

STUDIES ON MICIPSELLA BREVICAUDA LYONS, 1958, A FILARIID
OF THE BLACK-TAILED JACK RABBIT (LEPUS CALIFORNICUS
MELANOTIS MEARNIS), WITH NOTES ON THE HELMINTH
PARASITES OF THE JACK RABBIT

by

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TABLE OF CONTENTS

INTRODUCTION AND REVIEW OF LITERATURE.....	1
MATERIALS AND METHODS.....	8
<u>Micipsella brevicauda</u>	8
Microfilariae.....	8
Collection.....	8
Fixation, Staining and Measurement.....	10
Experimental Infections.....	11
Transplants.....	11
Field Exposures.....	12
Pathology.....	15
Host Specificity.....	15
Distribution of <u>M. brevicauda</u> in Kansas.....	16
Helminth Parasite Study.....	16
RESULTS AND DISCUSSION.....	17
<u>Micipsella brevicauda</u>	17
Microfilariae.....	17
Description.....	23
Other Microfilariae Occurring in <u>Lepus</u> spp.....	28
Experimental Infections.....	33
Transplants.....	33
Field Exposures.....	39
Pathology.....	40
Macropathology.....	40
Micropathology.....	43

Host Specificity.....	52
Distribution of <u>Micipsella brevicauda</u> in Kansas.....	52
Helminth Parasite Study.....	58
<u>Micipsella brevicauda</u>	58
Other Parasites.....	77
SUMMARY.....	90
ACKNOWLEDGMENTS.....	95
LITERATURE CITED.....	96

INTRODUCTION AND REVIEW OF LITERATURE

A number of parasite surveys of the leporid Lagomorphs have been made in the United States, Canada and foreign countries. These surveys have revealed few records of filarial nematodes in the leporids. Three species of filariids occurring in the Leporidae in the United States are; Micipsella brevicuda Lyons (1958), Dirofilaria scapiceps Leidy (1886), and D. uniformis Price (1957).

The type species, Micipsella numidica, was described by Seurat in 1917 from Lepus pallidior¹ and L. kabylicus¹ in Algeria. Kalantarian (1924) reported similar species from Lepus spp. in Armenia. Lyons (1958), studied the endoparasites of the black-tailed jack rabbit (Lepus californicus melanotis Mearns) in Kearny Co., Kansas, and report 50 percent of the hares to be infected with Micipsella brevicuda, a new species. His report constituted the first record of this genus in the United States. These nematodes were found in the abdominal cavity of the host.

Erhardova (1956), reported Micipsella numidica in Europe. He found the worms in the capillaries of the omentum in Lepus europeus. Ivashkin (1954) also found this parasite in Lepus tolai¹ in Mongolia.

¹ Now considered a subspecies of L. capensis. (All information on classification of L. capensis in this thesis was kindly provided by Dr. M. W. Setzer, Smithsonian Institution, U.S.N.M.)

Rao (1938) reported M. indica from L. nigricollis in India. This report appears to be the only account of this species in the literature.

Nevenic (1954) conducted an endoparasite study of 86 hares in Yugoslavia and reported no Micipsella species. Naumov (1944) reported no filariids in 528 hares (L. timidus and L. europeus) in the Vologda and Kirov Districts and in the Northern Caucasus in Russia. Call (1949), Archetti (1947), Franchini and Ganora (1931), Foley, Catanei and Vialatte (1926) and Balfour (1911) report microfilariae from hares but did not give an account of or a classification of adult worms. It is probable that these microfilariae were those of M. numidica.

Call (1949) reported microfilariae from three hares, (Lepus sp)? in Northern Eritrea. Archetti (1947) found microfilariae in L. tigrensis in Africa. Franchini and Ganora (1931) demonstrated microfilariae in L. aegyptius. Foley, Catanei and Vialatte (1926) while working in South Oranais, Algeria, found two hares L. sefranus (Thomas) infected with microfilariae. Balfour (1911) reported microfilariae from L. hawkeri (?) along with finding two adult parent worms in the body cavity.

Leidy (1886) found filarial worms under the skin of the hind foot of L. sylvaticus (Sylvilagus floridanus mallurus). He named them Filaria scapiceps (Dirofilaria scapiceps). The locality of Leidy's collection is not given, but Hall (1916) believed that the collection was probably made in Pennsylvania. Hall reported two other cases of this filariid, one from L.

campestris, locality not given, and another collected by Douthitt from S. fl. alcer at Sulphur Springs, Oklahoma.

In 1927, Harkin reported microfilariae in the blood of a rabbit from British Columbia, Canada. Alicata (1929) recorded a previously unreported case of D. scapiceps from a rabbit collected near Woodford, Virginia. The worms were found inside the tarsus of S. fl. mallurus. He also reported worms from inside the tarsus of what was probably the marsh rabbit, S. palustris, from Kingston, North Carolina. Schwartz and Alicata (1931a, and b) described microfilariae from the blood of L. washingtonii collected in Washington State. MacLulich (1937) found D. scapiceps in L. americanus in Ontario, Canada. He reported seeing microfilariae in the blood but did not describe them. Manweiler (1938) found this parasite in the snowshoe hare (L. am. phaeonotus) in Minnesota. About one-third of the hares examined by Manweiler were infected. This was the first report of this nematode in this hare.

Higby (1938) observed microfilariae in the blood collected from a snowshoe hare in Minnesota. He observed development of infective larvae in various wild caught mosquitoes which had fed on the infected hare. Later in 1943, he reported that 40 percent of the hares examined in Minnesota were infected. Except for one case, all nematodes were found in the tarsal bursa, a pocket, covered with tough connective tissue, below the distal end of the tibia on the anterior aspect of the tarsal joint. The exception noted was recovered from the posterior aspect of

the tarsal joint. He also described the microfilariae which were collected from the site of infection, the tarsal bursa. These were taken from a hare which was infected with eight female and seven male worms. Highby demonstrated that five species of Aedes mosquitoes were susceptible as intermediate hosts. Transmission through infected mosquitoes from the infected snowshoe hare to the domestic rabbit was accomplished. Sevelid (1942) in working with the snowshoe hare (L. americanus) in Maine, reported that 38 percent of the hares examined were infected with D. scapiceps. Llewellyn and Handley (1945) reported that 20 percent of the rabbits were infected with D. scapiceps in their study in Virginia. The nematodes were found in the intermuscular fasciae of the hind legs except for one from the subcutaneous fascia of the back just posterior to the shoulder. Penner, Dery, and Knuckles (1953) examined four cottontails (S. floridanus) from Connecticut. Three of the four rabbits examined contained microfilariae in the blood. However, adult worms were found in only one rabbit which were located in one hind foot near the Tendon of Achilles. These same workers (1953) also reported D. scapiceps from a cottontail rabbit collected in Massachusetts. Penner (1954) found one snowshoe hare (L. americanus) infected collected in Connecticut. Price (1954) found blood of Sylvilagus spp. infected with microfilariae in the Washington and Baltimore areas. Robinson (1954) in surveying the fauna of Georgia for filarial nematodes, reported filarial worms and microfilariae from S. fl. mallurus, but did

not name them. Crites and Phinney (1958) report findings of D. scapiceps in S. fl. mearnsi in the Zaleski State Forest in Ohio. The worms were located in the hind feet in the inter-muscular fasciae of the hock of the foot and at the joint between the fibia, tibia and tarsal bones. One hundred and sixty nematodes were removed from two reported infected rabbits.

Price (1957) found microfilariae in peripheral and cardiac blood in 11 of 124 rabbits (S. fl. mallurus (Thomas)) collected in Maryland. At autopsy of the rabbits, a mixed infection of D. scapiceps and unidentified filarial worms were found in the subcutaneous connective tissue of the dorsal and lateral surfaces of the body. D. scapiceps was found in the tarsal bursa.

In summary, filarial nematodes have been reported from the Leporidae in 13 states in the United States and two provinces in Canada. In the United States, Micipsella brevicauda is known apparently, only in Kansas. Dirofilaria scapiceps is known in Pennsylvania, Oklahoma, Virginia, North Carolina, Washington, Minnesota, Maine, Connecticut, Massachusetts, Ohio, and in Canada in British Columbia and Ontario. D. uniformis is apparently, known only in Maryland. One unknown form is reported from Georgia.

Several parasite surveys in Lepus sp. in the United States have been made, but filarial nematodes were not reported. McCampbell (1926) working with L. c. melanotis and L. townsendii in eastern Colorado; Vorhies and Taylor (1933) who studied L. alleni and L. californicus in Arizona; Ward (1934),

who in Oklahoma, studied the ecto and endoparasites of L. californicus; Philip (1938) studying the varying hare in Alaska; Philip, Bell and Larson (1955) who studied a population of L. c. deserticola in northern Nevada; Grundmann (1957) surveying small mammals, including L. c. deserticola, of the Great Salt Lake Desert of Utah; Adams (1959) who studied a population of snowshoe hares in northwestern Montana; and Lechleitner (1959) in working with parasites and diseases of the black-tailed jack rabbit L. californicus in the Sacramento Valley, California.

The pathology of filariid infections in man and animals has been reported in the literature. Pathology of filarial worms dwelling in the body cavity is minimum when compared with the tissue or lymphatic inhabiting forms.

The classical example of the latter is Wuchereria bancrofti (Cobbold, 1877), infecting man throughout the tropics and subtropics except in the Southwest Pacific (Manson-Bahr, 1936; Mackie, Hunter and Worth, 1954). The infection may be accompanied by important pathological changes related to the lymphatic system. These include inflammatory lesions, dilations, rupture and blocking of the lymphatics. The latter, if occurring in the limbs, often produces the characteristic elephantiasis.

Other tissue inhabiting forms known to infect man and animal include Onchocerca spp. producing the characteristic subcutaneous fibrous nodules, and the African eye worm, Loa

loa producing creeping eruption from worm migrations along with calabar swellings.

Among the body cavity dwelling forms, pathology is in general absent or at a minimum. Many of the reactions produced are those of a defense mechanism of the host against the parasite Tisseuil (1934a and b), Sarwar (1945), Scott and Gross (1945) and Vinnitsky (1951). Mansonella ozzardi (Manson, 1897) and Acanthocheilonema perstans (Manson, 1891) are found in the peritoneal cavity of man and are considered non-pathogenic. Setaria equina (Abildgaard, 1789) and S. cervi (Rud., 1819) are found in the peritoneal cavities of horses and cattle respectively, but are considered nonpathogenic to their hosts. Sarwar (1945) working with S. cervi in yearling buffalos, found endothelial cells, slight congestion and fibrous tissue surrounding the worms in a cross section of a portion of the muscular and serous membrane of the body cavity. These were thought to be normal host reactions to the migrating and developing worms.

Tisseuil (1934a and b) reported encapsulation of the filarial worms found in the peritoneal cavity of the opossum (Philander sp.) as a type of defense reaction of the host against the parasite. A comparable reaction was reported by Scott and Gross (1945) working with Litomosoides carinii in the cotton rat (Sigmodon hispidus).

Vinnitsky (1951) introduced live ascarids into the body cavity of rabbits and observed a rapid walling-off process of

the worms by the rabbit. This was followed by infiltration of leucocytes, phagocytosis and final resolution of the cyst. In the present study, apparently a comparable reaction, in part, occurs in the black-tailed jack rabbit (L. c. melanotis) against the peritoneal inhabiting filarial worm, M. brevicauda.

Preliminary studies on the helminth parasite burden of the black-tailed jack rabbit in Southwestern Kansas was initiated in 1956, (Lyons, 1958). The present study includes a continuation of this endoparasite survey. Classification and laboratory techniques, with minor exceptions, were those of Lyons (1958). Since similar methods were used in both studies, a true comparison can be made between the surveys.

The purposes and objectives of this study were to:

- (1) describe the microfilariae of M. brevicauda, (2) establish an infection of M. brevicauda in the laboratory white rabbit,
- (3) study the pathology of M. brevicauda, (4) establish the host specificity and geographical distribution of M. brevicauda in Kansas, (5) begin preliminary intermediate host studies, and
- (6) continue the helminth parasite study of the black-tailed jackrabbit in southwestern Kansas.

MATERIALS AND METHODS

Micipsella brevicauda

Microfilariae. Collection. The description of the microfilariae of Micipsella brevicauda is based on a study of 80 larvae from about 850 thick blood smears taken from the hares.

Blood was collected from the ears, heart and from the lungs when possible. Blood was collected from these different sites in order to: begin preliminary studies on periodicity, determine the best site of blood sample in determining infected hares, and determine if any morphological variations existed among the microfilariae with respect to the site of collection.

Heart blood was taken immediately after the hare was killed. Collections were made in two ml. amounts with a five ml. syringe using a 12 or 14 gauge needle. The blood was then put into 15 ml. centrifuge test tubes which contained crystals of potassium and ammonium oxalate as anticoagulant (Mackie, Hunter and Worth, 1954). At the laboratory, 10 to 18 hours later, the samples were subjected to a modified Knotts technique (Mackie, Hunter and Worth, 1954). The blood samples were laked by adding seven to 10 ml. distilled water. The samples were then centrifuged and the supernatant drawn off with pipette. The sediment was spread over two-thirds the area of a clean slide and allowed to air dry.

Ear blood was collected by pressing blood (few drops) from severed ears of each hare immediately after being killed onto a clean slide. The slides were then air dried.

Blood from the lungs was collected by removing a portion of one lobe and touching the cut surface to a clean slide. Care was taken to wash dissecting instruments before and after removal of lung tissue so as to avoid contamination with blood from other sources. The preparations were prepared as thick smears and air dried.

Fixation, Staining and Measurement. Several methods of staining were used in the study. Azur II and Eosin in normal saline (Feng, 1933), Wrights stain, as well as Methyl Green-Pyronin (Fulleborn, 1913) all proved unsatisfactory in the hands of the author. Very good results, however, were obtained by using Delafield's Hematoxylin (Mackie, Hunter, and Worth, 1954) and a modified Giemsa stain. Delafield's Hematoxylin was used to demonstrate anatomical features, particularly, the sheath. The modified Giemsa stain, Giemsa-Triton was prepared as follows. A phosphate buffered water solution pH 7.0-7.2 (Mackie, Hunter and Worth, 1954) using phosphate buffers was prepared. A surface active agent, Triton X-100¹ (Stock solution, 10 percent) was added to make a resultant buffered water-Triton solution of five-tenths percent. Triton X-100 was added to improve the staining qualities of the Giemsa stain and help prevent transfer of microfilariae between blood films during mass staining (Melvin and Brooke, 1955). The Triton-Giemsa stain was prepared by adding stock Giemsa stain to the buffered water-Triton solution in a ratio of 1:50. The dried blood smears were stained for 45 minutes, air dried and examined.

All slides were examined with a compound binocular microscope under low power, making five passes at random over the slide. If microfilariae were not found in five passes, the slide and the hare were considered negative. Smears made from

¹ Triton X-100, Rohm and Haas Co., Philadelphia 5, Penn.

ear, heart, and lung blood were all stained and examined by the before mentioned methods.

In describing the microfilariae of M. brevicauda, two different methods were used to measure and record the anatomical points (Price, 1957). The points were recorded as average distance in microns from the anterior end and as the percent distance of the total length of the larva from the anterior end of the specimen. An ocular micrometer was used in measuring the microfilariae. In establishing the correct association between the microfilariae occurring in the blood of the host and those from the uteri of the female worms (M. brevicauda), microfilariae were teased from the uterus, stained and studied.

Experimental Infections. Efforts were made to establish infections of M. brevicauda in the laboratory white rabbit. Two methods were employed in an attempt to establish infections: transplantation of worms from infected hare to rabbit, and field exposures of the rabbit to the possible natural intermediate host(s).

Transplants. During the course of the study, six rabbits received transplanted worms from infected hares. The worms, recovered from the hares for transplantation were suspended in sterile saline in sterile petri dishes from one-half to two hours before being transplanted. They were only superficially sexed before transplantation to avoid excess handling and damage to the worms. All tools and equipment used in the transplant operations were sterilized by appropriate methods. The

rabbits were anesthetized with Nembutal¹ in saline given intravenously in the ear. The surgery involved making an incision, two and one-half cm. in length along the linea alba, midway between the xiphisternum and anus. The worms kept in sterile saline were then placed, with the aid of a blunt forceps, into the peritoneal cavity. The muscle and skin layers were sutured with 000 Medium Chromic Surgical Gut U.S.P. A small amount of penicillin was administered between the sutured muscle layers and the skin to prevent infection. Also, each rabbit was given one cc. penicillin (300,000 units) intramuscularly after the operation. All rabbits survived the operations and no infections were noted at the incision sites.

After the transplantation, each rabbit was examined periodically for microfilariae by drawing blood from the ear veins or directly from the heart. Amounts taken varied from .75-4.0 cc. per rabbit. Diurnal and nocturnal blood samples were taken and studied so that the microfilaremia would not be missed in the event that the microfilariae showed a periodicity. Each rabbit was ultimately necropsied and examined.

Field Exposures. Three rabbits were placed in exposure cages (Plate 1, Figs. 1, 2) located in a field near Lakin, Kansas. This field harbored infected hares. They were exposed in order that they might pick up a natural infection from the natural intermediate host(s). The exposure cage was

¹Nembutal (Pentobarbital Sodium), Abbott Laboratories, N. Chicago, Ill.

EXPLANATION OF PLATE I

- Fig. 1. A side view of the exposure cage. The screened enclosure housed the rabbit during exposure.
- Fig. 2. An end view of the exposure cage, showing the screened enclosure and the cone-shaped screened ends permitting insects to enter through a hole in the apex and feed on the host rabbit.

PLATE I

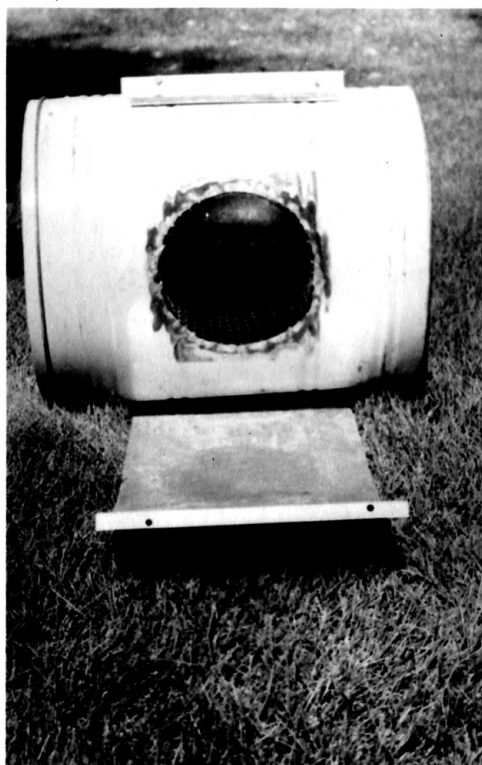


Fig. 1

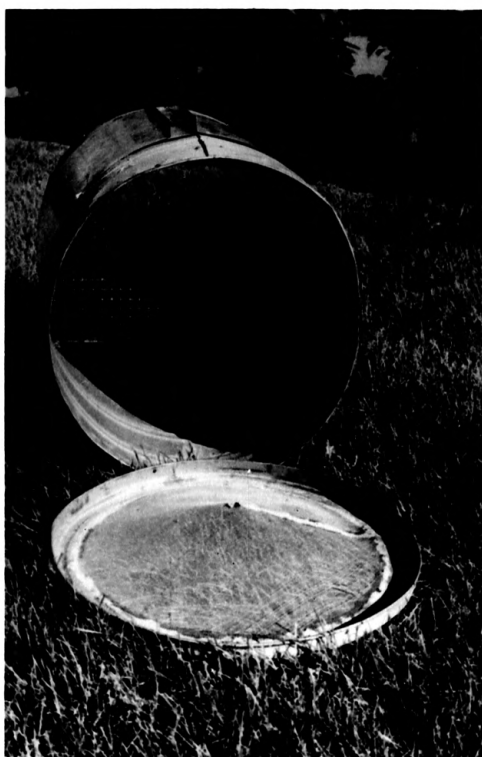


Fig. 2

designed so as to permit insects to enter but not to escape after feeding on the rabbit. Rabbit 4680 was exposed on August 8 and 9, 1958. Numbers 4681 and 4682 were exposed on August 16 and 17, 1958. After the exposures, the rabbits were taken back to the laboratory at Manhattan, Kansas for observations. Blood samples were taken periodically, processed, stained, and studied.

Pathology. Numerous cysts were recovered from the peritoneal cavity of the hares examined during the study. The cysts, yellow in color, ranged in size from .5-28 mm. (Plate IV, Figs. 5, 6). Upon recovery from the hares, the cysts were fixed in 10 percent buffered formalin. They were then appropriately prepared, imbedded, and sectioned at 4-10 microns. Sections were stained as follows: Von Kossa's stain for collagen fibers; Toluidine blue, (a metachromatic stain) for demonstration of tissue basophils; Hematoxylin and Eosin as a general stain; Giemsa stain, (Wolbach's modification) for collagen and other tissue elements; and Azure Eosin for demonstration of necrosis. Stains, their composition and staining procedures were those according to Gridley (1957).

Host Specificity. In order to determine the host specificity of M. breviscauda in southwestern Kansas, mammals other than L. c. melanotis were necropsied and examined. The majority of the mammals collected were killed with a .22 caliber rifle, a few were live trapped. All were examined for adult filariids only at either the laboratory at Manhattan, Kansas or at one of the field laboratories.

Distribution of M. brevicauda in Kansas. In order to determine the distribution of M. brevicauda infections in L. c. melanotis in Kansas, collections were made outside Kearny County (Table 4 and Plate VIII).

Except for the collections made in Dickinson and Stafford Counties, all hares taken were examined in temporary field laboratories. The hares taken in Dickinson and Stafford Counties were examined in the laboratory at Manhattan, Kansas. In addition to examining each hare for M. brevicauda, each was examined, where possible, for cysts and microfilariae. The methods used for examination of the blood for microfilariae were those previously described.

Helminth Parasite Study

Two hundred and sixty-nine hares (Lepus californicus melanotis Mearns) were examined for endoparasites during a 27-month period from October, 1957 through December, 1959. These hares were collected with a .22 caliber rifle within a 10 mile radius of Lakin, Kansas north of the Arkansas river in Kearny County. Monthly collections of 10 hares per month were made throughout the 27 monthly collecting periods. These hares were taken from larger monthly collections of 22-52 hares. The first 10 hares collected each collecting period which showed no apparent shot damage to the gastrointestinal tract and organs, were preserved in 10 percent formalin and saved for the endoparasite study. All hares were weighed,

measured, aged, and sexed by Dale Taylor, Department of Zoology, Kansas State University (Taylor, 1960).

The hares were divided into two age classes, adults and juveniles. Aging was based on size and condition of the reproductive organs and the epiphyseal closure of the humeri, (Taylor, 1960).

Methods used, with few exceptions in examination of the gastrointestinal tract and organs for parasites, and handling of parasites during identification were those of Lyons (1958). The exceptions noted were as follows: nematodes were examined directly from glycerine-alcohol without clearing in glycerine, cysticerci and coenuri were examined directly without clearing in lacto-phenol, and the tapeworms were cleared in lacto-phenol without dehydration in higher alcohols.

Classification and taxonomy of the parasites were those of Lyons (1958).

RESULTS AND DISCUSSION

Micipsella brevicauda

Microfilariae. In order to investigate the relationship between the microfilariae occurring in the blood stream of infected hares and those from the adult worms (M. brevicauda), larvae were teased from the uterus of freshly recovered adult worms, appropriately fixed, stained and examined. A statistical analysis (Table 9) showed that no difference, with one exception, existed between the larvae recovered from the blood of the host








and those from the uterus of the adult female worm. The exception noted was in the anal pore measurements where a significant difference existed. This significant difference may be explained by the fact that a small coefficient of variation existed in the anal pore measurements, which made it possible for the t-test to detect the small existing differences in the specimens. Thus it was concluded that the microfilariae occurring in the blood of the infected hares were those of Micipsella brevicauda. The statistical analysis was based upon 80 larvae from host blood and 20 from the uterus of adult female worms.

In the present study, a moderate degree of variation was noted in the absolute measurements between specimens. The variation among specimens was much greater between slides than among those on any one slide. Golvan (1956) studied the effects of variation of strength and kind of stain, time elapsed between making smears and staining, and variation in fixation on the morphology and orientation of the filarial larvae of Wuchereria spp., Mansonella ozzardi and Dipetalonema perstans in thick blood smears. He reported, even though the technique was kept constant, a variation existed between stained specimens within each and among the above mentioned species. This he contributed to a difference in the cuticle permeability among the different species. A high degree of permeability allowed for fast drying and, inversely, a more rapid staining, thus allowing for shrinkage and distortion of specimens. He

stated that all microfilariae did not shrink in the same way, thus giving each species their own characteristic conformation. Thus it can be concluded in the present study, since the technique for each stain was kept consistent, the variation in size of specimens was due in part to normal shrinkage occurring during the staining process.

Because of this variation in absolute measurements of the larvae, percent measurements of the anatomical points were also used. By the use of percent measurements, together with the absolute measurements (microns), a better description of the microfilariae could be made (Table 1). The use of percent measurements, since shrinkage was considered to be consistent within specimens, eliminated the great variation among specimens which earlier was found to exist in the absolute measurements. Golvan (1956) stated that retraction in no way hindered diagnosis of different species of filarial larvae. Microfilariae, because of various degrees of shrinkage, may differ widely as to absolute measurements, but differ less as to percent measurements because regardless of the degree of shrinkage, the anatomical points will remain in linear proportion to each other. As indicated earlier, various stains were used in staining of the microfilariae. Delafield's Hematoxylin was found to be the most reliable, and gave the best results. It consistently stained the sheath (Plate III, Fig. 3) and more clearly stained the anatomical points and nuclear column of the larvae. Giemsa (Giemsa-Triton) was found to be a good

Table 1. Comparison of microfilariae of *Micipsella brevicauda*, *M. numidica*, probable species of *Micipsella* and *Dirofilaria scapiceps*.

Anatomical points and items	<i>M. brevicauda</i> Lyons 1958			<i>M. numidica</i> Seurat, 1917			Microfilariae Call, 1949			Microfilariae Foley, et al., 1926			Microfilariae Balfour, 1911			<i>Mf. tigrensis</i> Archatti, 1947			<i>D. scapiceps</i> Highby, 1943		
	Absol.	%	No.	Absol.	%	No.	Absol.	%	No.	Absol.	%	No.	Absol.	%	No.	Absol.	%	No.	Absol.	%	No.
	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.	meas.
Total length	155.79* (+6.29)	---	100	110	---	---	110.76	---	10	97.5	---	6	95	---	---	189.3 (+6.0)	---	40	243	---	20
Sheath	181.75	---	57	unsheathed			unsheathed (in vivo)			unsheathed			not mentioned			unsheathed			present (no measurements given)		
Width	4.59 (+0.61)	---	98	4	---	---	3.55	---	10	4.28	---	5	---	---	---	4.4 (+0.3)	---	40	6	---	20
Cephalic space	3.75 (+0.69)	---	95	---	---	---	5.58	---	---	indistinct, groove like 5 no measurements	---	---	---	---	---	8.71	---	31	3	---	20
Nerve ring	40.38 (+2.83)	26.00 (+1.79)	94	---	---	---	31.13	27.9	7	27.5	27.8	4	---	---	---	51.68	27.3 (+1.3)	31	55.16	22.7	20
Excretory pore	54.88 (+2.93)	36.64 (+1.76)	100	---	---	---	40.0	36.3	10	39.0	40.2	5	40	42.1	---	66.44	35.1 (+1.7)	38	80.68	33.2	20
Excretory cell	61.99 (+2.96)	39.90 (+1.66)	94	---	---	---	---	---	---	---	---	---	---	---	---	78.75	41.6 (+1.3)	20	95.27	39.2	20
Innen Körper	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	134.14 (ant.)	55.2	9
Rectal cells																			(post.) 154.06	63.4	9
G ₁	102.39 (+4.02)	65.71 (+2.22)	91	---	---	---	69.9***	61.7	9	---	---	---	60***	63.1	---	125.2***	65.9	---	175.45	72.2	20
G ₂	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
G ₃	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
G ₄	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	159.58	84.3 (+1.8)	18	---	---	---
Anal pore	137.96 (+5.09)	88.44 (+1.83)	86	---	---	---	95.44	86.2	10	73.0	74.4	3	85	89.1	---	168.48	89.0 (+0.6)	38	204.36	84.1	20
Tail nuclei and arrangement																					
Stain and fixative	Dela. Hem. & Giemsa Alc. and ether			(not listed) (not listed)			Giemsa (not listed)			Giemsa (not listed)			Leishman (not listed)			Giemsa Alcohol			Aqueous Azur II. not given		
Host	<i>L. c. melanotis</i> Mearns			<i>L. pallidior</i> and <i>L. kabylicus</i>			Hares (<i>Lepus</i> sp.)?			<i>Lepus sefranus</i> Thomas (?)			<i>Lepus hawkeri</i> (?)			<i>Lepus tigrensis</i> (Blanford)			Snow shoe hare (<i>Lepus americanus</i>)		
Site larvae recovered	Blood from heart and lungs, and parent worms			uterus of female worm			Blood from internal organs and peritoneal fluid			peripheral blood			not given			Blood from internal organs			Lymph at site of infection in tarsal bursa		
Locality	Kearny Co. (Lakin), Kansas (U.S.A.)			Algeria			N. Eritrea, Africa			S. Oranais Algeria, Africa			Anglo-Egyptain Sudan			Eritrea, Africa			Minnesota (U.S.A.)		

* absolute measurements of the mean in microns, followed by the standard deviation of the mean in parenthesis.

** not applicable or data missing

*** not actual G₁ cells, see text

**** drawn from authors description

general stain, being more easily employed and requiring less time to perform than Delafield's Hematoxylin.

Hematoxylin base stains such as Hemalum and Delafield's (Guyer, 1953) have been employed by many workers for the study of filarial larvae: (Francis, 1916; Feng, 1933; Causey, 1939; Kotcher, 1941; Bell and Brown, 1945; Gibson, 1952; Mackie, Hunter and Worth, 1954; Hawking and Webber, 1955; Webber and Hawking, 1955; Iyengar, 1956; Newton and Wright, 1956; and Golvan, 1957).

Bell and Brown (1945) clearly demonstrated the presence of a sheath on the microfilariae of Litmosoides carinii by the use of alum Hematoxylin. Up to the time of their work, the authors indicate that the status of the sheath on the larvae of L. carinii was uncertain. Golvan (1957), working with larvae of Wuchereria bancrofti and W. malayi reported that hemalum stain consistently demonstrated the presence or absence of a sheath. He stated that the classic characteristic, the sheath, has lost some value because of the improper choice of stain when microfilarial descriptions are attempted. He indicated that Giemsa stains are good, general, morphological stains, but only rarely and inconsistently stain a sheath. The common blood stains such as Wrights are not suitable for demonstration and description of microfilariae in blood smears.

In the present study, no sheaths were found to be stained in Giemsa preparations. As indicated earlier, Delafield's Hematoxylin was observed to clearly stain the sheaths of the larvae of M. brevicauda.

In several instances, microfilariae from host blood were found to have no sheath when stained with Delafield's Hematoxylin. The larvae examined from the uterus of the female worms, as well as those recovered from the lymph in the peritoneal cavity of infected hares, were found to be sheathed. Kershaw (1948) found that the larvae of L. carinii, occurring in the pleural cavity of the cotton rat, lost their vitelline membrane (sheath) at birth and later through a "cuticle" molt, received another sheath which was demonstrated by most of the microfilariae in peripheral circulation. In other forms, the sheath was found to adhere close to the cuticle, thus making its presence hard to demonstrate. Similar observations were reported by Kershaw, (1949). These observations were made from Hematoxylin stained preparations.

From the data collected thus far, the absence of a sheath in some specimens, as found in the present study, is not fully explainable. It is quite possible that some degree of maturing takes place after parturition of the larvae which is characterized by an ex-sheathing process. However, many larvae were observed to be closely bound with a fine envelope which may have been an "early" sheath, not yet separated from the cuticle of the larvae.

The origin of the sheath as discussed in the literature by various workers, is not fully known. Some authors indicate that it is a mere stretching and extension of the vitelline membrane at parturition, whereas others indicate that it has

its origin as a molt from the cuticle of the larvae.

Description. The anatomical points or structures used in the description of the microfilariae of M. brevicauda (Table 1; Plate II) with few exceptions, were those of Price (1957). The exceptions noted are the rectal cells, G_2 to G_4 , and the Innen Körper which were not discernable in specimens of M. brevicauda. A camera lucida drawing and a photomicrograph of microfilariae from lung blood is given on Plate II; and Plate III, Fig. 3 respectively.

In the description of the microfilariae (below), the anatomical points or structures of the larvae are given as average distances in microns from the anterior end (absolute measurement) followed by, in parenthesis, the average percent distances of the total length of the larvae from the anterior end (percent measurement).

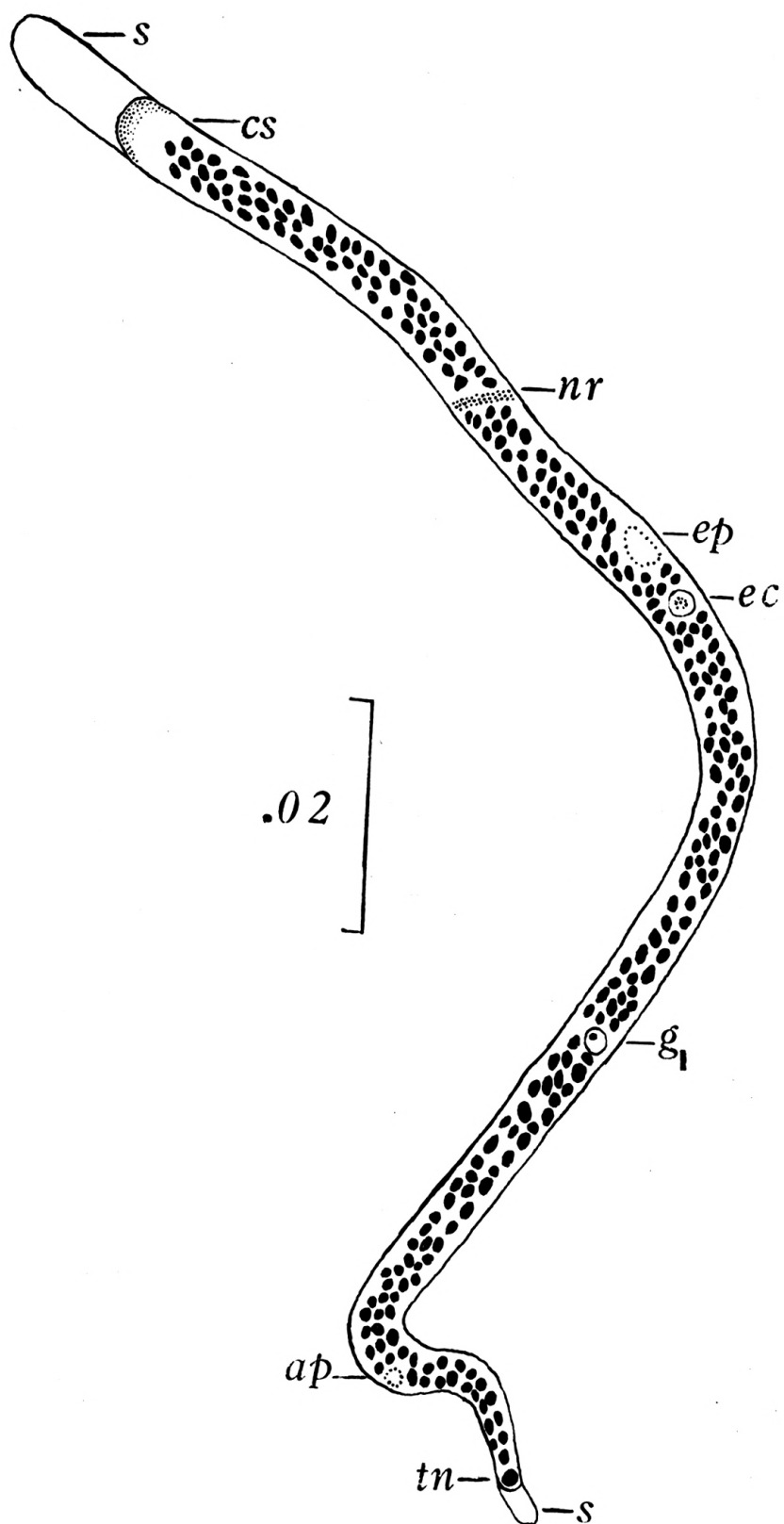
Microfilariae (Plate II; Table 1): 155.79 microns long; width, 4.59 microns; sheath, 181.75 microns; cephalic space, 3.75; nerve ring, 40.38 (26.00); excretory pore, 54.88 (36.64); excretory cell, 61.99 (39.90); rectal cells, G_1 102.39 (65.71); anal pore, 137.96 (88.44). Tail blunt, slightly tapered with moderately large tail nucleus at extreme tip. The last two or three tail nuclei in linear arrangement, smaller than the terminal nucleus. Innen Körper absent, genital cells G_2 , G_3 , G_4 not discernable, G_1 not readily discernable occurring at the break in the nuclear column posterior to the excretory cell. Nerve ring, excretory pore and anal pore appearing as breaks in

EXPLANATION OF PLATE II

A camera lucida drawing of a microfilaria of Micipsella brevicauda from lung blood of the host. The following symbols denote anatomical points. (scale in mm.)

ssheath
cscephalic space
nrnerve ring
epexcretory pore
ecexcretory cell
g_1first rectal cell
apanal pore
tntail nucleus

PLATE II



EXPLANATION OF PLATE III

Fig. 3. Photomicrographs of microfilariae of Micipsella brevicauda from lung blood of the host. (approximately 550X) The following symbols denoting anatomical points.

ssheath
cscephalic space
nrnerve ring
epexcretory pore
ecexcretory cell
g_1first rectal cell
tntail nucleus

Fig. 4. Photograph of a large coenurus (Multiceps spp.) located in the abdominal cavity of a hare. (The total length of the ruler is 158 mm.)

PLATE III



Fig. 3

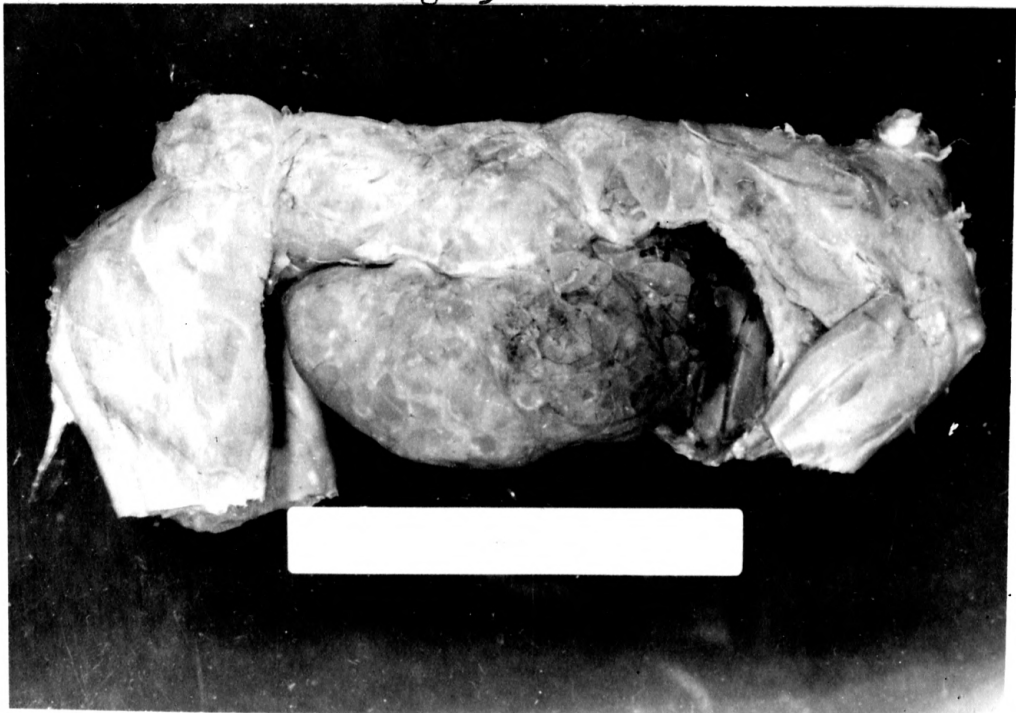


Fig. 4

the nuclear column.

Host: Lepus californicus melanotis Mearns

Location: blood stream and lymph of peritoneal cavity of host.

Locality: Kearney Co. (Lakin), Kansas

Other Microfilariae Occurring in Lepus spp. Seurat (1917) in the original description of the type species, Micipsella numidica, gave only a brief description of the microfilariae of that species (Table 1). He stated that the larvae measured 110 microns in length and four microns in width. The larvae were unsheathed, with a bluntly rounded tail. The larvae examined were taken from the uterus of female worms. He, however, did not give the techniques employed in the larval examination. Kalantarian (1924) gave no description of the microfilariae in his work. Ivashkin (1954) and Erhardova (1956) working with M. numidica in Mongolia and Europe respectively, gave no description of the microfilariae in their work.

Rao, M.A.M. (1938), reported M. indica, a new species from India, but did not give any detailed description of the microfilariae. He stated that the larvae were unsheathed, with a bluntly pointed tail. The technique upon which he based his brief description was not given.

Balfour (1911); Foley, Catanei and Vialatte (1926); Franchini and Genora (1931); Archetti (1947); Call (1949) gave accounts of microfilariae as recovered from Lepus spp. but gave no account of or a classification of the adult worms (Table 1).

Balfour (1911) reported microfilariae from L. hawkeri (?) along with finding two adult parent worms in the body cavity of the hares. Foley, Catanei and Vialatte (1926) while working in South Oranais, Algeria, found two hares L. safranusi (Thomas) infected with microfilariae. They indicated that their findings compared very closely to those of Balfour (1911) and Seurat (1917). They concluded that the microfilariae recovered from the infected hares were those of M. numidica. Franchini and Ganora (1931) demonstrated microfilariae in L. aegyptius. Archetti (1947) found microfilariae in L. tigris in Africa. Call (1949) reported microfilariae from three hares (Lepus spp.)? in Northern Eritrea. Since Seurat (1917) reported M. numidica from L. pallidior and L. kabylicus (now considered L. capensis) from Algeria, it is quite possible that above listed records of microfilariae are those of M. numidica (Table 1).

Table 1 lists a comparison among microfilariae of Micipsella species, probable Micipsella species (Archetti, 1947; Call, 1949; Foley et al., 1920; Balfour, 1911), and Dirofilaria scapiceps as occurring in Lepus species. The microfilariae as reported by Call (1949), Foley, Catanei and Vialatte (1926), and Balfour (1911), compare quite closely in measurements of total length and width to those of Seurat (1917). However, since data is lacking in Seurat's description, further identification of these unknown filarial larvae can not be made.

It is interesting to note the striking similarity between the morphology of the tail, and morphology and arrangement of the tail nuclei among the Micipsella and probable Micipsella species (Table 1). The large nucleus, located at the tip of the slightly tapered, bluntly rounded tail appears to be characteristic of Micipsella spp. microfilariae. The morphology and arrangement of the tail nuclei of the probable Micipsella spp. were drawn according to their authors description from text. In contrast, the tail nuclei of D. scapiceps (Table 1) according to Highby (1943), are four to seven in number, arranged in linear fashion, with the last nucleus located at the tip of the tail. The tail is moderately tapered, with the tail nuclei becoming progressively more attenuated toward the posterior end.

The presence or absence of a sheath in the microfilariae of M. numidica and probable Micipsella spp., apparently, is not clear (Table 1). Archetti (1947), Call (1949) and Foley et al. (1926) all indicate the larvae as being unsheathed. It is quite possible, since Giemsa stain was used in the staining of these microfilariae (Table 1), that a sheath was present but not detected. As discussed earlier, Giemsa has been proven to be unreliable as a consistent sheath stain. Yorke and Maplestone (1926) listed the larvae as being unsheathed in the generic description of the genus Micipsella. Seurat (1917), in the original description of M. numidica indicated that the larva were unsheathed. He, however, did not indicate the stain used and on what basis he made his description. As indicated

earlier, the microfilariae of M. brevicauda (Plate III, Fig. 3) upon staining with Delafield's Hematoxylin, clearly demonstrated a sheath. But no sheath was indicated when stained with Giemsa.

From the data collected thus far on the microfilariae of Micipsella spp., it appears that the generic description of the genus Micipsella (Yorke and Maplestone, 1926) should be amended to include sheathed larvae. It should prove challenging to secure adult worms and fixed thick blood smears from infected hares with M. numidica from Dr. Ivashkin and Dr. Erhardova, stain them with Delafield's Hematoxylin and more clearly establish the status of the sheath and morphology of microfilariae of M. numidica.

The status and position of the rectal cells, G_1 , G_2 , G_3 , G_4 in M. brevicauda and probable Micipsella spp. appears to be uncertain. In the present study the rectal cells, G_2 , G_3 , G_4 were not discernable. The G_1 cell, located on the anterior margin of the break in the nuclear column, posterior to the excretory cell, was difficult to observe in most specimens. The genital cells, apparently, are very small and fail to demonstrate special staining characteristics. The use of Methyl-green pyronin, Azur II and Eosin as well as Delafield's Hematoxylin and Giemsa stains, failed to clearly differentiate the rectal cells.

Archetti (1947) observed the G_1 in only four specimens (Micipsella spp.)?. The other genital cells were small, near

each other and arranged in a linear fashion. G_4 was located approximately 10 microns anterior to the anal pore. He did not list the locations of G_1 , G_2 , G_3 , nor the frequency at which he observed these structures.

Archetti (1947) reported finding an interruption in the nuclear column located anterior to G_1 at approximately 66 percent of the total distance of the larva from the anterior end. This break was oval in shape and stained light violet. He did not associate any name or function with this internuclear space. Call (1949) described a similar nuclear interruption located at approximately 62 percent of the total distance from the anterior end. He called it the "central spot", but associated no function or significance with it. A similar morphological characteristic was also listed by Balfour (1911).

In the present study, a similar nuclear interruption was noted in the microfilariae of M. brevicauda. The break in the nuclear column was noted immediately posterior and adjacent to G_1 (Plate 1). The significance or function of these nuclear interruptions is not known. It appears from the data collected thus far (Table 1), these interruptions are characteristic of Micipsella microfilariae.

It is worthwhile noting the close relationship of the percent measurements of the internuclear spaces among the larvae of M. brevicauda and those listed by Archetti (1947), Call (1949) and Balfour (1911) (Table 1). Since the rectal cell, G_1 occurs

near the internuclear interruption in the microfilariae of M. breviceauda, it was thought that comparable relation may exist in the probable species of Micipsella (Archetti, 1947; Call, 1949; and Balfour, 1911). For this reason and for the sake of simplicity, the measurements of the breaks in the nuclear column are listed as rectal cells, G_1 in Table 1.

In summary, the presence of a sheath; the internuclear space near G_1 ; the large terminal tail nucleus; and the slightly tapered, bluntly rounded tail appear to be characteristic of the microfilariae of the genus Micipsella. Since data are lacking in the description of the microfilariae of M. numidica and M. indica, the differentiation and species characteristics of the microfilariae of Micipsella species can not be listed at this time.

Experimental Infections. Transplants. In an effort to establish an artificial infection of M. breviceauda in the laboratory white rabbit, filarial worms were transplanted directly from infected hares to laboratory white rabbits (Table 2).

In only one instance were viable worms recovered at necropsy (Table 2). Rabbit 4679 received three worms of unknown sex on September 20, 1958. Seventy-six days post-transplantation, two viable female worms were recovered. No indication of the fate of the third worm was found.

Cysts were found in three of the rabbits during autopsy (Table 2). Two of the cysts (rabbits 4687 and 4688) were attached to the peritoneal wall between the site of incision made during transplantation and the anus. The third cyst (rabbit

4684) was found unattached in the body cavity, lying in the folds of the cecum.

These cysts appear to be the end results of the hosts defensive mechanism against the peritoneally introduced worms. Fragments of worms could be seen in the cysts collected from rabbits 4687 and 4688. These fragments were surrounded by yellowish granular material. The cyst collected from rabbit 4684 showed no worm fragments. No evidence of any gross pathology or after effects of the operations was noted in the rabbits at necropsy. A detailed histological study of these cysts is given in the section on Pathology. Victor (1958) conducted transplants of Setaria digitata into the peritoneal cavity of a rabbit. The rabbit received six male and 12 female worms through an abdominal incision. Blood samples taken every four hours up to 28 days revealed no microfilariae. No adult worms were found at necropsy, however, three small pea-sized nodules were found attached to the omentum. These were found to contain only some caseous material.

Webber and Hawking (1955) transplanted Dipetalonema digitatum and D. gracile from infected to non-infected monkeys. These filariids were found to occur in the peritoneal cavity of the host. Microfilariae were recovered as early as two and one-half months after transplant with the microfilaraemia persisting for six months post-transplantation. The adult worms were not recovered at necropsy. No pathology was reported as being caused by the worms in the natural infections.

Williams (1955) successfully transplanted Setaria cervi

Table 2. Transplants of worms (*Micipsella brevicauda*) from hares to laboratory white rabbits.

Rabbit No.	Transplants			Microfilariae (blood samples)*				Necropsy		
	Date	Number worms	Site	Avg. amt.	Number	Avg. time	First sample	Post-trans.	Findings	Findings
		Male : Female :		(cc.)		(days)	post-trans. (days)	(days)		
4677	9-19-58	3**	ear	1.24	6	21.8	24	neg.	201	no worms, cysts, or microfilariae
4679	9-20-58	1** 2	ear	1.05	4	18.7	11	neg.	76	two ♀ worms (viable), no cysts or microfilariae
4683	3-27-59	2 3	ear	1.78	2	59.0	99	neg.	267	no worms, cysts, or microfilariae
4684	3-28-59	2 4	ear	1.78	2	59.0	98	neg.	266	one cyst, no worms or microfilariae
4687	1-8-60	3 2	ear	2.0	5	2.5	3	neg.	15	one cyst, no worms or microfilariae
4688	1-9-60	2 12	ear and heart	2.87	8	2.3	3	neg.	21	one cyst, no worms or microfilariae

* Samples taken between transplantation and necropsy

** Sex unknown

from slaughtered cattle into the laboratory rabbit. Microfilariae were recovered as early as 56 hours after the transplant. Five adult worms were recovered eight days after the implant. The worms were suspended in saline from one to two hours before being used in the transplant.

Webber (1954) working with transplants of Litomosoides carinii in the cotton rat, found that worms kept in Ringer's solution more than one-half hour did not survive the transplant as well as those suspended for only a few minutes. Microfilariae appeared at a minimum of a few days and at a maximum of 49 days post-transplantation.

In some cases, she found that even though members of both sexes were transplanted, no microfilariae were found in the blood samples. Reasons given for this were: worms were dead, immature or encapsulated; microfilarial development in uterus of female interrupted by transplant; worms attacked by phagocytes and females not inseminated. In some cases upon autopsy, microfilariae were found in the pleural cavity but for some reason did not get into the blood stream.

She also stated that experiments showed that repeated insemination of female worms was not necessary for maintaining microfilaria in the host. The duration of the microfilaraemia and maximum number of microfilariae present were less in the transplants than in the normal infections. She concluded that the transplantation technique was harmful to the worms.

Wharton (1946) transplanted Litomosoides carinii from

infected to non-infected cotton rats. Three days after transplant, the worms were dead and in a mass which later became a syncytium of degenerating worms with a serous covering. Similar results were reported by Bertram et al. (1946); Kershaw, and Bertram (1948) and Wharton (1947). Microfilariae were found in the blood and pleural cavity for only about one week. Transplants were successful, however, when made to infected, and splenectomized rats.

Tisseuil (1935) transplanted into the peritoneal cavity of the opossum (Philander sp.), a male and female filarial worm (probably Cortiamosoides philanderi, Foster, 1939). Microfilariae were found on the 12th day after transplant. Necropsy 18 days post-transplantation revealed no worms or microfilariae. A large grayish nodule was found attached to the omentum which upon dissection and staining showed fragments of adult worms and microfilariae.

The white laboratory rabbit apparently possesses a strong and quick defensive mechanism in the walling off and removal of foreign material within its peritoneal cavity. Vinnitsky (1944, 1951) found that, while artificially inducing ascarids into the peritoneal cavity of various animals, the rabbit possessed a very rapid leucocyte exudation and granulation which led to a speedy death as well as encapsulation of the induced worms. The capsule then, in turn, was subject to resolution and in time was removed without trace.

The results of our experiments with M. brevicauda as compared to those of Williams (1955) are not fully explainable.

It is possible that the defense mechanism of the bovine is comparable to that of the white rabbit, therefore the worms (S. cervi) were not subjected to a great change when transplanted to the rabbit and were able to live for a period of time post-transplantation. Conversely, it appears that the defensive mechanism of the natural host (L. c. melanotis) is well tolerated by the worms (M. brevicauda), but are unable to withstand the more violent defensive mechanism of the experimental host, the laboratory rabbit. However, in our experiments (Table 2), it is interesting to note that viable worms were recovered from rabbit 4679, 76 days post-transplant, but none from rabbits 4687 and 4688 which were autopsied 15 and 21 days post-transplant respectively. The difference between these two above mentioned results may be attributed to the possibility that the worms were less viable in the latter two cases, and when subjected to handling during the transplant were killed and quickly walled off by the rabbit after the implant. The cyst, composed of degenerating worms, recovered near the site of inoculation would also tend to support this idea.

The viable worms recovered from rabbit 4679 contained no advanced stages of developing microfilariae in utero. It may have been that the transplant stunted the normal function of the reproductive system or the worms were not inseminated.

The absence of microfilariae in the blood stream (Table 2), is not fully explainable. Blood samples taken during the course of the infection as well as peritoneal fluid smears at autopsy,

failed to demonstrate microfilariae. Blood samples were taken during the day and night so the larvae would not be missed if they demonstrated a periodicity. Since the reproductive condition of the worms before the transplant was not known, it is uncertain if the transplant technique stunted the normal function of the reproductive system thus stopping reproduction indefinitely. It is also probable that microfilariae were liberated by the worms and were quickly walled off and resolved by the host. However, it must be kept in mind that in the case of rabbits 4677, 4683 and 4684, a relatively long lapse occurred between blood samples as well as between the first blood sample and the date of transplantation (Table 2), thus, the microfilaraemia could have been missed.

From data in Table 2, it appears that the filarial nematode, M. brevicauda does not adapt itself to the laboratory white rabbit.

Field Exposures. Three white rabbits were exposed in the field where known infected hares existed. These rabbits were exposed in order that they might receive a natural infection from the intermediate host(s). Periodic peripheral blood-samples taken from the ear, between the exposure and autopsy, revealed no microfilariae.

Rabbit 4680 was autopsied on July 6, 1959, 332 days after exposure on August 8-10, 1958. Rabbits 4681 and 4682 were exposed August 8-10 and 14-18, 1958, and autopsied July 6, 1959, 325 days post-exposure. No worms or cysts were found at

necropsy.

Mosquitoes were seen feeding periodically on the ears of the rabbits during the exposures.

In light of the results obtained, the white rabbit does not appear to be a suitable experimental host for M. brevicauda. However, it must be kept in mind, since the intermediate host is not known, nor the period of the year when it is active and present in the area, the true value of these results obtained through the exposures can not be evaluated.

It is quite probable that the intermediate host was not present during the exposure period in August, 1959; ectoparasites are serving as intermediate hosts which would have greatly reduced the chance for receiving the infection because of lack of close association between infected hare and rabbit; that the period of exposure was too brief to give the intermediate host an opportunity to feed on the rabbit; the intermediate host and the rabbit were incompatible (an intermediate host with a high degree of host specificity).

Pathology. Macropathology. As indicated earlier, many cysts (Plate IV) were recovered from the peritoneal cavity of the hares upon necropsy. The cysts ranged in size from .5 to 28 mm. in diameter. Most of the cysts were yellow, the smaller ones being round or oval and the larger ones usually more flattened. The smaller cysts, ranging in size from .5 to 5 mm. were located predominately in connection with the omentum. The larger ones were usually randomly located in the

EXPLANATION OF PLATE IV

- Fig. 5. Cysts (nodules, lesions) as recovered from the peritoneal cavity of the hares. (ruler is metric)
- Fig. 6. Filarial nematodes (M. brevicauda) partially or fully enveloped by the host in the form of cysts. (ruler is metric)

PLATE IV



Fig. 5

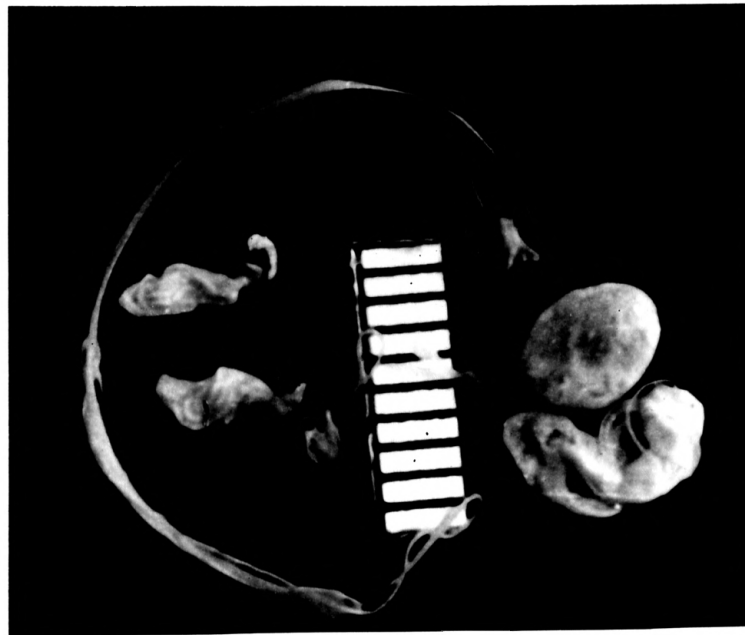


Fig. 6

body cavity. Many of the small to medium sized cysts were attached to the peritoneal wall and mesenteries by a fine stalk. No cysts or filarial worms were found in the pleural cavity of necropsied hares.

It is probable that many of these cysts, as recovered from the peritoneal cavity of infected hares, were produced when filarial worms died and were encapsulated by the host.

Micropathology. Histological examination of various lesions (cysts), stained with Azur Eosin and Von Kossa's, indicated that caseation necrosis (Plate VII, Fig. 12) and calcification (Plate V, Fig. 7) was present in various degrees. In some instances, the nodule (cyst) was encapsulated by dense irregular connective tissue. The lesions examined showed no consistency as to cell arrangement or type. Only a few giant cells or tissue basophils and eosinophils were noted. Since the presence of a large number of eosinophils is highly characteristic of nematode parasitic invasions, it is worthwhile noting the few eosinophils observed in these nodules. No explanation is offered for this irregularity. Many of the nodules were relatively acellular and aplastic, while others showed cellular organization demonstrating rows of cells parallel to the nodule or alternating with rows of cells undergoing necrosis. The cells undergoing necrosis were found adjacent to areas showing nothing but coagulation necrosis.

Microfilariae and adult worms were noted in various

EXPLANATION OF PLATE V

- Fig. 7. Cross section of a cyst from the peritoneal cavity of a hare showing calcification (dark center area, arrow) as stained with Von Kossa's stain.
(approximately 50 X)
- Fig. 8. Cross section of a cyst from the peritoneal cavity of a hare showing filarial worms undergoing necrosis.
(arrows point to sections of worms)
(approximately 50 X)

PLATE V



Fig. 7

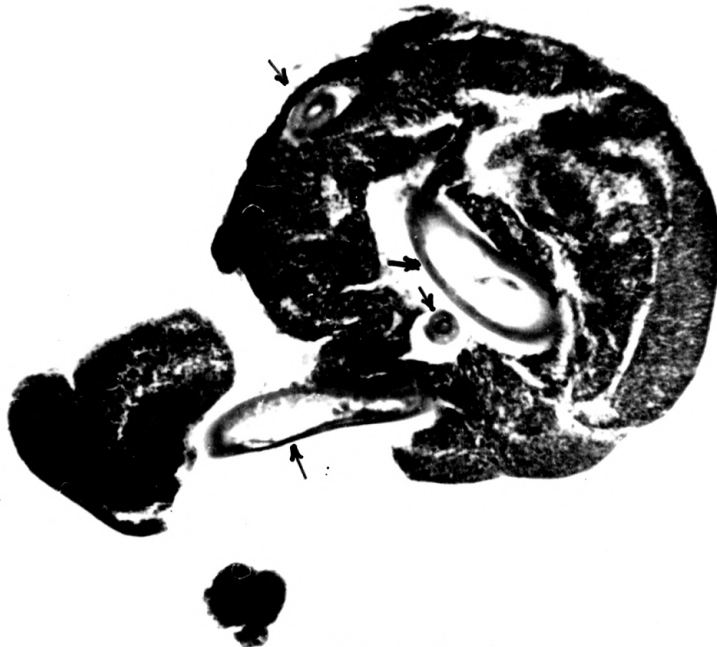


Fig. 8

EXPLANATION OF PLATE VI

- Fig. 9. Cross section of a cyst from the peritoneal cavity of an experimentally infected laboratory rabbit showing a capsule of dense irregular connective tissue (arrow) underlined by a few leucocytes and an area of caseation necrosis. (Hematoxylin and Eosin stain) (approximately 210 X)
- Fig. 10. Cross section of a cyst from the peritoneal cavity of a hare showing calcification (dark area) and microfilariae (center). (Hematoxylin and Eosin stain) (approximately 210 X)

PLATE VI

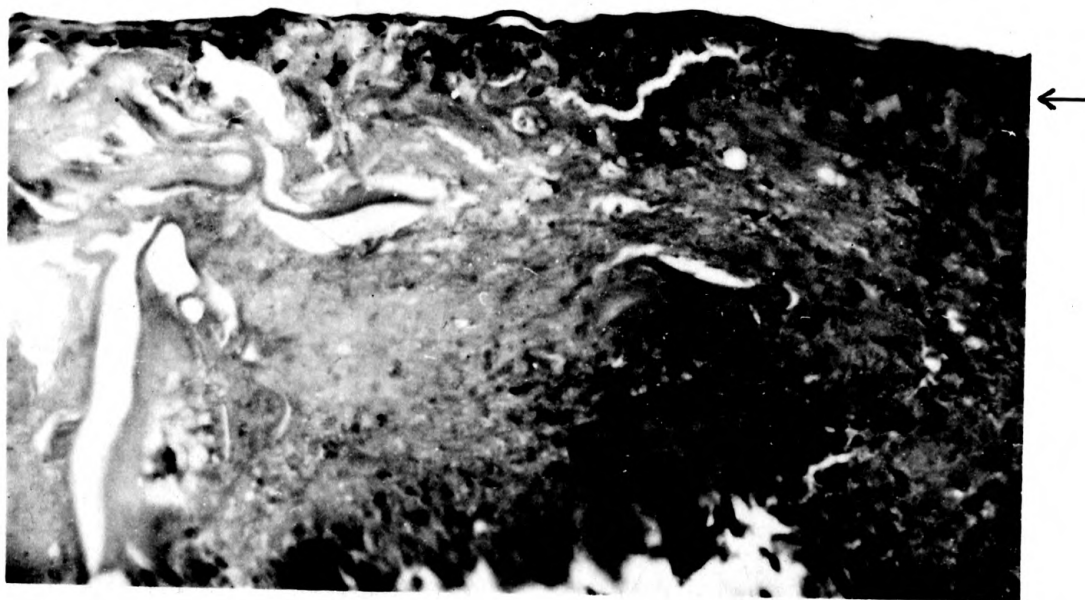


Fig. 9

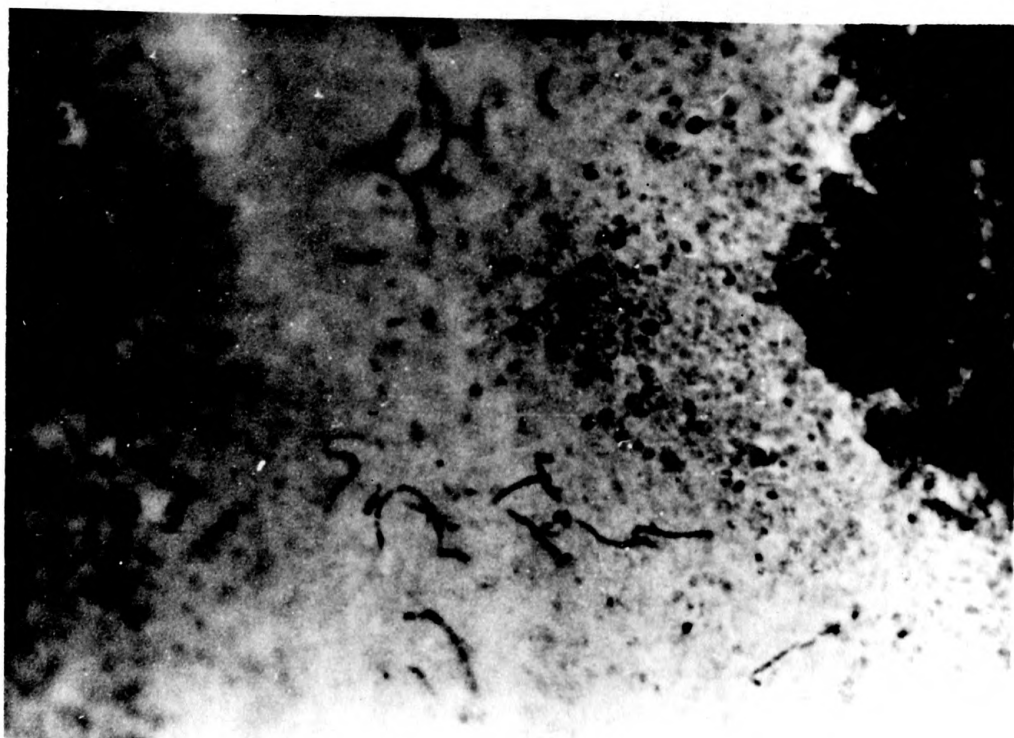


Fig. 10

EXPLANATION OF PLATE VII

Fig. 11. Cross section of a cyst recovered from the peritoneal cavity of a hare showing various cell types. (Hematoxylin and Eosin stain) (approximately 550 X)

Alymphocyte

Bgranular cell (tissue
basophil)

Cmacrophage

Dneutrophil

Fig. 12. Cross section of a cyst recovered from the peritoneal cavity of a hare showing caseation necrosis. (Hematoxylin and Eosin stain) (approximately 550 X)

PLATE VII

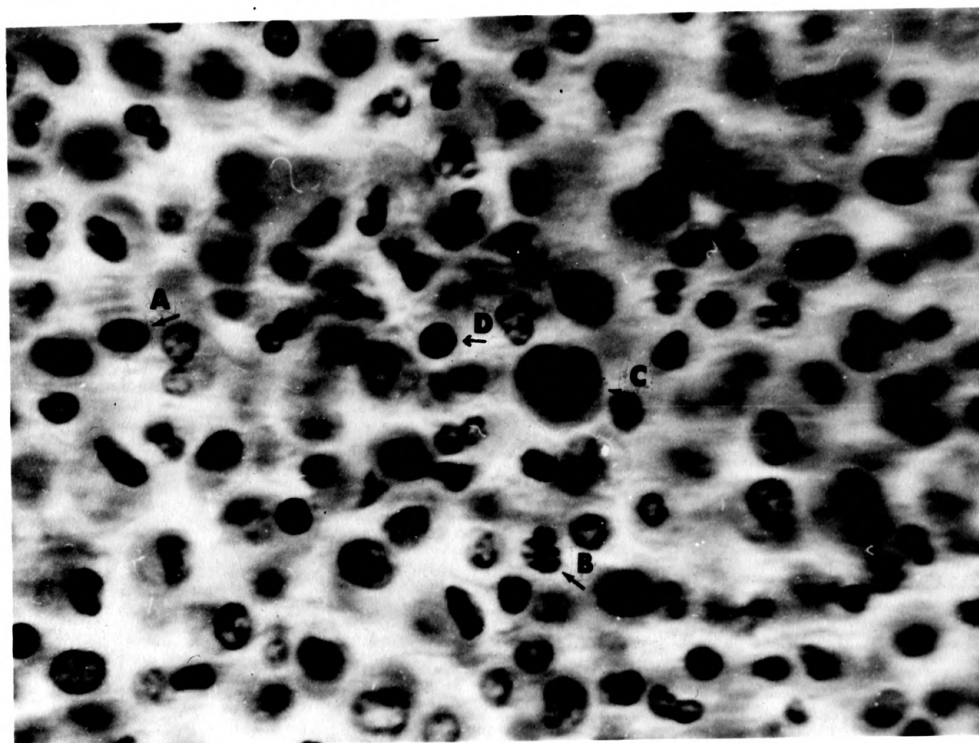


Fig. 11

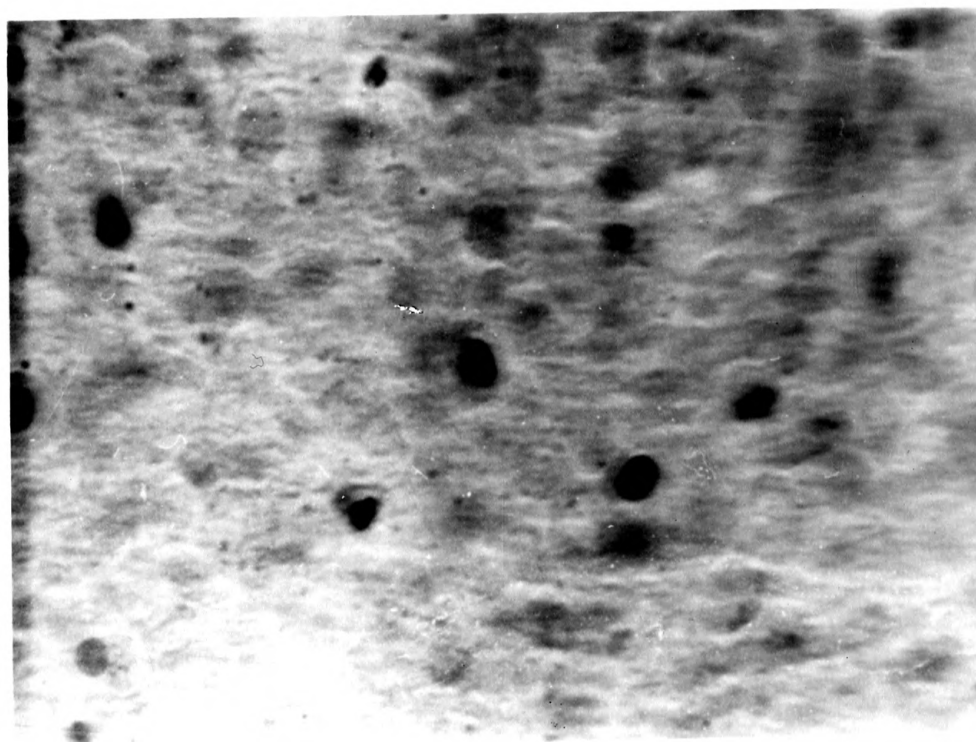


Fig. 12

nodules (Plate V, Fig. 8; Plate VI, Fig. 10). The adult worms in most cases were noted in various stages of necrosis.

Plate VII, Fig. 11, shows various cell types as found in a cross section of one of the nodules. A lymphocyte, granular cell (tissue basophil), macrophage and a neutrophil were noted as indicated by the lettered symbols, A, B, C, D, respectively.

As noted elsewhere, in an effort to establish an artificial infection of M. brevicauda in the laboratory rabbit, worms were transplanted from infected hares into the peritoneal cavity of the laboratory rabbit. The end result (Table 2) in most cases was a walling off process of the worms in the form of cysts by the rabbit.

Histological studies of a lesion (cyst) from rabbit 4684 indicated, upon Hematoxylin and Eosin staining, the presence of necrotic filarial nematode tissue and microfilariae. The main mass of the nodule consisted of caseation necrosis. The nodule was enveloped in a capsule of dense irregular connective tissue under which were located a few leucocytes (Plate VI, Fig. 9).

A second lesion from rabbit 4687 differed from the case cited above in that it was more of an inflammatory type reaction. Large numbers of lymphocytes and polymorphs were noted. A few granular cells were seen which probably were tissue basophils. A few fibroblasts and areas of caseation necrosis were observed. In contrast to the case observed above, this nodule was not encapsulated by a connective tissue envelope. This lesion was

connected to the peritoneal wall at various points by what appeared to be mostly collagen fibers and fibroblasts.

Vinnitsky (1944) introduced ascarid worms into the body cavities of various laboratory animals in an effort to study the peritoneal cavity defensive reaction of experimental hosts. He indicated that of the animals employed (guinea pigs, rabbits, cats and dogs) the rabbit possessed the most violent and quickest response to foreign material in the body cavity. It possessed a most impetuous leucocyte exudation and granulation which lead to an early death and encapsulation of the worms. Multiple coalescences between abdominal organs was also noted. In spite of a heavy band of polynuclear cells, which were found to surround the worms, no leucocytes were noted to have penetrated the cuticle. The leucocytes surrounding the necrotized ascarids were transformed in time, to a structureless necrotic mass. From the data collected thus far, the filarial nematodes (M. brevicauda) apparently are unable to withstand the defensive mechanism of the laboratory rabbit, thus making it an unsuitable experimental host.

At present, it is impossible to state the entire histopathological history of the lesions as found in the peritoneal cavity of the hares. Since the life-cycle is not known, the relation of the cysts to: the life span of the filarial worms; microfilarial migrations; and infective filarial larvae migrations as deposited by the intermediate host in relation to their termination as developing adult worms in the body cavity,

can not be explained at this time.

Host Specificity. To establish a clearer picture of the host specificity of M. brevicauda in Western Kansas, small mammals other than L. c. melanotis were necropsied (Table 3). It was thought that this filarial infection, when compared to that of D. scapiceps, might be found in the cotton tail rabbit (Sylvilagus spp.). The autopsy of 75 specimens revealed no filarial infections (M. brevicauda). Other mammals necropsied, locality of collection and percent infected are listed on Table 3.

It is worthy to note the host specificity of Dirofilaria scapiceps (Leidy, 1886). Penner et al. (1953) reported it from S. floridanus, MacLulich (1937) collected it from L. americanus, while Schwartz and Alicata (1931) reported its microfilariae from L. washingtonii. Hall (1916), Alicata (1931), and Crites and Phinney (1958) also report it from various subspecies of S. floridanus.

From the limited data collected (Table 3), it appears that M. brevicauda might have a relatively high degree of host specificity.

Distribution of Micipsella brevicauda in Kansas. From the data collected (Table 4), it appears that the infection of M. brevicauda in L. c. melanotis is limited to the western one-third of Kansas (Plate VIII). However, since no data has been collected in the northern portion of the western one-third, and sufficient data is lacking from the eastern half of

Table 3. Mammals, excluding Lepus californicus melanotis, examined for Micipsella brevicauda in Western Kansas.

Species Examined	County				Total No. : examined	Percent infected
	: Kearny	: Hamilton	: Lane	: Meade		
<u>Sylvilagus floridanus</u> and <u>S. audubonii</u>	60	6	8	1	75	0.0
<u>Cynomys ludovicianus</u>	0	70	4	0	74	0.0
<u>Dipodomys ordii</u>	12	0	0	0	12	0.0
<u>Taxidea taxus</u>	7	1	1	0	9	0.0
<u>Spermophilus</u> <u>tridecemlineatus</u>	2	1	3	0	6	0.0
<u>Vulpes velox</u>	2	0	0	0	2	0.0
<u>Peromyscus maniculatus</u>	2	0	0	0	2	0.0
<u>Didelphis marsupialis</u>	2	0	0	0	2	0.0
<u>Erethizon dorsatum</u>	1	0	0	0	1	0.0

Table 4. Counties in Kansas surveyed for Micipsella brevicauda in Lepus californicus melanotis. Approximate locality given by towns in parenthesis.

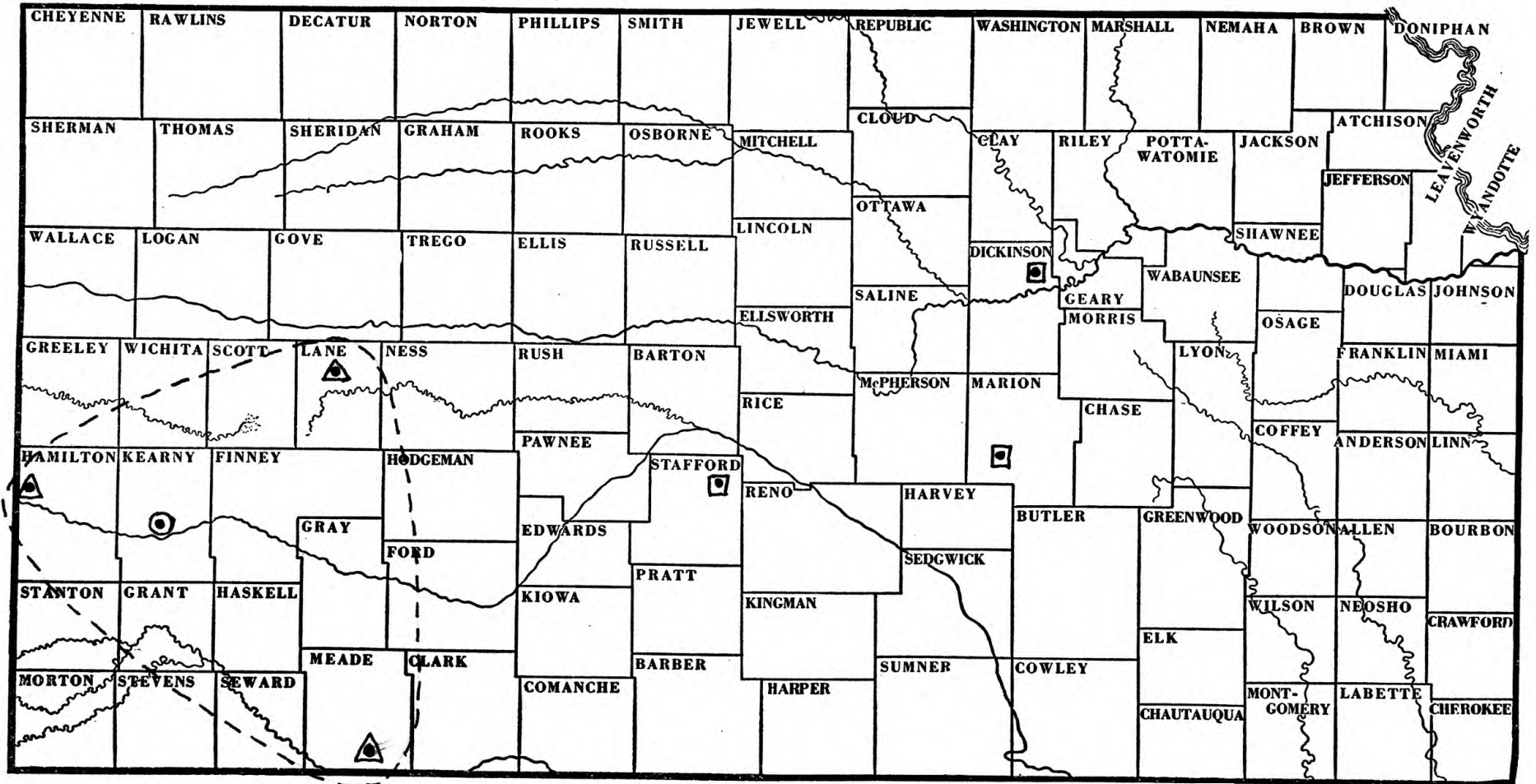
Locality	Date of survey	<u>M. brevicauda</u>		Cysts		Microfilariae (heart blood)	
		Number	Percent	Number	Percent	Number	Percent
		examined	infected	examined	infected	examined	infected
Hamilton (Coolidge)	Aug. 1959	21	14.3	21	9.5	17	35.3
Meade (Meade)	Aug. 1959	16	12.5	20	10.0	13	7.7
Lane (Dighton)	Jul. 1959	45	2.2	45	2.2	23	4.3
Marion (Hillsboro)	May to Sept. 1959	20	0.0	20	0.0	0	
Dickinson (West of Junction City)	Feb. 1960	4	0.0	4	0.0	0	
Stafford (Seward)	Sept. 1959	2	0.0	2	0.0	0	
Kearny (Lakin)	present study (Jun. 1958 - Jan. 1960)	728	10.71	728	8.93	358	12.59

EXPLANATION OF PLATE VIII.

Distribution of M. brevicauda infections in L. c. melanotis in Kansas.

- ⊙central study area (Lakin)
- ◻no M. brevicauda recovered
-indicates approx. endemic area
- △location of known infections

PLATE VIII



the state, the true picture of this filarial distribution is not known. It must also be noted that the collections from Hamilton, Meade, and Lane Counties which were made in the summer of 1959, if paralleled to those made in the Lakin study area, were made when the infection rate was at a lower level (Plate XI, Fig. 14). Therefore, it may be possible that the Lane County collection showing only a 2.2 percent infection in 45 hares examined, may be at the low phase of the seasonal fluctuation and not necessarily at a low overall infection rate of the hares in that area.

It is interesting to note the great difference in percent infection of microfilariae between the Hamilton and Meade County collections (Table 4). The number of hares examined in each collection differs only by five, but the percent infected with microfilariae differs by 27.8 percent. The percent infected with filariids varies only by less than 2.0 percent. The blood handling techniques, as described earlier, as well as the time of day the collections were made, were kept consistent in all collections as listed on Table 4. Since the techniques were kept consistent, the reason for this great difference, other than sample error, is not known. Other than the percent infected with microfilariae, the Hamilton and Meade County collections compare favorably to the Kearny County collection, the center of the study area.

Part of the collections made in the Hamilton and Meade Counties (Plate VIII) were made near the Colorado and Oklahoma

borders respectively. The percent infected in each area may indicate that this filarial parasite could also be found in eastern Colorado and northwestern Oklahoma.

Helminth Parasite Study

The 269 hares examined in this study were found to harbor no sheep or cattle parasites. No trematodes were found in this study. No adult nematodes were recovered from the kidneys, lungs or heart.

Table 5 shows the parasites and cysts recovered during the present study and the number and percent hares infected. The table includes a comparison of the percent hares infected of the present study with that of Lyons (1958).

All classification of parasites recovered during this study was according to Lyons (1958).

No parasites in addition to those reported by Lyons (1958), except microfilariae of M. brevicauda, were recovered in the present study.

It should be noted that only nine hares were examined in July 1958, thus accounting for only 269, instead of 270 hares which would have been examined during the 27 monthly collections of 10 hares each.

Micipsella brevicauda. A special study was made of the relationships among M. brevicauda, microfilariae in host blood and cysts as recovered from adult and juvenile hares (Tables 6, 7 and Plates IX, X). Data in Table 7 are shown by graph in Plates IX and X.

Table 5. Helminths, cysts, and microfilariae recovered from 269 hares in Kearny Co.
(Lakin), Kansas.

Item	: No. : :infected:	Percent infected		
		Bartel 1960	: Lyons 1958	(130 hares)
<u>Raillietina</u> spp.	147	54.6	66.0	(2)*
<u>Passalurus nonanulatus</u>	63	23.4	75.0	(1)
<u>Micipsella brevicauda</u>	62	23.0	50.0	(3)
<u>Nematodirus arizonensis</u>	30	11.1	5.0	(8)
<u>Multiceps</u> spp. (coenuri)	29	10.8	19.0	(4)
<u>Nematodirus</u> spp.	11	4.1	17.0	(5)
<u>Physaloptera</u> spp.	11	4.1	2.0	(9)
<u>Taenia pisiformis</u> (cysticerci)	10	3.7	7.0	(6)
<u>Dermatoxys veligera</u>	1	0.37	6.0	(7)
<u>Nematodirus leporis</u>	1	0.37	1.5	(10)
<u>Dirofilaria scapiceps</u> (234 hares)	0	0.0	---	**
Cysts	28	10.4	--	
Microfilariae (138 hares)	19	13.8	--	

* Indicates rank in Lyons' study

** Not examined for

Table 6. Incidence of Micipsella brevicauda, microfilariae, and (Lakin), Kansas.

Adults		Microfilariae (blood)						Cysts			
		Peripheral (ear)		Heart		Lung		Irrespective of site			
Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
examined	infected	examined	infected	examined	infected	examined	infected	examined	infected	examined	infected
728	10.71	257	5.83	358	12.59	209	15.78	425	14.82	728	8.93

cysts in the total number of hares examined in Kearny Co.

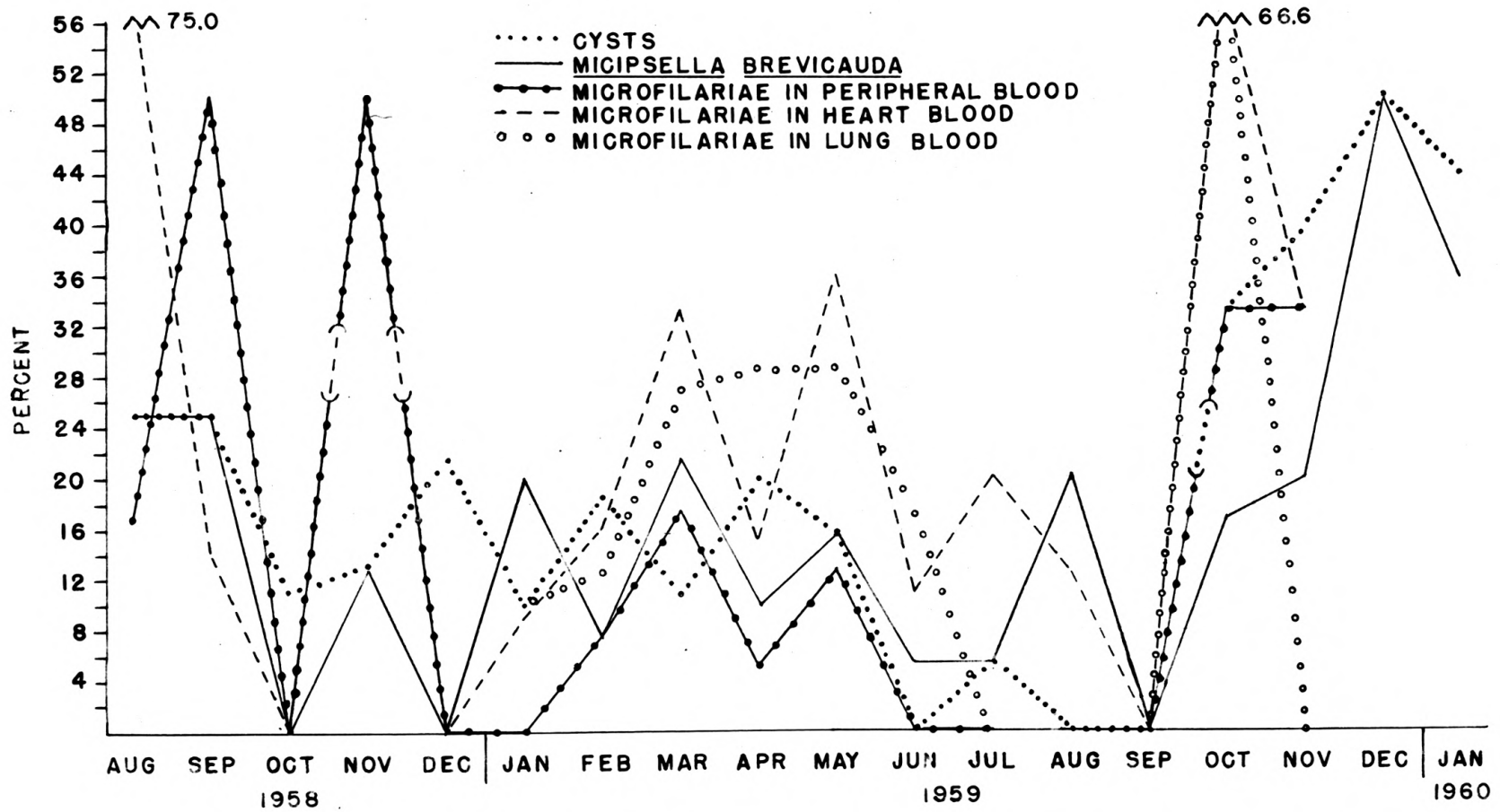
Table 7. Seasonal relationships between infections of adult and juvenile hares with cysts, microfilariae, and *Micipsella brevicauda*.

Date	Cysts						Microfilariae (blood)										<i>Micipsella brevicauda</i>			
	Adults			Juveniles			Peripheral (ear)		Heart		Lung		Lungs		Adults		Juveniles			
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
	examined	infected	examined	infected	examined	infected	examined	infected	examined	infected	examined	infected	examined	infected	examined	infected	examined	infected	examined	infected
Aug. 58'	12	25.0	40	5.0	6	16.7	14	0.0	4	75.0	19	10.5	--	--	--	--	12	25.0	40	2.5
Sep. 58'	12	25.0	31	3.2	2	50.0	15	0.0	7	14.3	19	0.0	--	--	--	--	12	25.0	31	0.0
Oct. 58'	9	11.1	36	0.0	1	0.0	8	0.0	3	0.0	22	4.5	--	--	--	--	9	0.0	36	0.0
Nov. 58'	15	13.3	32	3.1	2	50.0	14	0.0	6	50.0	21	0.0	--	--	--	--	15	13.3	32	3.1
Dec. 58'	14	21.4	14	7.1	7	0.0	8	0.0	12	0.0	13	0.0	--	--	--	--	14	0.0	14	14.3
Jan. 59'	30	10.0	0		18	0.0	0		22	9.1	0		20	10.0	0		30	20.0	0	
Feb. 59'	38	18.4	0		26	7.7	0		25	16.0	0		24	12.5	0		38	7.9	0	
Mar. 59'	37	10.8	1	0.0	17	17.6	0		21	33.3	0		26	26.9	0		37	21.6	1	0.0
Apr. 59'	30	20.0	1	0.0	19	5.3	0		26	15.4	0		21	28.6	0		30	10.0	1	0.0
May 59'	32	15.6	2	0.0	16	12.5	0		25	36.0	0		21	28.6	0		32	15.6	2	0.0
Jun. 59'	18	0.0	11	0.0	16	0.0	0		18	11.1	0		18	16.7	2	0.0	18	5.5	11	0.0
Jul. 59'	18	5.5	40	2.5	9	0.0	3	0.0	10	20.0	7	0.0	10	0.0	6	0.0	18	5.5	40.0	0.0
Aug. 59'	5	0.0	17	0.0	--	--	--	--	8	12.5	8	0.0	--	--	--	--	5	20.0	17	0.0
Sep. 59'	3	0.0	33	9.1	2	0.0	14	7.1	1	0.0	20	5.0	2	0.0	19	10.5	3	0.0	33	9.1
Oct. 59'	6	33.3	27	3.7	3	33.3	17	0.0	3	66.6	18	0.0	3	66.6	20	0.0	6	16.7	27	7.4
Nov. 59'	5	40.0	19	5.3	3	33.3	18	5.5	3	33.3	18	5.5	2	0.0	17	11.8	5	20.0	19	21.1
Dec. 59'	14	50.0	14	14.3	--	--	--	--	--	--	--	--	--	--	--	--	14	50.0	14	42.8
Jan. 60'	25	44.0	4	25.0	--	--	--	--	--	--	--	--	--	--	--	--	25	36.0	4	50.0

EXPLANATION OF PLATE IX

The total percent adult hares infected with cysts, Micipsella brevicauda, microfilariae in peripheral, heart and lung blood from August, 1958 through January, 1960.

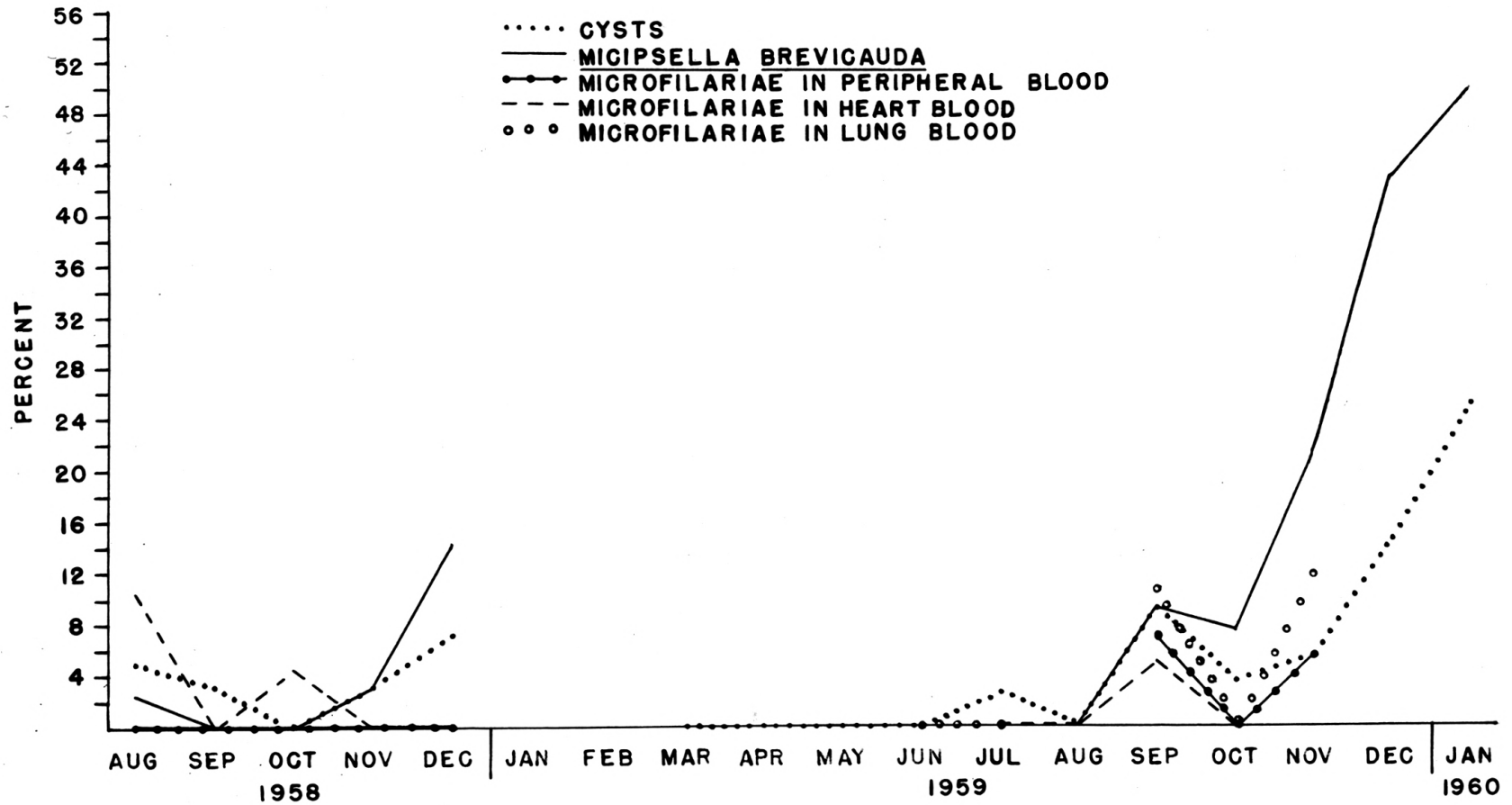
PLATE IX



EXPLANATION OF PLATE X

The total percent juvenile hares infected with cysts, Micipsella brevicauda, microfilariae in peripheral, heart and lung blood from August, 1958 through January, 1960.

PLATE X



A statistical analysis (Table 9) showed that there was a significant monthly correlation between; adult infected hares with microfilariae, and the presence of cysts; and adult infected hares with filarial worms, and the infection with microfilariae (Table 7, Plate IX). The correlation of the filarial worms, microfilariae in host blood and cysts in adult and juvenile hares is shown by graph in Plates IX and X. The data collected also indicated in some instances that: hares were infected with microfilariae but harbored no filariids or cysts; filariids were present but no cysts or microfilariae were recovered. The former instance may partially be explained by the fact that worms or cysts may have been overlooked at necropsy. The latter instance may indicate a unisexual infection or female worms in a non-reproductive state. The lack of cysts in the latter instances may indicate an early infection in which old or dead worms are absent or cysts were overlooked at necropsy.

Plates IX and X, clearly indicate the difference in the infection of adult and juvenile hares with cysts, M. brevicauda and microfilariae in peripheral, heart and lung blood. A comparison of data on Plates IX and X, clearly indicate that the infections in juvenile hares, (Plate X) with cysts, M. brevicauda and microfilariae do not make their appearance until August or September whereas the adult hares (Plate IX) which are present throughout the year, (Table 7) show some degree of infection each month. The juvenile picture (Plate X) can partially be explained by the fact that according to Taylor

(1960) the breeding season begins in January with the young appearing in February or March. Thus it can be concluded that the occurrence of cysts, M. brevicauda, and microfilaria do not appear until August or September. Taylor (1960) considered all hares collected in January capable of reproductive activity and therefore classified all hares collected after December as adults until the new born of the year appeared, which of course are considered as juveniles. One exception should be noted for January 1960 (Table 7) where four hares were collected which were considered as juveniles. This accounts for the juvenile hares (Plate X) as shown being infected in the month of January, 1960.

The probable time of infection of the juvenile hares and the time of appearance of the adult worms in early fall and winter months, along with cysts and microfilariae, will be discussed later in this discussion.

From the data collected (Table 7; Plate IX), it is apparent that samples of heart blood, more consistently and to a higher degree, diagnose infections of microfilariae in infected adult hares than do those from peripheral or lung blood. This may be explained in part, by the difference in the amount of blood taken at each site. The amount (2 cc.) taken from the heart is greater than that taken from the ears (peripheral) or lungs, which upon centrifuging would tend to pick up slight or moderate infections which may not be true of the latter two.

The high percent of the hares infected with microfilariae

as diagnosed by the lung blood samples, is not readily explainable (Table 6). It is probable that the microfilariae tend to congregate in the lungs, and even in slight infections may be found in the lungs when their presence in heart blood would be difficult to demonstrate. Hawking and McFadzean (1954) working with Wuchereria bancrofti and Dirofilaria aethiops, found that the lungs acted as a reservoir for microfilariae chiefly by day and to a lesser degree by night. Kartman (1953) suggested that microfilariae of Dirofilaria immitis were filtered from the peripheral circulation by the lungs. Here the possibility of being ingested by mosquitoes which were being employed as experimental intermeddiate hosts was slight.

Since the microfilarial periodicity, if present, is not known, and data from day time blood sampling is lacking, the role played by the internal organs such as the lungs in view of microfilarial concentration, is unexplainable at this point. Also, since the intermediate host is not known, the relationship of microfilarial concentration in lung and peripheral blood to the intermediate host is not known at this time.

The percent incidence of M. brevicauda shows a definite seasonal as well as monthly fluctuation (Plate XI, Fig. 14). The peak of winter and early spring fluctuation, although not as definite from December, 1958 to March, 1959, are definitely indicated in a comparable period in 1957-1958 and December 1959. Data collected since the termination of the study included in this thesis indicated a slight drop in January, 1960 with a steady

increase through February up to approximately a 70 percent infection in March, 1960. Lyons (1958) reported a similar peak infection during the winter and early spring months of 1956-1957. Basing his results on a bi-monthly sample of 20 hares collected from December, 1956 to June, 1957, he found a peak incidence of 80 percent of the hares infected in December, 1956 with a gradual decline down to 10 percent in August, 1957. The percent incidence from July through September, 1957 were based on monthly collections of 10 hares each.

The summer and fall months, June through November, show a low tide in the infection of M. brevicauda (Plate XI, Fig. 14). Lyons (1958) reported a low point in the seasonal fluctuation as occurring in August, 1957 with 10 percent infected. In the present study, low points were noted in October and November of 1958 and in July, 1959 with no hares infected (Plate XI, Fig. 14). As indicated in the graph (Plate XI, Fig. 14), the infection rate gradually decreases through the spring months and gradually increases in the late fall or early winter to reach a peak in the January collections.

A difference is noted between the percent infected hares from the total number examined as compared with the sample of 10 examined each month (Plate XI, Fig. 14). Statistical analysis (Table 9) shows a significant difference between the results of these two samplings. However, the results given by these two samples appear to follow each other quite closely in trends of monthly and seasonal fluctuations. The reason for this difference is thought to be a sampling error.

EXPLANATION OF PLATE XI

- Fig. 13. Number of adult and juvenile hares infected or noninfected with Micipsella brevicauda in each of the 27 monthly collecting periods.
- Fig. 14. Percent hares infected with Micipsella brevicauda per total number examined and per sample of 10 examined in each of the 27 monthly collecting periods.
- Fig. 15. Total percent hares infected with Raillietina spp. and Passalurus nonanulatus in each of the 27 monthly collecting periods.

PLATE XI

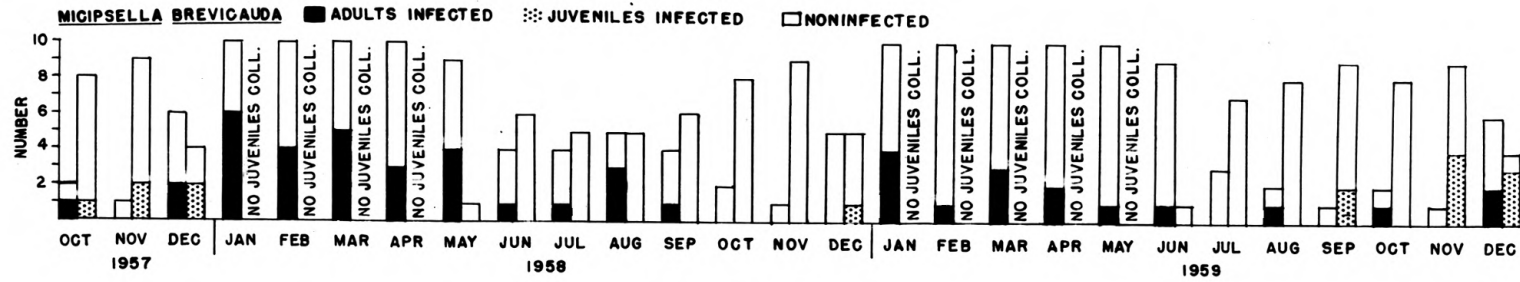


FIG. 13

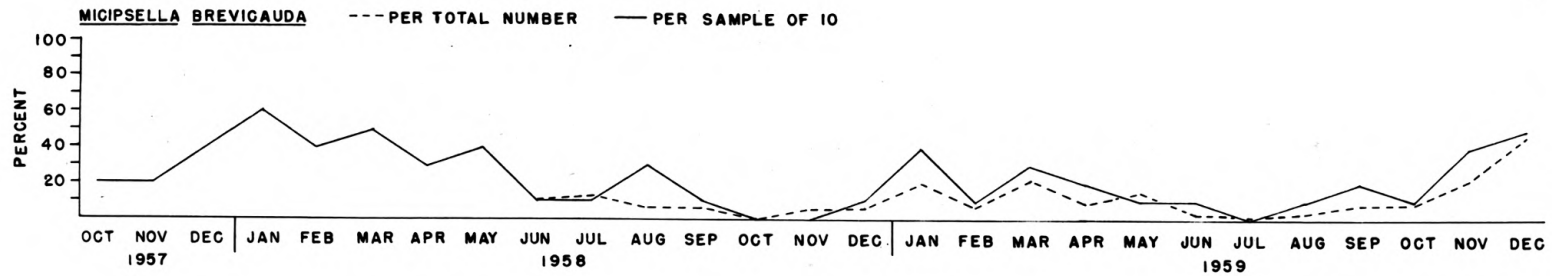


FIG. 14

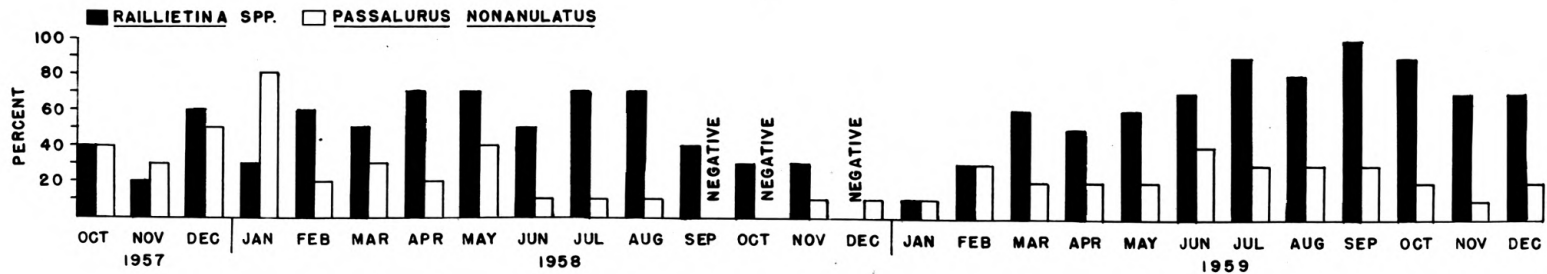


FIG. 15

The number of infected and non-infected adult, and juvenile hares was calculated for each of the 27 monthly collecting periods (Plate XI, Fig. 13). For example, in October 1957 two adult and eight juvenile hares were collected. One adult and one juvenile hare were infected. The number of adult infected hares reached a peak of infection in January 1958 and 1959 with a steady decline into the early summer months of June and July of the respective years. Lyons (1958), basing the incidence on bi-monthly collections of 20 hares from December, 1956 to June, 1957, found the peak incidence in April with a decline down to no infected adult hares in the August collection. Taylor (1960), according to the aging technique used, considered all hares collected in January of 1958 and 1959 as adult hares.

With breeding season beginning in early January, juvenile hares did not make their appearance in the monthly collections until May in 1958 and June, 1959 (Plate XI, Fig. 13). Lyons (1958) reported one juvenile infected hare in August with a gradual increase to two infected hares in December, 1957. In the present study, only one juvenile infected hare was collected in 1958 which was in the December collection. The number of infected juvenile hares in 1959 was higher than in 1958 (Plate XI, Fig. 13). Two hares were infected in September, 1959 with peak infection in November of four hares, dropping back to three infected hares in December of the same year. The increase in infected juvenile hares in the fall and winter collections of 1959 over the same period in 1958, when compared to the data in

Plate XI, Fig. 14, may indicate the increase as due, in part, to a sampling error. However, as indicated by Plate XI, Fig. 14, a true over all increase in infections of hares did occur in the fall and winter collections of 1959 as compared to 1958.

It is interesting to note that more juvenile hares were collected in July, August and September of 1959 than in the same period in 1958. Thus it appears that this increase in juvenile hares would explain the increase of infected juvenile hares in the 1959 collection. However, Taylor (1960) reported no significant change in the adult juvenile ratio of the hares collected in the above mentioned periods, except in the September collection, 1959. In this collection, a very high percent of the hares collected (92 percent) were juveniles. This may account for the relatively early and high percent of the juvenile hares infected in the September collection. Since the ratio of adult and juvenile hares collected in October, November and December 1959 is relatively constant as compared to 1957 and 1958, it can be concluded that a natural increase in the infection of M. brevicauda occurred in the above mentioned period.

From the data collected (Plate XI, Fig. 13) it appears that the degree of juvenile infections in the fall and early winter months are a good indication of the percent infected hares which will occur in the winter and spring of the following year. In October, November and December, 1957 one, two and two hares were

infected respectively. A peak infection of six hares occurred in January, 1958, declining down to four in May. In 1958, only one juvenile hare was infected, which was in the December collection. A detectably fewer number of hares were infected in January through May in 1959. In contrast, a large number of the juvenile hares collected from September through December, 1959 were infected with M. brevicauda (Plate XI, Fig. 13). Data collected since the termination of the study included in this thesis from January through March, 1960, shows three animals infected in January with a gradual increase to seven infected hares in March. A similar relationship was also shown by Lyons (1958).

The time of the year at which the hares contract the infection of M. brevicauda, the life span of the filarial nematode and the time required for the development of the microfilariae to the infective larval stage in their intermediate host(s) is not known. It is suggested that the infection is picked up by the hares during the summer or early fall months of the year, requiring four to six months to develop to maturity and appear in the hares during the winter of the same year or early spring of the following year. This theory is based on the results gained by a preliminary intermediate host study conducted during the summer months, 1959, where summer active mosquitoes were observed as heavy, primary feeders on the hares.

However, if one considers the presence of microfilariae in peripheral blood as an indication of their availability to the

intermediate host, the data collected (Table 7 and Plate IX) tends to suggest another possibility. The incidence of worms, consequently, also the microfilariae in adult hares are at their peak during the late winter and spring months. The chance that the intermediate host, providing it takes a blood meal from peripheral blood, would have to pick up the infection of microfilariae while feeding, would be much greater in the above mentioned period than during the summer or fall months as suggested earlier. Juvenile hares, according to the data collected (Table 7, Plate X), would not act as a source of microfilariae until the fall or winter of the year and then only to a small degree. On this basis then, it is postulated that the intermediate host contracts the infection in late winter and early spring, transmits it to the new born of the year which are then found to carry the infection as juvenile hares in the early fall or winter of the same year and as adults in the following year. This however, would necessitate winter and early spring active blood sucking arthropods.

Since no concentration technique was used on the small sample of peripheral blood, it is quite possible that slight to moderate infections were missed. Thus a greater percent of the hares in late spring and summer may be infected which would make possible the role of the intermediate host(s) being played by blood sucking, summer active forms. However, it may be possible that early spring forms of blood sucking insects exist in the area which would then be present when the microfilariae are

found in large numbers in the peripheral blood as mentioned earlier.

Preliminary intermediate host studies during the summer of 1959 showed mosquitoes as being heavy feeders on the ears of the hares during early and late evening hours of the day. The number of hares infected with ticks and fleas in the Lakin study area as found during this study from June, 1958 through December, 1959 were at a very minimum.

El-Rawi (1957) conducted an ectoparasite study of the black-tailed jack rabbit (L. c. melanotis) in Kearny Co. Kansas. He reported a heavy infestation of Dermacentor parumapertus (84 percent) and Hoplopsyllus gracialis (20 percent) on the hares examined. He however, does not state where, in relation to the present study, his animals were collected. It is highly probable that his collections were made south of the Arkansas River, south of Lakin, Kansas. It should then be kept in mind, since this large natural barrier (river) exists between his probable collection area and the present study area as well as different type of vegetation and farm land, a true reference of his ectoparasite findings reflected onto the present study can not be made.

In adult hares, the monthly correlation between cysts and the presence of the filarial worms a month previous approaches statistical significance (Table 9). Thus it appears that when an increase in infection of filarial worms occurs, the infection of cysts increases accordingly a month later (Plate IX). This

may indicate, since the cysts have been associated with the filarial worms and microfilariae (section, Pathology), the life of the filarial nematode and microfilariae may be approximately one month. However, in view of data collected thus far, as discussed above, the author suggests that the above postulated life span of one month is not truly suggestive of the actual life span of this filarial nematode.

Until further studies are made on the origin and development of the cysts as occurring in the peritoneal cavity of the host, their true relationship to filarial worms and microfilariae as suggested by the statistical analysis, can not be known.

Table 8 lists the monthly sex ratios of M. brevicauda as based on 161 worms collected from 91 infected hares over a 28 month collecting period from October, 1957 to January, 1960. From the data collected it was found that a 1 to 1.33 male to female ratio existed. A statistical analysis (Table 9) showed that no significant difference existed between the male to female ratio through the 28 monthly collection periods (Table 8).

Other Parasites. Taenia pisiformis Block, 1780. Three and seven-tenths percent of the hares examined were infected with larval tapeworms (cysticerci), T. pisiformis (Table 5). The number of cysticerci recovered from each hare ranged from one to six. The cysticerci were found on the mesenteries of the stomach, cecum, large intestine, liver and adjacent to the spinal cord. Lyons (1958), (Table 5), reported a seven percent incidence in 130 hares examined.

Table 8. Sex ratio of Micipsella brevicauda.

Months	<u>M. brevicauda</u>		
	Male	Female	Ratio
January	9	12	1 - 1.33
February	10	16	1 - 1.60
March	13	10	1 - 0.77
April	6	8	1 - 1.33
May	0	4	0 - 4.00
June	0	1	0 - 1.00
July	0	2	0 - 2.00
August	2	2	1 - 1.00
September	5	1	1 - 0.20
October	4	5	1 - 1.25
November	1	6	1 - 6.00
December	19	25	1 - 1.31
Total	69	92	1 - 1.33

Multiceps spp. According the Erickson (1944) and Clapham (1942), the larvae of M. serialis and M. packii, both occurring in the lagomorphs, can not be distinguished from each other. Therefore, the present study refers to these coenuri as Multiceps spp.

Ten and eight-tenths percent of the hares examined, having one to two coenuri, were found to be infected (Table 5). The highest percent incidence was found in May, 1959, with five out of 10 animals carrying the infection.

The largest cyst recovered measured 155 mm. in length and 92 mm. in diameter (Plate III, Fig. 4). The cyst proper was located within the body cavity and extended from the diaphragm posteriorly almost to the anus. A portion of the cyst proper extended through the muscles of the back on the right side at the hips, and extended anteriorly to the ribs. Another large cyst was found located on the hip of a hare which measured 100 mm. in diameter and 50 mm. in thickness. Neither hare seemed affected by the large cysts. The digestive tract of the former was not distended, which may have been due to the fact that it may not have fed before being collected. The majority of the cysts recovered were attached to the musculature within the body cavity. Grundman, Parker, Stagg (1955) reported a huge cyst from a hare in Utah, but gave no measurements.

Lyons (1958) reported 19 percent incidence of Multiceps spp. in his study. The peak infection occurred in September, 1956 with 40 percent infected.

Raillietina spp. One hundred and forty-seven hares were infected with the tapeworm, Raillietina spp., representing a 54.6 percent infection rate. Lyons (1958) reported a 66 percent incidence of this tapeworm (Table 5). He reported that three hares were infected with tapeworm spp. which were specimens that were not suitable for identification. In the present study, since no other genus of tapeworms was recovered by Lyons (1958) and during the present study, all tapeworms were considered as Raillietina species. The number of species present in this study is not known. The identification of several specimens proved to be R. (Paroniella) retractilis (Stiles, 1895), Honess and Winter (1956).

The highest number of tapeworms recovered in one animal was 78, which was collected in September, 1959, of which nearly all were immature forms.

The identification of the specimens was made largely on the basis of the presence of hooks on the suckers and single-pored proglottids which is characteristic of the genus Raillietina, Wardle and McLeod (1952).

The number of infected and non-infected adult and juvenile hares was calculated (Plate XII, Fig. 17) as well as the percent incidence for each of the 27 monthly collecting periods. (Plate XI, Fig. 15).

A seasonal as well as a monthly fluctuation in the incidence of tapeworms is clearly indicated in Plate XI, Fig. 15. The peak infection is not quite as evident in 1958 as in 1959. However,

EXPLANATION OF PLATE XII

- Fig. 16. Number of adult and juvenile hares infected or noninfected with Passalurus nonanulatus in each of the 27 monthly collecting periods.
- Fig. 17. Number of adult and juvenile hares infected or noninfected with Raillietina spp. in each of the 27 monthly collecting periods.

PLATE XII

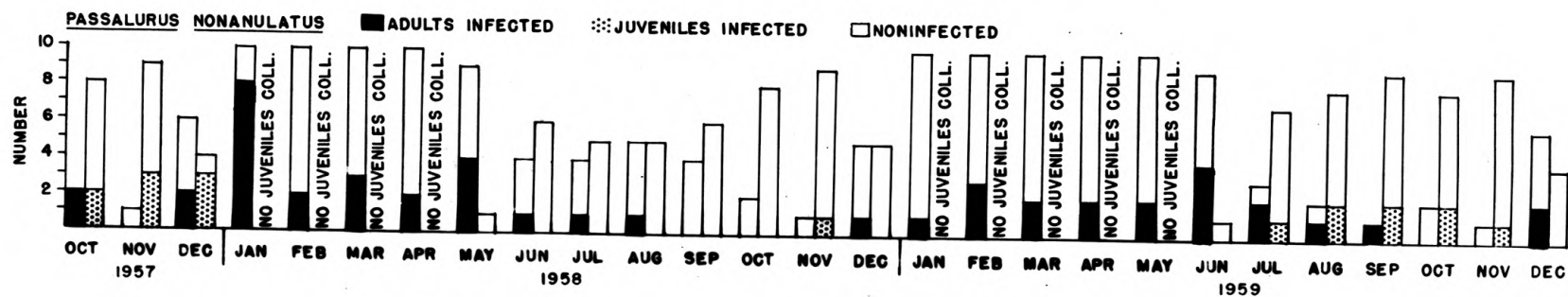


FIG. #16

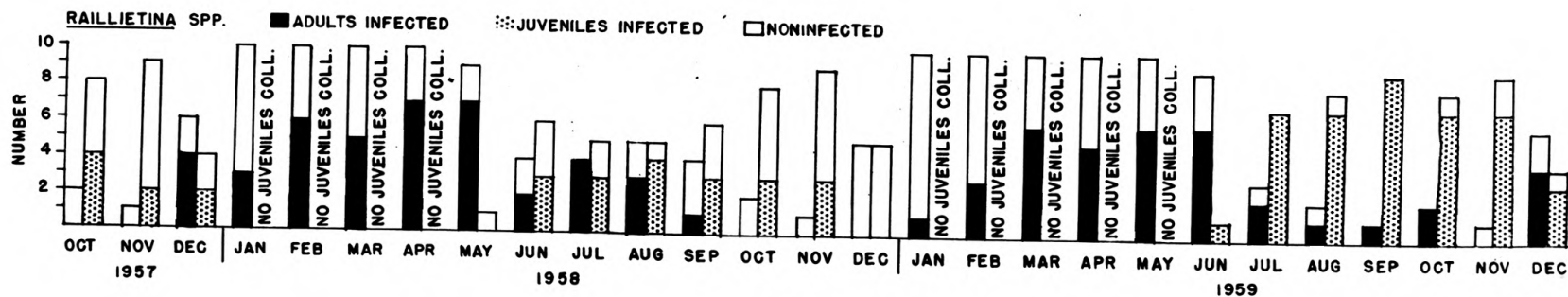


FIG. #17

a definite seasonal fluctuation can be seen in 1958 and 1959, with low level infections occurring during the winter months of the year. Lyons (1958) basing the results on bimonthly collections of 20 hares from December, 1956 through June, 1957 found a comparable seasonal fluctuation.

Plate XII, Fig. 17 shows the infection rate in juvenile and adult hares. No great change in the number of infected adult hares is noted between the 1958 and 1959 collections. Lyons (1958) reported a comparable infection rate in 1956 and 1957 in adult hares. However, a detectable difference in the juvenile hare infection rate in late summer and fall of 1959 is noted over the same period in 1958. Nearly all juvenile hares collected from June to November, 1959 were infected. A comparable period in 1958, and in October and November, 1957 indicated a fewer number of the hares infected. Lyons (1958) reported a comparable number of hares infected in September and October, 1956. No collection was made in November, 1956. In contrast, he found nearly all the juvenile hares collected from June through September, 1957 infected.

Thus it appears that, from the data collected thus far (Lyons, 1958; and present study), the infection rate of tapeworms (Raillietina spp.) in juvenile hares demonstrate a cyclic infection rate every two years with the peak incidence occurring in the late summer and fall months of the year. A high percentage of the juvenile hares collected in summer and fall of 1957 and 1959 were infected, whereas a fewer number were infected in the same

period in 1956 and 1958. It should be noted that a higher percent of juvenile hares were collected in September, 1959 than in a same period in 1958. Even though a higher percent of juvenile hares was collected in 1959 (Plate XII, Fig. 17), a true over all infection of tapeworms did occur in 1959 (Plate XI, Fig. 15).

Since the intermediate host is not known, and its relationship and dependency upon ecological and environmental conditions is not known, the reason for the fluctuations of this parasite can not be given at this time.

Passalurus nonanulatus Skinker, 1931. This species is closely related morphologically to P. ambiguus (Rudolphi, 1819) Dujarden, 1845. Lyons (1958) conducted a careful study of the species occurring in black-tailed jack rabbit and concluded that the species present were P. nonanulatus.

Since no other pinworm has been recovered during the present study, all immature pinworms were considered to be of the same species.

Twenty-three and four-tenths percent of the hares examined harbored this parasite (Table 5). The cecum and large intestine were the most common locations for this nematode. In a few instances they were recovered from the small intestine, which may have been ingested during coprophagy (Lechleitner, 1957). Lyons (1958) found 75 percent of the hares infected during his study (Table 5). He reported a median of 178 worms and a mean of 2,271, with one hare harboring approximately 48,000 worms. In the present study, the median and mean were 150 and 276.2

respectively with one maximum number of worms recovered from one hare being 19,710. It should be noted that these results in the present study were obtained by a dilution technique (Lyons, 1958) and not by actual count.

For each of the 27 monthly collecting periods, the total percent incidence (Plate XI, Fig. 15) and the number of infected and non-infected adults and juveniles was calculated (Plate XII, Fig. 16).

A seasonal as well as a monthly fluctuation of this parasite can be seen on Plate XI, Fig. 15. A low incidence in the seasonal fluctuation is noted in the fall months of the years, 1957, 1958 and 1959. Lyons (1958) noted a similar low incidence period in 1956 and 1957. A peak incidence, although not definite, is noted during late spring in 1959. A major incidence peak occurred in January 1958 with a gradual increase from November, 1957 and a sharp decrease to February, 1959 and again a minor peak in May of the same year. Lyons (1958) basing his findings on a bimonthly sample of 20 hares from December, 1956 through June, 1957, found a peak incidence of 100 percent of the hares infected in the February and April, 1957 collections, with a decline to 40 percent in the September collection of the same year.

Other than the high infection rate of eight out of 10 adult hares infected in January, 1958, the adult hare infection rate of 1958 is comparable to that of 1959 (Plate XII, Fig. 16). Lyons (1958) found the peak infection rate in April, 1957 with all of

the 20 adult hares collected harboring the pinworms. The infection rate dropped to one out of two hares collected infected in the September collection, 1957. Thus the adult infection rate of pinworms dropped in 1958 and 1959 as compared to that of 1957 (Lyons, 1958).

An increase in the infection rate of juvenile hares (Plate XII, Fig. 16) is noted in the late summer and fall of 1959 over a comparable period in 1958. Lyons (1958) reported a gradual increase in the infection rate of juvenile hares from September, 1956 to eight of 12 hares examined in December, 1956. A similar increase was noted from one infected hare in June, 1957 (Lyons, 1958) to three infected hares in four examined in December, 1957 (Plate XII, Fig. 16).

The fall in the infection rate in juvenile hares in 1958 and the rise in 1959 is not fully explainable. Since the infection of P. nonaulatus may be direct, the coprophagous habit of the hares (Lechleitner, 1957) may play an important role in retroinfection, and in passing the infection from adult hares to their offspring, (Lechleitner, 1957). Lapage (1956) lists P. ambiguus as having a direct life cycle with no intermediate host needed, which may also be true for P. nonanulatus.

Studying the contents of the stomach and small intestine of young hares should prove enlightening to the role played by coprophagy in the infection of hares with the pinworm, P. nonanulatus.

In the present study, as well as that of Lyons (1958), no

adverse effects were noted at autopsy of the hares due to presence of the large number of pinworms as sited in the above cases.

Dermatoxys veligera (Rudolphi, 1819) Schneider, 1866. One hare (thirty-seven hundredths percent incidence) was found to be infected with this nematode (Table 5). This specimen was recovered from the large intestine. Since a dilution technique was used on contents of the cecum and large intestine for the diagnosis of pinworm infections, slight infections may have been missed, thus accounting for the low infection rate as stated above.

Lyons (1958) reported slight infections of one to two worms per hare, with a six percent infection rate as found in the autopsy of 130 hares.

Physaloptera spp. Eleven hares (four and one-tenths percent) were found harboring this nematode (Table 5). Four juvenile and seven adult hares were infected. One adult hare harbored 33 worms. All but a few of the specimens were recovered from the stomach, the remainder were found in the small intestine. Lyons (1958) reported a two percent infection rate in his study, one animal having six worms while the other two hares harbored two worms each.

Identification of species of Nematodirus was made primarily on the characteristics of the bursa and spicules of the male worms (Lyons, 1958). Whenever only female worms were found in an animal, they were classified as Nematodirus spp. Lyons (1958) found that the measurements of the female worms were so similar

that differentiation of female worms as to species (N. arizonensis and N. leporis) was impossible. In no case was there more than one species of male worms found in any single animal. So whenever both male and female worms were found in a hare, all female worms were considered to be of the same species as that of the male worms.

Nematodirus arizonensis Dikmans, 1937. A high percentage, 11.1 percent, of the hares was found to harbor this nematode. Lyons (1958) found five percent of the hares infected, harboring from one to 52 worms per animal. In the present study, the maximum number of worms recovered from one animal was 139. Twenty-three animals were infected in 1959, whereas only five were infected in 1958. The data collected indicate a peak infection rate occurring in late fall through the winter months in 1958 and 1959.

Nematodirus leporis Chandler, 1954. Only one hare (thirty-seven hundredths percent) was found to be infected with this worm (Table 5). Two hares (one and five tenths percent) were reported as being infected by Lyons (1958).

Nematodirus spp. Pieces of worms and female worms with no accompanying males were classified as Nematodirus spp. Four and one-tenths percent (11 hares) harbored these worms (Table 5). Seventeen percent of the hares examined by Lyons, 1958, was infected with Nematodirus sp. It is probable that most of these worms were N. arizonensis.

Dirofilaria scapiceps (Leidy, 1886). During the present study (Table 5), the tarsal bursa of the hind legs of 234 hares

were examined for D. scapiceps. This examination was carried out so as to more clearly indicate the origin of the microfilariae which were found circulating in the blood of the hares. During the entire study, no specimens or microfilariae of D. scapiceps were recovered.

Microfilariae of Micipsella brevicauda. Thirteen and eight-tenths percent of 138 hares examined was found infected with microfilariae. These 138 hares were a portion of the 269 hares examined during the helminth parasite survey (Table 5). The microfilariae recovered were irrespective of site of blood sample. Thus, hares were diagnosed as positive if microfilariae were recovered from ear, heart or lung blood samples. In only a relatively few cases were blood samples recovered from each site from each hare examined.

It is interesting to note the close correlation between the percent incidence of infected hares with filarial larvae in the 138 hares examined during the helminth parasite study (Table 5), and the sum total number of hares examined, 425, throughout the entire study (Table 6). In the helminth study, 13.8 percent of the 138 hares examined was found to be infected (Table 5), whereas in the over all total number examined, which includes the data on Table 6, 14.82 percent were infected. The percent infected hares with adult worms varied greatly between the above two mentioned samples. In the 269 hares examined in the helminth study (Table 5), a 23 percent infection rate was noted, whereas in the sum total of 728 hares examined (Table 6), 10.71 percent were infected. As indicated earlier, a significant difference

was noted between the sample of 10 per month and the total number examined each month in the M. brevicauda infection rate (Plate XI, Fig. 14), which was due apparently to a sampling error. The previously mentioned difference, as noted in comparison of Table 5 and Table 6, is also thought to be due to a sampling error.

Cysts. Twenty-eight hares were found to be infected with cysts as recovered from the peritoneal cavity of the hares (Table 5). No cysts were recovered from the pleural cavity. This represents a 10.4 percent infection. A comparable eight and ninety-three hundredths percent infection rate is noted from 728 hares examined throughout the entire study (Table 6).

The histological description and discussion of the possible relationship of the cysts to M. brevicauda and microfilaria is noted elsewhere.

SUMMARY

The present study was initiated to study the filarial nematode, Micipsella brevicauda, a parasite located in the abdominal cavity of the black-tailed jack rabbit (Lepus californicus melanotis Mearns) in Kearny Co. (Lakin) Kansas. The study was also made as a continuation of a survey of the helminth parasite fauna occurring in jack rabbits in southwestern Kansas.

Microfilariae of M. brevicauda were collected and described from host blood and uteri of parent worms. The presence of a sheath as indicated by staining with Delafield's

Hematoxylin; a slightly tapered, bluntly rounded tail with a large nucleus at the tip as well as an internuclear interruption near the rectal cell G_1 was found to be characteristic of the microfilariae of M. brevicauda. Sufficient data were lacking from other Micipsella spp. microfilariae to demonstrate species identification and differentiation.

Transplants were made of the filarial nematode from infected hares to the laboratory white rabbit. The laboratory rabbit was found to be an unsuitable experimental host. The rabbit, as shown by other workers, possesses a violent defensive mechanism toward foreign material within its body cavity. The end result in most cases was a rapid walling off and encapsulation of the transplanted worms. Laboratory rabbits exposed in the endemic area failed to contract the infection of M. brevicauda.

Numerous cysts were recovered from the peritoneal cavity of hares infected with M. brevicauda. Histological studies indicated that many of the cysts were the end products of the host defensive reaction against the nematodes and microfilariae. The staining of tissue sections with Azure Eosin Von Kossa's indicated the presence of various amounts of caseation necrosis and calcification. The lesions (cysts) examined showed no consistency as to cell arrangement or type. The necropsy of various small mammals other than L. c. melanotis in the endemic area in southwestern Kansas indicated that the infection of M. brevicauda was limited to the hare. From data collected thus far, the distribution of the infection of M. brevicauda in the hares apparently is limited to the western one-third of Kansas and its presence in eastern

Colorado and northwestern Oklahoma is highly probable.

Two hundred and sixty-nine jack rabbits were examined for helminth parasites. The hares were collected within a 10 mile radius of Lakin, north of the Arkansas River, in Kearny County, Kansas during 27 monthly collecting periods from October, 1957 through December, 1959. The heart, lungs, liver, kidneys, gastrointestinal tract, body cavity, musculature of the hares were examined for endoparasites.

A significant positive correlation was noted between adult hares with microfilaraemia and the presence of cysts, as well as between adult hares with microfilaraemia and the presence of filarial worms. A positive correlation approaching significance was noted between adult hares infected with filarial worms and the presence of cysts. The infections of M. brevicauda, cysts and microfilariae in juvenile hares did not make their appearance until the late summer or fall months of the year. The male to female ration of M. brevicauda based on 161 worms during the study was 1 to 1.33.

The percent incidence of M. brevicauda, cysts and microfilariae as recovered from the sample of 10 hares per month (269), compared favorably to the total number hares examined (728) throughout the study.

Micipsella brevicauda and Passalurus nonanulatus showed the highest percent incidence in the winter months. Raillietina spp. showed a peak infection rate in the summer months.

Following is the percent incidence of each parasite found

in this study: Raillietina spp. 54.6 percent, Passalurus non-anulatus 23.4 percent, Micipsella brevicauda 23.0 percent, Nematodirus arizonensis 11.1 percent, Multiceps spp. 10.8 percent, Nematodirus spp. 4.1 percent, Physaloptera spp. 4.1 percent, Taenia pisiformis (cysticerci) 3.7 percent, Dermatoxys veligera 0.37 percent, Nematodirus leporis 0.37 percent, Dirofilaria scapiceps (234 hares) 0.0 percent, microfilariae of M. brevicauda (138 hares) 13.8 percent. Cysts were recovered from 10.4 percent of the 269 hares examined. No trematodes were recovered in this study.

Table 9. Statistical Analyses according to Snedecor, 1957.

Item	Statistical test	Test statistic	Level of significance (.05 level)	Conclusion
Male : Female ratio of <u>M. brevicauda</u> vs. months of the year	Contingency Chi-square	13.88	19.68	nonsignificant
Sample of 10 hares examined per month vs. total number hares examined per month for <u>M. brevicauda</u> .	Wilcoxon Matched-Pair	34	46	significant
Adult infected hares with <u>M. brevicauda</u> vs. presence of cysts.	Linear Correlation	.4329	.456	approaches significance
Adult infected hares with <u>M. brevicauda</u> vs. microfilariae in host blood.	Linear Correlation	.5818	.468	significant
Adult infected hares with microfilariae vs. presence of cysts.	Linear Correlation	.5082	.468	significant
Microfilariae of host blood vs. microfilariae of . parent worms (<u>M. brevicauda</u>).	Series of t-tests	---	---	nonsignificant *

* significant difference on anal pore only, see text

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STUDIES ON MICIPSELLA BREVICAUDA LYONS, 1958, A FILARIID
OF THE BLACK-TAILED JACK RABBIT (LEPUS CALIFORNICUS
MELANOTIS MEARNIS), WITH NOTES ON THE HELMINTH
PARASITES OF THE JACK RABBIT

by

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A review of the literature indicated few reports of filariids in the leporid Lagomorphs. Three species of filariids have been reported in the United States: Micipsella brevicauda Lyons (1958), Dirofilaria scapiceps Leidy (1886), and D. uniformis Price (1957).

The present study was initiated to study the filarial nematode, Micipsella brevicauda, a parasite located in the abdominal cavity of the black-tailed jack rabbit (Lepus californicus melanotis Mearns) in Kearny Co. (Lakin) Kansas. The study was also made as a continuation of a survey of the helminth parasite fauna occurring in jack rabbits in southwestern Kansas.

Microfilariae of M. brevicauda were collected and described from host blood and uteri of parent worms. The presence of a sheath as indicated by staining with Delafield's Hematoxylin; a slightly tapered, bluntly rounded tail with a large nucleus at the tip as well as an internuclear interruption near the rectal cell G_1 was found to be characteristic of the microfilariae of M. brevicauda. Sufficient data were lacking from other Micipsella spp. microfilariae to demonstrate species identification and differentiation.

Transplants were made of the filarial nematode from infected hares to the laboratory white rabbit. The laboratory rabbit was found to be an unsuitable experimental host. The rabbit, as shown by other workers, possesses a violent defensive mechanism toward foreign material within its body cavity. The end result in most cases was a rapid walling off and encapsula-

tion of the transplanted worms. Laboratory rabbits exposed in the endemic area failed to contract the infection of M. brevicauda.

Numerous cysts were recovered from the peritoneal cavity of hares infected with M. brevicauda. Histological studies indicated that many of the cysts were the end products of the host defensive reaction against the nematodes and microfilariae. The staining of tissue sections with Azure Eosin and Von Kossa's indicated the presence of various amounts of caseation necrosis and calcification. The lesions (cysts) examined showed no consistency as to cell arrangement or type. The necropsy of various small mammals other than L. c. melanotis in the endemic area in southwestern Kansas indicated that the infection of M. brevicauda was limited to the hare. From data collected thus far, the distribution of the infection of M. brevicauda in the hares apparently is limited to the western one-third of Kansas and its presence in eastern Colorado and northwestern Oklahoma is highly probable.

Two hundred and sixty-nine jack rabbits were examined for helminth parasites. The hares were collected within a 10 mile radius of Lakin, north of the Arkansas River, in Kearny County, Kansas during 27 monthly collecting periods from October, 1957 through December, 1959. The heart, lungs, liver, kidneys, gastrointestinal tract, body cavity and musculature of the hares were examined for endoparasites.

A significant positive correlation was noted between adult hares with microfilaraemia and the presence of cysts, as well as

between adult hares with microfilaraemia and the presence of filarial worms. A positive correlation approaching significance was noted between adult hares infected with filarial worms and the presence of cysts. The infections of M. brevicauda, cysts and microfilariae in juvenile hares did not make their appearance until the late summer or fall months of the year. The male to female ratio of M. brevicauda based on 161 worms during the study was 1 to 1.33.

The percent incidence of M. brevicauda, cysts and microfilariae as recovered from the sample of 10 hares per month (269), compared favorably to the total number hares examined (728) throughout the study.

Micipsella brevicauda and Passalurus nonanulatus showed the highest percent incidence in the winter months. Raillietina spp. showed a peak infection rate in the summer months.

Following is the percent incidence of each parasite found in this study: Raillietina spp. 54.6 percent, Passalurus nonanulatus 23.4 percent, Micipsella brevicauda 23.0 percent, Nematodirus arizonensis 11.1 percent, Multiceps spp. 10.8 percent, Nematodirus spp. 4.1 percent, Physaloptera spp. 4.1 percent, Taenia pisiformis (cysticerci) 3.7 percent, Dermatoxys veligera 0.37 percent, Nematodirus leporis 0.37 percent, Dirofilaria scapiceps (234 hares) 0.0 percent, microfilariae of M. brevicauda (138 hares) 13.8 percent. Cysts were recovered from 10.4 percent of the 269 hares examined. No trematodes were recovered in this study.