NEMATODES OF THE MOUSE

MUS MUSCULUS LINNE

With Two Tables and Five Plates

- by -

Edgar M. Matthias Medlar

Lincoln, Nebr.
1912.

TABLE OF CONTENTS.
########

#####

INTRODUCTION

As early as 1782 Goeze and Werner noted parasites for the mouse, Mus musculus Linne, and since that time a large number of authors have noted and described both ecto - and endo - parasites harbored by the mouse, so that quite a formidable array is charged up against the gregarious little animal. However, in the large majority of cases, the descriptions of all the parasites, in so far as I have had occasion to examine, were very brief and inaccurate. In many instances, the descriptions were so meagre, that, had the parasite been placed before one, one would not have been able to recognize it from the description. All of the descriptions examined were incomplete in the details of the organology and in but one case, that of Marchi (1871), was the description of the histological structure and development undertaken. Also, but few of the authors made drawings of the parasites described and thus made it all the more difficult to ascertain a definite comprehension of the parasites which they described. Thus, while quite a large number of parasites have been described, the entire field needs to be carefully worked over, more detailed drawings need to be made, and a more complete and accurate description of each species of parasite should be given. As far as

can be ascertained but little work has been done on the parasitic fauna of Mus musculus in the United States, and none whatsoever has been done on the nematode fauna of musculus in the civinity of Lincoln, Nebraska. This article will include the data obtained from the investigation of the nematode fauna of Mus musculus in vicinity of Lincoln; a detailed morphological description of the nematode, Acuaria muris Gmelin 1790, together with a discussion of its taxonomic position; the organology of Oxyuris obvelata Rudolphi 1819 - of Oxyuris tetraptera Nitzsch 1821, and of Acuaria submucosa, new species; and a list of all nematodes reported to date for Mus musculus.

TECHNIQUE

The mice examined were all personally collected in various parts of the city of Lincoln. The common "out-of-sight" mouse trap was found to be the best for procuring the mice, despite the fact that the mice thus caught were not examined in a perfectly fresh state, though in nearly every case the mice were examined within twenty-four hours after being killed. A trap made after the fashion of the Marty trap, so successfully used in catching rats alive, was also used, but with very poor

success, as only six out of a total of one hundred-five mice were thus caught. The mice were all caught between the months of September and June in 1908, 1909, and 1910.

In examining the mice, the abdomen and thorax were carefully cut open, and the edges were then firmly pinned back in a dissecting pan to completely display the viscera. The following routine was followed in examining the viscera; bladder, rectum and caecum, intestines, ~~st~~ stomach and esophagus, liver, abdominal cavity, kidneys, heart, lungs, thoracic cavity, muscles of the diaphragm and thigh. Blood examinations were made in a number of cases, both with stained and unstained specimens, but no flagellates or other blood parasites were found. Also, in the majority of the cases, the intestinal contents were carefully examined for protozoan parasites, but none were found. This of course does not necessarily mean that either intestinal protozoa or blood parasites are entirely absent in the mice of this vicinity, but it does lead one to the conclusion that they are infrequent.

The thick and impermeable cuticula of the nematode renders the technique of staining, sectioning and mounting especially difficult and rather unsatisfactory. Numerous technique methods were experimented with but

only those found to be of value, together with a few of the best recommended with which satisfactory results were not obtained, will be mentioned in this paper.

The best killing and fixing agents were found to be lacto-phenol, alcoholic glyerin and 3% formol. For toto mounts lacto-phenol used steaming hot, not boiling, proved very efficient as it killed the worms quickly with but a very slight amount of shrinkage or distortion, and in addition cleared the worms very nicely so that the internal structure and relationships could be very easily differentiated. Alcoholic glycerin composed of 10 cc glycerin in 90 cc of 70% alcohol was found to be an excellent killing and fixing fluid if used at just the boiling point, killing and fixing the worms fairly straight and without much shrinkage or distortion. The solution however did not clear the worms without allowing the alcohol to slowly evaporate, when the worms cleared up and were as good for study in toto as those killed in lacto-phenol. 3% formol used either hot or cold killed and fixed the worms fairly well, so that they were very satisfactory for study in sections, but the worms killed and fixed in it could not be satisfactorily cleared with either lacto-phenol or alcoholic glycerin to make toto mounts.

In making toto mounts the worms were almost invariably cleared in lacto-phenol, though fairly good toto mounts were obtained by clearing in evaporated alcoholic glycerin, then placing in pure glycerin. The worms were mounted in Fol's glycerin jelly and after this set firmly they were sealed with asphaltum. As the relationship of the various structures, and in large measure the cell structure could be made out easily in the unstained specimens, few attempts were made to obtain stained toto mounts.

For histological sections the best results were obtained with ordinary paraffine embedding, using from 52° to 55° C. paraffine. Fairly perfect serial sections were thus obtained with practically normal histological arrangement, the sections being cut from .01 to .025 mm thick. The cuticula was in many cases however a very serious handicap, especially in case the worm was allowed to remain in xylol, cedar oil or oil of Bergamot for longer than one to two hours when the cuticula became very tough and almost impossible of sectioning. Sections obtained from celloidin embedding were excellent for histological study, but it was impracticable to obtain serial sections from this method.

Several methods for softening the cuticula were tried, but because of the small size of the worms and

the thickness of the cuticula, they were not altogether satisfactory, as the normal histology was greatly distorted. The best results were obtained by allowing the worms to macerate in cold solutions of eau de Labarraque (Lee, 1905: 309) diluted with 8 to 10 volumes of water for from 30 minutes to 2 hours. This softens the cuticula without very marked deleterious action upon the softer parts and makes the cuticula very permeable to stains. Good results were not obtained with eau de Javelle (Lee, 1905: 309) though the same technique was used as above. Caustic soda, caustic potash, and nitric acid were all used but their action was too severe upon the softer parts. For staining sections on the slide the technique used by Goldschmidt (1903:4; 1905:43) was found very efficient. Delafield's haemotoxyln counterstained with eosin brought out the general nuclear structure especially well, the chromidial granules so prominent in some specimens, and the histology of the intestine, testis, and ovary. Heidenhain's iron haemotoxyln was very useful in the study of the structure of the esophagus, circumesophageal,nerve ring, and the individual nerve cells with their processes. Grenacher's borax carmine counterstained with indigo carmine gave excellent results for the histological study of the

nerve cells, intestine, and reproductive organs, but, for the histological study of other structures, the indigo carmine was too diffuse to be of value. The method followed with this stain was to stain in toto with the borax-carmine, then dehydrate, clear, embed and section, and then counterstain the sections mounted on the slide with a .5% solution of aqueous indigo carmine in 95% alcohol from 2 to 5 minutes. Borax-carmine alone gave good results, when used in staining in toto for sectioning, or when used to stain sections, and was very useful in the study of the development of the ova in the uteri. Mayer's hacmalum counterstained with eosin gave results very similar to those of Delafield's haemotoxyln counterstained with eosin.

The nematodes were very erratic in taking the stain in toto, some taking the stain with great avidity requiring only an hour or so to stain very densely while others would not take the stain for days. Borax-carmine proved to be the most penetrating stain, for it often stained worms that the other stains could not penetrate. For study in toto the stained worms were far inferior to the unstained as they could not be cleared satisfactorily in alcoholic glycerin or lacto-phenol, and though cleared by oil of cedar, or Bergamot when mounted in balsam or

gum damar they became opaque and almost useless for study. However, the worms stained in toto gave very satisfactory results for study in sections and the tedious time-consuming method of stainging sections on the slide was thus abolished.

Several attempts were made to use the Raman J Cajal (Lee 1905:443) and the Golgi slow method (Lee 1905: 435) of impregnating nervous structures, but both proved useless in studying the nervous structure of these nematodes. The hemotoxyln acid-fuchsin and picric acid stain employed by Goldschmidt (1903: 4) gave fair results with the nervous tissue, but not nearly as satisfactory as the iron hemotoxyln, or the borax-carmine counter-stained with indigo carmine.

DATA

A careful record of every mouse examined was kept, in which the date of the examination, size and sex of the mouse, and the kinds and number of parasites found with the organ or organs infected was noted. A total of 105 mice were examined from various portions of Lincoln that the various data obtained might be representative of the mice of this vicinity. The mice were examined in all the months between September and June in 1908, 1909, and 1910. No difference was noted with regard to

the percent or degree of infection during the different months, or years. Both young and adult mice were examined, and it was found that the young mice were much less infected than the adults, and also that the young mice when infected contained a fewer number of parasites than the adults. Of the 105 mice examined 77 were males and 28 were females; of the 29 cases of infection 21 were males and 8 were females, thus showing that the males were infected slightly more than the females. The following table gives the number of mice examined and the number of cases of infection from each locality, together with the parasite or parasites found and the organ or organs infected.

Place	No. of mice	No. of inf.	Nematodes		Cestodes		Cysticerci		Diptera Larvae	
			No. of inf.	Org. inf.	No. of inf.	Org. inf.	No. of inf.	Org. inf.	No. of inf.	Org. inf.
House	23	0								
Store	9	4	4	Cecum St. 1 Cec. 3					1	Body Cavity
Store	9	7	5	St. 2			2	Liver		
Store	26	4	3	Cec. Cec.1			1	"		
Store	6	6	6	St. 5			1	"		
Store	7	1					1	"		
Store	3	1	1	St.						
House	1	1			1	int.				
House	1	0								
Hospital	19	5	5	Cec.2 St. 3			1	"		
House	2	0								
Total	105	29	24		1		6		1	

From the above table it will be noted that 31% of musculus were infected with some sort of an endo-parasite. Of those infected 82% were infected with nematoda; 20% were infected with the larval form of Taenia crassicola, Cysticercus fasciolaris; 3% were infected with cestoda, the only species found being Taenia pussilla Goeze 1782; and 3% were infected with the larvae of some species of Diptera. It will also be noted that 86% of all parasites were found somewhere in the digestive tract. Of the total number of infections there were but three cases of double infection, that is, infection with more than one species whether nematode or other endo-parasite. Two of these cases of double infection were with Cysticercus fasciolaris, 2 in the first case and one in the second, there also being 2 nematodes in the first case and 24 in the second. The third case of double infection was in the case of a mouse examined within 12 hours after being killed. Upon opening the abdomen 9 larvae were found wandering over the diaphragm, liver and stomach; in this case there were also found 4 nematodes. The larvae found in the case just mentioned were larvae of some species of Diptera and must have been contained in the stomach of the mouse at the time of killing, later boring its way

thru the stomach wall into the abdominal cavity. The cysts containing the Cysticereus fasciolaris were all at the surface of the liver, varying from 1 mm. to 15 mm. in diameter; 8 Cysticerci were obtained from the six cases of infection. The following table gives the 24 cases of nematode infection with the species; the number of specimens in each case, together with the number of males and females; the organ infected in each case; and the total number of each species of nematode obtained.

No.of Mouse	Species Nematode	No. of Nematodes	No.of Males	No.of Females	Organ Infected	Total No. Nematodes
22	Acuaria muris	1		1	Stomach	
28	" "	1		1	"	
37	" "	2	1	1	"	
43	" "	8	2	6	"	
71	" "	14	5	9	"	39
72	" "	4	1	3	"	
73	" "	1	1		"	
74	" "	3		3	"	
81	" "	2	1	1	"	
83	" "	3		3	"	
19	Oxyuris tetraptera	5	1	4	Caecum	
					Rectum	
34	"	22	10	12	Caecum	
35	" "	1		1	"	
45	" "	13	3	10	"	47
55	" "	1		1	"	
64	" "	1		1	"	
77	" "	4	1	3	"	
18	Oxyuris obvelata	2		2	Caecum	
					Rectum	
49	" "	24	2	22	Caecum	
87	" "	9		9	"	60
					Rectum	
95	" "	24	1	23	Caecum	
102	" "	1		1	"	
98	Acuaria submucosa	18	11	7	Stomach	
	" "	11	5	6	"	29

With the exception of Acuaria submucosa, new species, which is found embedded in the submucosa of the stomach wall, the nematodes were all found lying free in the lumen of the digestive tract in the midst of the food contents. On examining the above table it will be noticed that the degree of infection varies greatly, even in a sirgle species. Also it will be noted that the female parasites are much more numerous than the male parasites, with the exception of Acuaria submucosa, where the males outnumber the females.

No attempt was made to keep a record of the ecto-parasites, because in most cases the mice had been dead long enough before examination to give ample opportunity for their escape, thus making the record very inaccurate, if not absolutely worthless. Such a record would, of course, be of importance, if blood flagellates were found and it was desired to work out their life cycle, as has been so completely done for the blood flagellate, Trypan-asoma Lewisi, of the rat . However, in such a case it would be necessary to use different methods in procuring the mice and then an accurate record would be possible.

ACUARIA MURIS GMELIN 1790

1782: Ascaris teretes Goeze, 1782a, 76 - partim.
1782: Lumbricus muris domestici minoris 1782a, 10, pl. 8, figs. 1- 7-
1790: Ascaris muris (Werner, 1782) Gmelin 1790a, 3032.
1791: Ascaris obtusa (Werner, 1782) Froelich, 1791a, 88 - 93, pl. 3, figs. 16-17; Rudolphi, 1809a, 156, 170-172; Olfers, 1816a, 64.
1803: Fusaria muris (Werner, 1782) Zeder, 1803a, 106.
1819: Spiroptera obtusa (Froelich, 1791) Rudolphi, 1819a, 27, 249-250: Bremser, 1824a, 126; 1824c, pl. 2, fig. 19-24: Nordmann, 1840a, 661; Dujardin, 1845a, 89-90: Diesing, 1851a, 214-215: Baird, 1853a, 9: Giebel, 1857a, 268: Molin, 1860b, 934; Schlotthauber, 1860a, 125: Cobbold, 1861a, 121: Eberth, 1863a, 64: Beneden, 1866a, 209: Leuckart, 1867b, 113, fig. 85-87; 1868a,366: Marchi, 1871a, 1-30, pl. 1-2, fig. 1-29: Leuckart, 1876a, 514, 614; 1879a, 102: Cobbold, 1879b, 316: deBonis, 1882a, 121; Leuckart, 1886d, 76: Jackson, 1886a, 685: De Witz, 1892b, 143: Anacker, 1893i, 345: Shipley 1896a, 161: vonLinstow, 1904f, 6.
1866: Filaria obtusa (Rudolphi, 1819) Schneider, 1866a, 79, 80, 82, 83, 84, 93, 97, 204, 216, 217, 234, 235, 253; pl. 5, fig. 4; pl. 22, fig. 11: Muhling, 1898b, 51.
1897: Filaria muris (Werner, 1782) Stossich, 1897v, 52.

In reading the literature concerning this species of nematode, I was much vexed at the very meagre descriptions, and the disagreement of the various authors as to the proper taxonomy of the species, as well as the chaotic condition of nematode taxonomy in general. In numerous cases I would have been unable to recognize this species from the descriptions given except for the length, spirally coiled tail of the male, and the habitat. This led to the careful examination, as far as possible, of the

original descriptions and taxonomy, so that the true taxonomic position of this species could be, if possible, definitely and accurately determined.

Goeze (1782a: 76) mentioned this species, including it with a large number of species of intestinal nematodes under the head of Ascaris teretes. He gave no description of the worm but mentions its having been discovered in the stomach of a male mouse and in the intestine of a field mouse. However, it is doubtful if the species found in the intestine of the field mouse is the same as that found in the stomach of the common house mouse, Mus musculus.

Werner (1782a: 10) was the first to describe the species placing it in the genus Lumbricus and designating it by the polynomial name Lumbricus muris domestici minoris. His description was very meagre and crude, and according to Froelich (1791a, 92) he described the female worm as the male.

Gmelin (1790a, 3032) gave a very brief and inaccurate description of the same species described by Werner and changed the name from the polynomial Lumbricus muris domestici minoris Werner 1782 to Ascaris muris Gmelin 1790.

Froelich (1791a, 88-93) gave a much more ex[illegible]

description, though his description was superficial and full of errors. From the drawings which he made of the male worm no definite conclusions could be drawn because of their small size and lack of detail. Judging from the horny obtuse tail and the two spicules of the male ("Das Mammchen mit einen hornformigen stumpfen schwanz-spitze; vor dieser eine wulstige Oeffnung, aus der meist zween Stacheln hervorstehen.") and the broad,,obtuse tail of the female ("das Weibschen am Hinterende sehr stumpf, breitlicht"), the species would seem to be identical with the species later described as Spiroptera obtusa Rudolphi 1819. However, Froelich described the head as having but three papillae ("der Kopf stumpf, knorpelartig, mit drey sehr deutlichen Warchen besetzt") and mentioned no lips. This would lead one to the conclusion that he had simply made a lateral study of the head, when scarcely more than three lips would be visible. He failed to mention the papillae present at the base of the lips, the position of the vulva in the female, and the presence of alae and papillae on the tail of the male. He changed the species to the genus Ascaris and from the polynomial name of Werner's to Ascaris obtusa Froelich 1791.

Zeder (1803a, 106) very briefly described the

species and renamed it Fusaria muris (Lumbricus muris domestici minoris Werner 1782a), no additions being made to the structure as given by Froelich.

Rudolphi (1809a, 170-172) described the species much more completely and accurately than the preceding authors and retained the name Ascaris obtusa Froelich 1791. However, (1819a, 249-250) on closer study of the species he discovered that there were six instead of three papillae ("Caput vere sexpapillare, ut olim retuli, quo certior fierem, anticam partem abscidi, et quas verme integro jam numeraveram singulas vidi papillas"), as designated by Froelich, that the mouth was round ("Os orbiculare") instead of trident as in the Ascarides, and that the tail of the male bore alae ("alae tennes, sed ipsa caudae pars, quam includunt, concava est.") Because of these differences from the Ascarides he placed the species in his new genus Spiroptera and accordingly changed the specific name to Spiroptera obtusa Werner 1782. In his later and more complete description he did not mention the presence of papillae at the base of the lips or on the tail, or the position of the vulva in the female. He gave the shape of the eggs as elliptical ("Ova elliptica, parva"), but gave no dimensions. The length of the worms he gave as varying from 6 to 20

lines, but did not give the length of the male and female separately.

Dujardin (1845a: 89-90) gave a much more accurate and detailed description of the species than had previously been given, though the material for study at his command was very meagre ("J'ai pu etudier deux exemplaires de cet helminthe envoye par le Museum de Vienne a celui de Paris.") He described the mouth as being surrounded by six lobes, ("bouche orbiculaire, entouree de six lobes") but did not give the arrangement or shape of the lobes in detail. In his description was the first mention of the transverse striations of the cuticula ("tegument marque de stries transverses, pen distinctes, ecartees de 0. mm 0046------."), which is a very constant feature of this species. The tail of the male was described more accurately, especially the cuticular alae ("munie de deux ailes membraneuses, soutennes chacune par trois on quatre petit cotes et recourbes en dessous"), though the tree or four supporting ribs he speaks of in connection with the alae have not been described by other authors, and certainly are not present in the specimens which I have examined; in all probability what he took to be supporting ribs were the anal papillae, for he does not mention them in his description. The

spicules, which had not formerly been measured, were given as 1.05 mm. long by .026 mm. broad at the base, for the larger spicule, and as .86 mm. long by .023 mm. broad at the base for the smaller spicule. The situation of the vulva in the female was first noted in this description ("Vulve situee aux trois huiticmes environ de la longueur, a 3 mm. de la bouch, et a 22 mm. 2 de lextremite caudale;"). The length of the eggs was given as from .046 mm. to .048 mm. The species was placed in the genus Spiroptera Rudolphi, 1819, under the specific name Spiroptera obtusa Rudolphi 1819.

Diesing (1851a; 214-215) again described the species briefly adding nothing further, but later (1861) he placed the genus Spiroptera under his family Spiruridae.

Molin (1859a: 934) described the species and was the first to note the six anal papillae ("alis conspicuis usque ad apicem caudalem extensis, singula papillis 6 fungiformibus exornata") but evidently he did not note that they were paired; he also failed to note the single papilla on the anterior lip of the anus, and failed to state the situation of the papillae with regard to the anus.

Schneider (1866a, 97) was the first to call the "papillae" of the head lips ("Kopf mit sechs Lippen

besetzt") and he gave an accurate description (80, 97) of the lips, together with am end view of the head (Fig. V, fig. 4) showing the lips in position and in relation to the four papillae ("Hunter den Lippen 4 Papillen"), which he is the first to note, at the base of the lips. He also was the first to note the inequality of the two cuticular alae on the tail, as well as the rough, cellular appearance of their ventral surface ("Breite Bursa mit ungleich entwickelten Randern. Die innere Flache der Bursa mit Langsreihen erhabner viereckiger Feldchen besetzt!") The six pair of anal papillae were also accurately placed ("und 2 stehen hinter dem Aster, 1 etwas mehr nach aussen. 3-6 stehen auf der langern Seite der Bursa, in grossern abstanden,"), and the single papilla on the anterior lip of the anus noted ("am vordern Asterraude eine unpaare Papille.") The proportion of the lengths of the spicular was given as 3 to 4, and the position of the vulva in the female was placed at 16 mm. from the anterior end. As Schneider did not consider the genus Spiroptera Rudolphi 1819 sufficiently different from the genus Filaria Mueller 1787 to warrant its retention, ("Die Gattung Filaria, nach der von mir aufgestellten Diagnose, umfasst hauptsachlich 2 Gattungen Rudolphi, Filaria und Spiroptera. Filaria und Spiroptera