1887) related to the sex and age categories of the hosts

Host age	Males		Females		Total	
	Examined	% infested	Examined	% infested	Examined	% infested
Juvenile	12	8.33	3	33.33	15	13.33
Subadult	9	22.22	12	16.67	21	19.05
Adult	31	14.29	39	10.26	70	10.00
Totals and averages	52	11.54	54	12.96	106	12.26

25.0%, 40.0%, 14.3% and 16.7%, respectively. This yielded none for squirrels collected in winter and spring, 7 (17.9%) for summer and 6 (12.8%) for fall. Therefore, the occurrence of this species on squirrels appears to be determined by seasonal influences.

Few mites of this species occurred on the squirrels in the density study. One male had one mite on the back for a density of 0.0090 per square centimeter or a total body density of 0.0023 per square centimeter. Two females were infested, one host with 1 on the legs for a density of 0.0087 per square centimeter and 3 of "uncertain location", for a total body density of 0.0100 per square centimeter; and the other host had 1 on the back (0.0067 per square centimeter), 1 on the legs (0.0084 per square centimeter), 1 on the head (0.0160 per square centimeter) and 3 were listed as "uncertain location" for a total body density of 0.0152 per square centimeter.

Clark (1956) found this species in gray squirrel nests in Maryland and Washington, D. C. It was more common in nests which had been in use for some time, but was not seen in high numbers. I also found this species in squirrel nests in the present study.

Haemogamasus ambulans (Thorell, 1872)

In my survey of 106 gray squirrels for ectoparasitic arthropods the mite, Haemogamasus ambulans, was found on 32 (30.2%) hosts. According to Allred and Beck (1966) who differentiated between morphological forms within the H. ambulans species complex as A, B, C and D forms, variations in the present study included only the C and D forms. Table XXXIX shows the percentages of infestation related to the sex and age

TABLE XXXIX. Percent gray squirrels infested with Haemogamasus ambulans (Thorell, 1872) related to the sex and age categories of the hosts

Host age	Males		Females		Total	
	Examined	% infested	Examined	% infested	Examined	% infested
Juvenile	12	50.00	3	100.00	15	60.00
Subadult	9	44.44	12	16.66	21	28.57
Adult	31	29.03	39	20.51	70	24.29
Totals and averages	52	36.54	54	24.07	106	30.19

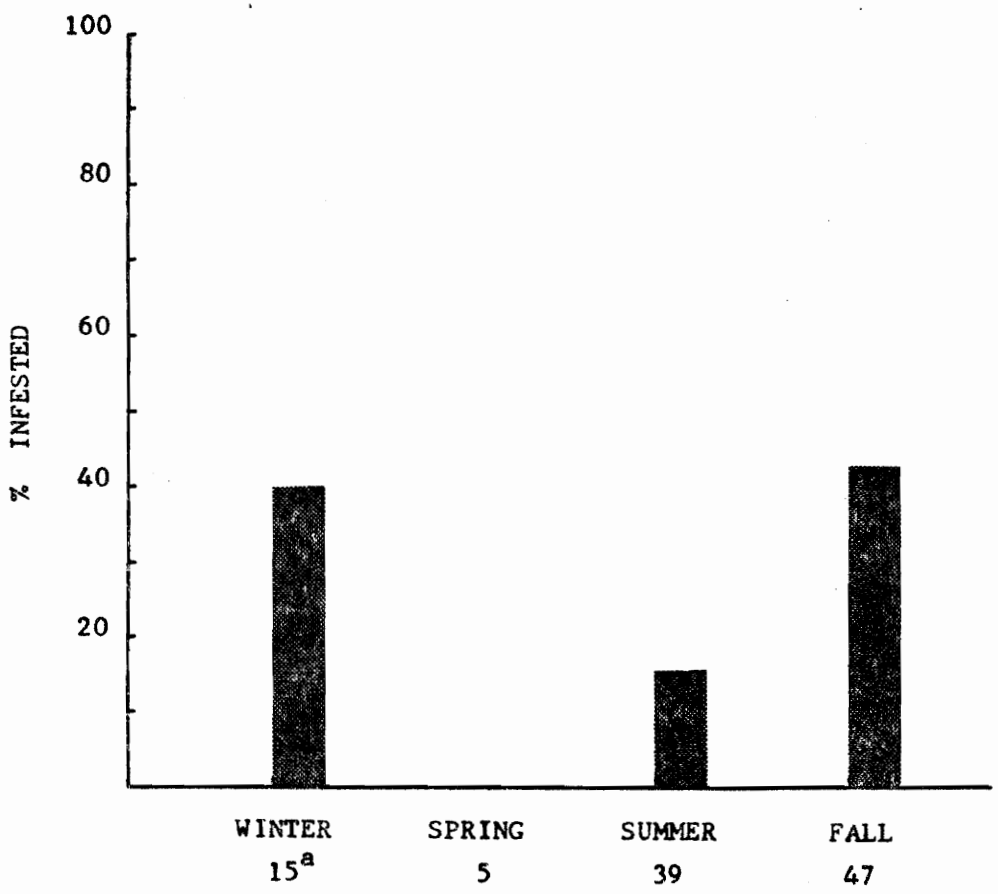
categories of the hosts. Viewing the totals of both sexes in each age class, it appears that the juvenile squirrels have the highest percentage (60.0%), with subadults second (28.6%) and adults least (24.3%). For the totals of all age classes of each sex, it also appears that the males (36.5%) may be more frequently infested than the females (24.1%).

Figure 25 illustrates the seasonal incidence of H. ambulans on the squirrels in this study. No squirrels were infested with this species in spring (0.0%); 15.4% were infested in summer, with increases to 42.6% in the fall. The winter sample is very similar to the fall sample, but shows a slight decline (40.0%).

The density studies of 24 adult gray squirrels yielded 8 (33%) infested with H. ambulans (4 males, 33%; 4 females, 33%). These percentages are very similar to that provided in the general survey. Total parasite counts of this species on individual hosts ranged from 1 to 21 (average 8.0) on infested males and from 8 to 130 (average 39.8) on infested females. The average for all infested hosts was 23.9 parasites per host.

This mite was recovered from all body regions, except the head, in the density study. This species, however, shows definite preference for the tail region over the other areas. Numbers on the tail region of infested males ranged from 0 to 11 (average 4.0), whereas the densities ranged from 0.0000 to 0.2707 per square centimeter (average 0.1062). On the tail of infested females, mite numbers ranged from 3 to 47 (average 16.7), whereas the densities ranged from 0.0912 to 1.7091 per square centimeter (average 0.5705). The tail averages for all infested

FIGURE 25. Percent squirrels infested with Haemogamasus ambulans (Thorell, 1872) correlated with seasons



a/ Number examined

hosts were 10.8 mites per host tail and 0.3384 per square centimeter.

No H. ambulans occurred on any other body regions in the males. Two females had them on the back, in which mite numbers were 1 and 2, respectively, with densities of 0.0061 and 0.0117, respectively. One host had 1 H. ambulans on the legs for a density of 0.0083 per square centimeter; and one host harbored 2 H. ambulans on the underparts, for a density of 0.0204 per square centimeter. Table XL summarizes the results of the density studies concerning this species.

Clark (1956) reported that H. ambulans was the most common acarine parasite in squirrel nests in Maryland and Washington, D. C. I also found these in squirrel nests in southwestern Virginia. Keegan (1951) indicated the distribution of this species to be Nearctic, Palearctic and Oriental. In North America it has been reported from the rodent host genera Tamiasciurus, Sciurus, Glaucomys, Neotoma, Microtis, Clethrionomys and Thomomys (Keegan, 1951).

Clark (1956; 1958) showed that H. ambulans may serve as the intermediate host and aid in the transmission of Hepatozoon griseisciuri to gray squirrels.

Another mite belonging to the same genus was found on one host in the general survey and was identified as Haemogamasus sp. (probably H. pontiger).

Echinolaelaps sp.

One specimen of Echinolaelaps sp. was recovered, from a subadult female gray squirrel collected in February on a campus woodlot, out of the 106 squirrels surveyed (0.94%). Members of this genus occur on

TABLE XL. Numbers and average densities of Haemogamasus ambulans (Thorell, 1872) related to body locations on adult gray squirrels

Host body region	4 males		4 females		Total	
	Total number	Avg. densities[1]	Total number	Avg. densities	Total number	Avg. densities
Tail	16	0.1062	67	0.5705	83	0.3384
Underparts	0	0.0000	2	0.0051	2	0.0025
Back	0	0.0000	3	0.0045	3	0.0022
Legs	0	0.0000	1	0.0021	1	0.0011
Head	0	0.0000	0	0.0000	0	0.0000
Uncertain location	16	---	86	---	102	---
Entire body totals and averages	32	0.0167	159	0.0352	191	0.0259

1/ Number per square centimeter of skin surface

rodents throughout the world. The rarity of this mite on the gray squirrels in this study suggests it to be either accidental or uncommon in occurrence. The lack of additional specimens prohibited specific identification.

Family Macronyssidae

Hirstionyssus sp.

One specimen of Hirstionyssus sp. was found on a single (0.94%) subadult female gray squirrel collected in the month of June in the town of Blacksburg. Species of this genus have been reported on small rodents, carnivores and insectivores in North America, Europe, Africa and Asia. One species was implicated as a potential vector of hemorrhagic fever in Korea (Traub, et. al., 1954). According to Baker, et. al. (1967), the life histories are unknown for any species of the genus Hirstionyssus. Strandtmann and Morlan (1953) suggested that these mites probably spend most of their time off the host.

Family Rodentopididae

Unidentified mites

These small mites (Figure 26) were found on two squirrels (1.89%). Most were attached to the hairs, some on the skin and a few were found actually embedded in the skin. The numbers recovered were 2,272 on one host and 223 on the other.

Dr. Don Johnson of the Institute of Acarology at Ohio State University, by way of correspondence, told me that these mites belonged to the family Rodentopididae which were known to occur on rodents in central

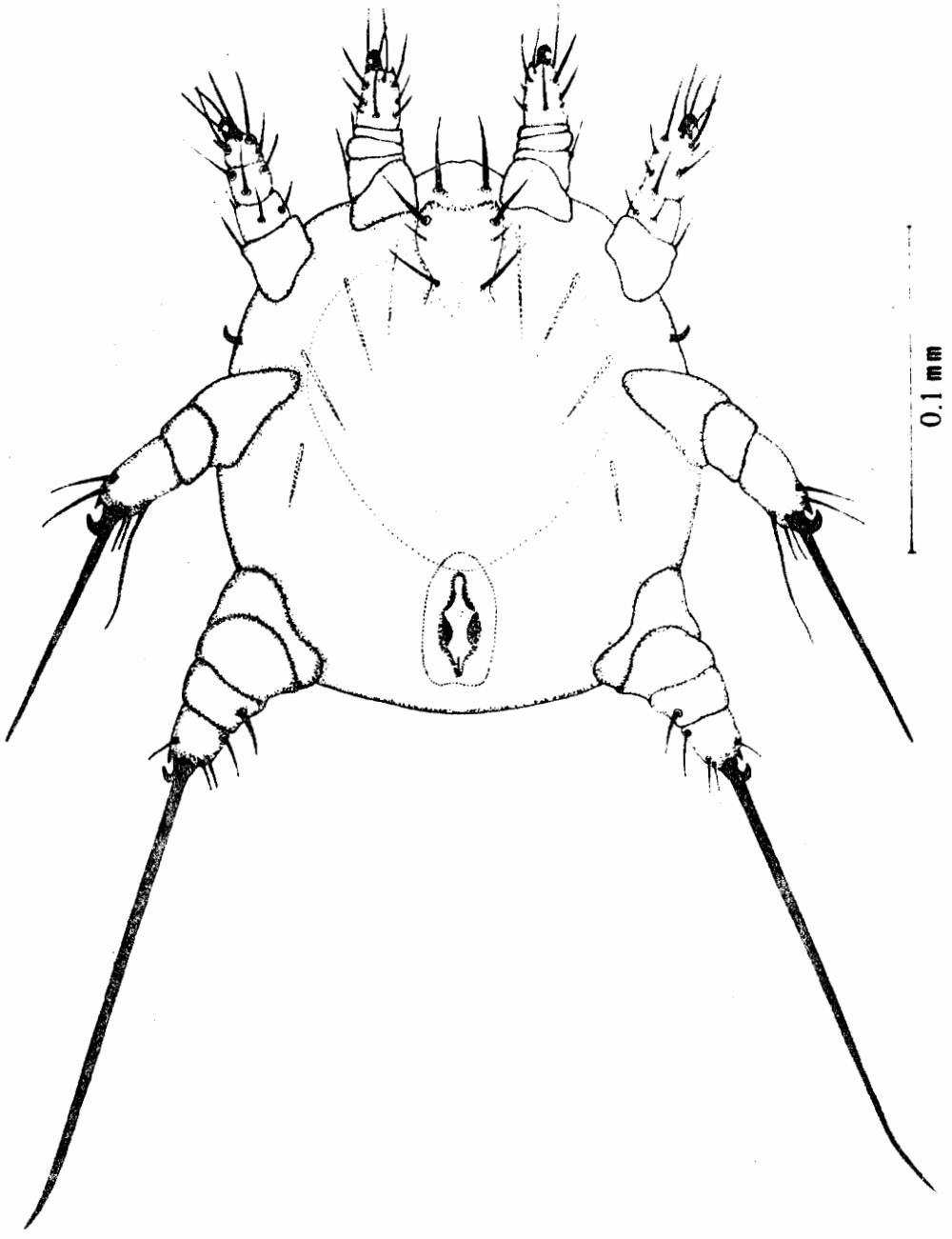


FIGURE 26. Unidentified mite

Africa. I was unable to locate this family name in the literature. Dr. Dorald Allred of Brigham Young University also examined these mites and could not be certain as to their taxonomic location. He also said that he was not familiar with the family Rodentopididae. Specimens were sent to two other experts in the field, one in England, who would not even guess at an identification, and one in Canada who said these mites belonged to the family Myobiidae. In further correspondence with Dr. Allred, he said these mites were definitely not of the family Myobiidae. Therefore, there is much uncertainty with regard to the taxonomic location of this mite. Due to the problems encountered with identification and because I am not an experienced acarologist, further study was not continued. It is likely these mites represent a new genus and possibly may require a new family designation also. For the present, I assign it the tentative family designation of Rodentopididae.

The sketch of the mite (Figure 26) was made from photomicrographs, sketches and notes and is included here for future reference. The body dimensions averaged about 0.153 mm in length by 0.109 mm in width. The long spines on Leg III averaged 0.075 mm in length while those on Leg IV averaged 0.143 mm in length.

Family Trombiculidae

Chiggers

In my general survey of 106 gray squirrels, 15 (14.15%) squirrels were infested with chiggers. Although specimens were not counted in that survey, chiggers appeared to occur in low numbers. The infested squirrels included 7 males and 8 females. On a seasonal basis, 1

(6.7%) of 15 examined in winter harbored chiggers; 1 (20.0%) of 5 in spring harbored them; 2 (5.1%) of 39 in summer harbored them; and 11 (23.4%) of 47 in fall harbored them. Therefore, it appears that chiggers are more prevalent during the fall months than at other times of the year.

The density survey yielded three (2 males; 1 female) hosts (12.5%) infested with chiggers of the 24 examined. This percentage is nearly that of the general survey. The parasite numbers on each host were 3, 5, and 80, respectively (average 29.3). The average densities of chiggers on these animals were 0.0060, 0.0100 and 0.1786 per square centimeter, respectively. Body regional preferences of these parasites could not be properly assessed by my present data, although I can say that chiggers occurred on the head region of all three infested animals. The greatest density of chiggers occurring on an individual host was found on the legs.

Most of the chiggers observed in this study belong to the genus Trombicula. One species was identified with the T. microti group.

I assume that chiggers instigate some degree of annoyance and discomfort to squirrels but I cannot estimate the effects of this on the well-being of the animal.

Incidental organisms

A number of organisms were found in the pelage of several squirrels which were not considered to be parasitic. Apparently these were inadvertantly caught in the fur of the host as their activities brought them in contact with them. These organisms are listed in Table XLI.

TABLE XLI. Arthropods found to be incidentally present in the pelage of gray squirrels in southwestern Virginia

Mites	Insects
Oribatella sp.	Trogoderma sp.
Oppia sp.	Lecanium sp.
Nothrus sp.	Cinara sp.
Galumna sp.	
Tumidalyus americana	
Scapheremaeus sp.	
Melichares dentriticus	
Macrocheles sp.	
Tyrophagus putrescentiae	
Pseudoparasitus sp.	
Speleognathidae (genus?)	
Parasitidae (genus?)	
Ceratozitidae (genus?)	
Camisiidae (genus?)	

4. Nest-funnel Survey

After a period of about four months from the time of installation of the nest-funnels in the two woodlots, I began monitoring them each month and examining the contents of the attached collecting jars for arthropods. This project was begun on 30 July 1968 and was continued regularly until 30 July 1969. During this time I found that only one squirrel nest was constructed in one of the six experimental nest-funnels located in the Crumpacker woodlot, but no squirrels were observed during the trips in which collections were made. This single nest-funnel was used by a squirrel or squirrels for about two months during which time I collected the following organisms: Orchopeas howardii, Ixodes marxi and Haemogamasus ambulans, which are parasitic on squirrels, and Hypoaspis sp., a mite which is apparently predaceous in habit. This nest-funnel was later abandoned and apparently not used again during the study period. A number of common flying and crawling arthropods were found in the collecting jars and examinations in this woodlot were discontinued after several months without finding any parasitic species.

In the Prices Fork woodlot, nest litter was placed by squirrels in all of the six experimental nest-funnels during the study period. However, none were continually occupied throughout the period of study. On occasions, one or two squirrels were occupying a nest-funnel at the time of the examination. During the spring months, I found that several nest-funnels were being used by nesting birds: two families of starlings and two families of sparrow hawks. When these were encountered, the eggs and litter placed by the birds were removed.

In the planning stages of this project, I anticipated that once a squirrel nest was established in the nest-funnel, it could then be monitored for some time to yield a broad spectrum of nest-inhabiting arthropods. This situation was not realized in the present study because individual nest-funnels were not occupied more than a few months. Also, it was thought that comparisons might be made between the two woodlots under study. This, too, was not realized in the present study, since the Crumpacker woodlot apparently had few squirrels and apparently only one nest-funnel was ever used.

In spite of the numerous problems and complications which befell the project, I was able to prepare a list of the arthropods which occurred in the nest-funnels located in the Prices Fork woodlot while they were being used regularly by gray squirrels. Table XLII lists these organisms and indicates their apparent status in the ecology of the nest, i.e., whether or not they are parasitic or predaceous. Those whose status could not be determined are included under the heading "Miscellaneous". Eight species are indicated as parasites and all of these were confirmed as parasites on gray squirrels in my ectoparasite survey. I did not collect any parasitic organisms in the nest-funnels that I had not found previously on squirrels.

Nest-funnel occupation and the occurrence of squirrel ectoparasites were recorded for the Prices Fork woodlot. Two (33.3%) nest-funnels in January yielded 150 organisms (average 75.0); 2 (33.3%) in February yielded 146 (average 73.0); 1 (16.7%) in March yielded 63; 6 (100%) in April yielded 135 (average 22.5); 5 (83.3%) in May yielded 709 (average

TABLE XLII. List of the arthropod inhabitants of squirrel nest-funnels collected from six locations in Prices Fork woodlot on the campus of Virginia Polytechnic Institute and State University, July, 1968-July, 1969

Organism collected	Proposed dietary habits		
	Parasitic	Predaceous	Misc.
Insecta			
Ceratophyllidae			
Orchopeas howardii	X		
Hoplopleuridae			
Neohaematopinus sciuri	X		
Hoplopleura sciuricola	X		
Enderleinellus longiceps	X		
Simuliidae			
Simulium sp.	X		
Acari			
Ixodidae			
Ixodes marxi	X		
Amerosiidae			
Ameroseius sp.		X	
Laelaptidae			
genus?			X
Haemogamasus ambulans	X		
Androlaelaps casalis	X		
Macrochelidae			
Macrocheles sp.		X	
Uropodidae			
genus?			X
Cheyletidae			
Cheyletus sp.		X	
Oribatulidae			
genus?			X
Acaridae			
Tyrophagus sp.		X	
Ascidae			
Blattisocius sp.		X	
Galumnidae			
Galumna sp.			X
Glycyphagidae			
Ctenoglyphus sp.		X	
Mesostigmata			
family? genus?			X

141.3); 3 (50.0%) in June yielded 323 (average 107.6); 5 (83.3%) in July yielded 31 (average 6.2); 1 (16.7%) in August yielded 22; 1 (16.7%) in September yielded 39; 6 (100%) in October yielded 830 (average 138.3); 4 (66.7%) in November yielded 306 (average 76.5); and 2 (33.3%) in December yielded 479 (average 239.5). From these limited data is suggested that the months of January through April and July through September are periods of low numbers of nest-inhabiting arthropods and May through June and October through December are periods of high arthropod intensities in the nest-funnels.

The control nest-funnels, which had one-fourth-inch-mesh hardware cloth obstructing the entrance hole to exclude squirrels, did not yield any squirrel parasites in the collecting jars. However, many flying and crawling arthropods and a few predaceous mites did find their way into the control nest-funnels and subsequently into the collecting jars.

A number of other arthropods, not listed in this study, were regularly found in the collecting jars. These included bees, wasps, flies of many species, various beetles, caterpillars, centipedes, roaches, moths and spiders. These were more numerous during the warmer months of the year. Assuming that the same variety and number of these organisms may find their way into the many natural squirrel nests and denning sites, it is likely that they constitute a certain physical annoyance to the squirrels by their presence.

Clark (1956) reported the following ectoparasites in squirrel nests in Washington, D. C. and Maryland: the flea, Orchopeas howardii;

the ticks, Ixodes marxi and Dermacentor variabilis; and the mites, Haemogamasus ambulans, Androlaelaps casalis and Ornithionyssus bacoti.

Pathology

Even though some squirrels demonstrated relatively high numbers of ectoparasitic organisms, I found no serious pathological conditions resulting from them. The attachment of ticks caused small localized irritations, but I never observed any signs of secondary infection resulting from this. The bites of fleas and lice and some penetration of the epidermis by small mites probably caused some discomfort to the host. Probably the scratching activities of the hosts cause more damage to the skin and fur than the ticks, fleas or lice themselves. The most extensive gross pathology caused by any ectoparasite was observed with the large fly maggot, Cuterebra sp., which formed huge lesions in the skin of its host, but rates of infestation were low in this study. The pathology of Cuterebra sp. in squirrels has already been properly elucidated by Payne and Cosgrove (1965).

Conclusions and Summary

In my observations of parasitic arthropods on gray squirrels, the following organisms and percentages of occurrence were shown by my general survey of 106 squirrels: Bruelia rotundata (1.9%); Neohaematopinus sciuri (81.1%); Hoplopleura sciuricola (54.7%); Enderleinellus longiceps (67.9%); Cuterebra sp. (7.6%); Simulium sp. (1.9%); Orchopeas

howardii (73.6%); Ixodes marxi (22.5%); Androlaelaps casalis (12.3%); Haemogamasus sp. (0.9%); Haemogamasus ambulans (30.2%); Echinolaelaps sp. (0.9%); Hirstionyssus sp. (0.9%); unidentified mites, family Rodentopididae; (1.9%), and chiggers, Trombiculidae, (14.2%).

A study was conducted to determine body surface areas of 24 adult squirrels (12 males and 12 females) using flat tracings of the skin from the various body regions and computing the area with a planimeter. These body regions were macerated with 5% sodium hydroxide and total counts of the ectoparasites were made. These data showed that the back constituted 35% of the total body surface area, followed by legs (25%), underparts (19%), head (14%) and tail (7%). The total densities of all ectoparasite species yielded the highest parasite densities on the back, with decreasing densities on the legs, head, underparts and tail. Also male hosts demonstrated the highest numbers of ectoparasites and the greatest densities.

Neohaematopinus sciuri infested both male and female hosts, showing about equal percentages of infestation. Also, percentages of infestation between age categories were not significantly different. The highest seasonal percentage of squirrels infested were those collected during winter. Density determinations for this species were highest on the back region, with decreasing densities occurring on the legs, underparts, tail and head.

Enderleinellus longiceps was shown to be more prevalent on male hosts and the highest average percentage occurred within the subadult age group. According to seasonal occurrence, this species was most

prevalent on squirrels collected during winter. The density study showed preference by this species for the head region, with decreasing densities on the underparts, back, legs and tail.

Hoplopleura sciuricola was more frequently encountered on male squirrels and the greatest percentage of hosts infested, according to age category, were subadults. Seasonal incidences showed highest percentages among squirrels collected during summer. The density study showed the highest density for this species occurred on the legs, with decreasing densities on the back, head, underparts and tail.

Orchopeas howardii was more frequently encountered on male hosts, but frequencies of infestation appeared to decrease with the age of the host, being highest among the juveniles. The density study showed highest density with this species on the tail, followed by legs, back, underparts and tail. However, the data were not considered conclusive due to the fact that this species exhibited a marked tendency of becoming separated from the host during handling manipulations in which more than half of the total collected could not, with certainty, be assigned to a body region.

Ixodes marxi was shown to be slightly more frequent on female hosts, but not significantly so. It also was more prevalent among the juvenile hosts. This species was not found on any squirrels collected during winter and spring, but exhibited maximum prevalence among the squirrels collected in fall. The density study yielded decreasing densities from tail to head, back, underparts and legs. However, I did not consider these results conclusive since one host harbored a

majority of the total ticks collected and thus influenced the averages.

Androalelaps casalis was about equally prevalent on males and females, and apparently there were no significant differences between the percentages of the age classes. The highest percentage of squirrels infested with this species occurred in the month of August, and it only occurred on squirrels collected during the months of July through October.

Haemogamasus ambulans showed its highest prevalence among male squirrels and the juveniles appeared significantly higher than the other age categories. According to seasonal occurrence, H. ambulans infested the greatest percentage of squirrels during the fall, but this was not significantly greater than the winter sample. No squirrels examined during spring were infested. The density study showed that this species had a definite preference for the tail region over other body regions, with decreasing densities on the underparts, back and legs. None occurred on the head.

A small mite was recovered in this study which remains unidentified, but was tentatively placed in the family Rodentopididae. An illustration and brief description was included.

Chiggers (fam. Trombiculidae) were present on several hosts, being most prevalent during spring and fall. Males and females harbored them in nearly equal percentages.

A number of arthropods occurred in the fur of squirrels which were not considered to be parasitic. A list of these was included.

Modified nest-funnels were constructed and placed on trees in two campus woodlots and monitored monthly. One woodlot yielded only one occupation of a nest-funnel and was used only temporarily. Another woodlot yielded occupation of the six experimental nest-funnels during the year study period, but none were used continually throughout that time. I presented a list of arthropods occurring in these nests when they were occupied and indicated their apparent ecological role in the nest. Eight species were listed as parasitic, all of which were shown to be parasites on squirrels by my other survey. No parasites were found in these nests that had not been formerly found on the squirrels I surveyed.

I observed no prevalence of serious pathological conditions in this study resulting from parasitic arthropods.

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ADDENDUM

I. Observations on the Parasites of North Carolina Gray Squirrels During the 1968 Emigration

On September 26-27, 1968, a trip was made to the western mountain region of North Carolina to observe and collect emigrating gray squirrels, Sciurus carolinensis carolinensis Gmelin, 1788, which had become victims of road kills and drownings. The chief purpose of this expedition was to collect reasonably fresh-killed specimens in order to determine the degree in which they were parasitized and to seek causes for the emigration. Obviously one of the factors inherent in the emigration activity was a large squirrel population. This factor provided an excellent opportunity to study the parasites of these animals. Flyger (1969) and Citron (1968) have released descriptions of the events of the gray squirrel emigration of 1968 in North Carolina and other areas.

A review of the literature on parasites from this host shows a substantial list recorded from North Carolina squirrels in comparison with most other localities. Harkema (1936) recorded the nematode Heligmodendrium hassalli (Price, 1929), the larval cestode Taenia taeniaeformis (Batsch, 1786), the lice Hoplopleura sciuricola Ferris, 1921, and Neohaematopinus sciurinus (Mjoberg, 1910), the mites Androlaelaps glasgowi (Ewing, 1925) and Trombicula sp., the tick Ixodes hexagonus-cookii Packard, 1869, and the flea Orchopeas howardii (Baker, 1895). Allison (1948) listed the myiasis-producing fly maggot Cuterebra sp., chiggers, Trombicula sp., and the flea, Orchopeas howardii. Shaftesburg (1934)

reported the flea, Pulex irritans (Linn., 1758), and Brennan and Wharton (1950) reported the chigger, Trombicula whartoni Ewing, 1929.

Upon meeting with Assistant District Game Protector Supervisor A. O. Ray and Game Protector R. W. Beard, the escord proceeded to Cheoah Lake and other areas within easy driving distance of Waynesville, N. C., to visit the areas of maximum squirrel activity to collect freshly killed specimens. The dead squirrels were placed in plastic bags and kept on ice until further processing could be made. The physical data on each squirrel was taken and recorded. The visceral organs were preserved in 10% formalin for later inspection. In the laboratory the visceral organs were dissected and the organs and contents washed in a #325 screen until the water ran clear. This material was then examined under a dissection microscope. The parasitic organisms were counted and stored in alcohol. Cardiac blood was taken for making blood smears, and 1-2 ml was added to 10 ml of 2% formalin for examination by Knott's (1939) concentration technique (modification by Herman and Price, 1955). Fecal pellets were added to 2% potassium dichromate solution in a volumetric manner and samples examined in a Petroff-Hausser counting chamber to make coccidial oocyst counts. The remainder was retained to allow time for sporulation of coccidia.

In order to recover as many ectoparasites as possible, the plastic bag was washed and the fluid examined and each squirrel was combed and brushed over a white enamel pan. The arthropods thus recovered were counted and preserved in alcohol.

Since, unfortunately, the peak of the squirrel emigration activity

had apparently passed by the time of collection, only nine fresh specimens (seven females and two males) were recovered, although numerous decaying and mutilated carcasses were still to be seen along highways and floating in reservoirs. The physical data on these hosts are tabulated in Table I.

The examination of the visceral organs of these nine squirrels showed that they harbored six species of nematodes. The number of species per host ranged 0 to 5, with an average of 3.1 species per host. The total number of nematodes per host ranged 0 to 914, with an average of 208.3 nematodes per host. Böhmiella wilsoni Lucker, 1943, was recovered from the stomach of one host; seven hosts harbored Citellinema bifurcatum Hall, 1916, in the small intestine; eight hosts harbored Heligmodendrium hassalli (Price, 1929) in the stomach, small intestine and cecum; Strongyloides robustus Chandler, 1942, occurred in the duodenum of eight hosts; one squirrel harbored Trichostrongylus calcaratus Ransom, 1911, in the small intestine; and Enterobius sciuri Cameron, 1932, occurred in the ileum, cecum and large intestine of three hosts. One juvenile squirrel did not harbor any nematodes.

The only pathological consequences from nematode infestation that was evident was erosion of the intestinal villi caused by attachment of the hookworms Heligmodendrium hassalli and Citellinema bifurcatum when they occurred in large numbers. Also slight damage was incurred by Strongyloides robustus penetration and entanglement in the villi, mostly in the upper duodenum. However, the latter did not appear to cause a major pathological problem because of the few worms encountered. No other pathological conditions resulting from nematode infestation were

TABLE I. Gray squirrels, Sciurus carolinensis carolinensis Gmelin, 1788, collected in western North Carolina during the fall emigration, September 26-27, 1968

Host number	Sex	Age	Weight (gms)	County	Manner collected	Locality
1	Male	Subadult	482	Graham	Drowned	Cheoah Lake
2	Female	Adult	545	Graham	Drowned	Cheoah Lake
3	Female	Subadult	491	Graham	Drowned	Cheoah Lake
4	Female	Adult	510	Graham	Drowned	Cheoah Lake
5	Female	Subadult	489	Graham	Drowned	Cheoah Lake
6	Female	Juvenile	430	Graham	Drowned	Cheoah Lake
7	Female	Juvenile	352	Buncombe	Roadkill	Sardis Road
8	Female	Subadult	430	Haywood	Roadkill	Rt. 19, Canton
9	Male	Subadult	453	Mitchell-McDowell lines	Roadkill	Linn Gap, Blue Ridge Parkway

evident, although one oxyurid, Enterobius sciuri, occurred in very high numbers.

The examination of Wright's stained blood smears and blood concentrates revealed the gametocytes of Hepatozoon sp., apparently H. griseisciuri Clark, 1958, encysted in the monocytes. Five hosts (55%) were diagnosed positive from the stained smears, while six hosts (66%) were positive as revealed by the blood concentrates. The numbers of gametocytes (estimated) ranged from scarce to very numerous. No microfilaria were observed by any of these blood examination techniques.

The microscopic examination of fecal material demonstrated the presence of intestinal coccidia, Eimeria sp., in all nine squirrels. Estimates of coccidial oocyst numbers ranged from 72,000 to 328,000 oocysts per gram of feces, with an average of 190,500. The cysts of the flagellate, Giardia sp., were observed in the feces of one host. An estimate of the number of cysts per gram of feces was 96,000. No gross pathology resulting from coccidia or flagellate was evident in these observations.

Despite the fact that six of these squirrels had been victims of drowning and three of roadkills and none was collected immediately at death, at least six species of arthropods were recovered from the fur of these animals. The squirrel flea, Orchopeas howardii, and the louse, Neohaematopinus sciuri Jancke, 1932, were recovered from all nine squirrels. The warblefly, Cuterebra sp., infested five squirrels and occurred under the skin of the neck, the back, the shoulder, the sides of the body and on the head behind the ear. No more than two of these large

maggots occurred on an individual host. The mite, Androlaelaps glasgowi (Ewing, 1925), occurred on two hosts, and Rostrozetes sp. (Fam. Haplozetidae), an oribatid, occurred on one host. Chiggers of the Family Trombiculidae occurred on four hosts. The ectoparasite infestation appeared to be light; however, the numbers of chiggers were high on two hosts. They may have been responsible for the reddish spots on the host skin which were observed on the belly and underparts.

Table II provides a summary of the parasites recovered from North Carolina gray squirrels in this study. The figures pertaining to Strongyloides robustus are very conservative, since these worms were deeply entangled in the villi of the gut wall and many were difficult to remove without breakage. No digestion techniques were employed for their recovery.

From unpublished data on the parasites of five gray squirrels collected from Summit, North Carolina, September 25, 1968, furnished by Dr. F. E. Kellogg of the Southeastern Cooperative Wildlife Disease Study, the following parasites and percent incidence (in parentheses) are listed: Böhmiella wilsoni (29%), Rictularia sp. (20%), Citellinema bifurcatum (80%), Heligmodendrium hassalli (100%), Eimeria sp. (100%) and Neohaematopinus sciuri (60%). All of the parasites reported above were found in the present study except one, Rictularia sp. In addition, the present report lists at least ten species not reported from Summit, North Carolina. Quentin (1969) has recently separated the nematode genus, Rictularia Froelich, 1802, into two genera, Rictularia and Pterygodermatites Wedl, 1861, and Lichtenfels (1970) considers the

TABLE II. Parasites recovered from nine gray squirrels, Sciurus carolinensis carolinensis Gmelin, 1788, in western North Carolina, September 26-27, 1968

Parasite	Percent squirrels infested	Intensity Average (range)
PROTOZOA		
Hepatozoon griseisciuri	66	--- (few to many)
Eimeria sp.	100	190,500 (72,000-328,000)[1]
Giardia sp.	11	69,000[2] (---)
NEMATODES		
Heligmodendrium hassalli	89	63.2 (5-284)
Citellinema bifurcatum	78	32.5 (1-76)
Bohmiella wilsoni	11	3.0 (---)
Trichostrongylus calcaratus	11	3.0 (---)
Enterobius sciuri	33	350.6 (42-800)
Strongyloides robustus	89	10.3 (2-28)
ARTHROPODS		
Orchopeas howardii	100	7.4 (1-19)
Neohaematopinus sciuri	100	8.8 (1-24)
Cuterebra sp.	55	1.6 (1-2)
Androlaelaps glasgowi	22	18.5 (6-31)
Rostrozetes sp.	11	1.0 (---)
chiggers (Trombiculidae)	44	62.7 (1-154)

1/ Oocysts per gram of feces
2/ Cysts per gram of feces

species from the gray squirrel to be Pterygodermatites parkeri Lichtenfels, 1970.

In order to implicate parasitism as a cause or factor in gray squirrel emigration, one must compare data on parasites in migrating squirrels with data obtained from the same host population during static conditions. Unfortunately, sufficient data are not available for making any notable conclusions.

Allison (1948) stated that 69.8% of 53 squirrels examined between April 1 and October 1 were infested with Cuterebra sp. in North Carolina. This does not significantly differ from the 55% incidence in the present study.

Harkema (1936) recorded monthly data from September, 1934 to May, 1935, on parasites of a total of 53 gray squirrels from Durham County, N. C. Five of the parasite species he listed were found in the present study. He listed only one species of nematode, Heligmodendrium hassalli, which occurred in six of seven squirrels examined in September and averaged 110.3 worms per infestation. Of the 53 squirrels examined, 92% were infested with H. hassalli, averaging 78 worms per host. The present study yielded a slightly lower percentage and infestation burden (Table II). Perhaps this was influenced by the presence of other nematode species, since five additional species occurred in these squirrels. Heligmodendrium hassalli occurred in the largest numbers (284 worms) in a squirrel which had only one other species present with it and in the smallest numbers (5 worms) when in association with four other species.

The other four species from Harkema's work found in common with the present study were ectoparasites. He reported chiggers, Trombicula sp., occurring on 43% of the squirrels in September and averaging 7 per host. Of the 53 hosts, 51% were infested with chiggers, averaging 6 per host. The incidence of infestation does not differ significantly from 44% in the present study, but our average figure of 67.2 mites per squirrel was considerably higher. Androlaelaps glasgowi occurred on 29% of the hosts collected in September and 13% of those for the 9 month period with averages of infestation 0.6 and 0.3, respectively, in comparison with 22% and 18.5 per infestation in the present study. Neohaematopinus sciuri was given as 14% for September and 51% for the year, with infestations averaging 2.7 and 28.8, respectively, in comparison to 100% and 8.8 in the present study. Orchopeas howardii occurred on 29% of the September squirrels and 51% of the year sample, with infestations averaging 0.7 and 3.7, respectively, in comparison with 100% and an average of 7.4 fleas in the present study.

These comparisons show that apparently a greater variety of nematodes were present in the squirrels during the emigration activities and either higher percentages of ectoparasite infestation and/or higher numbers on the hosts were usually significant. Apparently these circumstances were the result of a high increase in the squirrel population and their increased movements on the ground. There is no evidence from this study that parasites had any influence upon the initiation of squirrel emigration activities. There is a great need for additional data on the parasites of squirrels under normal conditions and further studies should be undertaken in the event of another emigration.

The following organisms appear to be the first published records as parasites of gray squirrels in North Carolina: Hepatozoon griseisciuri, Eimeria sp., Citellinema bifurcatum, Böhmiella wilsoni, Trichostrongylus calcaratus and Strongyloides robustus. Enterobius sciuri was first described from the introduced American gray squirrel in Scotland (Cameron, 1932) and has subsequently been reported from the flying squirrel, Glaucomys volans volans (Linn., 1758) and the fox squirrel, Sciurus niger rufiventer St.-Hilaire, 1803, in Michigan, Ohio and Wisconsin by Rausch and Tiner (1948). Apparently this is the first report of this species in the gray squirrel for North America and substantially increases its distribution much farther south of its previous reports in other hosts. Giardia sp. appears to be a new host record.

We wish to extend special thanks to Messr. A. O. Ray and R. W. Beard for their helpful assistance in securing specimens, to Drs. R. I. Sailer, E. W. Baker and K. C. Emerson for identification of the ectoparasites, and to Dr. F. E. Kellogg for permission to use his unpublished data.

PROTOZOAN, HELMINTH AND ARTHROPOD PARASITES OF THE GRAY SQUIRREL IN SOUTHWESTERN VIRGINIA

BY: James Conn Parker

(Abstract)

A comprehensive, qualitative and quantitative survey of parasites of 176 gray squirrels, Sciurus carolinensis pennsylvanicus Ord, 1815, was conducted from September, 1966, to July, 1969, in southwestern Virginia. Most of the hosts came from Montgomery County, some being taken also from Giles and Craig counties. Of that total, 167 were examined for coccidia and intestinal flagellates; 84 were examined for blood parasites; 175 were examined for helminths and 106 for ectoparasitic arthropods.

The incidence of coccidiosis was 91% in which five species were described and identified with the genus, Eimeria. The sporozoan, Hepatozoon griseisciuri Clark, 1958, occurred in the blood as evidenced by a 58% incidence in stained blood smears and 79% in blood concentrates. Excysted gametes of this species were kept in vitro for four days without any apparent union or multiplication. The cysts of the flagellate, Giardia sp., are reported for the first time in this host and occurred in 5% of those examined. Gray squirrels, hamsters, white mice and a chipmunk were successfully infected with cysts obtained from a naturally infected squirrel. In vitro cultivation of the trophozoites was partially successful. Cultures were maintained up to 21 days.

In examinations for helminths, 3% of the hosts harbored adult cestodes of which two species were identified and 62% harbored nematodes of which 12 species were identified. From habitats designated "town, woodlot, forest fringes and remote forest," the highest incidence of

nematode infestation occurred in the forest fringes sample. Nematodes were generally more prevalent among male squirrels than females, and the average high incidence occurred during the spring. Numbers of worms per infection were generally higher in summer. The most common species were Citellinema bifurcatum Hall, 1916, (45%), Strongyloides robustus Chandler, 1942, (28%), and Böhmiella wilsoni Lucker, 1943, (14%).

The arthropod survey indicated 97% of the hosts were infested. Four species of lice, two flies, one flea, one tick and six mites were recovered. The greatest percentages of hosts infested, based on individual parasite species, were generally those collected during winter. The most common arthropods were Neohaematopinus sciuri Jancke, 1932, (81%), Orchopeas howardii (Baker, 1895), (74%) and Enderleinellus longiceps Kellogg and Ferris, 1915, (67%). Twenty-four adult squirrels (12 male, 12 female) were examined for total ectoparasites using a hair dissolving technique. Also the surface areas of these squirrels were calculated by body regions so that parasite densities could be determined. The results showed the highest densities generally occurred on the back, followed by legs, underparts, head and tail. Parasite densities were generally greater on males. The preferences by the various species for certain body regions were determined from density data. A number of incidental arthropods were also encountered in these studies. Modified nest-funnels were constructed and placed in campus woodlots and monitored for a year. A list of the arthropod species occurring in these nests is given.

Several new host records, a number of distributional records and one new species were evidenced in this study.