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PROCEEDINGS OF THE HELMINTHOLOGICAL SOCIETY OF WASHINGTON

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The Pathogenicity of the Common Sheep Tapeworm, *Moniezia expansa*

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The tapeworm, *Moniezia expansa*, occurs in sheep and certain other ruminants throughout the world and, because of its large size, has probably been known to man since sheep became domesticated. Much uncertainty and difference of opinion still exist among sheepmen, veterinarians, parasitologists, and others concerning the role of this parasite as a pathogen. There are several reasons for this state of affairs. Heavy infections have been observed in apparently healthy sheep, and, conversely, moderate to severe clinical symptoms, and even death, in some cases, have been ascribed to this parasite when no other cause was apparent. Until recently it was impossible to obtain experimental proof of pathogenicity or lack thereof, because the life cycle of the parasite was not known. Transmission by oribatid mites was first reported by Stunkard (1937). Some reports of pathogenicity have appeared in connection with the successful use of anthelmintics for expulsion of the worms from sheep and the coincident alleviation of various clinical conditions. Reports of this kind, particularly in regard to use of lead arsenate as an anthelmintic, were recently summarized by Foster and Habermann (1948). There have been only two reports, exclusive of the present one, of experiments designed to determine the possible pathogenicity of *Moniezia* by means of experimentally induced infections, namely, those of Shorb (1939) and Hansen et al. (1950). The purpose of this paper is to present a reasonably complete survey of the literature on this subject and the results of experimental work which have been published previously only in abstract form by us (1949).

REVIEW OF LITERATURE

A summary of the widely scattered literature on this subject is not now available, and an attempt is made here to bring these reports together. Opinions expressed and observations reported in the past may be conveniently summarized under three categories as follows: (a) General statements in various publications, particularly in well-known books on veterinary medicine and helminthology; (b) field observations and case reports many of which are concerned with the use of ruminant teniacides; and (c) experimental work not primarily concerned with anthelmintic medication. Many of the observations on the effect of *M. expansa* infections on ruminants were made before the life cycle of the parasite was known, and before reasonably adequate information on the effects of other parasites, such as gastrointestinal nematodes, coccidia, and other pathogens, was available. Moreover, little or no information derived from experimentally induced infections was available to the earlier workers.

General statements.—The following writers, among others, have considered *M. expansa* a serious pathogen of domestic ruminants under certain conditions:

[1] A considerable part of this work was done with the technical assistance of Mr. C. E. Runkel.

Curtice (1890), Neumann (1905), Daubney (1923), Skriabin and Schultz (1934), Neveu-Lemaire (1936), Monnig (1947), and Hutyra, Marek, Manninger, et al. (1949). The symptoms ascribed to infections by this parasite vary slightly in detail and wording in the different publications of these authors, but may be summarized for all of them as follows: Light infections, especially in older animals, usually do not cause serious symptoms, but because of the large size of the worms, even small numbers on rare occasions may cause various manifestations of disease. Heavy infections in young animals cause serious digestive disturbances, diarrhea, and sometimes constipation; stunting, emaciation, edema, anemia, and considerable loss of wool may occur. Death may ensue after a time as a result of the cumulative effects of the tapeworms or suddenly after a period of convulsions or acute toxemia. The specific cause has been variously ascribed to reduction in available nourishment for the host as a result of the utilization of food by the rapidly growing worms, elimination of waste products and toxins by the parasites, and mechanical blockage of the alimentary tract by masses of worms. Skriabin and Schultz (1934), for example, assert that in Russia enormous losses are caused annually by moniezioses, and that in certain areas, in unfavorable years, a mortality of 80 percent of the young animals has occurred.

On the other hand, the opinion that *M. expansa* is seldom the cause of serious disease of sheep, with the reservation that reliable information on the subject is lacking, has been expressed by Cameron (1934), Clunies Ross and Gordon (1936), Dikmans and Shorb (1942), and Morgan and Hawkins (1949). Some of these authors believe that in supposed losses or serious effects thought to be due to tapeworms, less obvious and more injurious parasites or other pathogens may have been present but were undetected. Furthermore, it has been expressed that until more proof is forthcoming that experimentally induced infections can cause definite injury to the hosts, and that satisfactory evidence is brought forward of improvement in condition of sheep or alleviation of symptoms following removal of tapeworms only by anthelmintics under controlled conditions, it will not be possible to reach any reliable conclusions concerning the pathogenicity of this tapeworm. In this connection, a recent statement by Gordon (1950) follows: "There is little doubt that heavy infestations (of tapeworms) in young sheep exposed to malnutrition are of some consequence." To the writers' knowledge, no work to support this statement has been published.

Field observations and case reports.—Reports of original observations in the field in which *M. expansa* and its close relative, *M. benedeni*, were thought to be the primary cause of disease have appeared for almost a century, from 1855 to 1950. However, their total number is surprisingly small, considering the cosmopolitan distribution of the parasites. These reports are summarized in chronological order.

Cox (1855) reported the loss in England of hundreds of lambs that were under his care. Before death they went off feed, became emaciated, and developed diarrhea. On postmortem examination of the lambs, large quantities of tapeworms, which he considered the cause of the deaths, were recovered. No mention was made of other parasites present, if any, but reference was made to an unusually severe winter preceding the losses and to the poor nutritional condition of the lambs. Some years later—in 1877—Cross reported from England the relief of scouring in lambs when large quantities of tapeworms were removed by administering a ruminant teniacide then in use. The same year there appeared an anonymous report of heavy losses in a large flock of lambs grazed in Central Park, New York City. Many lambs in this flock became sick for two or three days, developed convulsions, turned around in circles, and dropped dead. Only one lamb was examined postmortem and contained enough tapeworms to fill a

12-ounce measure. No other significant details were mentioned in this report. Neumann (1905) cited these reports as evidence of tapeworm pathogenicity. In the discussion of the general paper by Daubney (1923), J. F. Craig spoke of a similar disease outbreak in lambs and ewes which he had observed; diarrhea occurred and death of some of the lambs was preceded by convulsions. One lamb was autopsied and "a large tract of the intestines was almost completely impacted with tapeworms." A Mr. Martin, also participating in the discussion of Daubney's paper, said that "as regarded tapeworms, he did not think much of it."

Since these early observations on alleged tapeworm disease under natural conditions, there has been, to the writers' knowledge, a dearth of reports of a similar nature up to the last decade, when a revival of reports, somewhat reminiscent of the earlier ones, occurred. McCulloch and McCoy (1941) reported treating diarrheic, unthrifty lambs with lead arsenate, which removed "enormous numbers" of tapeworms, and two months thereafter the owner reported the lambs were making better gains than in previous years. Baywater (1942) treated a flock of sheep, many of which showed clinical symptoms of parasitic gastritis (gastroenteritis) accompanied by diarrhea, with a two percent solution of copper sulphate. In his own words: "The results were spectacular. Within an hour of being dosed the animals began to pass large numbers of tapeworms and thereafter recovery was rapid, . . ." No mention was made of other parasites which may have been present and may have been affected by the medication. Likewise, Radeleff (1944) reported a mortality, resulting from monieziasis, as high as 50 percent in herds of kids and calves and 25 percent in lambs, and prompt relief of symptoms and reduction of losses by use of lead arsenate as a ruminant teniacide. He made no mention of any other parasites or pathogens which may have been present in the animals. Further tests of the value of lead arsenate in treatment of lamb scours, and simultaneous removal of quantities of *M. expansa* from lambs, were reported by Habermann and Carlson (1946). A few scouring lambs were treated, tapeworms were effectively removed, scouring ceased shortly after treatment, and the condition of the animals improved. No other cause of the scouring other than tapeworms was observed. These workers did not note any signs of convulsions and sudden deaths, as reported by some other authors.

Two reports have appeared recently in which acute symptoms and sudden deaths in sheep were ascribed to tapeworms. Tableman (1946) observed the death of several lambs on a farm in Illinois, supposedly as a result of heavy tapeworm infections. He stated that "the outstanding characteristics of these cases were: (1) the absence of stomach worms or intestinal roundworms, (2) the excellent condition of the lambs, and (3) the fact that all the lambs died in convulsions. . . . The cause of death in these cases was probably an intoxication due to absorption of metabolic products of the worms—or was it?" Four dead lambs were examined on the farm, and, although it was stated that no parasites other than tapeworms were present, a very rare phenomenon for sheep, no mention was made of a microscopic examination of the contents of the alimentary tract or feces or other diagnostic procedures. Lafenêtre (1948) reported heavy death losses in sheep in numerous flocks pastured in southern France between Beziers and the Pyrenees Mountains. Some of the affected flocks were locally owned, while some wintered in this area but came from summer pastures in the mountains of the Republic of Andorra and the provinces of Pyrénées-Orientales and Ariège. The disease, affecting sheep of all ages, was characterized by sudden onset and rapid progress. Some 250 to 300 deaths occurred among 3,000 to 3,500 animals. The affected animals isolated themselves from the flock, stood stiff-legged with heads down, and mucoid saliva flowed from their lips. The mucous membranes were normal or congested. Body temperatures were normal, but diarrhea was often

seen with abundant and fetid feces. Terminally, convulsions occurred, and the animals became comatose and died. Recovery from the disease was exceptional. Animals killed during the acute phase of the disease showed no macroscopic lesions, tapeworms were present in all cases, some animals had a "few strongyles", and no liver flukes were found. Bacteriological and other diagnostic procedures gave negative results. Further observations were presented, supposedly to support the thesis that tapeworms were the primary cause of these losses. Some 85 animals left from an affected flock were sold for slaughter. These were examined for tapeworms, and "massive" (?) infestations of six to ten tapeworms were observed. Except for a few animals, the carcasses rated top grade. Also, medication of several flocks with copper sulphate was tried, following the dosage recommended by Skriabin and Schultz (1934). The author noted that treatment had been instituted in these flocks when the number of death losses was declining, and that losses had ceased in some flocks spontaneously. It was postulated that tapeworms may cause disease in sheep as a result of teniatoxins produced, or massive infections may favor infection by pathogenic organisms not yet known. In 1948, shortly after Lafenêtre's paper appeared, an anonymous editorial was published, which referred to Lafenêtre's report and certain other selected references, and the suggestion was made that the current view of certain authorities that tapeworms of sheep are of little consequence should be restudied.

A recent report by Link et al. (1950) was summarized by the authors as follows: "An extensive outbreak of *Moniezia expansa* infection in dairy calves is reported. Out of a herd of 79, 19 had died, and 42 were emaciated, lacked vigor, and grew slowly. Treatment with lead arsenate removed the tapeworms and permitted the calves to make an uneventful recovery." The animals were anemic, pot-bellied, and had profuse diarrhea. Pneumonia was present and was considered a probable contributory cause of death. An autopsy was performed on one calf only, and areas of congestion were observed in the lungs and the small intestine. From the latter organ, which was markedly inflamed, 32 tapeworms were recovered. No other details were given regarding the affected animals, except that at the time of medication the yards and calf barn were cleaned and the calves moved to another pasture.

Experimental work.—As already stated, exclusive of the work reported herein, there have been only two reports of work on experimentally induced, pure tapeworm infections of lambs. Shorb (1939) fed four lambs 51 to 203 cysticercoids of *M. expansa* each, over variable periods of time. Three lambs became infected, but no significant clinical symptoms developed during two to two and one half months. Weight gains of the infected lambs were slightly less than those of the controls, but these were not considered significant by the author because of the small number of animals employed. The three infected lambs contained 4, 9, and 39 tapeworms, respectively, at autopsy. Hansen et al. (1950a) fed one lamb 5 cysticercoids, another lamb 60, and kept one uninfected lamb as a control. Later (1950b) in an abstract they stated that six lambs were infected. It was concluded on the basis of this work that infected lambs were retarded in growth, and it was reported that there was some depression in haemoglobin and haematocrit values in comparison with the control lamb or lambs.

Three other reports of an experimental nature are of interest. Freeborn and Berry (1934) studied the weight gains of naturally infected lambs in comparison with the gains of exposed but uninfected lambs (?), and no significant differences were observed. When these lambs were assigned numerical grades at the time of slaughter, the negative controls graded higher than lambs made negative for tapeworms by treatment and those that were infected. No mention was made of other parasites which may have been, and probably were, present in many of the animals.

Shorb (1940) recorded the grades made by a group of 71 tapeworm infected, seven-month-old lambs. Most of these had *Thysanosoma actinioides* infections, a few had *M. expansa* only, and some had both species. No significant grade differences were noted between parasitized and non-parasitizd animals. Hawkins (1946) studied the effect of *M. expansa* infections in a flock of ewes and their lambs over a four-year period. Heavy infections were acquired by the lambs in May or June and were usually lost by August or September. No distinct symptoms could be ascribed to tapeworms alone, and infected and uninfected lambs appeared much the same. It was concluded that for tapeworms alone treatment was not justified, because of the lack of marked symptoms or lesions and spontaneous loss of worms.

MATERIALS AND METHODS

From the preliminary work of Shorb (1939) it was clear that the major limiting factor in making a study involving a larger number of experimentally infected animals than had been used by previous investigators, was the ready availability of several thousand cysticercoids of *M. expansa*, which had to be obtained from oribatid mites. It was also necessary to have this source of infective material available at the time young, parasite-free lambs were also available for the experiment. Details of the method used in this Bureau for securing adequate infective material have been described elsewhere by Kates and Runkel (1948). Because over 3,000 cysticercoids were required for the work reported herein, and the best yield of cysticercoids that could be expected from previous mite dissections was somewhat less than 100 from an equal number of mites collected, it was necessary to collect over 4,000 mites, mainly of the species *Galumna virginiensis*, from a contaminated plot before dissecting the cysticercoids therefrom and starting the experiment. Therefore, mites were collected for five to six weeks before the experiment was begun. This was done by means of a battery of modified Berlese funnels. The mites collected were placed in 50 cc weighing bottles with a small piece of moist filter paper on the bottom, and the bottle tops tightly inserted. Most of the mites remained alive in these bottles until dissected one to six weeks later, and were readily available as a source of cysticercoids when needed.

When enough mites had been collected from the contaminated plot, and parasite-free lambs were available, dissection of mites for cysticercoids was begun, as described by Kates and Runkel (1948). The living cysticercoids were accumulated in physiological saline solution in small watch glasses until enough had been obtained to infect one or more lambs, and then they were placed in small gelatin capsules partially filled with moist, pulverized small animal feed. This small capsule was then placed within a slightly larger one and given to the lamb with a balling gun. Cysticercoids were thus fed lambs within a few hours of the time they were removed from the mites. A total of 16 lambs were fed 121 to 411 cysticercoids per lamb. The total dose was given to each lamb as a single feeding, except that two lambs were given two feedings separated by only two days in one case and three days in another. Although it was not possible to feed the desired numbers of cysticercoids to all the lambs on the same day, infection of all lambs was accomplished during the first week of the experiment after the initial weights of the animals were taken and rations established.

The plan of the experiment was as follows: 16 lambs, two to three months of age and previously weaned, were fed cysticercoids in numbers indicated in Tables 1 and 2. These lambs were divided into two groups of eight each. One group was killed and autopsied approximately one month after infection (Table 1), and the other group approximately two months after infection (Table 2). A third group of four lambs, of approximately similar age and weight, was not infected

TABLE 1.—*Postmortem results on lambs approximately one month after being fed cysticercoids.*

Lamb	Cysticercoids Fed	Scolex Count at Autopsy	Percent Development	Volume of Moniezia in Dilute Formalin (quarts)	Volume of Moist Worms by Displacement (cc)	Variation in Worm Length (cm)	Variation in Worm Development[a]	Weight Gains of Lambs (lbs.)
1	150	114	76	0.50	70	37 to 270	Immature-Gravid	5.50
2	165	75	45	1.00	160	All worms fragmented	do	14.50
3	142	67	47	0.75	100	67 to 420	do	8.50
4	216	53	24	1.00	140	50 to 360	do	11.75
5	160	89[b]	55	0.66	80	4.3 to 180	do	6.00
6	166	56	53	0.50	57	22 to 360	do	6.00
7	121	9	7	0.50	55	175 to 420	All Gravid	8.00

[a] Eggs or proglottids had not appeared in the feces of the lambs before autopsy.
[b] Nine of these tapeworms were less than 30 cm long.

TABLE 2.—*Postmortem results on lambs approximately two months after being fed cysticercoids.*

Lamb	Cysticercoids Fed	Feces Positive[a]		Day of Autopsy	Scolex Count at Autopsy	Worm Material Recovered in Feces			Weight Gains of Lambs (lbs.)
		First Day	Last Day			Total cc (centrifuged)	Estimated Percent		
							Gravid	Non-Gravid	
8	411	30th	63rd	65th	0	104	29	71	10
9	334	31st	51st	66th	0	40	11	89	12.75
10	357	32nd	61st	66th	0	173	39	61	10.5
11	344	40th	68th	68th	0	117	69	31	9.5
12	256	34th	.....	65th	68[b]	200	49	51	13.5
13	250	34th	57th	63rd	0	34	16	84	6.5
14	264	33rd	56th	63rd	0	83	14	86	9

[a] For non-gravid or gravid proglottids or pieces of strobilae; non-gravid material usually passed in feces before gravid material or eggs.

[b] Apparent volume of tapeworms in dilute formalin one pint; volume of moist worms by displacement 80 cc. No terminal proglottids present, no eggs in feces; only one piece of strobila contained a few eggs, all other worms non-gravid. Intact strobilae with scoleces varied in length from 5 to 250 cm.

and used as controls. The reason for having both one-month and two-month groups concerned the fact that in the living animal it is difficult to determine the size of infections established, not only because of the often rapid spontaneous loss of tapeworms from heavily infected animals, but also because of the poor correlation between the production of eggs and proglottids, or both, and the degree of infection. From preliminary infections of several lambs fed variable numbers of cysticercoids, it was observed that tapeworms were not usually lost spontaneously during the first month of infection. Therefore, the one-month group was considered primarily as an "infection control" group, which gave information on the average degree of the infection established by the method employed and the number of cysticercoids fed. Also, this group afforded an opportunity to observe effects of heavy infections during the major part of the prepatent period when the number of tapeworms and quantity of tapeworm material were known.

The infected lambs were observed from day to day for clinical effects of the infections, and once eggs or proglottids began passing in the feces, the animals were bagged and all feces collected and examined daily for tapeworm material until the experiment was terminated. The course of tapeworm infections in lambs cannot be followed to any great advantage by fecal examinations for eggs or by proglottid counts. Therefore, the only practical method found for following quantitatively the course of infection in these lambs was to obtain all feces eliminated during patency and collect therefrom all tapeworm material, individual proglottids, and pieces of strobilae, expelled by the lambs.

The infected animals were autopsied with care to remove the tapeworms intact, if possible. This was usually successful, but in lamb 2 (Table 1) not one intact tapeworm was obtained although 75 scoleces and a large quantity of broken strobilae were recovered. All intact tapeworms recovered postmortem were allowed to relax in water and were then measured. The relative quantities of material thus recovered from the lambs were determined in two ways, as follows: (1) All tapeworms recovered from each lamb were placed in quart fruit jars in dilute formalin, allowed to settle, and the quantity estimated as a pint, quart, or fractions thereof. Results obtained by this rather unsatisfactory method are recorded in the tables only because similar means were employed by certain other workers to measure quantities of tapeworms recovered postmortem or after treatment. (2) Volume of moist tapeworm material was also determined by displacement as follows: Tapeworms in dilute formalin were poured from the container onto a coarse screen and the excess fluid drained off over a uniform period of five minutes, the tapeworms not being allowed to dry. Thereafter, they were transferred to a graduated cylinder containing a known volume of water and the tapeworm volume determined by the water displaced. Volume determinations of the proglottids and fragments of strobilae recovered from dung of lambs of the two-month group were made by centrifugation at 1,000 rpm for two minutes in graduated centrifuge tubes. This method was better than the water displacement method for this material because the material measured consisted of large numbers of individual proglottids and small pieces of strobilae, much of which was in poor condition after being removed from the dung.

The lambs were fed measured quantities of a maintenance diet of hay and grain, so that each group had available the same feed in equal quantities per lamb per day. Any uneaten feed was removed daily and weighed before the next daily allowance was given. Each group of lambs was kept in a concrete-floored pen, half of which had shelter and half did not. All debris was removed daily from the pens, which were then thoroughly washed with hot water. All lambs were weighed weekly, and the total weight of feed eaten was recorded on a weekly basis and for the entire experiment.

RESULTS

Of the 16 lambs that were fed large numbers of cysticercoids, failure to establish infection occurred in only two, one in the one-month group (Table 1) and one in the two-month group (Table 2). The reason for these two failures is not clear. A total of 14 lambs, therefore, were successfully infected experimentally seven in the one-month group and seven in the two-month group. The data on the two uninfected lambs are not included in the tables, as their weight gains were intermediate between the maximum and minimum weight gains of the infected lambs, and the writers believe they contributed nothing to the results of the experiment.

One-Month Group.—Table 1 summarizes the results obtained from the seven lambs killed approximately one month (30 to 34 days) after 121 to 216 cysticercoids were fed per lamb. No tapeworms or eggs were observed in the feces of the lambs up to autopsy. The percentage of tapeworms recovered at autopsy, on the basis of the cysticercoids fed, varied from 7 to 76. The average percentage of development for all lambs was 41. The numbers of tapeworms developing had no close relationship to the number of cysticercoids fed. The actual numbers of tapeworms recovered varied from 9 to 114, the average being 66, indicating that heavy experimental infections were established by the method employed.

At autopsy it was immediately apparent that there was great variation in development of the tapeworms in the six lambs having over 50 worms each, although cysticercoids were fed to each lamb at approximately the same time. Complete tapeworms, with terminal segments, recovered from lambs 1 to 6 varied in development from small, immature specimens to large, gravid ones. The smallest complete worm recovered from lamb 5 was only 4.3 cm long, while the largest worm was 180 cm long. The largest complete worm from any of the lambs was 420 cm long. The nine tapeworms recovered from lamb 7, which had the smallest number at autopsy, were more uniform in size, and all were in the same stage of development, with gravid terminal proglottids. When the volume of moist tapeworms recovered from each lamb was determined by displacement, there was little correlation between the volume and the scolex count. The 9 worms from lamb 7 had a volume of 55 cc, that of the 56 worms from lamb 6 was only 57 cc, while the 114 worms from lamb 1 had a volume of only 70 cc. Thus, in lambs 1 to 7 the heaviest infection by volume was only about three times that of the lightest infection, namely, 160 cc against 55 cc; by numbers, the heaviest infection was almost 13 times the lightest infection, namely, 114 against 9. Therefore, the data in Table 1 show that when large numbers of tapeworms are present in lambs, their growth is irregularly retarded, and when few worms are present they develop more or less uniformly. Therefore, these observations indicate that in pathogenicity studies volume of tapeworms appears to be a more reliable criterion of degree of an infection of *M. expansa* than number of individuals present. It is difficult to think of the relatively small, unarmed scolex of this species as an important factor in causing injury to the host.

Lambs of the one-month group did not show any effects of their relatively heavy tapeworm infections during the experiment. At autopsy a considerable portion of the small intestines of these small lambs appeared to be filled with tapeworms, but there were no observable injurious effects on the intestines or other organs. There was considerable variation in weight gains of the lambs, but these did not seem to be related to the degree of infection. It is of interest to note, but probably not significant, that lambs 2 and 4, having the heaviest infection by volume of worms, made the greatest gains. The average gains for both the infected and control groups, during the same experimental period, were

identical, namely, 8.6 pounds per lamb, and the feed consumption per lamb for the two groups was also the same.

Two-Month Group.—Table 2 and Figure 1 summarize the data on the seven infected lambs observed for about two months. These lambs were fed somewhat larger numbers of cysticercoids than the one-month group, the total per lamb varying from 250 to 411. Infections in the seven lambs were shown by the presence in their feces of individual proglottids or pieces or strobilae, beginning

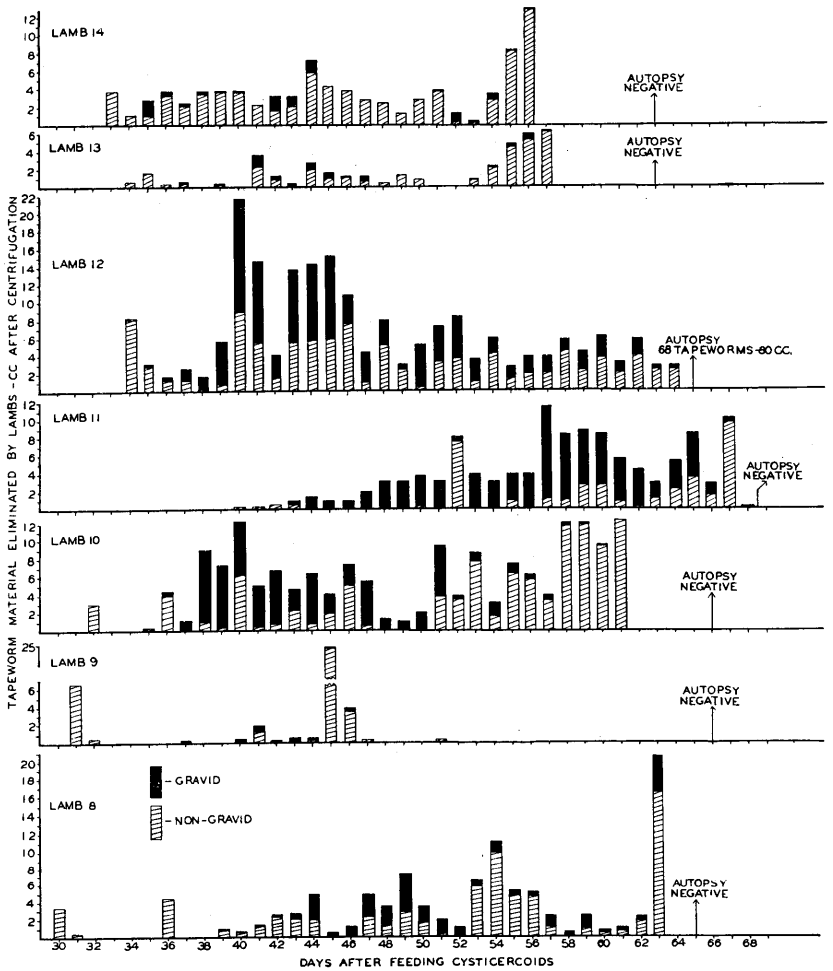

FIG. 1. Detailed summary of the tapeworm infections established in each of the lambs of the "two-month group" (Table 2), showing the daily output of tapeworm material by volume in the feces, and the extent of the patent period of infection and tapeworms recovered, if any, from lambs at autopsy.

on the 30th to 40th day after cysticercoids were fed. Six of the lambs ceased passing tapeworm material in their dung between the 51st and 68th days after infection. Lamb 12 was still passing some proglottids in the feces on the 65th day, when it was killed and examined. This lamb was the only one of the group retaining some of its tapeworms to the time of autopsy, 68 tapeworms being re-

covered at that time. The other six lambs were tapeworm-free when autopsied 63 to 68 days after cysticercoids were fed. Therefore, six of the lambs of this group spontaneously expelled their tapeworms at some time during the second month after infection between the 30th and 68th days.

An accurate check was obtained on the infections, even though at autopsy six of the lambs were tapeworm-free. All the tapeworm material passed in the feces of the lambs was collected daily and measured volumetrically. Table 2 gives the total volume of material obtained after centrifugation from each lamb during the patent period. This method of measuring the quantity of tapeworm material expelled in the feces is roughly comparable to the displacement method employed for the one-month group (Table 1), but because the material collected from the feces is not in as good condition as similar material collected directly from the small intestine at autopsy, the figures in table 2 are probably lower than they would be for fresh tapeworms. The seven lambs passed 40 cc to 200 cc of tapeworm material each during patent periods of 20 to 33 days. These totals show that heavy parasitism was established in all cases. In addition, as already stated, lamb 12 retained 68 tapeworms, amounting to 80 cc by displacement, when autopsied 65 days after infection, making a total of 280 cc of tapeworm material from this lamb.

Tapeworm material passed in the feces was examined to ascertain how much consisted of gravid segments and strobilae and how much was non-gravid; considerable quantities of non-gravid strobilae is indicative that more or less complete tapeworms were being expelled. Table 2 shows that in six of the lambs more than 50 percent was non-gravid; in one lamb only 31 percent was non-gravid. The data show that whole tapeworms were being spontaneously lost from time to time during the patent period, even though it was not possible to identify scoleces in 24-hour fecal collections.

Figure 1 shows the daily record of tapeworm material expelled from each lamb. The first material expelled was almost entirely non-gravid, which showed a tendency early in the infection toward expulsion of entire immature worms or parts thereof. In lambs 9, 13, and 14, the percentages of non-gravid material passed in the dung were respectively 89, 84, and 86, indicating that for these lambs most of the tapeworms were expelled before reaching the gravid stage. In lamb 9, over 60 percent of the total tapeworm material recovered during patency was expelled on the 45th day after cysticercoids were fed. In lambs 13 and 14 the tapeworms were expelled over a more or less extended period.

Figure 1 also shows that the greatest quantities of gravid segments and strobilae were expelled from lambs 8, 10, 11, and 12. Lamb 11 was the only one of the group which expelled more gravid than non-gravid material, and had the longest prepatent period (40 days) for its tapeworm infection. The tapeworms in this lamb probably became more firmly established than in the others, and a larger proportion of the worms were able to reach the gravid stage before being expelled. However, many worms were probably expelled on the 51st day and the remainder on the 67th day after infection, as indicated by the large quantities of non-gravid material expelled during these two days. In lambs 8, 9, 10, 13, and 14, all of which spontaneously expelled their entire infection before autopsy, the mass of tapeworms was lost as follows: Lamb 8, on the 63rd day after infection; lamb 9, on the 45th day; lamb 10, between the 58th and 61st days; lamb 13, between the 55th and 57th days, and lamb 14, during the 55th and 56th days.

These observations on relatively heavy infections show a strong tendency for spontaneous expulsion of all tapeworms from the host. However, there is evidence that this is not necessarily true in relatively light infections, for in one infection, produced by feeding only a few cysticercoids to a lamb, elimination of gravid

proglottids in the feces began after the usual prepatent period and continued at a relatively steady pace for about ten and one half months, when the animal was killed. At that time two large gravid specimens of *M. expansa* were recovered from the small intestine.

There was no evidence of clinical effects of parasitism in the seven lambs of this group during the entire experiment. As shown in Table 2, there was considerable variation in weight gains of the lambs, but this was not related to variations in degree of infection, as determined volumetrically. In fact, lamb 12, which expelled the greatest volume of tapeworm material, and still retained 68 tapeworms having a volume of 80 cc, made the greatest gain. In contrast, lamb 13, which expelled the least tapeworm material and was tapeworm-free at autopsy, made the smallest gain. It is believed, however, that these data on individual gains are of value only in showing that the heaviest infections did not depress the gains of the lambs; they are not interpreted to mean that heavy infections have favorable effects on growth or on feed utilization by lambs. The average gain of the infected lambs during the experiment was 10.25 pounds, while the control lambs gained an average of 10.75 pounds. The total feed consumption of the control lambs averaged one half pound higher than that of the infected lambs. The slightly higher average gain by the controls than by the infected lambs is not significant.

DISCUSSION

Pathogenicity of Moniezia.—The literature on the pathogenicity of the *Moniezia* spp. to ruminants, especially sheep, which has been summarized earlier in this paper, does not afford a strong case that tapeworms alone are the cause of clinically acute disease and death of the host. Most of the reports of tapeworm disease resulting from natural infections leave much to be desired, especially in regard to detailed accounts of the disease conditions observed by the various authors and the possible causative organisms, which may have been present in the animals. The nature of the disease conditions reported in the past for sheep and cattle and ascribed to tapeworms will probably never be determined with certainty. It is safe to conclude, however, that the acute symptoms and death losses reported as caused by tapeworms by Cox (1855), Cross (1877), Anonymous (1877), Lafenêtre (1948), Link et al. (1950), Radeleff (1944), Skriabin and Schultz (1934), Tableman (1946), and others are not confirmed by critical observations, involving both experimental and naturally acquired infections. For instance, it is not possible to take seriously, on the basis of available evidence today, the claims of Skriabin and Schultz (1934) that tapeworms caused a mortality as high as 80 percent of young animals in certain areas in Russia, and that of Radeleff (1944), who claimed that moniezioses caused a mortality of 50 percent in kids and calves and 25 percent in lambs in Texas. Neither of these reports was documented with data to show that tapeworms were the sole cause of these losses. Many similar reports must also be discounted because of insufficient supporting data.

The writers of this paper believe that a strong case can be made today, on the basis of evidence now available, that *Moniezia* infections of sheep are relatively innocuous, even when the parasites are present in large numbers in young lambs. This belief is supported by the work of Shorb (1939), Hawkins (1946), that of the writers of this paper and to some extent by the work of Hansen et al. (1950). Shorb made the first report on experimental infections, employing a small number of lambs, and no significant evidence of tapeworm pathogenicity was obtained. Hawkins carefully studied an experimental flock of ewes and their lambs during a four-year period under conditions very favorable to the develop-

ment of heavy natural tapeworm infections in the lambs, and reported that no distinct symptoms ascribable to tapeworms alone were observed. The lambs usually developed heavy infections in May or June, which usually were spontaneously lost by August or September. The writers of this paper produced infections in 14 young lambs, most of them being the heaviest experimental tapeworm infections thus far reported. However, no injurious effects were observed. Hansen et al. also infected experimentally six lambs, using smaller numbers of cysticercoids per lamb than those used by the present writers. They reported only slight retardation in growth and slight depression of haemoglobin and haematocrit values in the infected animals, which they considered significant. In none of this admittedly limited experimental work has any sign appeared that heavy tapeworm infections in lambs can cause serious disease, with acute symptoms and death. Furthermore, no proof has been presented by anyone that gross or histopathological changes, or both, in the small intestines or other organs accompany heavy infections of *M. expansa* alone. Certainly, no such changes were observed by the present writers in 14 infected lambs, seven of which were known to retain from 53 to 114 tapeworms at autopsy.

This does not mean, however, that there is conclusive proof that *Moniezia* infections of lambs and older animals are at all times innocuous and can be ignored. The contention of Gordon (1950) that heavy tapeworm infections of young, malnourished sheep are of some consequence should be investigated further. Also, the assertion by such an authority as Monnig (personal communication) that young lambs before weaning show the most serious effects of heavy tapeworm infections should be thoroughly investigated.

Variation in growth and development of M. expansa in heavy infections.— Measurements and observations of complete tapeworms obtained at autopsy of lambs fed large numbers of cysticercoids approximately one month earlier, showed great variation in size and degree of development. As all the cysticercoids were fed at approximately the same time, in most cases as a single dose, it was of interest to note that complete worms obtained from these lambs varied in length from less than two inches to six or more feet, and in development from small worms having a scolex and only a few immature proglottids to very large worms with strobilae consisting of hundreds of proglottids, many of which were mature and gravid. If this great variation in growth of tapeworms in heavy infections is a common phenomenon in sheep, as it seems to be, it is obvious that the actual scolex count of tapeworms at autopsy does not give an accurate quantitative picture of the infection in individual cases. Therefore, in estimating the degree of infection a more accurate method appears to be a measurement of volume of tapeworm material by some appropriate method, rather than by a count of the scoleces. Data presented in Table 1 show that the scolex count in different lambs varied greatly, from 9 to 114, but the volume of tapeworm material varied only from 55 to 160 cc. In addition, the lamb having the largest number of worms did not have the greatest volume of tapeworm material.

The great variation in development of tapeworms in heavy infections has been ascribed by some authors to the so-called "crowding effect", which has not been fully elucidated. The suggested explanations of this phenomenon of retarded growth in heavy infections were summarized by Reid (1942) as follows: "(1) that a local immunity is developed in the host, (2) that insufficient food is available for all tapeworms, (3) that excretory products of the worms inhibit growth, (4) that an actual physical crowding takes place. There is some evidence against certain of these suggestions but no positive supporting evidence is available."

Recently, Read (1951) agreed with Reid (1942) in that previously suggested

causes of the "crowding effect" are unsupported by evidence, and stated "that the hypothetical limiting factor in the crowding effect is probably not a food substance obtained from foodstuffs ingested by the hosts." He suggested that the substance now known to be present in small, variable quantities in the small intestine, which may fulfill the criteria for a growth limiting factor on tapeworms, is oxygen. This explanation is hypothetical and supported by a minimum of evidence, but deserves as much consideration as the others.

Spontaneous loss of infections.—Hawkins (1946), Hansen et al. (1950), and others have noted the tendency of spontaneous loss of tapeworms from sheep. This phenomenon has been demonstrated as a fairly consistent one in heavy infections by the data presented in Table 2 and Figure 1. Six of seven lambs spontaneously lost their tapeworms some time during the second month after acquiring them; one lamb did not, retaining 68 parasites on the 65th day after infection. These observations and those of others show that sheep tapeworms (*M. expansa*) in heavy infections apparently lead a precarious existence in the small intestine of lambs, and may be expelled before or soon after they reach their full development. Infections of a few worms, or rarely heavy infections, may be retained by sheep for long periods. This evidence of spontaneous loss of *M. expansa*, especially in heavy infections of lambs, should be taken into consideration in all critical work on tapeworm anthelmintics, and adds further support to the contention that this parasite is of little consequence as a pathogen.

SUMMARY

1. The important literature on the pathogenicity of *Moniezia* spp. to ruminants, especially that of *M. expansa* to sheep, is briefly summarized.

2. Heavy infections of *M. expansa* were induced experimentally in 14 lambs by feeding 121 to 411 cysticercoids per lamb.

3. Seven of the parasitized lambs were autopsied approximately one month after being fed cysticercoids, and from 9 to 114 tapeworms were recovered from each lamb. Six of these lambs had over 50 tapeworms each. The volume of moist tapeworm material varied from 55 to 160 cc per lamb. The tapeworms obtained from the lamb having only 9 parasites were all large and gravid, while those from the other six lambs varied greatly in size and in development—from immature to gravid specimens.

4. Seven of the infected lambs were observed for approximately two months before they were killed and examined for tapeworms. Six were tapeworm-free at autopsy, while one lamb still retained 68 tapeworms of variable sizes. All these lambs expelled rather large quantities of tapeworm material in the feces, much of it being non-gravid pieces of strobilae, and some probably were complete worms although not identifiable as such.

5. The infections did not result in any observable injurious effects to the lambs, or any significant retardation in growth in comparison with that of the controls.

LITERATURE CITED

ANONYMOUS. 1877. Enzootic amongst lambs. Amer. Vet. Rev. **1**: 147–148.

———. 1948. Ovine and caprine monieziasis. Editorial, J. A. V. M. A. **113**: 270–271

BAYWATER, H. E. 1942. Copper sulphate tolerance of sheep. Vet. Rec. **38**: 380.

CAMERON, T. W. M. 1934. The internal parasites of domestic animals. London.

CLUNIES ROSS, I, and GORDON, H. McL. 1936. The internal parasites and parasitic diseases of sheep. Sydney.

COX, W. 1855. Taenia in lambs. Veterinarian, London **28** (332), 4. s. **1**: 471–472.

CROSS, J. G. 1877. Tapeworms in lambs, causing scour. Ibid. **50**: (595), 4, s. **23** (271): 471–472; notes by editors p. 472; (596), 4. s. (272): 552.

CURTICE, C. 1890. The animal parasites of sheep. U. S. Dept. Agric., Washington.
DAUBNEY, R. 1923. The adult tapeworms of sheep, particularly those occurring in Great Britain. Vet. Rec. 3: 679–686.
DIKMANS, G. and SHORB, D. A. 1942. Internal parasites of sheep and goats. U. S. Dept. Agric. Yearbook, Keeping Livestock Healthy, Washington pp. 859–903.
FOSTER, A. O., and HABERMANN, R. T. 1948. Lead arsenate for removal of ruminant tapeworms. J. A. V. M. A. 113: 51–54.
FREEBORN, S. B. and BERRY, L. J. 1934. Observations on the sheep tapeworm, *Moniezia expansa,* in California. Ibid. 85: 611–616.
GORDON, H. McL. 1950. Some aspects of parasitic gastro-enteritis of sheep. Austral. Vet. Jour. 26: 14–28.
HABERMANN, R. T. and CARLSON, F. N. 1946. Lead arsenate relieves scouring in lambs due to tapeworm infestation. Vet. Med. 41: 306–310.
HANSEN, M. F., KELLEY, G. W., and TODD, A. C. 1950. (a) Observations on the effects of a pure infection of *Moniezia expansa* on lambs. Trans. Amer. Microscop. Soc. 69: 148–155; (b) Effects of a pure infection of the tapeworm *Moniezia expansa,* on lambs. Jour. Parasitol. 36 (6, sec. 2): 45.
HAWKINS, P. A. 1946. Studies of sheep parasites. VII. *Moniezia expansa* infections. Ibid. 32 (6, sec. 2): 14.
HUTYRA, MAREK, MANNINGER, *et al.* 1949. Special pathology and therapeutics of the diseases of domestic animals. Chicago, Vol. II, 5th ed. p. 284.
KATES, K. C. and RUNKEL, C. E. 1948. Observations on oribatid nite vectors of *Moniezia expansa* on pastures, with a report of several new vectors from the United States. Proc. Helminthol. Soc. Wash. 15: 19–33.
———— and GOLDBERG, A. 1949. Experimental tapeworm (*Moniezia expansa*) infections in young lambs. Jour. Parasitol. 35 (6, sec. 2): 38.
LAFENÊTRE, H. 1948. A propos d'une enzoitie de monieziose ovine. Bull. Acad. Vet. France 21: 152–156.
LINK, R. P., DEVINE, N. D., DANKS, A. G. and WOELFFER, E. A. 1950. Moniezia infection in a calf herd. J. A. V. M. A. 117: 52–53.
MCCULLOCH, E. C. and MCCOY, J. E. 1941. Treatment of ovine teniasis with lead arsenate. Ibid. 99: 496–497.
MONNIG, H. O. 1947. Veterinary helminthology and entomology. Baltimore.
MORGAN, B. B. and HAWKINS, P. A. 1949. Veterinary helminthology. Minneapolis.
NEUMANN, L. G. 1905. Treatise on the parasites and parasitic diseases of domesticated animals. London, 2nd ed. (English).
NEVEU-LEMAIRE, M. 1936. Traité D'Helminthologie Médicale et Vétérinaire. Paris.
RADELEFF, R. D. 1944. Lead arsenate an effective taeniacide for domestic ruminants. Vet. Med. 39: 453–454.
READ, C. P. 1951. The "crowding effect" in tapeworm infections. Jour. Parasitol. 37: 174–178.
REID, W. M. 1942. Certain nutritional requirements of the fowl cestode *Raillietina cesticillus* (Molin) as demonstrated by short periods of starvation of the host. Ibid. 28: 319–340.
SHORB, D. A. 1939. Preliminary observations of the effect on sheep of pure infestation with the tapeworm, *Moniezia expansa.* Proc. Helminthol. Soc. Wash. 6: 77–79.
————. 1940. A note on the effect of tapeworm infestation on the condition of sheep. Vet. Med. 35: 180–181.
SKRIABIN, K. I. and SCHULTZ, R. E. 1934. La lutte contre les monieziose. Invasions des moutons par les vers rubanes. Bull. Office Internat. Epizoot. 8: 355–378.
STUNKARD, H. W. 1937. The life cycle of *Moniezia expansa.* Science 86: 312.
TABLEMAN, H. G. 1946. Taeniasis in lambs. Vet. Med. 41: 455–456.

Tests with a Phenothiazine-Salt Mixture as a Growth Stimulant for Sheep

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That the administration of a phenothiazine-salt mixture to sheep is often followed by an increased gain in weight of the treated animals has been demonstrated by Britton and Miller (1944), Seghetti and Marsh (1945), and others. So far as the writers have been able to ascertain, this effect has been observed only in conjunction with roundworm control, and the increased gains in weight have been attributed to the anthelmintic action of the phenothiazine. Apparently no attempts have been made to determine whether phenothiazine possesses growth stimulating properties in the absence of nematode infections of consequence. The present report describes experiments which clarify this problem.

DATA

The observations were made for two consecutive years, starting in November, 1949. Lambs were made available by Professor P. E. Neale of the Animal Husbandry Department of New Mexico State College.

In each year 40 lambs, ranging in age from 8 to 10 months, were divided into 4 groups of 10 each, and placed in dry lots. Their diet consisted of alfalfa cubes. Some of the lambs in each group came from the range and some came from dry lots or small irrigated pastures. Two groups of the lambs had available to them throughout the test period a mixture consisting of 1 part phenothiazine and 9 parts common salt, while the other two groups received only salt.

During the first year one experimental group (Lot 6), with controls (Lot 5), was on test for 25 days, while the other experimental group (Lot 8), with controls (Lot 7), was on test for 46 days. Corresponding periods for the second year were 33 days (Lots 6 and 5) and 47 days (Lots 8 and 7).

Differential egg counts, based on the descriptions published by Kates and Shorb (1943), were made on individual lambs at the beginning and end of each test. They indicated the presence of only small numbers of nematodes of the following species: *Haemonchus contortus*, *Trichostrongylus* spp., *Nematodirus spathiger*, and *Strongyloides papillosus*.

All lambs were weighed individually at the beginning and end of each test. During the first year the average daily gains in weight in pounds were as follows: Lot 6, 0.5; Lot 5, 0.52; Lot 8, 0.4; and Lot 7, 0.49. During the second year the gains were: Lot 6, 0.41; Lot 5, 0.48; Lot 8, 0.41; and Lot 7, 0.56. Thus, in each instance greater gains in weight were made by lambs receiving salt only than by lambs receiving the phenothiazine-salt mixture. Treatment of the data by analysis of variance showed that the differences during the first year were not significant. Those of the second year were found to be significant, but this was due to the fact that 3 lambs in the groups receiving the phenothiazine-salt mixture for some unknown reason made gains in weight far below the average.

CONCLUSIONS

It is concluded on the basis of these experiments that a mixture containing 1 part phenothiazine and 9 parts common salt does not, in itself, possess properties capable of stimulating the growth of sheep. It seems probable, therefore, that the increased growth rates which have been observed by other workers following the administration of this mixture are due solely to the anthelmintic action of the drug.

LITERATURE CITED

BRITTON, J. W. and MILLER, R. F. 1944. The practical application of anthelmintic medication of lambs. J. Am. Vet. Med. Assoc. **104**: 170–172.

KATES, K. C. and SHORB, D. A. 1943. Identification of eggs of nematodes parasitic in domestic sheep. Am. J. Vet. Res. **4**: 54–60.

SEGHETTI, L. and MARSH, H. 1945. Control of intestinal parasitism in lambs by winter treatment of ewes as compared with the use of phenothiazine in salt in summer. Am. J. Vet. Res. **6**: 159–164.

Notes on the Trematode Genus *Glypthelmins* Stafford, 1905

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Much of the controversy concerning the validity of species assigned to the trematode genus *Glypthelmins* Stafford, 1905, may be considered to have resulted from: (1) the inadequate generic characterization; (2) insufficient information available to investigators who have reviewed or commented on the species of the genus; and (3) the close relationship of other genera of the subfamily Plagiorchiinae Pratt, 1902, to *Glypthelmins*. It is the purpose of this paper to present a brief historical review of the genus and to propose suggestions which might aid in clarifying the systematics of this group.

The genus *Glypthelmins* was erected by Stafford (1905) with the species *Distomum quietum* Stafford, 1900. As genotype, it may be considered that the inadequate description given by him laid the groundwork for much of the confusion concerning the validity of species later assigned to the genus. Recognizing the controversy regarding the uncertainty of species assigned to *Glypthelmins*, Miller in 1930 redescribed the type species *G. quieta* (Stafford, 1900). Although he listed seven species [*G. quieta; linguatula* (Rud., 1819) Travassos, 1924; *parva* Travassos, 1924; *repandum* (Rud., 1819) Travassos, 1924; *elegans* Travassos, 1926; *staffordi* Tubanqui, 1928; and *californiensis* (Cort, 1919) Miller, 1930] he recommended that the genus be revised, since it was apparent that some of the species did not fit the genus as originally described by Stafford. Olsen (1937), accepting Miller's emendation to include species without, as well as with, pharyngeal glands, presented a key to the genus *Glypthelmins* to which he added two species, *G. subtropica* Harwood, 1931, and *G. shastai* Ingles, 1936. Caballero (1938), in his review of the genus and the presentation of his key, considered ten valid species—those listed by Miller and Olsen and one additional, *G. rugocaudata* (Yoshida, 1916) Yahata, 1934. (This species had been returned to the genus *Endiotrema* Looss, 1900, by Syôgaki in 1937.) *G. palmipedis* (Lutz, 1928) was omitted from the keys of Olsen and Caballero. Review of the literature revealed that this species was briefly described as *Haplometra palmipedis* Lutz, 1928; Travassos (1930), after having examined the original material of Lutz, transferred it to the genus *Glypthelmins*. In 1941, de Freitas described two new species, *G. simulans* and *G. proximus*. In this same work, de Freitas redescribed *G. palmipedis* and *G. subtropica*. The addition of these new species brought the total number assigned to the genus to thirteen.

Following the suggestion of Miller (1930) that the genus be revised, Rankin (1944) made a systematic study of "hundreds of specimens from about seventy-five individual hosts." Employing the keys of Olsen and Caballero, Rankin stated, "Attempts to identify specimens found by the use of these keys failed." Using characters relating to the metraterm, uterine coils, and vitellaria, he concluded that

G. quieta, elegans, linguatula, and *repandum* were valid and that *G. californiensis, parva, rugocaudata, shastai, staffordi,* and *subtropica* were synonyms of the type species.

Cordero (1944) described as new *G. festina* and *G. sera.* Ruiz (1949) removed *G. elegans* to the genus *Choledocystus* Pereira and Cuocolo, 1941, and regarded *C. eucharis* Pereira and Cuocolo, 1941, and *C. vesicalis* Ruiz and Leão, 1942, as synonyms of *Choledocystus elegans* n. comb., which, because of the law of priority, became the genotype. Byrd (1950) accepted Rankin's revision but removed *G. repandum* to the genus *Microderma* Mehra, 1931, retaining as valid *G. quieta, linguatula,* and *elegans.* Apparently, Byrd considered *Glypthelmins* a more suitable genus for *elegans* than *Choledocystus. Glypthelmins africana* Dollfus, 1950, was the more recent species assigned to the genus.

The brief historical background of the species which have been assigned to *Glypthelmins*[1] indicates that the characters used in classification of the genus should be more explicit. From the examination of available types and paratypes assigned to this genus, a study of literature, and discussions with several investigators who have worked with the genus, two alternatives appear possible for correcting the systematics of the group: (1) create a new genus for the South American species; or (2) amend the genus so as to include these species.

It was pointed out by de Freitas (personal communication) that the species of *Glypthelmins* are divisable into two groups:

a. species with peripharyngeal glands and without uterine coils developed in the pretesticular zone.

b. species without peripharyngeal glands and with uterine coils developed in the pretesticular zone.

On the basis of point *b,* a new genus could be created to include the species *G. linguatula, repandum, palmipedis, simulans,* and *proximus.* The species *repandum* does not belong in the genus *Microderma,* since that genus is characterized by the absence of an esophagus and the possession of a coiled seminal vesicle. To the first group *a* belong *G. quieta, californiensis, rugocaudata, shastai, staffordi,* and *subtropica.* The species *G. parva* might be considered intermediate, since it does not have peripharyngeal glands nor uterine coils in the pretesticular zone. This species appears to have been described from specimens which were not properly fixed; consequently, proper interpretation of its morphological details has been difficult. The species *elegans* need not be considered since it has been removed from the genus.

Employing the characters used by Rankin in his determination of valid species of *Glypthelmins,* Caballero (personal communication) and the writer found the species *G. simulans* and *G. sera* to be synonyms of *G. linguatula,* the species *G. proximus* to be a synonym of *G. repandum,* and that the species *G. festina* could be placed in the genus *Choledocystus,* since it possesses generic characters making it resemble very closely the type species, *C. elegans.* It must be pointed out that in making this study only the characters listed by Rankin were employed in deriving the above conclusions.

Apparent generic relationships of the adult trematodes alone are often not substantiated when larval characters are considered. McMullen (1931), and Byrd, Parker, and Reiber (1940) have shown the advantage of studying the excretory system of a group in attempting to determine its systematic position. Baer (1924) pointed out, "If the excretory systems were better known, it might perhaps be shown that the relationship between various families can be established by the

[1] Rankin (1944) and Ruiz (1949) offer extensive historical accounts of most of the species assigned to this genus.

shape of the excretory vesicle.'' In this same work, Baer diagrammed what he considered the ontogeny of the excretory vesicle, showing that the remotely different Y-shaped and I-shaped vesicles are derived from a common stem and that similarity does exist during certain stages of its evolution. The genera, *Choledocystus* and *Astiotrema* Looss, 1900, appear to be phylogenetically related to *Glypthelmins* when a general morphological study of these groups is made. It has probably been due to this close relationship that specimens have been erroneously assigned to these groups. The excretory vesicle in *Astiotrema* is Y-shaped, in *Glypthelmins* it is I-shaped, and in *Choledocystus* there is a reduction in the branches of the Y so as to make the appearance of the vesicles in the form of an I. *Choledocystus,* therefore, may be considered as being in a state of transition intermediate between *Astiotrema* and *Glypthelmins.* Ruiz (1949) made a similar analogy, but apparently considered *Choledocystus* closer related to *Plagiorchis* Lühe, 1899, than *Astiotrema.*

It has been shown that the genus *Glypthelmins* has been complicated by species which do not fit the genus as originally described by Stafford. This, to a large extent, has been due to the inadequate description of the characters of the genus which should readily serve to distinguish this genus from others of the subfamily Plagiorchiinae. Two alternatives have been suggested to correct the systematics of this group and to insure that differentiation of *Glypthelmins* from other genera is sufficiently explicit. Until additional material becomes available so that a more comprehensive morphological study may be continued, the writer feels no justification for making revisions at this time.

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LITERATURE CITED

BAER, J. G. 1924. Description of a new genus of Lepodermatidae (Trematoda) with a systematic essay on the family. Parasit. **16**: 22–31.

BYRD, E. E. 1950. *Allogyptus crenshawi,* a new genus and species of digenetic trematode (Plagiorchiinae) from the chameleon. Trans. Amer. Mic. Soc. **69**: 280–287.

————, PARKER, M. V., and REIBER, R. J. 1940. A new genus and two new species of digenetic trematodes, with a discussion on the systematics of these and certain related forms. J. Parasit. **26**: 111–122.

CABALLERO Y C., E. 1938. Revision y clave de las especies del genero *Glypthelmins.* An. Inst. Biol., Mexico. **9**: 121–149.

CORDERO, E. H. 1944. Dos nuevas especies de tremátódos del genero *Glypthelmins* de los bactracios del Uruguay. An. Acad. Brasil. Cienc. **16**: 1–8.

DE FREITAS, J. F. TEIXEIRA. 1941. Sobre alguns tremátódeos parasitos de rãs. Rev. Brasil. Biol. **1**: 31–40.

DOLLFUS, R. PH. 1950. Trematodes recóltés au Congo Belge par le Professeur Paul Brien (mai-août 1937). Ann. Mus. Congo Belge. C, Ser. V, **1**: 1–136.

LUTZ, A. 1928. Estudios sobre tremátódeos observados en Venezuela. In: Estudios de Zoologia y Parasitologia Venezolanas. pp. 101–125.

MCMULLEN, D. B. 1937. A discussion of the taxonomy of the family Plagiorchiidae Lühe, 1901, and related trematodes. J. Parasit. **23**: 244–258.

MEHRA, H. R. 1931 c. On a new trematode *Microderma elinquis,* n.g., n.s. Parasit. **23**: 191–195.

MILLER, E. L. 1930. Studies on *Glypthelmins quieta* Stafford. J. Parasit. **16**: 237–243.

OLSEN, O. W. 1937. A systematic study of the trematode subfamily Plagiorchiinae Pratt, 1902. Trans. Amer. Mic. Soc. **56**: 311–339.

RANKIN, J. S. 1944. A review of the trematode genus *Glypthelmins* Stafford, 1905, with an account of the life cycle of *G. quieta* (Stafford, 1900) Stafford, 1905. Trans. Amer. Mic. Soc. **63**: 30–43.

RUIZ, J. M. 1949. Considerações sobre o gênero *Choledocystus* Pereira and Cuocolo, 1941. (Trematoda, Plagiorchiidae.) Rev. Brasil. Biol. **9**: 167–174.

STAFFORD, J. 1900. Some undescribed trematodes. Zool. Jahrb. Sys. 13: 399–414.

———. 1905. Trematodes from Canadian vertebrates. Zool. Anz. 28: 681–694.

TRAVASSOS, L. 1930. Pesquisas helmintologicas realizadas em Hamburgo. I. Genero *Haplometra* Looss, 1899 (*Trematoda: Plagiorchiidae*). Mem. Inst. Oswaldo Cruz. 23: 163–168.

Removing Nematodes from Soil

J. R. CHRISTIE[1] and V. G. PERRY[2]

The investigation of plant parasitic nematodes has always been hampered by lack of a simple and efficient technique for removing these organisms from the soil. When working with species that feed on the surface of roots without penetrating the tissues, some method of determining their abundance, distribution, and habits in the soil is an absolute necessity. Our present ignorance of the enormous importance of nematodes in this category is largely attributable to lack of a means for securing such pertinent information. The two methods most commonly used for removing nematodes from soil are sieving and the Baermann technique. Both have been described in various publications, the most recent description being that of Goodey.[3] What is needed, however, is a method whereby the nematodes can be obtained in clear water reasonably free from debris; sieving does not accomplish this nor does the Baermann technique, as this method is ordinarily employed. The writers have been using a combination of these two methods with very good success and have found it exceedingly useful. Such a combination seems an obvious and logical procedure but it does not appear to be in use, nor does Goodey (loc. cit.) mention such a possibility. In view of this, and believing that others will find the method useful, the writers present the following description.

Equipment.—Two sieves are needed, a coarse one of about 20 to 24 meshes to the inch that will allow the nematodes to pass through but will retain the coarser components of the soil, and a fine one that will retain the nematodes. When it is not necessary to retain the very small forms, such as larval stages, a sieve with 150 meshes to the inch may be adequate. When it is desirable to retain as many as possible of the small forms, a sieve with 200 meshes to the inch should be used. Even with such a sieve many of the very small nematodes will be lost, but with it the writers have had no difficulty in removing larvae of the root knot nematodes (*Meloidogyne* spp.) and of the citrus nematode (*Tylenchulus semi-penetrans* Cobb) in large numbers. For special purposes, sieves of silk bolting cloth, as described by Cobb[4] and Goodey (loc. cit.) may be found useful. For the sieving operation a piece of rubber hose or tubing is attached to a faucet. The operation can be accomplished somewhat more efficiently if the free end of the hose is attached to a device that will throw a fine spray over a small area. Sprinklers used for moistening clothes before ironing serve this purpose very well. The hose should be firmly

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[3] Goodey, T. 1949. Laboratory methods for work with plant and soil nematodes. Tech. Bull. 2, Nematology Dept., Rothamstead Expt. Sta., Min. Agr. and Fisheries.

[4] Cobb, N. A. 1918. Estimating the nema population of the soil. Agr. Technology Cir. 1, U. S. Dept. Agr., Bur. Plant Indust.

attached, to both the faucet and the sprinkling device, otherwise it will be blown off when pressure is applied.

The Baermann apparatus consists of a funnel, the snout of which is inserted into a short piece of thin-walled rubber tubing equipped with a clamp. Supports are provided for as many funnels as are needed. The writers use glass funnels 6 inches in diameter across the top. Cloth sacks (Fig. 1) are prepared from a fairly heavy weight, closely woven muslin. These are fitted in the form of small skullcaps and each is finished with a hem around the edge. Rings about 5½ inches in diameter are made from number 10 galvanized iron wire. The ends forming a ring abut but are not soldered or otherwise fastened together. A ring is inserted into the hem of a cloth sack and the sack placed in the top of a funnel. Suitable receptacles such as buckets and beakers, paddles for roiling soil, and a block of wood for rapping the side of a sieve, complete the necessary equipment.

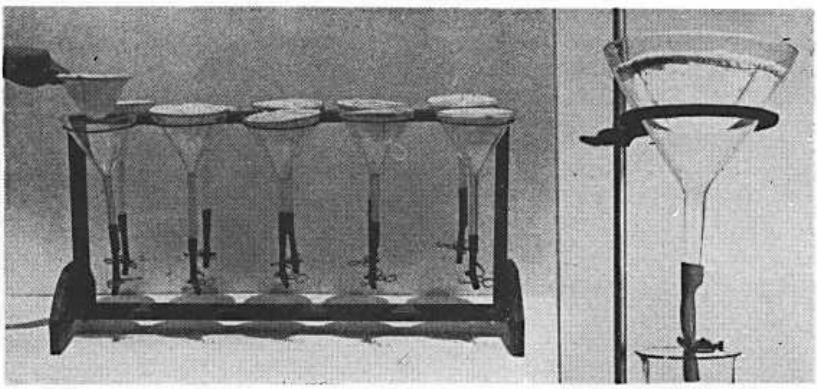

FIG. 1. A set of ten Baermann funnels used for removing nematodes from soil. Funnel on right shows sack in place and how cloth is sewn.

Procedure.—Thoroughly wet the fine sieves and place the coarse sieve on top of it. Place the sample of soil in a suitable container, add water equal to about three or four times the volume of soil, and thoroughly roil. Allow the container to stand a few seconds while the sand and heavier particles settle, then carefully pour off the water into the sieves. If this operation is repeated three times, most of the nematodes will be removed from the sand. Wash the material caught in the coarse sieve with a spray of water to carry through any nematodes that may have been caught on it, then discard. In the same manner, wash the material caught in the fine sieve, which contains the nematodes, to carry through colloidal matter and fine particles and continue until the water from the sieve runs clear, then wash the material to the side of the sieve and into a beaker. When the sieve is tilted for removing this material it is sometimes helpful to direct a spray, without too much force, against the bottom from the outside. When the water does not run through the sieve, rap it sharply on the side with a block of wood.

Pour tepid water into the funnel until the cloth sack is partly filled but still has enough room to receive the contents of the beaker without overflowing. Roil the contents of the beaker and pour it *into this water, not onto the cloth.* After about five minutes, open the clamp and draw off a few milliliters of water. This will remove most of the debris that may have been washed through the cloth sack during the filling operation. Next, allow the material thus prepared in the funnel to stand where it will not be disturbed or jarred. The usual practice is to set up the funnels during late afternoon and draw off the nematodes the following morn-

ing. However, if vigorous, living specimens are needed for experimental purposes, set up the funnels in the morning and draw off the nematodes every hour or two.

Do not use cheesecloth in the funnels. Just as many nematodes will burrow through a more closely woven fabric and far less debris will go through. In cool weather fill the funnels with water heated to a temperature of about 90° F.

For sandy soils, one-pint samples are a convenient and satisfactory size. Clay soils are much more difficult to sieve but the difficulties are not insurmountable. When working with such soils it may be necessary to modify the sieving procedure somewhat and to use smaller samples of soil, or to divide a sample and sieve each part separately.

Standard sieves, as fine as 200 meshes to the inch, can be purchased from companies that sell laboratory supplies. Very satisfactory sieves can be made by cutting the bottoms from tin pans and soldering on copper screening. The sides of such sieves should be painted with asphalt varnish or some similar waterproof paint. Square sieves made in this way are very convenient because the material caught in them can be poured from a corner. Fine sieves should always be thoroughly washed and dried immediately after using.

Attempts to Transfer *Plasmodium berghei* Vincke and Lips to Domesticated Animals[1]

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INTRODUCTION

Plasmodium berghei was first described in 1948 by Vincke and Lips from the red blood cells of a wild tree rat, *Thomnomys surdaster*, in the vicinity of Elisabethville, Belgian Congo. Vincke and Lips were able to transfer the parasites by blood inoculations to *Rattus rattus*, *R. frugivorus*, *R. rattus alexandrinus*, and white mice. They were unable to transfer the organism to guinea pigs and rabbits. Adler and associates (1950), working with *P. berghei* at the Hebrew University in Jerusalem, found this organism infective for the golden hamster, *Mesocricetus auratus*, and the field vole, *Microtus guntheri*.

According to Vincke and Lips, the insect vector is probably *Anopheles dureni* Edw., a mosquito native to the area in which the parasitized rats were captured. This mosquito is very difficult to raise in the laboratory, and so far as the present writer is aware passages through the mosquito to rats have not been successful.

Since *P. berghei* can easily be maintained in laboratory rats it is particularly useful for the testing of antimalarial drugs. For this reason the Division of Tropical Medicine, National Institutes of Health, requested the Bureau of Animal Industry, U. S. Department of Agriculture, for permission to bring this organism into the United States. This Bureau is charged by law with supervision over the introduction of disease organisms into the United States and their distribution within this country after introduction. Permission to do so was granted. When this became known, other research organizations interested in the study of malarial organisms and the chemotherapy of malaria, requested that *P. berghei* be placed at their disposal. Before acceding to these requests, the Division of Tropical Diseases,

[1] The writer wishes to express his thanks to Miss Nancy Allen, formerly of the Division of Tropical Diseases, National Institutes of Health, for her assistance in carrying out the work reported in this paper.

National Institutes of Health, consulted the Bureau of Animal Industry. The Bureau, charged with protecting the livestock industry from diseases and disease-producing organisms not present in the United States, and mindful of the disastrous results that on previous occasions have followed the introduction into this country of foreign pests and their later dissemination, accidental or otherwise, hesitated to grant permission for the distribution of *P. berghei* to laboratories other than those of the National Institutes of Health. It was pointed out that there was no information available on the susceptibility of domestic animals to *P. berghei,* even though it was recognized that the various species of *Plasmodium* are, generally speaking, rather definitely host specific. It was decided that such information should be obtained concerning the possible transmission of *P. berghei* to domestic animals before permission for the distribution of the organism to research laboratories could be granted. In accordance with that decision the experiments described in this paper were carried out.

EXPERIMENTAL PROCEDURE AND RESULTS

The parasitized blood used in these experiments was obtained from white rats, *Rattus rattus.* Each rat was inoculated intravenously (i.v.) with about 5,000 parasites. On the sixth day after inoculation the rats were bled out and destroyed. Blood smears were made and checked for parasites. This heavily parasitized blood was used to inoculate experimental animals.

Two young lambs were each inoculated i.v. with 4 cc of blood from the aforementioned white rats. One young pig was inoculated i.v. with 5 cc of blood and another was given 1.5 cc of blood subcutaneously and 3 cc intraperitoneally. Temperature readings were taken on all inoculated animals beginning on the 4th day after inoculation and continuing through the 20th post-inoculation (P.I.) day. Thick and thin smears of peripheral blood were taken every other day, beginning on the 4th day and continuing through the 20th day, and then every 4th day until the 41st day. All blood smears were negative for parasites.

In order to determine whether any inapparent or subpatent infection was present in the lambs or pigs, blood was drawn on the 20th P.I. day and on the 41st P.I. day and injected i.v. into four young white rats. Two rats were injected with blood from each animal, each rat receiving 0.2 cc of blood. All the rat recipients were observed for 3 weeks following the subinoculations. Blood smears were examined daily for parasites, and at no time did they show any evidence of infection with *P. berghei.*

A similar procedure was carried out using a calf and 2 young goats. The amount of blood used for the initial inoculation varied somewhat from that used in the first group of animals. The calf was inoculated i.v. with 6 cc of heavily parasitized rat blood. The first goat received 6.5 cc and the second received 9.5 cc of rat blood i.v. The first subinoculation into white rats from these test animals was made on the 21st P.I. day. The second subinoculation was made on the 35th P.I. day. No evidence of infection was noted in the rats. Observations on the calf and 2 goats were discontinued on the 35th P.I. day. At no time during the experiment did any of the test animals show signs of infection with *P. berghei,* as determined by repeated examinations of blood smears.

A third group of animals, consisting of 2 puppies and 2 kittens, was inoculated with heavily infected rat blood. One puppy was inoculated i.v. with 2 cc of heavily parasitized rat blood and the second puppy was inoculated i.v. with 4 cc of heavily parasitized rat blood. The same procedure of subinoculation was used on all test animals, except that in this group the first subinoculation was made into white mice on the 6th P.I. day. The second subinoculation was made into

white rats on the 25th P.I. day. The only exception was in the case of the second puppy. That animal showed symptoms of distemper soon after the inoculation of rat blood; therefore, the first subinoculation was made on the 3rd P.I. day. The puppy died of distemper on the 8th P.I. day. At no time did any of the animals in this group show evidence of infection with *P. berghei.* All the subinoculated rats and mice were negative for parasites. Observations on the first puppy were discontinued on the 31st P.I. day and on the kittens on the 35th P.I. day.

The last group of animals used consisted of 5 ten-day-old chicks. Each bird was inoculated i.v. with 0.1 cc of heavily parasitized rat blood. These animals were observed for 32 P.I. days without evidence of *P. berghei* infection, as determined by daily examination of blood smears.

SUMMARY AND CONCLUSIONS

A group of domestic animals, consisting of 2 lambs, 2 pigs, 1 calf, 2 puppies, 2 kittens and 5 chicks, was inoculated i.v. with blood of white rats heavily parasitized with *P. berghei.* The inoculated animals were observed for clinical symptoms of parasitosis with negative results. Thick and thin blood smears were made and found negative for parasites. Subinoculations were made to check for any inapparent or subpatent infections; all results were negative.

Since attempts to transfer *Plasmodium berghei* to the animals used in these experiments were unsuccessful, it is reasonable to conclude that the hosts listed were not susceptible to infection with this parasite.

LITERATURE CITED

ADLER, S., VOELI, M. AND ZUCKERMAN, A. 1950. Behavior of *Plasmodium berghei* in some Rodents. Nature **166** (4222): 571: Sept. 30.

VINCKE, I. H. et LIPS, M. S. 1948. Un nouveau plasmodium d'un rongeur Sauvage du Congo, *Plasmodium berghei n.*sp. Annales soc. Belge de Med. Trop., **28** (1): 97–104.

Observations on the Length of Dormancy in Certain Plant Infecting Nematodes

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Nematode-infected plant materials collected at various times have been kept at room temperatures at the Salt Lake City, Utah, station of the Division of Nematology to determine the length of dormancy of the nematodes. Corder [1933, J. Parasitol. **20** (2) 1: 104] and McBeth [1937, Proc. Helminth. Soc. of Washington **4** (2): 53] published reports on this material, and the present examination, made in the fall of 1949, constitutes the third report. The reader is referred to Corder (*loc. cit.*) for samples discarded. Samples no. 19–32 are new.

A small portion of each lot of material was soaked in unchlorinated water over night. The nematodes were then picked out and placed on a slide in a drop of water containing a few sand grains, and retained in a moist chamber for several days. The specimens were observed for movement and those showing no activity were taken from the moist chamber and placed on a celluloid slide. When cut with an eye knife the body contents gushed from live nematodes as if they were under pressure, while those of dead specimens oozed only slightly, if at all. Many specimens which did not become active in the moist chamber were found

TABLE 1.—*Results of examination of dormancy samples, 1949*

Sample No.	Species	Host	Locality and Date	No. Years Dormant	No. Specimens Examined	Percentage alive by:	
						Cutting	Movement
3.	*Ditylenchus dipsaci*	*Dipsacus fullonum*	Santa Clara Co. Cal., 1926	23	60	1.7	0
4.	" "	*Allium sativum*	San Benito Co. Cal., 1926	23	22	0	0
5.	" "	*Plantago lanceolata*	Santa Cruz Co. Cal., 1927	22	45	4	0
6.	" "	*Dipsacus fullonum*	Molalla, Oregon, 1927	22	60	58	0
7.	" "	*Avena sativa*	Santa Cruz Co. Cal., 1928	21	60	53	0
10.	" "	*Hypochoeris radicata*	Salem, Oregon, 1929	20	18	100	100
17.	*Tylenchus balsamophilus*	*Balsamorrhiza sagitatta*	Wellsville, Utah, 1925	24	24	4	0
18.	*Ditylenchus dipsaci*	*Medicago sativa*	Midvale, Utah, 1933	16	50	0	0
19.	*Ditylenchus phyllobius*	*Solanum elaeagnifolium*	Verda Valley, Ariz., 1930	19	4	0	0
20.	*Anguina agrostis*	*Festuca rubra commutata*	Corvallis, Oregon, 1945	4	100	100	100
14.	*Anguina tritici*	*Triticum aesticum*	Madison, Wis., 1922	27	125	90	85
21.	" "	" "	Fulton County, Ga., 1940	9	100	100	100
22.	" "	" "	Ellicott City, Md., 1929	20	180	41	0
23.	" "	" "	Arlington, Va., 1928	21	180	17	0
24.	" "	" "	Pulaski, Va., 1939	10	150	100	100
25.	" "	" "	Pulaski, Va., 1938	11	200	96	84
26.	" "	" "	Pulaski, Va., 1938	11	200	100	100
27.	" "	" "	Leesburg, Va., 1923	26	200	0	0
28.	" "	" "	Arlington, Va., 1924	25	200	0	0
29.	" "	" "	Arlington, Va., 1928	21	200	100	100
30.	" "	" "	Wilkesboro, N. C., 1921	28	200	100	100
31.	" "	*Secale cereale*	Timberville, Va., 1919	30	200	0	0
32.	" "	" "	Arlington, Va., 1924	25	200	0	0

alive when cut. Several discrepancies occurring between McBeth's results (*loc. cit.*) and those reported herein are probably due to this cutting technique which he did not use. Results are given in Table 1.

It is well known that certain species of nematodes can remain dormant for long periods of time, and various investigators have reported on this dormancy, the longest period recorded being that for *Tylenchus polyhypnus* Steiner & Albin revived from rye after 39 years by Steiner and Albin [1946, Journal of the Washington Academy of Sciences **36** (3): 97–99].

The 1949 examination showed the wheat nematode, *Anguina tritici* (Steinbuch), alive after 28 years. This corroborates the verbal statement to Steiner (*loc. cit.*) by Prof. Dr. Heinrich Simroth that the wheat nematode was revived after 28 years, and the published record by Needham [1775, in Observ. Phys., Hist. nat. e. Arts **5**: 226–228] that Baker had revived the wheat nematode after 27 years. However, Goodey (1923, Jour. Helminth. **1**: 47–52), and Byars (1920, U. S. Dept. of Agric. Bul. **842**: 1–40) found that *A. tritici* would not revive after 10 years and considered this the longest period of dormancy for that nematode. The dormancy period for *A. tritici* in wheat is variable (see Table 1) and undoubtedly depends on the maturity of the specimens, the time of year collected, the locality, and the storage conditions.

The 1949 examination showed *Ditylenchus dipsaci* (Kühn) alive after 23 years, in fullers teasel, *Dipsacus fullonum*. This constitutes the longest period of dormancy reported for that genus.

All samples which contained living specimens were returned to storage for future examination.

A New Stage in the Life Cycle of the Golden Nematode *Heterodera rostochiensis* Wollenweber[1]

JOYCE W. HAGEMEYER[2]

Heterodera rostochiensis is one of the most important of the plant parasitic nematodes. In England and parts of Europe it is the most serious pest of potatoes. *H. rostochiensis* has been studied by many workers but there is still much to be learned concerning its life history. This paper will deal with only one phase of the life cycle—the development of the first stage larva and its molt to the second and infective stage while inside the egg.

Strubell (1888) was the first to report a molt occurring inside the egg of *Heterodera schachtii* Schmidt. His work was confirmed by Chatin in 1891. Neither of these men considered it to be a separate stage. Raski (1950) established the molt in the egg as a separate stage. A molt has been reported in the egg of the root knot nematode, *Meloidogyne* sp. by Nagakura (1930). Christie and Cobb (1941) also observed this molt. Jensen (1950) has found a molt in the egg of *Pratylenchus vulnus*. Chitwood and Buhrer (1945) reported that they could find no molt in the egg of *Heterodera rostochiensis*.

Since the molt in the egg had been observed in several closely related species it seemed likely that it also occurred in *H. rostochiensis*. It was decided that the

[1] This work was carried on at the Golden Nematode Research Laboratory, Hicksville, New York with funds supplied by the Dept. of Plant Pathology, Cornell University.

[2] Graduate Student, Division of Entomology and Parasitology, University of California, Berkeley.

The author wishes to thank Dr. Bert Lear, of the Dept. of Plant Pathology, Cornell University, for making the photomicrograph.

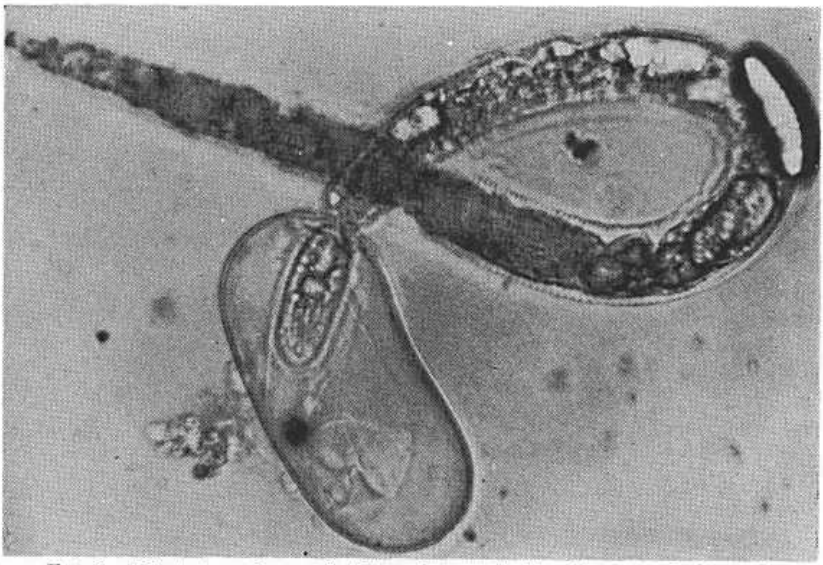

FIG. 1. First stage larva of *Heterodera rostochiensis* ruptured from the egg while molting.

most favorable material to examine would be cysts containing eggs with both embryonic and fully developed larvae present. It was found that such cysts were usually yellow in color. Several yellow cysts were opened up and their contents examined under an oil immersion lens. Many eggs were found which demonstrated the molt. By pressing lightly on the coverslip as suggested by Raski (1950) it was possible to rupture the egg shell without damaging the larvae. Figure 1 is a photomicrograph of a molting larva which has been ruptured from the egg.

The first stage larvae have very little resemblance to the succeeding stages. The head region is undifferentiated; no spear or sclerotized structures are present. The esophagous is just beginning to take form (Fig. 2A). As the larvae molt,

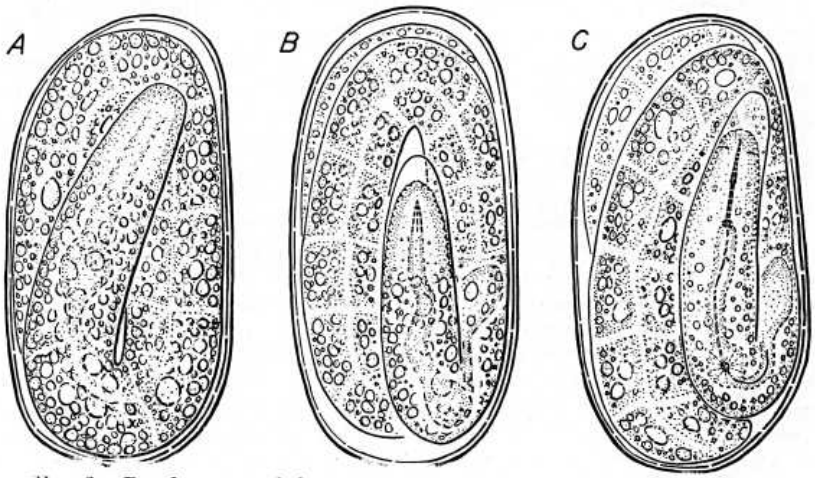

FIG. 2. Development of first stage larva of *Heterodera rostochiensis*. A—First stage larva inside the egg. B—Beginning of first molt. C—First molt, advanced stage. × 600.

the prorhabdions of the spear develop and annulations appear on the head. (Fig. 2B). As the molt progresses the remainder of the spear is formed, the head becomes set off and its sclerotized framework appears (Fig. 2C). The esophagus likewise completes its differentiation. The first stage larval cuticle now has been completely cast off and the second stage larvae complete their development in the egg after which they are ready to hatch.

Due to the presence of this molt, descriptions of the life stages of the Golden Nematode should be revised. It is probable that the life cycle of *Heterodera rostochiensis* is similar to the life cycle of *Heterodera schachtii* as reported by Raski (1950).

REFERENCES CITED

CHATIN. JOANNES. 1891. L'Anguillule de la betterave (*Heterodera schlachtii*) Min. Agr. (France) Bull. Ann. **10**: 457–506.

CHITWOOD, B. G. and BUHRER, EDNA M. 1946. The life history of the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber under Long Island, New York conditions. Phytopath. **36**: 180–189.

CHRISTIE, J. R. and COBB, GRACE S. 1941. Notes on the life history of the root-knot nematode, *Heterodera marioni*. Proc. Helm. Soc. Wash **8**: 23–26.

JENSEN, H. J. 1950. The biology and morphology of the root lesion nematode parasitic on walnuts in California. Ph.D. Thesis, Univ. of Calif.

NAGAKURA, KWAIICHIRO.1930. Ueber den Bau und die Lebengeschichte der *Heterodera radicicola* (Greeff) Muller. Jap. Jour. Zool. **3**: 95–160.

RASKI, D. J. 1950. The life history and morphology of the sugar-beet nematode, *Heterodera schachtii* Schmidt. Phytopath. **2**: 135–152.

STRUBELL, ADOLPH. 1888. Untersuchungen über den Bau und die Entwecklung des Rübennematoden, *Heterodera schachtii* Schmidt. Bibliotheca Zoologica **1**: 1–52.

The Recovery of Encapsulated, Infective Larvae of *Trichinella spiralis* Relatively Free of Muscle Tissue

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Two techniques for the diagnosis of trichinosis and the recovery of trichina larvae have been in use since their initial descriptions by Farre (1835) and by Thornbury (1897). The first, described by Farre, is a simple press preparation in which small pieces of muscle tissue, suspected of containing trichinae, are compressed between two plates of glass and examined under a microscope for the characteristic cysts of the parasite. The second, described by Thornbury and elaborated by Ransom (1916), makes use of an artificial digestive solution to dissolve muscle tissue surrounding the cysts and to weaken the cyst wall. This results in freeing the contained larvae, which, in the liquid medium, are easily detectable. The artificial digestion method, while requiring more time than the press preparation, gives more accurate results.

This comparative accuracy was shown by Schwartz (1938) in a quantitative determination of the efficiency of the sampling and subsequent direct microscopic examination of pork muscle tissue. He found that when less than one larva per gram of muscle tissue was present only one out of 11 positive samples (found positive by the digestion technique), examined microscopically three times, showed trichina larvae. When the infection was characterized by as many as three larvae per gram of muscle tissue, only approximately 50 per cent of the known positives showed trichinae on direct microscopical examination.

For many years there has been a need for a more rapid method for a positive

diagnosis of trichinosis. This more rapid method should be at least as accurate as the digestion technique. The procedure outlined herein[1] was found to be as reliable and much more rapid than the digestion technique. The technique is briefly as follows.

DESCRIPTION OF TECHNIQUE

Meat to be examined is ground through a food chopper or cut into small pieces. From 10 to 25 gms. are placed in a Waring blendor with enough cold water to cover the meat. The blendor is run for about four minutes. When small amounts (10 to 15 gms.) are used, the contents of the blendor are diluted with water and poured through a 10-mesh screen (see Fig. 1) into a funnel inserted into a large separatory

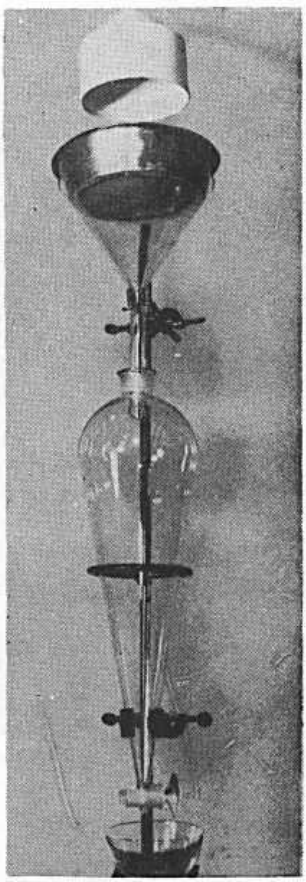

FIG. 1. Apparatus for the recovery of trichina larvae and cysts from blended muscle tissue.

funnel. The screen is washed with fine streams of water from an inverted Buchner funnel connected to a cold water tap, and the washings collected in the separatory funnel. The fibrous connective tissue remaining on the screen is discarded. After the material has sedimented for 30 minutes, the stopcock of the separatory funnel

[1] While this manuscript was in preparation the author's attention was directed to an abstract by Fallis (1943) in which is described briefly a technique of obtaining migrating young larval nematodes similar to the method outlined herein.

is opened and a specimen is collected in an examination dish. An N/10 solution of sodium hydroxide is added to the content of the dish as a clearing agent. Search is now made under a stereoscopic, wide field microscope for parasites either free or usually within their cysts (Fig. 2).

When larger amounts of meat are to be examined or the blended material is especially gelatinous or tends to flocculate, sedimentation in the separatory funnel is dispensed with and washing by screening is employed. The blended material is screened through the 10-mesh screen to remove large pieces of connective tissue. A sedimentation jar large enough to fit under the 10-mesh screen is used to receive the blended material and screen washings. After the screen has been washed, the contents of the sedimentation jar are poured onto a standard screen of 200 mesh

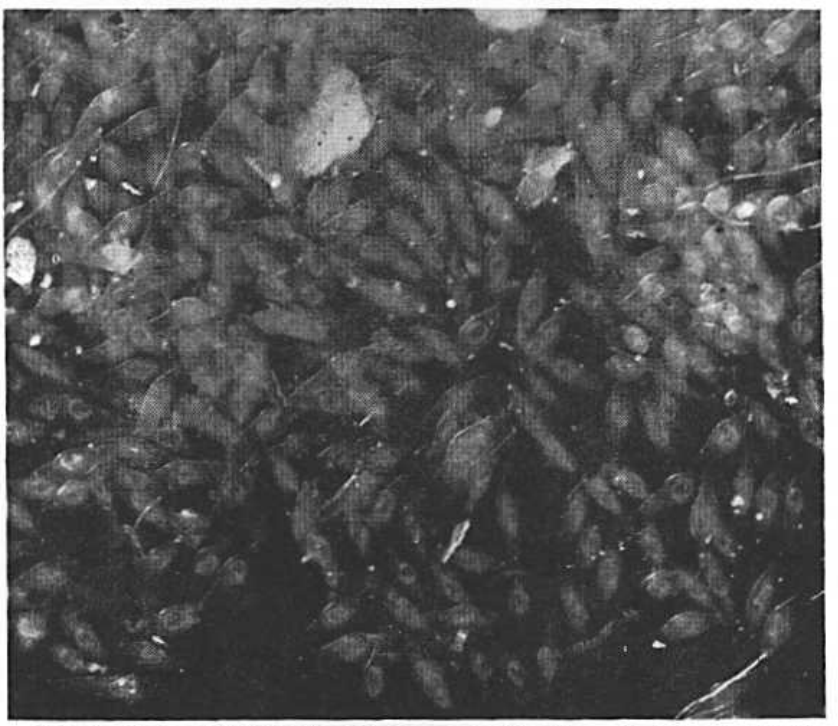

FIG. 2. Cysts of *Trichinella spiralis* recovered by the method described in the text.

to the inch, a mesh sufficiently fine to prevent the passage of larvae and cysts. After washing and screening, the contents of the screen are washed into an examination dish and the dish examined for parasites if such is desirable, or, if the object is the recovery of large numbers of cysts, the contents are counted by the dilution technique.

The technique of the digestion method employed in these experiments is as follows: The meat is ground through a food chopper and a weighed amount is placed in the digestive fluid. The formula used for the digestive solution is given by Schwartz (1939), who states that for digesting a one-half pound sample, the digestive fluid was made up as follows: scale or spongy pepsin U.S.P., 5 to 6 gms.; hydrochloric acid, 10 cc.; water, 600 cc. The water for the digestive fluid should be at a temperature of 37° C. Digestion is carried out in an incubator at 37.5° C.

with periodic stirring of the digest. After about 18 hours, the supernatant fluid is decanted and the material in the digesting jar poured through a 50-mesh screen, to remove any undigested particles, into a sedimentation vessel. Water is added and after 30 minutes' sedimentation the supernatant is decanted and the sediment poured into a conical test glass. The concentrated sediment of the test glass is examined after 30 minutes for possible larvae of *Trichinella spiralis.*

EXPERIMENTAL DATA

To test the reliability of the blendor method—the procedure just outlined, the following experiment was performed. Thirteen young pigs were each fed 5 to 100 cysts of *Trichinella spiralis* (Table 1). The cysts in small pieces of tissue were carefully dissected from infected hog meat, counted, and administered to the young pigs by mouth. In 30 days after infection, the animals were killed and the diaphragm and its pillars were examined for trichinae. One pillar was examined

TABLE 1.—*Number larvae recovered from the diaphragm of pigs, 30 days after infection, by the digestion and the blendor methods.*

Pig No.	Larvae Fed	Trichinae recovered from the pillars of diaphragm by:						Trichinae recovered from fibrous diaphragm by digestion		
		Blendor method			Digestion method					
		Weight (gms.)	No. recovered	Larvae per gram	Weight (gms.)	No. recovered	Larvae per gram	Weight (gms.)	No. recovered	Larvae per gram
2002	5	26	0	0	18	0	0	104	0	0
1971	10	28	0	0	20	0	0	96	0	0
1973	15	22	1	.04	18	1	.05	89	1	.01
2004	20	37	0	0	32	0	0	115	0	0
1981	25	28	3	.1	16	2	.12	102	15	.15
1972	30	16	13	.81	23	3	.13	83	10	.12
2001	35	19	4	.21	26	11	.42	83	9	.11
1985	40	42	11	.26	41	19	.46	125	48	.38
1984	45	26	10	.38	22	37	1.68	112	57	.51
2003	50	14	47	3.35	17	51	3.0	52	200	3.8
1979	55	41	29	.7	33	18	.54	127	145	1.1
1983	60	7	9	1.28	9	11	1.22	81	105	1.3
1974	100	21	0	0	25	2	.08	102	4	.04

by the peptic digestion method and the other by the blendor method. The remainder of the diaphragm was digested and the number of larvae recovered recorded. As can be seen from Table 1, no larvae were recovered by either method from the diaphragms of three pigs fed 5, 10, and 20 cysts, respectively. Larvae were recovered from the remaining 10 pigs, fed 15 to 100 cysts of the parasite, using both methods for their discovery. No significant differences were noted in the efficiency of the two methods in detecting trichina larvae from very light infections.

In two other experiments designed to compare the number of larvae or cysts recovered from infested flesh by the digestion and blendor methods, two lots of meat were used, one comparatively lightly infected and the other heavily infected. Ten 25-gm. samples were used for each method. For the digestion method, the 10 samples, equally divided between the two lots of meat, were digested, screened and diluted. Numbers of larvae in each sample were estimated by dilution count and recorded (Table 2). The second 10 samples, equally divided between the two lots of meat, were blended for four minutes each and, because of the large amount of blended material, were treated by passing first through a 10-mesh screen and then

through a 200-mesh screen as described above. The contents of the 200-mesh screen were washed into a dish and the cysts and larvae recovered were counted, using the dilution technique (Table 2). Although the average number of larvae recovered from each lot of meat is somewhat greater for the digestion method, the averages for the two groups are within 10 per cent of each other.

TABLE 2.—*Showing the number of larvae or cysts per gram of pork recovered by digestion and blendor techniques from 25-gram samples of either lightly or heavily infected meat*

	Larvae recovered from:			
	Lightly infected meat by:		Heavily infected meat by:	
	Blendor technique	Digestion technique	Blendor technique	Digestion technique
	72	98	660	744
	104	130	660	826
	80	87	864	906
	93	73	660	773
	93	73	799	756
Total	442	461	3643	4005
Average	88	92	729	801

The question arose as to the viability and infectivity of the encapsulated larvae obtained by use of the blendor. In order to answer this question, 20 rats were selected and divided into two groups of 10 each. The first group was infected with decapsulated larvae obtained by digesting infected meat. The second group

TABLE 3.—*Number of adult worms or larvae recovered from rats fed either 1,000 decapsulated larvae or 1,000 encapsulated larvae of* T. spiralis *each*

Rats killed 5 days after infection				Rats killed 29 days after infection	
	Adult worms recovered			Larvae recovered	
Rat No.	Female	Male	Total	Rat No.	No.
			RATS INFECTED WITH DECAPSULATED LARVAE		
1	80	40	120	6	2,500
2	120	60	180	7	4,000
3	80	0	80	8	6,000
4	40	0	40	9	5,000
5	20	0	20	10	19,000
			RATS INFECTED WITH ENCAPSULATED LARVAE		
1	139	74	213	6	23,000
2	120	20	140	7	13,000
3	120	0	120	8	23,000
4	160	40	200	9	22,000
5	440	20	460	10	24,000

was infected with encapsulated larvae obtained by blending infected meat. Each rat of the two groups received 1,000 larvae (estimated by dilution technique). Five days after infection, five rats from each group were killed and the small intestine of each was opened and examined for adult trichinae. The worms found were counted by the dilution technique and recorded (Table 3). Twenty-nine

days after infection the remaining 10 rats of the two groups were killed and the carcass of each was ground in a food chopper and digested. Decapsulated larvae resulting from this procedure were counted by the dilution technique and recorded (Table 3). The experiment shows evidence that the encapsulatd larvae obtained by use of the blendor were not only as vigorous as decapsulated larvae obtained by the digestion technique, but were also superior in their infectivity.

DISCUSSION AND CONCLUSIONS

The recovery of larvae from muscle tissue suspected of harboring trichinae is of the utmost importance from the standpoint of diagnosis. Schwartz (1938) in a discussion of the diagnosis of trichinosis, states that "a positive diagnosis of trichinosis in human beings can be made with certainty only when the parasites are actually found in the patient, usually following a biopsy." In the human or in the animal subject the tissue for examination is excised with a knife or with a harpoon. The harpooning of food animals for biopsy specimens was developed by the Germans and has been described by Leuckart (1860) and by Althaus (1864). Friedreich (1862) was apparently the first to use the harpoon to demonstrate trichinae in the living human muscle. The press preparation is extensively used to examine biopsy specimens owing to the small pieces of biopsied flesh available. If larger amounts of muscle tissue can be secured, the peptic digestion method will give the more accurate and conclusive results. In experiments reported herein, the blendor method equals the digestion method in accuracy in detecting infections of small intensity, and approximates it in the recovery of larvae from larger infections. In addition, the larvae recovered from the blendor process seem to be of greater infectivity than those recovered from the peptic digestive procedure. Whether this is due to the greater vitality of the larvae from the blendor process or to their single exposure to gastric juice was not ascertained.

In favor of the new method is the comparatively short time required for its completion. Accurate examinations can be made in about five minutes with the screen-washing technique and the 200-mesh screen and from 35 to 50 minutes with the sedimentation technique. The digestion technique ordinarily requires 18 hours.

SUMMARY

The first and simplest method for the examination of muscle tissue for the presence of trichinae—the press preparation—was described the year the trichina worm was discovered and named. Sixty-two years later the digestion method was described. Nineteen years after its description, Ransom elaborated the method into the accurate technique of modern procedure. A new method of quickly determining the presence of *Trichinella spiralis* in muscle tissue has been described. The recovery of large numbers of trichina cysts from heavily infected meat is possible with this method. In employing it, a choice of sedimentation, which requires 35 to 50 minutes and a small amount of blended material, and of screening, which requires about 5 minutes and the use of either gelatinous or voluminous blended material, is offered.

Experimental evidence is given that the new technique is equal to the digestion technique in its ability to detect infections of small intensity and is approximate to it in the numbers of larvae recovered from heavier infections. Larvae so recovered by the new technique seem to possess greater infectivity than those recovered by the digestion method.

LITERATURE CITED

ALTHAUS, J. 1864. On trichina disease, its prevention and cure. Med. Times and Gaz., London, 1 (Apr. 2): 362–364, (Apr. 9): 390–392.

FALLIS, A. M. 1943. Use of the Waring blendor to separate small parasites from tissues. (Abstract) Canad. J. Pub. Health, 34 (1): 44.
FARRE, A. 1835. Observations on the *Trichina spiralis*. Lond. Med. Gaz., 17 (Dec. 12): 382–387.
FRIEDREICH, N. 1862. Ein Beitrag zur Pathologie der Trichinenkrankheit beim Menschen. Arch. für Path. Anat., 25 (2F), 5 (3–4: 399–413.
LEUCKART, R. 1860. Untersuchungen uber *Trichina spiralis*. Leipzig. 57 pp.
RANSOM, B. H. 1916. Effect of refrigeration upon larvae of *Trichinella spiralis*. Jour. Agric. Research, 5 (18): 819–854.
SCHWARTZ, B. 1938. Trichinosis in swine and its relation to public health. Jour. Amer. Vet. Med. Assoc., 92 (3): 317–337.
————. 1939. Freedom from viable trichinae of frankfurters prepared under Federal meat inspection. Proc. Helminth. Soc. Wash. 6 (2): 35–37.
THORNBURY, F. L. 1897. The pathology of trichinosis. Original observations. Univ. Med. Mag., 10 (2): 64–79.

Wellcomia evaginata (Smith, 1908) (Oxyuridae: Nematode) of Porcupines in Mule Deer, *Odocoileus hemionus*, in Colorado

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and

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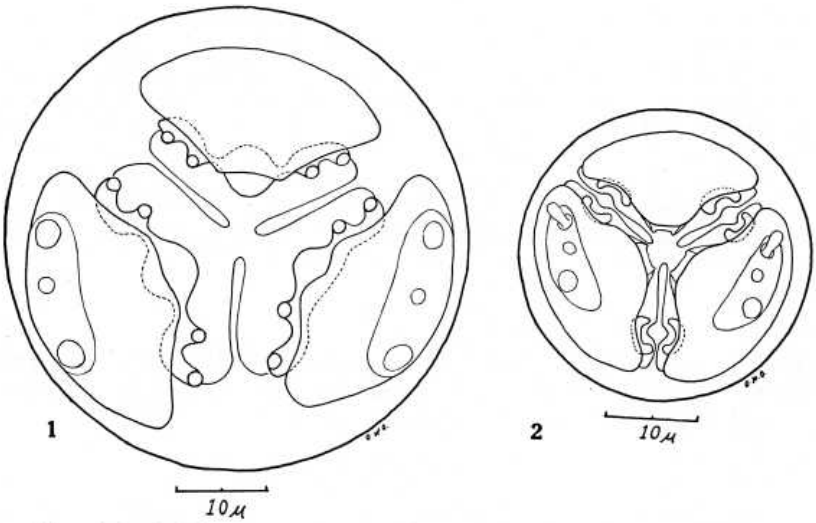
Species of the nematode genus *Wellcomia* are parasites of rodents. Porcupines are both commonly and heavily parasitized by them. Approximately 30,500 of these nematodes were collected from one pregnant and apparently healthy porcupine, *Erethrizon epixanthum*, taken near Gunnison in western Colorado. With such heavy infection of *Wellcomia* occurring in porcupines, it is apparent that large numbers of eggs are produced and scattered over areas where these animals forage. In regions where both porcupines and deer occupy the same range, the latter are exposed to infection by these parasites.

During the course of a study of the parasites of the mule deer in Colorado, two individuals from the Gunnison area were found to be infected with nematodes of the same type as those in porcupines. There were approximately 20 and 70 worms, respectively, in the deer. Both male and female nematodes were present; the females were large but not gravid. Comparison of the specimens from the deer and porcupine revealed similar morphological details.

Following a study of the species of *Wellcomia* Sambon, 1907, it was the opinion of Mao (1939: Ann. Parasitol. Comp. et Hum. 17: 336–354) that the members of this genus found in American porcupines, *Erethrizon dorsatum* and *E. epixanthum*, is different from *W. evoluta* (Linstow, 1899) from the Malayan porcupine, *Hystrix brachyura*. His conclusion was based on two points, namely, (1) that Linstow's description is too vague to make a positive identification and, therefore, the name *W. evoluta* should be retained for the species from *Hystrix brachyura*, and (2) that the great difference in geographic distribution of the hosts is justification for considering the nematodes as being different species. For the species of *Wellcomia* reported from *Erethrizon* by Smith (1908, Univ. Penn. Med. Bull. 20: 266–267), Hall (1916, Proc. U. S. Nat. Mus. 50: 70–74), and Jellison (1932, Trans. Am. Micro. Soc. 52: 43–44), he assigned the name *W.*

[1] Appreciation is expressed by the authors to Mr. Gilbert Hunter, Game Manager, Colorado Game and Fish Department, for making this study possible.



FIGS. 1–2. *Wellcomia evaginata* from deer. 1—En face view of large non-gravid female. 2—En face view of adult male.

evaginata (Smith, 1908). The specimens reported in this paper from both deer and porcupines are designated as *W. evaginata*. This is the first record known to the authors of *W. evaginata* occurring in deer.

Hall (loc. cit.) identified as *Oxyurus evoluta* Linstow, 1899 nematodes collected from *Erethrizon epixanthum* and *E. dorsatum*. He described and figured the mouth and lips. His description and figure, however, do not agree. He stated that "there are three broad lips (Fig. 80) and between them three other inter-

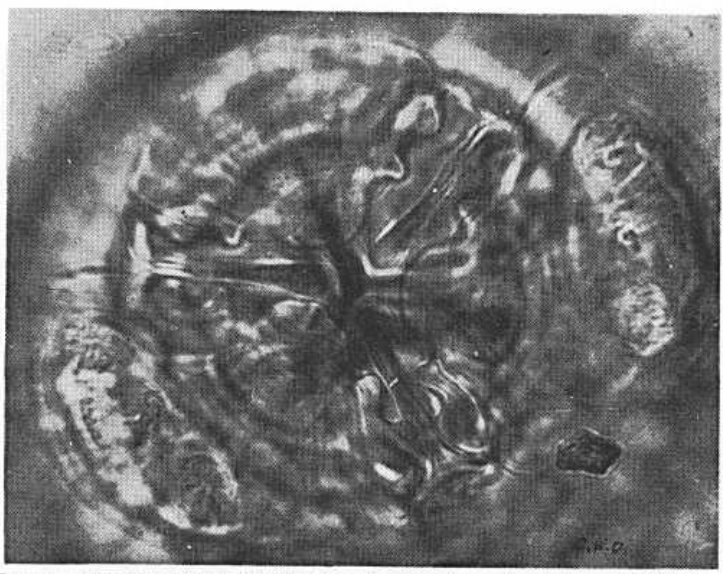


FIG. 3. Unretouched photomicrograph of en face view of gravid female *Wellcomia evaginata* from porcupine.

mediate lip structures projecting nearer to the median longitudinal axis.'' His figure does not show the intermediate lips mentioned in the description. Moreover, examination of our specimens from both deer and porcupines showed labial structures very different from those figured by Hall. It is the opinion of the writers that Hall's mounts of the head were not good and, therefore, did not show the true nature of these structures.

Inasmuch as no accurate figures of the mouth and lips of *W. evaginata* exist, the character of these structures in our material is shown by drawings of a large non-gravid female (Fig. 1) and an adult male (Fig. 2) from deer, and a photomicrograph (Fig. 3) of a gravid female from the porcupine.

Studies on the Helminth Fauna of Alaska. VIII. Some Cestode Parasites of the Pacific Kittiwake (*Rissa tridactyla* Ridgway) with the Description of *Haploparaxis rissae* n. sp.

EVERETT L. SCHILLER[1]

Thirty-seven adult Pacific kittiwakes (*Rissa tridactyla* Ridgway) were collected at St. Lawrence Island, Bering Sea, Alaska, during the latter part of August, 1950. Parasitological examinations revealed that 25 (70%) of these gulls contained cestodes representing three genera and four species. *Tetrabothrium erostre* (Lönnberg, 1889), previously recorded from this host, was found in two of the birds. This species has been adequately described in the literature and therefore will not be considered further here.

The majority of the gulls positive for cestodes harbored both *Anomotaenia larina* (Krabbe, 1869) and *A. micracantha* (Krabbe, 1869). Although these species have also been previously recorded from the kittiwake, they are poorly known and incompletely described. The present specific identifications were made primarily on the basis of rostellar hook number, size, and shape, together with cirrus sac size. A redescription of each species, resulting from a study of this material, is presented.

Several cestodes of the genus *Haploparaxis* Clerc, 1902, were recovered from the small intestine of a single host. These worms constitute an hitherto unknown species which is herein described as new.

Anomotaenia micracantha (KRABBE, 1869)

(Figs. 1–2)

Diagnosis.—Dilepididae. Length of strobila 100–120 mm.; maximum width 2 mm., attained at posterior end of strobila. Scolex 325 μ in diameter. Evaginated rostellum 288 μ in length by 72 μ in diameter. Knob-like apex of rostellum bears 20–22 hooks measuring 25–32 μ in length and disposed alternately in two rows. Suckers unarmed, very muscular, directed forward, 160 μ in diameter. Strobila 160 μ wide immediately posterior to base of scolex. Genital pores irregularly alternate. Genital ducts pass between ventral and dorsal excretory canals. Cirrus sac about 216 μ long by 24 μ in width. Vas deferens tightly coiled prior to juncture with cirrus sac. Protrusible unarmed cirrus 8 μ in diameter. Testes 16–20 in number contained within boundaries of excretory canals posterior to all female reproductive organs. Testes 72 μ in diameter in mature proglottids. Ovary located in anterior part of proglottid aporal to median line and composed

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of numerous short thick lobes. Vitelline gland lies ventral to ovary, prominent in middle of proglottid about 240 × 130 μ. Piriform seminal receptacle lies just anterior to vitelline gland and measures 90 × 75 μ in mature segments. Saccate uterus fills entire proglottid within boundaries of ventral excretory canals when gravid. Eggs about 40–45 μ in diameter; hooks of onchosphere, 16 μ in length.

Host.—*Rissa tridactyla* Ridgway.

Locality.—St. Lawrence Island, Bering Sea, Alaska.

Habitat.—Duodenum.

One slide, No. 47088, containing an entire specimen has been deposited in the Helminthological Collection of the U. S. National Museum.

Anomotaenia larina (Krabbe, 1869)

(Figs. 3–4)

Diagnosis.—Dilepididae. Length of strobila about 80 mm.; maximum width 3 mm. attained at posterior end of strobila. Scolex 500 μ in diameter. Suckers unarmed, weakly muscled, about 216 μ in diameter. Evaginated rostellum 360 μ in length and 144 μ in diameter. Rostellum provided with 20–22 hooks, 96–110 μ in in length and arranged alternately in two rows. Strobila about 390 μ in diameter immediately posterior to scolex. Genital pores irregularly alternate. Genital ducts pass between dorsal and ventral excretory canals. Cirrus sac 240–288 μ long by 72 μ in greatest diameter. Cirrus unarmed, protrusible, about 10 μ in diameter. Testes about 30 to 40 in number measure 72 μ in diameter in mature proglottids. Testes all situated posterior to ovary within boundaries of ventral excretory canals. Ovary occurs in anterior part of proglottid. Ovary deeply lobed with long finger-like projections which extend to excretory canals on either side. Vitelline gland, located directly posterior to ovary in middle of proglottid, measures about 180 × 95 μ in mature proglottids. Uterus sacculate and develops by lateral branching and enlargement, filling entire proglottid when gravid. Eggs about 32–36 μ in diameter. Ventral longitudinal excretory canals 12 μ in diameter; dorsal canals 8 μ in diameter.

Host.—*Rissa tridactyla* Ridgway.

Locality.—St. Lawrence Island, Bering Sea, Alaska.

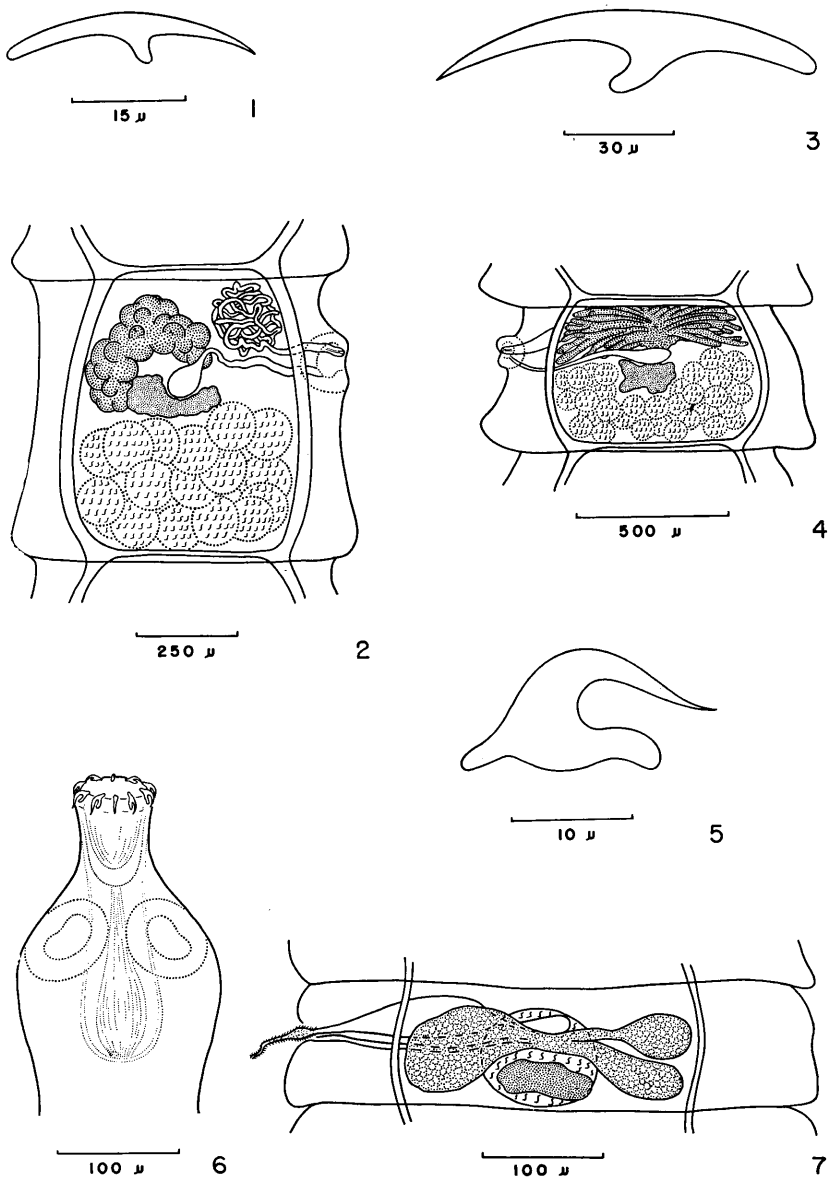
Habitat.—Duodenum.

One slide, No. 47087, containing an entire specimen, has been deposited in the Helminthological Collection of the U. S. National Museum.

Haploparaxis rissae n. sp.

(Figs. 5–7)

Diagnosis.—Hymenolepididae. Length of strobila about 20 mm.; maximum width 600 μ. Scolex 170 μ in diameter. Suckers 75 μ in diameter, unarmed, strongly muscled and directed forward. Evaginated rostellum 225 μ long and 72 μ in diameter at apex. Rostellum provided with 10 hooks, 21 μ in length. Strobila 130 μ wide immediately posterior to base of scolex. Genital pores unilateral and dextral. Cirrus spinose. Muscular cirrus sac 160 × 35 μ. Saccate external seminal vesicle 50 μ long by 20 μ in greatest diameter. Single ovoid testis 95 × 80 μ in mature proglottids and located in middle of segment. Ovary usually trilobed, 230 μ long, contained within boundaries of excretory canals. Vitelline gland, 75 × 30 μ, situated ventral and posterior to ovary, usually between posterior lateral lobes. Vagina about 5 μ in diameter; position variable, occurring posterior, directly ventral, or anterior to cirrus sac, and approaching ovary dorsally. Seminal receptacle not observed. Uterus sacculate. Completely gravid segments not observed. Ventral longitudinal excretory canals measure 6 μ in diameter.



FIGS. 1–7. Morphological details of some cestode parasites of the Pacific kittiwake. 1—Rostellar hook of *Anomotaenia micracantha*. 2—Mature proglottid of *A. micracantha* (ventral view). 3—Rostellar hook of *A. larina*. 4—Mature proglottid of *A. larina* (ventral view). 5—Rostellar hook of *Haploparaxis rissae* n. sp. 6—Scolex of *H. rissae*. 7—Mature proglottid of *H. rissae* (ventral view).

Host.—*Rissa tridactyla* Ridgway.

Locality.—St. Lawrence Island, Bering Sea, Alaska.

Habitat.—Duodenum.

Type.—One slide, No. 47086, containing an entire specimen, has been deposited in the Helminthological Collection of the U. S. National Museum.

Discussion.—Six of the 25 known species of the genus *Haploparaxis* occurring in birds (24 as reported by Schiller, 1951, and one since described, *H. galli* Rausch, 1951)[2] possess a spinose cirrus. These are: *H. fuliginosa* Solowiow, 1911; *H. veitchi* Baylis, 1934; *H. clerci* Yamaguti, 1935; *H. scolopacis* Yamaguti, 1935; *H. xemae* Schiller, 1951; and *H. galli* Rausch, 1951. Of these, *H. rissae* n. sp. most closely resembles *H. clerci* and *H. xemae*. *H. xemae* is the only member of this group reported from lariforms. A critical comparison of these species with *H. rissae* resulted in the following major differences:

H. clerci Yamaguti, 1935, (parasitic in Charadriiformes) has a strobila of much greater length (130 mm.), and a considerably shorter rostellum (63–96 μ), which bears hooks of a characteristically different shape. This species differs, as well, in having an elongate ovary which extends laterally beyond the excretory canals when fully developed.

H. xemae Schiller, 1951, (parasitic in Lariformes) differs in the structure of the cirrus sac (extends past the median line of the proglottid), the shape of the ovary (subspherical), and in the greater size of the rostellar hooks (25 μ).

LITERATURE CITED

KRABBE, H. 1869. Bidrag til Kundskab om Fuglenes Baendelorme. Dansk. Vidensk. Selsk. Skr., *naturvid. math. Afd.* 8: 249–363.

RAUSCH, R. 1951. Studien an der Helminthenfauna von Alaska. IV. *Haploparaxis galli* n. sp., ein Cestode aus dem Schneehuhn, *Lagopus rupestris* (Gmelin). Zeitschr. Parasitenk. 15: 1–3.

SCHILLER, E. L. 1951. Studies on the helminth fauna of Alaska. I. Two new cestodes from Sabines gull (*Xema sabini*). J. Parasit. 37: 266–292.

[2] The writer misplaced a data sheet during the preparation of an earlier manuscript (1951) relative to this group which later resulted in an erroneus report by Rausch of 23 species of the genus *Haploparaxis* occurring in birds.

A Note on the Cell Inclusions of *Syringolaimus smarigdus* Cobb, 1928

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Numerous specimens of the marine nematode, *Syringolaimus smarigdus*, were collected from the common mud snail, *Nassa obsoleta*, at Woods Hole, Massachusetts, in the summer of 1950. The nemas occur in great abundance among the algae on the shell of the snail, confirming Cobb's report of this association (J. Wash. Acad. Sci., 18 (9): 249–253, 1928). There is an encrusting orange alga (genus *Ralfsia?*) which adheres closely to the shell of the snail and a filamentous green alga which forms a thick felt over the orange alga. Cobb noted the bright green intestinal cell inclusions in *S. smarigdus* and designated them *granulum viride intestinalis* (sic). He maintained that the nemas obviously feed upon the orange alga, since the intestinal content of the syringolaims has the same color as the alga. However, for several reasons we feel that the cell inclusions are due to pigment from the green alga. Firstly, there is an obvious difference in color between the orange alga and the cell inclusions. Secondly, the orange pigment in the intestine is uniform in density and color intensity from the esophagus to the anus. Thirdly, the majority of specimens examined had no orange pigment or

only scattered patches. Fourthly, masses of green algae were often observed in the intestine. Finally, chemical tests showed that the cell inclusions were not derived from the orange alga. The standard tests for chlorophyll using concentrated sulfuric and nitric acids and potassium iodide were run. No blue color resulted, but when concentrated sulfuric and nitric acids were diffused slowly under the cover slip as the nematodes were being observed under oil immersion a remarkable color change took place. The cell inclusions slowly passed from bright green to dark green, dark blue, red, orange, pink and eventually became colorless. In the same time the bright orange pigment of the intestinal lumen remained unchanged but the color gradually faded to a pale orange.

These tests are suggested for the demonstration of similar cell inclusions in other alga-eating nematodes. For example, *Oncholaimium oxyure* (Ditl., 1911) var. *domesticum* (Chitw., 1938) n. comb. (syn. *O. oxyuris* var. *domesticus* Chitw., 1938), cultured for an entire year in an unaerated aquarium on filamentous green algae, showed no color changes of the olivaceous sphaeroids under similar tests. Perhaps the recent techniques of chromatography (vid. Ann. N. Y. Acad. Sci., **18** (9): 141–326, 1948) could be effectively applied to the precise determination of pigment cell inclusions in certain nematodes, since the limit of identification of some tests is often below one gamma.

The Morphology of *Ascaris laevis* Leidy 1856, and Notes on Ascarids in Rodents[1]

JACK D. TINER

During the summer of 1949 Dr. Asa Chandler, Rice Institute, Houston, Texas, informed the writer that he had obtained a bottle of ascarids from a woodchuck *Marmota* [*Arctomys*] *monax* in Pennsylvania.[2] At about the same time Dr. Robert Rausch, Alaska Health and Sanitation Activities, U. S. Public Health Service, collected several *Ascaris* sp. from ground squirrels *Citellus richardsoni* on the Arctic Slope of Alaska. Inasmuch as neither of these groups of roundworms could be identified, Dr. Chandler generously loaned his specimens to the writer for comparison. It is now evident that there is a distinct species of *Ascaris* naturally parasitic in North American woodchucks and ground squirrels.

Leidy (1856) described an *Ascaris laevis* from the woodchuck. Inasmuch as he stated "lips prominent" in his description, it seems probable that he was dealing with a member of the Ascaroidea. Leidy's measurements indicate that *A. laevis* is shorter and wider than *Ascaris columnaris* which he named and redescribed in the immediately preceding paragraph of the same paper. Consequently, it appears that he was dealing with a species which he regarded as distinct from, but possibly similar to, *A. columnaris*. The writer has often observed that the woodchuck and ground squirrel ascarid is proportionately of greater diameter than either the *Ascaris* spp. of North American carnivores or *Ascaris lumbricoides*. In female specimens of the North American members of the genus *Ascaris* the mucronate tail which Leidy noted on *A. laevis* has been observed only on the species

[1] A contribution from the Department of Zoology, University of Illinois, Urbana, Ill.

[2] These specimens were collected by the Pennsylvania Mammal Survey, which is a Pittman-Robertson Project, and is a cooperative feature between the Pennsylvania Game Commission, the U. S. Fish and Wildlife Service, and the Carnegie Museum, Pittsburgh. The worms were made available for study by Dr. J. Kenneth Doutt, Curator of Mammals, Carnegie Museum.

from the woodchuck. The following original description of *A. laevis* quoted in its entirety from Leidy (1856) was very probably based on a specimen of the woodchuck *Ascaris:*

> "113. ASCARIS LAEVIS Leidy. Body cylindrical, narrowing at the extremities. Head naked; lips prominent. Tail conical, mucronate. Length of female 3½ inches, breadth 1½ lines. Male not seen.
>
> "A single specimen was obtained by Mr. Packard, from the intestine of *Arctomys monax.*"

No type locality was specified and no mention was made of the disposal of the type specimen of *A. laevis.* Neither Stiles and Hassall (1894) nor Walton (1927) made any reference to it in their listings of the Leidy collection specimens. Presumably the original specimen and any accompanying labels or notes have been lost. Dr. D. H. Wenrich of the Zoological Laboratory, University of Pennsylvania, (personal correspondence, 1950) stated that there are a considerable number of Leidy's specimens at the University of Pennsylvania, but that he could not find any record of the specimen or the host of *A. laevis.* Dr. E. W. Price, U. S. Bureau of Animal Industry has informed the writer that there is no type specimen of *A. laevis* in the U. S. N. M. collection, and Dr. A. C. Walton, Knox College, writes that he did not encounter this species when he restudied the Leidy collection.

Owing to the apparent scarcity of adult *Ascaris* collected from rodents, Rausch and Tiner (1948) considered the possibility that the specimen which Leidy described as *A. laevis* might have been an accidental *Ascaris lumbricoides* or *Ascaris columnaris* which happened to mature in the woodchuck's intestine. However, a redescription based entirely on the specimens from *Marmota monax* in Pennsylvania is possible, and is given below:

Ascaris laevis LEIDY 1856

Cervical alae absent. Oesophagus without ventriculus, and intestine without projections or diverticula.

Female.—Length 80–100 mm., width 2.4–2.8 mm., in middle of body. Esophagus, 4.5–5.1 mm. long, and .59–.71 mm. wide in posterior one fourth. Nerve ring .75–.90 mm., excretory pore 1.12–1.16 mm. (Fig. 6), and vulva 20–28 mm. from anterior end. Anus .56–.83 mm. from posterior end of body (Figs. 5 and 7). Eggs .067–.069 mm. long by .051–.054 mm. wide (Fig. 4).

Male.—Length 30–48 mm., width 1.2–1.8 mm. in middle of body. Esophagus 2.45–3.64 mm. long with a maximum width of .315–.500 mm. in its posterior one fourth. Nerve ring .44–.55 mm., and excretory pore .63–.73 mm. from anterior end. Spicules equal or subequal, .36–.55 mm. long. Cloaca .375–.410 mm. from posterior end (Fig. 1). Five pairs post-cloacal papillae, of which most anterior two are doubled (Fig. 2). About 50 pairs pre-cloacal papillae in irregular rows beginning beside cloaca on each side and running anteriorly. Roughened patches of cuticle present just anterior to and just posterior to cloaca, extending into cloaca on its posterior wall (Fig. 1). Cuticle on sides of worm in cloacal region forms slight caudal alae (Fig. 2).

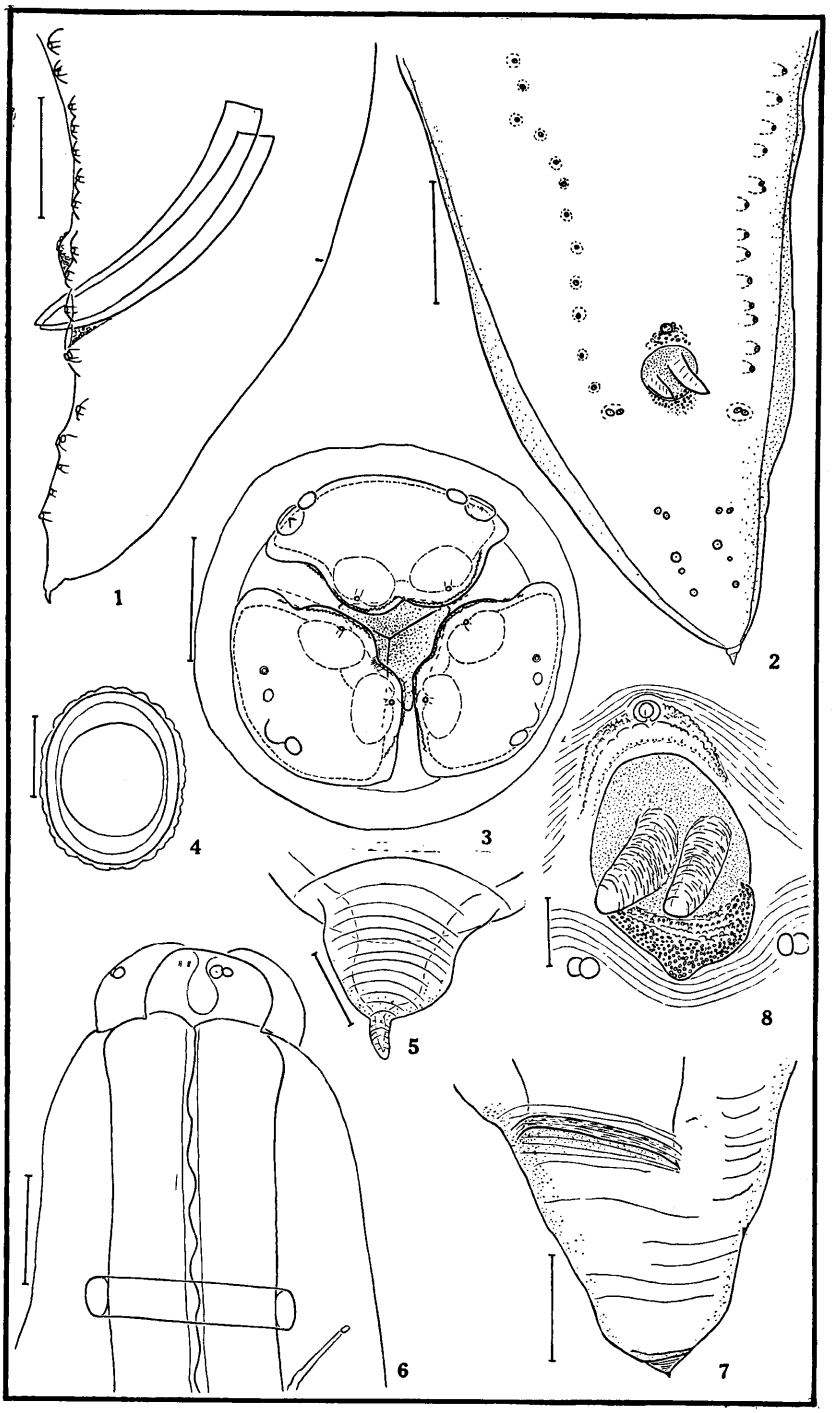
Hosts.—*Marmota monax in* Pennsylvania, and *Citellus parryi barrowensis* in Alaska.

Localities.—Benson Swamp, 5 mi. East of Columbus, Warren Co., Pennsylvania; Anaktuvuk Pass (Tolugak Lake), Alaska, and Umiat, Alaska.

Location.—Small intestine.

Specimens.—Neosyntypes, Carnegie Museum Number NR-CG 2108; Alaskan specimens, U. S. National Museum Helminthological Collection No. 47359.

When the ordinary morphological criteria are used, the nearest relatives of *A. laevis* might be *A. columnaris* in North American skunks, badgers, and raccoons,



Ascaris schroederi McIntosh 1939 from an Asiatic panda in the New York Zoological Park, and *Ascaris gulonis* Gmelin 1790 of the wolverine *Gulo luscus* (L. 1766).[3] The male of *Ascaris laevis* differs from males of these carnivore parasitizing ascarids by having smaller and less extensive roughenings in the vicinity of the cloaca. It also differs from the male *A. lumbricoides* which has no pericloacal cuticular roughenings. The spicule length of *A. laevis* (.36–.55 mm.) can be used to separate it from *A. schroederi* for which the original description gives the spicule length as .60 mm., and from *A. gulonis* in which the writer has measured spicules .60–.63 mm. long. However, the spicule lengths of *A. laevis* would coincide with those of *A. columnaris*. Measurements of numerous specimens of *A. columnaris* from skunks and raccoons indicate that its spicules range from .45 to .55 mm. long. The ''.8–.9 mm.'' recorded for the length of the spicules of *A. columnaris* by the writer in a previous note (Tiner and Chin, 1948) was evidently measured through a 10× instead of a 6× ocular and should have been multiplied by a factor of .6 to give .48 to .54 mm., which falls in the correct range.

Probably the size of the host species to which an ascarid is adapted as an intestinal parasite can be correlated to some extent with the maximum size of the worm. Specimens of *A. laevis*, a parasite of rodents, tend to be shorter than those members of the genus parasitic in carnivores, or in man and pig (*A. lumbricoides*), or in cattle (*A. vitulorum*). Maximum lengths of *A. laevis* of either sex measured by the writer were less than maximum lengths of specimens of the corresponding sex of other *Ascaris*. One of the female worms from the *Umiat* squirrel measured 116 mm. long. McIntosh (1939) described the female of *A. schroederi* as being 125 mm. long, Goodey and Cameron (1923) reported a length of 225 mm. for female *A. columnaris*. *Ascaris gulonis* females measured by the writer have been 196–245 mm. long. Various textbooks have given the length of mature female *A. lumbricoides* as 200 mm. to 350 mm., though in different units (cf. Chandler, 1949; Faust, 1949; and Belding, 1942). Bhalerao (1935) gives 300 mm. for the maximum length of females of *A. vitulorum*.

It was noted that Linsdale (1946) reported a length of 135 mm. for an *Ascaris* in the California ground squirrel *Citellus beecheyi* which he tentatively called *Ascaris columnaris*, but which more probably is a variant of *A. laevis*. Through the courtesy of Dr. Linsdale it was possible to examine several of these ascarids from *C. beecheyi* collected on the Hastings Natural History Reservation of the University of California. Male specimens measured up to 72 mm. long, thus exceeding the lengths of the above described Pennsylvania male specimens. The spicules were similar in shape to other specimens identified as *A. laevis*, and ranged from .410 to .540 mm. long. The left spicule was usually a little shorter than the right. About 49 pre-cloacal papillae were present on each side. The pericloacal roughenings of the male were inconspicuous like those of *A. laevis*. These roughenings were prominent on male ascarids from raccoons at the Hastings Natural History Reservation which were subsequently loaned by Dr. Linsdale.

[3] The writer has examined ascarids collected from the wolverine by Dr. Robert Rausch in Alaska and by Mr. H. F. Quick at Ft. Nelson, B. C. Thanks are expressed to Mr. Merle Kuns for forwarding the British Columbia specimens to the writer.

FIGS. 1–7. Morphology of *Ascaris laevis*. Scales for Figs. 1, 2, and 3 represent 200 μ; for Figs. 4 and 5, 30 μ; for Figs. 6 and 7, 300 μ; for Fig. 8, 50 μ. 1—Lateral view, posterior extremity of male. 2—Ventral view, posterior extremity of male. 3—*En face* view of female. 4—Egg. 5—Tip of tail, female (same specimen as Fig. 7). 6—Anterior end of female. 7—Tail of female. 8—Perianal region of male.

The identifications of *A. lumbricoides* from a fox squirrel reported by Rausch and Tiner (1948) and from a muskrat by Tiner and Chin (1948) are not affected by proof of the existence of *A. laevis*. Both records are based on male worms which were more than twice the maximum known length of mature *A. laevis* males, and both possessed greater numbers of pre-cloacal papillae than the writer has been able to count on *A. laevis*. Spicules of these worms were much longer than those known for either *A. laevis* or *A. columnaris*, and no pericloacal roughenings were found.

Through the courtesy of Mr. Kuns, Department of Biology, Purdue University, three additional vials of ascarids from fox squirrels were examined. Two additional records of *A. lumbricoides* in *Sciurus niger rufiventer* have resulted, and are listed in the following paragraphs:

One vial contained a male and fragments of a female worm, and was labelled: "Ascarids × Fox sq. #216F Collected by C. G. Fredine during fall and winter of 1946. Passed in feces." The male specimen was 96 mm. long, had spicules 1.4 mm. long, and in excess of 40 pre-cloacal papillae. An exact count of these last mentioned was not possible because of infoldings of the cuticle of the curled posterior end of the worm. Pre- and post-cloacal roughenings were completely absent. Mr. Kuns (personal correspondence, 1949) supplied the following information: The squirrel was captured in Benton Co., Indiana, September, 1946, and kept in the wildlife laboratory in a wire mesh cage until it died March 30, 1947. Ascarids were found in the cage by Mr. Fredine at different times between October and December, 1946; although there was no apparent exposure to *Ascaris* eggs during captivity.

A second vial from fox squirrel 245 F, Cunningham Wildlife Study Area, Tippecanoe Co., Indiana, April 14, 1947 contained a single male 90 mm. long in which the spicules were .86 (left) and .94 (right) mm., there were at least 45 pre-cloacal papillae on either side, and pre- and post-cloacal roughenings were absent. The writer considers this specimen as also belonging to the species *A. lumbricoides*.

A third female ascarid, passed by a captive fox squirrel, Tippecanoe County, Indiana was 125 mm. long and 2.5 mm. wide. This specimen closely resembled the other ascarids from Indiana squirrels examined by the writer, and is most probably *A. lumbricoides*.

Mr. Kuns writes that there are no records as to the area in Benton Co. in which squirrel 216 F was captured, but that squirrel 245 F was killed at the edge of a 40-acre woodlot, and that pigs were present around farm buildings in the opposite corner of the woods about 300–400 yards away. In view of the distances of several miles sometimes traveled by fox squirrels during their lifetime (Allen, 1943), and the rapidity with which they moved into Allen's study area from other nearby woodlots to restore an experimentally destroyed population, it appears probable that, previous to capture, each of the above three squirrels had been exposed to *A. lumbricoides* eggs on soil frequented by infected pigs.

Thanks to Mr. James Gilford, collector, an additional record of *Ascaris lumbricoides* in the muskrat *Ondatra zibethica* has also resulted. This host species was first listed for *A. lumbricoides* by Tiner and Chin (1948). A single muskrat was taken April 11, 1951 from the same area where two infected individuals were collected among the 21 reported on in the previous paper, and a male worm 128 mm. long and 2.1 mm. wide with spicules 1.14 mm. long was recovered. The exact locality was at the origin of the Embarrass River, 1¾ miles south of the center of the City of Champaign, Ill. on U. S. Highway 45. The University Pig farm is on the same watershed about ½ mile downstream and the stream runs within 300 ft.

of an area pastured by pigs at that point. It is possible, of course, that the infected muskrats had moved in from other farms further downstream.

To date we still lack evidence that *A. lumbricoides* which become accidental parasites of rodents can be of epidemiological significance in spreading eggs (see Rausch and Tiner, 1948). There is evidence that parasitic animal species do change from one host to another (sometimes phylogenetically unrelated) species of host whenever two such hosts happen to occupy the same geographical area and when the food habits of one happen to be such that there is repeated exposure to the parasites of the other. Some workers are of the opinion that the porcine variety of *A. lumbricoides* was acquired by pigs during their long association with man and constant exposure to the infective eggs. Evidently a similar process of exposure is now taking place with native North American rodents selecting out ascarids capable of maturing in their intestinal tracts. However, human and pig intestines do not differ markedly in size, and there is no reason to suppose that a size barrier existed to impede the change of a strain of *A. lumbricoides* from the one environment to the other. A process of selection toward a shorter and smaller variety would be expected to accompany permanent adaptation of any *A. lumbricoides* progeny to the various wild rodent species which are now known to be occasional hosts.

LITERATURE CITED

ALLEN, D. L. 1943. Michigan fox squirrel management. Dept. Conservation, Lansing, Mich., Game Div. Publ. **100**. 404 pp.

BELDING, D. L. 1942. Textbook of clinical parasitology. Appleton-Century-Crofts, New York.

BHALERAO, G. D. 1935. Helminth parasites of the domesticated animals in India. The Imperial Council for Agricultural Research. Scientific Monograph No. 6. Delhi. 365 pp.

CHANDLER, A. C. 1949. Introduction to parasitology. 8th ed. J. Wiley & Sons, New York.

FAUST, E. C. 1949. Human helminthology. 2nd ed. Lea & Febiger, Philadelphia.

GOODEY, T. and CAMERON, T. W. M. 1923. Observations on the life history of *Ascaris columnaris* Leidy, a nematode parasite of the skunk. J. Helminthol. **1**: 1–8.

LEIDY, JOSEPH. 1856. A synopsis of entozoa and some of their ectocogenera observed by the author. Proc. Acad. Nat. Sci. Phila. **8**: 42–58.

LINSDALE, J. M. 1946. The California ground squirrel. Univ. Calif. Press, Berkeley and Los Angeles. 475 pp.

MCINTOSH, ALLEN. 1939. A new nematode *Ascaris schroederi*, from a giant panda *Ailuropoda melanoleuca*. Zoologica **24**: 355–357.

RAUSCH, R. and TINER, J. D. 1948. Studies of the parasitic helminths of the North Central States. I. Helminths of Sciuridae. Am. Midl. Nat. **39**: 728–747.

STILES, C. W. and HASSALL, A. 1894. A preliminary catalogue of the parasites contained in the collections of the U. S. Bureau of Animal Industry, U. S. Army Medical Museum, Biological Department of the University of Pennsylvania (Coll. Leidy) and in Coll. Stiles and Coll. Hassall. Vet. Mag. **1**: 245–253, 331–354.

TINER, J. D. and CHIN, T. H. 1948. The occurrence of *Ascaric lumbricoides* L. 1758 in the muskrat *Ondatra zibethica* L. J. Parasitol. **34**: 253.

WALTON, A. C. 1927. A revision of the nematodes of the Leidy collection. Proc. Acad. Nat. Sci. Phila. **79**: 49–163.

Counting *Nematodirus spathiger* Eggs in Sheep Dung[1]

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During the course of certain experimental work, already reported in part in abstract by Kates and Turner (1949) on *Nematodirus spathiger*, a trichostrongylid nematode parasite of sheep and other ruminants, accurate counts of the parasite eggs in the host's feces were made in order to follow the normal course of infection in the experimental animals. This presented an opportunity of testing the accuracy of the egg count method employed, the modified direct centrifugal flotation (D.C.F.) method of Stoll (1930). Sheep feces, containing eggs of *N. spathiger* only, was used. The work of Stoll involving the D.C.F. method, as applied to sheep dung, was concerned only with eggs of *Haemonchus contortus*. The purpose of this paper is to present egg count data obtained from 42 different fecal specimens containing variable numbers of *N. spathiger* eggs.

MATERIALS AND METHODS

It has been reported by Kauzal (1933), Kates (1947) and others that nematodes of the genus *Nematodirus* produce relatively few eggs, and that relatively low egg counts are encountered even in heavy infections. Consequently, a dilution egg count method, as described by Stoll or a modification thereof, would not be practical for counting *Nematodirus* eggs. As stated by Stoll "high counts naturally favor the dilution method, low counts the D.C.F." Therefore, only D.C.F. counts were made on feces containing *Nematodirus* eggs only.

The fecal specimens were obtained rectally from five lambs experimentally infected with *N. spathiger*, and stored in an electric refrigerator in small stoppered bottles until used. The D.C.F. egg count method employed was slightly, but not essentially, modified from that of Stoll. It was preliminarily determined that about 99 to 100 percent of *Nematodirus* eggs were recovered when a total of five successive coverslips were examined from each D.C.F. preparation. In some cases, however, negative coverslips were obtained before the fifth one. Therefore, routinely in the fecal egg counts reported herein, five successive coverslips from each preparation were examined for eggs, and the total number so obtained was considered arbitrarily to be all, or 100 percent, of the eggs in the fecal sample examined. Duplicate preparations and counts were made from each fecal sample, so that a total of ten coverslips were examined for each specimen. The numbers of eggs counted on the original and duplicate D.C.F. preparations of each specimen were totaled for each of the five successive coverslips per tube, and their respective percentages of the sum total of all eggs counted were calculated. The original and duplicate egg counts of the first coverslips and of the totals of all five coverslips were plotted against a fitted correlation line to show the normal variation between original and duplicate counts on the same fecal sample.

RESULTS AND DISCUSSION

In Fig. 1A, a scatter diagram of the original counts is plotted against the duplicate counts obtained on the first of five coverslips of the 42 separate samples. A correlation line has been fitted to the series. The original counts should equal the duplicate counts in a perfect correlation; therefore a simple linear equation was applied. This was expressed by the formula $Y = a + bX$, and calculated to be

[1] Report of a study made under the Research and Marketing Act of 1946.

[2] The writer expresses his appreciation to Dr. A. O. Foster for helpful advice in formulating the statistical data.

$Y = 0.238 + 1.067X$. The average variation between the number of eggs obtained on the first original and the first duplicate coverslip was 9 percent, ranging from a low of 1 percent to a high of 43 percent, with the greater number below 15

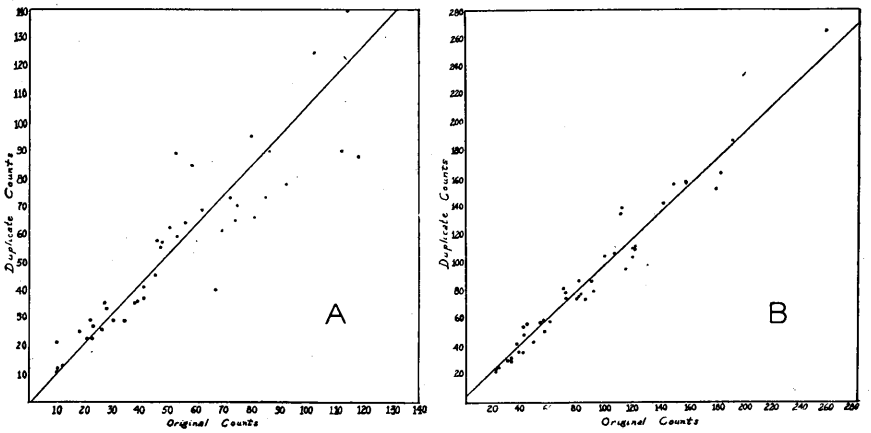

FIG. 1. *Nematodirus spathiger* eggs in sheep dung. A—Comparison of original and duplicate egg counts on first coverslips only. B—Comparison of original and duplicate egg counts, totaled on all coverslips.

percent. A scatter diagram showing the relationship between the total eggs recovered on the five original and the five duplicate coverslips of each sample is also represented in Fig. 1B. In this figure the derived equation was $Y = 3.34 + 0.94X$. Total counts from the five original and five duplicate coverslips used in these data

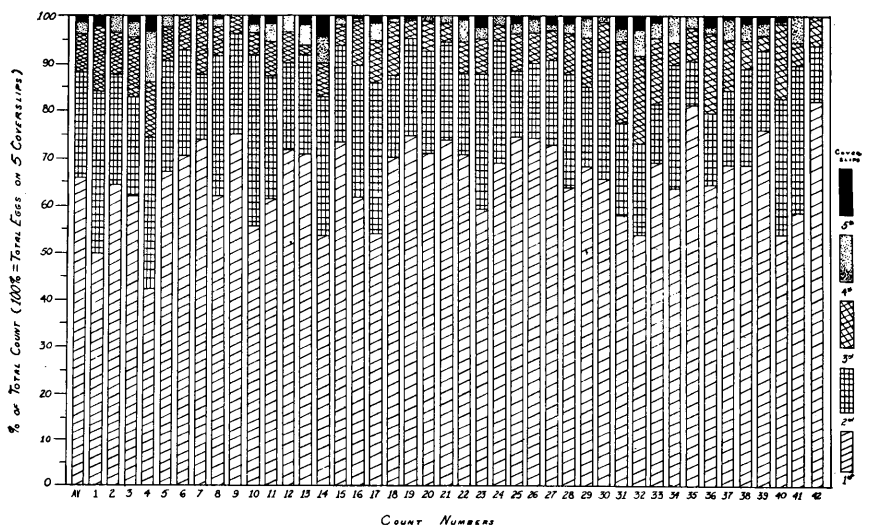

FIG. 2. Results of counting *Nematodirus spathiger* eggs in 42 sheep fecal samples by means of the D.C.F. method. Graph shows the total of duplicates of 5 successive coverslips from each sample.

did not vary over 10 percent, except in one instance in which the duplicate count differed from the original count by 12 percent; the majority varied less than 5 percent.

All the egg counts are summarized in Fig. 2. These data show that the average percentage of eggs obtained on the first coverslips was 66, varying from 50 to 81 percent on different fecal samples; on the second coverslips the average was 22 percent, varying from 9 to 36 percent on different specimens. The average percentage of eggs retained on the third coverslips was 8.2, ranging from 1 to 18 percent. Eleven samples showed an absence of eggs after the fourth coverslip, the average percentage of eggs on this coverslip being 2.7 and varying from 0 to 10 percent. Two samples were negative on the fourth coverslips and were not included in the calculation of percentage of eggs on the fifth coverslips. Twenty-nine of the 42 fecal samples counted were found to have eggs adhering to the fifth coverslips, the percentage averaging 1.1 and varying from 0 to 4 on different samples.

The total number of eggs counted for each of the samples in duplicate varied from 44 to 524, the average number being 174. These figures represent the number of eggs per one-fifth gram of feces. Thirty-one of the fecal samples contained over 100 eggs, or more than 500 eggs per gram.

Farr and Luttermoser (1941) using eggs of the chicken nematodes, *Ascaridia lineata* and *Heterakis gallinae,* obtained from 78 to 90 percent on the first coverslip, depending upon the type of flotation medium employed. However, physical differences between poultry and sheep dung and the different parasite eggs they studied may have been influential in their high reccovery. Kates (1947) stated that with careful use of the D.C.F. technique over 90 percent of various helminth ova could be obtained on the first coverslip. Although *Nematodirus* ova were included in his data, they constituted less than 7 percent of the total differential egg counts on parasites of six genera. As eggs were counted on only one coverslip of duplicate samples by this worker, the data presented herein show that his counts of *Nematodirus* eggs probably represent only about 66 percent on the average of those actually present in the fecal samples. Stoll noted that in one group of tubes negative for *Haemonchus* eggs on the third coverslip, 90.1 percent of the eggs occurred on the first coverslip and 9.9 percent on the second. Three other groups containing 173 tubes had 1 percent or less on the third or fourth coverslip or had none on four or more coverslips. When these three groups were combined, 87.3 percent of the *Haemonchus* eggs were on the first coverslip and 11 percent on the second, the total for both being 98.3 percent.

The percentage of *Nematodirus* eggs recovered on the first coverslip by the writer was lower than that of *Haemonchus,* as reported by Stoll, and that of eggs of the poultry nematodes reported by Farr and Luttermoser. A reason for this may be the large size and possibly greater specific gravity of the *N. spathiger* eggs. These particular eggs vary from 181 μ to 230 μ in length and 91 μ to 170 μ in width. *H. contortus* measure 65 μ to 82 μ long and 39 μ to 46 μ wide (Kates and Shorb 1943); *Ascaridia lineata,* 75 μ to 80 μ long and 45 μ to 50 μ wide; and *Heterakis gallinae,* 63 μ to 71 μ long and 38 μ to 48 μ wide (Morgan and Hawkins 1949). Stoll was of the opinion that *Nematodirus* eggs would be "demonstrable with greater facility" than those of *Haemonchus* because of the larger size of the former. However, results reported here show the opposite to be true. It is quite possible that the large *Nematodirus* eggs become crowded on the coverslip and in lifting the cover from the tube a number may be retained by the fluid. Also there is a certain amount of human error regardless of the exactness with which a sample is examined. Assuming the correct amount of salt solution has been added to the tube, the writer has found that the greatest source of error is in the removal of the coverslip from the tube. At times there will be encountered a slight adhesion between the ground lip of the tube and the coverslip, resulting in a none-too-smooth

pickup. As a result of this movement a small drop of liquid may fall from the coverslip into the tube, causing a loss of some eggs on that particular sample.

Some of the duplicate counts on the first coverslips were not as close as desired. As shown in Fig. 1A there was greater variation between duplicate counts in samples containing the higher number of eggs. These data bear out Stoll's statement that counts by the D.C.F. method are less accurate in the samples containing the higher number of eggs than in those containing a comparatively low number. In Fig. 1B, however, the total counts of the original and duplicate coverslips were more nearly equal than those of the first counts only, indicating that the eggs not picked up on the first coverslip were recovered on the succeeding ones. This is in agreement with Stoll's findings that tubes with low yields on the first coverslip "uniformly compensate" on succeeding ones.

Counts were made to the fifth coverslip in the work reported herein, but this is obviously too laborious for ordinary diagnostic work. A majority of *N. spathiger* eggs are recoverable on the first count, but it is desirable to count the eggs on at least three coverslips to approximate the total number per gram in the stool of the sheep examined. As can be observed in Fig. 2, over 96 percent, on the average, of the total eggs obtained on five coverslips were on the first three.

SUMMARY

The efficiency of the direct centrifugal flotation method was studied in regard to counting *Nematodirus spathiger* eggs in sheep dung. Duplicate preparations and counts were made from each of 42 fecal samples, utilizing five coverslips per tube.

It was found that an average of 66 percent of the total eggs counted on five coverslips were recovered on the first coverslip, 88 percent on first and second combined, 96.2 percent on three consecutive coverslips, 98.9 percent on four, and the remainder on the fifth. The total eggs obtained from all five coverslips was considered to be a 100 percent of the eggs in the fecal sample, as preliminary counts of a sixth coverslip were generally negative.

It is concluded, therefore, that if it is desired to count more than 90 percent of the *N. spathiger* eggs in a fecal sample by the D.C.F. method, the eggs on at least three coverslips from each preparation should be counted in duplicate.

LITERATURE CITED

FARR, M. M. and LUTTERMOSER, G. W. 1941. Comparative efficiency of zinc sulfate and sugar flotation for the simultaneous flotation of coccidial oocysts and helminth eggs. Jour. Parasitol. 27 (5): 417–424.

KATES, K. C. 1947. Diagnosis of gastro-intestinal nematode parasitism of sheep by differential egg counts. Proc. Helm. Soc. of Wash. 14 (1): 44–53.

———— and SHORB, D. A. 1943. Identification of eggs of nematodes parasitic in domestic sheep. Amer. Jour. Vet. Res. 4 (10): 54–60.

———— and TURNER, J. H. 1949. Observations on the pathogenicity of *Nematodirus spathiger* in lambs. (Abstract) Jour. Parasitol. 35 (6), Sect. II: 13.

KAUZAL, G. P. 1933. Seasonal incidence of gastro-intestinal parasites of fat sheep in New South Wales. Australian Vet. Jour. 9 (5): 179–186.

MORGAN, B. B. and HAWKINS, P. A. 1949. Veterinary Helminthology. Burgess Pub. Co. 283–287.

STOLL, N. R. 1930. On methods of counting nematode ova in sheep dung. Jour. Parasitol. 22 (1): 116–136.

Some Alcohols and Hydrocarbons as Nematocides

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INTRODUCTION

A number of chemicals were tested with the intention of confirming the theory of Chitwood that chemicals which do not dissolve or dissolve in (penetrate) beeswax are probably of very little value as nematocides, while those which do dissolve (penetrate) beeswax readily may be of some value provided they have other favorable characteristics.

In discussing possible nematocides, the principles of soil fumigation should be considered. Plant parasites may be in the roots, loose in the soil, or in the form of eggs enclosed in a mucoid mass. In any case the parasites are covered by a film of moisture, and, therefore, a fumigant must: disperse through the soil, penetrate all barriers, kill, and leave no phytotoxic residue.

Kühn (1881) introduced carbon disulfide as a nematocide, and it was used by Bessey (1911) against root-knot nematodes. Bessey found that it could not be used with safety around living trees. At the same time, he tried potassium sulphocarbonate, which was not only unsuccessful but also very expensive. No practical development followed for many years. Mathews (1919) discovered nematocidal properties in chloropicrin, and this chemical was developed by Johnson and Godfrey (1932) as a soil fumigant. Taylor and McBeth (1940) found that methyl bromide appeared to be an effective soil nematocide for use against root-knot and free-living nematodes. Carter (1943) discovered the very effective mixture of 1–3 dichloropropylene, 1–2 dichloropropane. Meanwhile, various workers were trying to find practical applications in the control of nematodes. Chitwood (1939) showed that the physical constants, including boiling point, vapor pressure, cholesterol solvency, and water solubility are correlated with efficacy and are apparently limiting factors. Soil texture was proven by Thorne (1939) to be an important factor. Temperature and moisture also seem to be factors determining the efficacy of soil treatment, according to McClellan, Christie, and Horn (1949). Hoshino and Godfrey (1932) found that the minimum period of time for killing the larvae of plant nematodes in water at 40° C. is 2 hours and 7.5 minutes, and for eggs it is 4.5 days $\pm$ 0.5 days. Christie (1945) found that Larvacide (chloropicrin) is more effective when infected roots have undergone decay, but with D-D and Dowfume G decay does not appear to be so important. Based on killing range as a criterion, the most pronounced nematocidal action was exhibited by mixtures containing dichloropropane (D-D and Dowfume N), by mixtures containing methyl bromide (Dowfume G and Iscobrome No. 1), by carbon disulfide, by ethlene chlorobromide and allyl bromide. Ellis et al (1949) found in field experiments that root-knot was effectively reduced and yields were markedly increased by soil treatment with uramon. Although soil treatment with uramon is known to be highly effective in control of root-knot, its use often results in stunted plants and poor yields.

PHYSIOLOGY

The egg is the most resistant stage since it is completely covered by a vitelline membrane. Regulation of the environmental contact with the nema or nemic

[1] A contribution from the Department of Biology, The Catholic University of America, Washington, D. C. This paper, prepared under the direction of Dr. Benjamin G. Chitwood, is based on Part I of the author's dissertation submitted in partial fulfillment of the requirements for the degree of Master of Science. Sincere thanks are offered to Dr. B. G. Chitwood and Mr. W. F. Simpson.

embryo is apparently governed by a "lipoidal" membrane in the egg and a thermolabile membrane in the larva or adult (Chitwood, 1938a). The vitelline membrane of eggs of all the investigated ascarid species has been proven to dissolve in substances capable of dissolving lipoids. The vitelline membrane is, therefore, of lipoid nature, and only those substances which will dissolve the vitelline membrane of the eggs are capable of penetration (Zawadowsky, 1928). Timm (1950) showed that the vitelline membrane in the living egg of *Ascaris lumbricoides* var. *suis* is similar to myricyl palmitate. He mixed equal amounts of the vitelline membrane wax and refined beeswax (myricyl palmitate) and found it to melt at 68° C. to 70° C. Chitwood (1951) observed crystallization of the vitelline membrane in the eggs of *Meloidogyne javanica*. These crystals were very similar in form and optical behavior to those produced by refined beeswax. Crystals of both origins become optically extinct and amorphous on standing. Chitwood (1950) pointed out that the egg shell is commonly covered by a series of mucoids which are hygroscopic. This material tends to prevent drying and reduce the penetration of many chemicals, such as fat solvents. In the presence of oxygen, this material commonly turns orange (possibly quinone tanning) on the surface, and is, therefore, less permeable to water.

EXPERIMENTAL PROCEDURE

The test organism used in this experiment was *Rhabditis strongyloides* reared on agar cultures containing bacteria and a small piece of uncooked meat. The chemicals which gave the best results against *R. strongyloides* were then tested against the root-knot nematode *M. javanica*. By this method of using *R. strongyloides* as a screening organism, it was easy to eliminate the chemicals which would be useless for *M. javanica*. In most cases, it was considered that if the chemical did not kill the free-living *R. strongyloides*, it would not bring death to the root-knot nematode.

A series of alcohols was tested for possible nematocidal activity, on a water dilution basis at three different temperatures: 20°, 30°, and 36° C. for a duration of two hours. A series of Wasserman tubes containing 1 cc. quantities of dilutions of the following percentages of various alcohols: 50, 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005, and 0.001 was set up for each of the three different temperatures. About twenty-five specimens of *R. strongyloides* including adults and larvae of both sexes were placed in each of the tubes. Two test tubes containing water were used in each series as controls. The series of tubes was then placed in water baths at the particular temperatures for a two hour period. At the end of this period, the series of tubes was removed and examined for the effect of the chemicals. The contents of each tube was emptied into a 20×8 mm. watch glass and examined under a binocular dissecting scope for the number of dead and living of the adults and larvae. A number of mature female worms, both living and dead, were mounted and examined under oil immersion lens to determine the effect on the embryonated eggs. The alcohols which gave the best results in the above method were tested against *M. javanica* in a similar manner. Results of this test are shown in Table 1.

The hydrocarbons and the hydrocarbon derivatives were tested by a different method because they were only slightly soluble in water. Other solvents or emulsifiers could have been used, but they were considered unnecessary in this method of testing. In testing the various hydrocarbons and derivatives, an effort was made to simulate the problems of a nematocide as much as possible. One half cubic centimeter of water was placed in a watch glass, which in turn was placed in a 50×30 mm. stender-dish. To the watch glass were added: free nematode larvae,

TABLE. 1.—*Minimum lethal concentration in per cent against* Rhabditis strongyloides (*water dilution*)

Alcohol	20° C.	30° C.	36° C.	Nematocidal rating
Allyl	1	0.1	0.1	Good
Amyl	1	0.5	0.5	Good
3-hexanol	0.5	0.1	0.1	Good
Hexanol	*	0.5	0.5	Moderate
2-pentanol	1	5	1	Moderate
n-butyl carbinol	1	1	0.5	Moderate
2-pentanol-4-methyl	1	1	1	Moderate
Methyl n-butyl carbinol	*	*	0.5	Moderate
Ethyl	5	1	1	Poor
Methyl	5	5	1	Poor
Sec-butyl	5	5	1	Poor
n-propyl	5	5	5	Very poor
Isopropyl	5	5	5	Very poor
n-butyl	5	5	5	Very poor
Against *Meloidogyne javanica*				
Amyl	1+	1+	1	Good
Allyl	5	5	1	Good
3-hexanol	5	5	5	Moderate
2-pentanol	5	5	5	Moderate
Hexanol	*	*	*	Poor
2-pentanol-4-methyl	*	*	*	Poor
n-butyl carbinol	*	*	*	Poor
Methyl n-butyl carbinol	*	*	*	Poor

* Did not kill nematodes at highest concentration in water.

free eggs, and a mucoid egg mass of *M. javanica*. Since the hydrocarbons and their derivatives were effective against *R. strongyloides*, the testing against *M. javanica* is emphasized here. Amounts of 1, 0.5, 0.1, 0.05, and 0.01 cubic centimeters of the chemical were then added to the stender-dishes and lids were put on and sealed with vaseline. After a period of two hours at 24° C. the watch glasses

TABLE 2.—*Minimum lethal concentration in cc. against* Rhabditis strongyloides *at 24° C. (vapor method)*

Chemical	Amt. in 30 cc. stender-dish	Nematocidal rating
Cyclohexyl bromide	0.01	Good
Cyclohexyl chloride	0.01	Good
Allyl acetone	0.01	Good
Cyclohexane	0.1	Moderate
Petroleum hexane	1	Poor
Synthetic hexane	1	Poor
Lindane	*	Very poor
Benzene hexachloride	*	Very poor
Against *Meloidogyne javanica*		
Cyclohexyl bromide	0.01	Good
Cyclohexyl chloride	0.01	Good
Allyl acetone	0.01	Good
Cyclohexane	0.1	Moderate
Petroleum hexane	1	Poor
Synthetic hexane	*	Very poor

* Did not kill with highest concentration used.

were removed and examined under a binocular dissecting scope and the number of larvae, dead and living, was recorded. The eggs were then mounted and examined under oil immersion lens for the effect of the chemicals. Allyl acetone, a compound which is neither an alcohol nor a hydrocarbon, was also tested in the same manner as the hydrocarbons. Results of this test are shown in Table 2.

A beeswax solvency test was performed on each of the chemicals used in this experiment. One hundred milligrams of beeswax was placed in each test tube and one cubic centimeter of the chemical was added every 24 hours until the beeswax was either dissolved or a total of twelve cubic centimeters of the chemical had been added. It was found that the chemicals which gave the best results as possible nematocides either dissolved or emulsified beeswax at 24° C. in proportions of 1 to 6 to 1 to 10.

Of the alcohols tested, sec-butyl, allyl, and amyl almost completely dissolved the beeswax; hexanol, 3-hexanol, and 2-pentanol produced a slight change; and ethyl, methyl, isopropyl, n-propyl, n-butyl carbinol, 2-pentanol-4-methyl, and methyl n-butyl carbinol gave no change. Twelve cubic centimeters of the above alcohols was used in each test. All the hydrocarbons dissolved the beeswax and gave a cloudy emulsion. Only six cubic centimeters of each of the chemicals was required. Twelve cubic centimeters of allyl acetone produced only a slight change in the beeswax.[2]

SUMMARY

Among the alcohols tested as possible nematocides, amyl and allyl were the most toxic, followed by 3-hexanol and 2-pentanol. The following alcohols were least toxic: methyl, butyl, n-propyl, sec-propyl, isopropyl, hexanol, ethyl, 2-pentanol-4-methyl, normal butyl carbinol, and methyl n-butyl carbinol.

Among the hydrocarbons tested, the best results were obtained with cyclohexane, cyclohexyl chloride, and cyclohexyl bromide. The least toxic were: petroleum, hexane, synthetic hexane, lindane, and benzene hexachloride. Allyl acetone tested as favorably as the best hydrocarbons and their derivatives.

From this experiment it can be seen that the chemicals which dissolved beeswax also tested favorably as nematocides, with the exception of allyl acetone which did not dissolve beeswax.

LITERATURE CITED

BESSEY, E. A. 1911. Root-knot and its control. U. S. Dept. Agric. Bur. Plant Ind. Bull. No. 217: 1–89.

CARTER, W. 1943. A promising new soil amendment and disinfectant. Science 97 (2521): 383–384.

CHITWOOD, B. G. 1938a. Further studies on nemic skeletoids and their significance in the chemical control of nemic pests. Proc. Helm. Soc. Wash. 5 (2): 68–75.

————. 1941. Soil treatments with volatile liquids for control of nematodes. Phytopath. 31: 818–824.

———— and CHITWOOD, M. B. 1939. Treatment of soil for the control of the bulb or stem nematode. Third Internat. Cong. Microbiol. N. Y. 169–170.

———— and ————. 1950. An Introduction to Nematology, Section I—Anatomy. Monumental Printing Co., Baltimore, Maryland. 186–187.

CHITWOOD, M. D. 1951. Notes on the physiology of *Meloidogyne javanica* (Nematoda: Heteroderidae). J. Parasitol. 37 (1): 96–98.

CHRISTIE, J. R. 1945. Some preliminary tests to determine the efficacy of certain substances when used as soil fumigants against the root-knot nematode, *Heterodera marioni* (Cornu) Goodey. Proc. Helm. Soc. Wash. 12 (2): 14–19.

[2] Detailed tables of all the experiments may be found in the original thesis in the library of The Catholic University of America, Washington, D. C.

ELLIS, D. E., CLAYTON, C. N. and OWENS, R. G. 1949. Effects of soil treatments with uramon and certain fumigants upon plant growth and incidence of root-knot. Phytopath. 39: 590–597.

HOSHINO, H. M. and GODFREY, G. H. 1933. Thermal death point of *Heterodera radicicola* in relation to time. Phytopath. 23: 260–270.

JOHNSON, M. O. and GODFREY, G. H. 1932. Chloropicrin for nematode control. Indust. and Eng. Chem. 24: 311–313.

MATHEWS, D. J. 1919. Report on the work of the W. B. Randall research assistant. Expt. and Res. Sta., Chestnut, Herts, Ann. Rpt. 5: 18–21.

MCCLELLAN, W. D., CHRISTIE, J. R. and HORN, N. L. 1949. Efficacy of soil fumigants as affected by soil temperature and moisture. Phytopath. 39 (4): 272–283.

TAYLOR, A. L. and MCBETH, C. W. 1940. Preliminary tests of methyl bromide as a nematocide. Proc. Helm. Soc. Wash. 7 (2): 94–96.

THORNE, G. 1939. Some factors governing the success of chemical treatment of soil for nematode control. Proc. Helm. Soc. Wash. 6 (2): 60–62.

TIMM, R. W. 1950. The chemical composition of the vitelline membrane of *Ascaris lumbricoides v. suis.* Science 112 (2920): 167–168.

ZAWADOWSKY, M. M. 1928. The nature of the egg shell of various species of *Ascaris* eggs. Trans. Lab. Expt. Biol. Zoopark Moscow 4: 201–206.

Minutes

Two Hundred Ninety-third to Three Hundredth Meetings

The 293rd meeting was held at the Lee House, Washington, D. C. on October 11, 1950, and was a dinner in celebration of the 40th Anniversary of the Society. Dr. F. G. Brooks, a corresponding member, was transferred to non-resident membership. The following were elected to membership: Mr. B. B. Babero, Mr. V. G. Perry, Mr. E. L. Schiller, Mr. W. A. Uricchio, Lt. T. A. Jackowski, Jr., Miss R. A. Buckner, Dr. S. Goto, Dr. P. V. Gustavson, and Dr. J. F. Kent. Dr. Foster acted as toastmaster for the evening, introducing the following speakers: Dr. Paul Bartsch, Dr. W. W. Cort, Dr. E. B. Cram, Dr. G. Steiner and Dr. B. Schwartz.

The 294th meeting was held at the Zoological Division Laboratory, Bureau of Animal Industry, Beltsville, Maryland, on November 22, 1950. The following were elected to membership: Dr. M. C. Meyer, Miss Ruth H. Bancroft, Dr. Benjamin F. Lownsbery, Mrs. Joyce W. Lownsbery, and Dr. C. A. Loos. Papers were presented by Dr. R. Rausch, Dr. D. A. Shorb, Mr. J. T. Lucker, Mr. J. R. Elsea, Dr. G. Dikmans, and Dr. K. C. Kates.

The 295th meeting was held at McMahon Hall, Catholic University of America, on December 13, 1950. The officers elected to serve during the year 1950 were: Miss Marion M. Farr, President; Dr. Leon Jacobs, Vice President; Mr. A. L. Taylor, Recording Secretary; Miss Edna M. Buhrer, Cor.-Secretary and Treasurer. Papers were presented by Dr. C. G. Durbin, Dr. Thomas Burch, Dr. B. F. Lownsbery, Mr. A. C. Tarjan and Dr. E. G. Reinhard.

The 296th meeting was held at McMahon Hall, Catholic University of America, Washington, D. C. on January 18, 1951. The following new members were elected: Dr. George Dubois, Mr. Henry F. Mengoli, Mr. John V. Owens, Dr. W. D. Valleau and Dr. Richard A. Chapman. Dr. L. A. Spindler was elected to represent the Society as a vice-president of the Washington Academy of Science, succeeding Dr. Price. The society voted to commemorate the deaths of the following members: Dr. H. E. Ewing, Dr. Banner Bill Morgan and Dr. A. B. Hardcastle. Papers were presented by Father R. W. Timm, Mr. William A. Uricchio, Miss Rita Buckner, Mr. J. V. Owens, and Mr. Donald Valentine.

The 297th meeting was held at McMahon Hall, Catholic University of America, Washington, D. C., on February 21, 1951. Mr. James W. Ingalls, Jr., and

Dr. Dewey J. Raski were elected to membership. Papers were presented by Dr. F. D. Enzie, Dr. L. R. Sinclair, Mr. Ralph Barr, and Dr. C. G. Durbin. Short notes were presented by Dr. B. G. Chitwood and Dr. E. E. Wehr.

The 298th meeting was held at the Auditorium of the National Institutes of Health, Bethesda, Maryland, on March 21, 1951. On the recommendation of the Executive Committee, it was voted that: 1. Annual dues are not be increased. 2. Subscription to future members of the proceedings, starting with volume 19 in 1952 is to be $3.00 for subscribers residing in the United States and $3.25 for subscribers residing in foreign countries. 3. Prices for back numbers of the proceedings to non-members are to be $3.00 domestic and $3.50 foreign for volumes 1, 2, 3 and 4, and $1.75 domestic and $2.00 foreign for volumes 5 to 18, inclusive. Papers were presented by Dr. P. P. Weinstein, Dr. J. R. M. Innes, Drs. Josephson, Greenberg and Taylor, and Mr. Goldman.

The 299th meeting of the Helminthological Society of Washington was held at the Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland on April 20, 1951. Dr. William F. Mai, Mr. Harry Herlich and Mr. David T. Clark were elected to membership. An invitation from Dr. Paul Bartsch to hold a meeting at his home was accepted by the Society. Dr. W. W. Cort introduced the following speakers: Mr. Eli Chernin, Mr. A. R. Barr, Mr. R. C. Wallis, Mr. R. E. Thorson, Mr. R. H. Foote and Dr. G. F. Otto.

The 300th meeting was a picnic held at "Lebanon", the home of Dr. and Mrs. Paul Bartsch near Lorton, Va., on June 16, 1951. Mr. A. S. Evans, Dr. Bert Lear, Mr. Julius Feldmesser and Dr. Alfred Edward A. Hudson were elected to membership. Dr. N. R. Stoll, formerly a corresponding member, was transferred to nonresident membership.

A. L. TAYLOR
Recording Secretary

Report of the Brayton H. Ransom Memorial Trust Fund

FUNDS ON HAND, Jan. 1, 1950	$1696.17
RECEIPTS: Interest rec'd in 1950	59.23
DISBURSEMENTS: Expenses and grant to Helminthological Society of Washington	31.00
BALANCE ON HAND, Dec. 31, 1950	1724.40

ELOISE B. CRAM,
Secretary-Treasurer

HENRY ELLSWORTH EWING

1883–1951

Dr. Henry Ellsworth Ewing, retired entomologist and life member of the Helminthological Society of Washington, died January 5, 1951.

Dr. Ewing was born in Arcola, Illinois, February 11, 1883. He received his A.B. degree from the University of Illinois in 1906 and his M.A. from the same institution in 1908. The Ph.D. degree was conferred upon him in 1911 by Cornell University.

From 1911 to 1914, Dr. Ewing was assistant Entomologist for the Oregon Agricultural Experiment Station; from 1914 to 1919, he taught Entomology at the Iowa State College; and from 1919 until his retirement in 1945, he was a specialist on arachnids and lice in what is now the Bureau of Entomology and Plant Quarantine of the U. S. Department of Agriculture.

Dr. Ewing was a member of many scientific societies, including the American Association for the Advancement of Science; Entomological Society of America; Association of Economic Entomologists; American Society of Mammalogists; American Society of Parasitologists (Pres. 1944); American Society of Ichthyologists and Herpetologists; Biological Society of Washington; Washington Academy of Sciences; Entomological Society of Washington; and others. He was elected to membership in the Helminthological Society of Washington, October 18, 1924, was its President in 1931, served as a member of the Editorial Committee of its Proceedings from 1934 to 1946, and was elected to life membership November 21, 1945. From the time of his election to membership until poor health prevented, Dr. Ewing rarely missed a meeting of the Society and took an active interest in its activities.

Dr. Ewing was a tireless worker and made many important contributions to the systematics of lice and parasitic arachnids. His publications in the field of parasitology consisted of one book and about 90 papers and notes.

In his death parasitology and entomology in general have lost a distinguished scientist and his fellow workers a friend. The Helminthological Society extends to his family its sincerest expression of sympathy.

E. W. PRICE

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