STUDIES IN LIFE HISTORY AND CONTROL OF HOG LUNGWORMS.

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This paper is a supplement to a preliminary report published two years ago in these Proceedings, and deals further with the life history and bionomics of the two hog lungworms *Metastrongylus apri* and *M. brevivaginatus*. These worms are typical of the superfamily Strongyloidea, which comprises a widely prevalent and abundant group of vertebrate parasites.

The males of this superfamily may be identified by a conspicuous caudal bursa, which is a broad membranous organ supported usually by six paired and one unpaired costae. These costae, or rays, extend outward from a common center very much like the fingers of a hand, and form an efficient clasping apparatus used in copulation. Two spicules, variously modified in structure, are also present in the males. These are attached to the seminal vesicle by bulb-like expansions and doubtless also serve in copulation. The females are invariably larger than the males, and show the more generalized nematode characteristics. The vulva is posterior to the middle of the body, while the anus is terminal. In those genera which comprise the lungworms of this discussion, the females are all viviparous. Both sexes possess a mouth with six lips or papillae, but the buccal capsule is generally missing. The following list comprises most of the strongyloid worms that have been reported from domesticated animals in this and other countries:

Horse ..... *Dictyocaulus arnfieldi* Railliet and Henry 1907.

Cattle ..... *Dictyocaulus viviparus* (Bloch 1782) Railliet and Henry 1907.
Metastrongylus pulmonaris Sluiter and Swellengrebel 1912.
Syngamus laryngeus Railliet 1899.

Sheep and goats..... *Dictyocaulus filaria* Railliet and Henry 1907a.
Synthetocaulus rufescens Railliet and Henry 1907a.

Swine ..... *Metastrongylus apri* Henry 1907.
M. brevivaginatus Railliet and Henry 1907.

Dog ..... *Haemostrongylus vasorum* (Railliet 1906) Neveu-Lamaire 1912.

Cat ..... *Synthetocaulus abstrusus* (Railliet 1898) Railliet and Henry 1907.

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TECHNIQUE.

It may be appropriate at this point to give briefly the technique which was developed, and which proved of value in conducting these studies. Most authorities on the subject of lungworms still maintain that it is impossible a raise worms in the laboratory that are taken directly from the lungs. The writer desires to correct this statement, which undoubtedly has discouraged investigation along this line, by asserting that such statements have no basis in fact. Hog lungworms, taken directly from the lungs, can be readily grown in soil, provided a few simple precautions are observed.

Material. The strongyles of this discussion were procured at a local slaughter house, and no difficulty was experienced at any time in obtaining them in quantity. The lungs containing these parasites were readily distinguished by the anaemic infarcts, which are very characteristic, and which make identification an easy matter. Such infested areas were cut out, and the pieces of tissue were placed into glass jars in which they were carried to the laboratory. It is best to dissect out these parasites the same day they are obtained, but they may be left over night in a refrigerator, providing the temperature is a few degrees above freezing. The worms lie, as a rule, in numbers at the terminal portions of the smaller bronchia. Best results are obtained if these bronchia are cut open with finely pointed scissors, when practically the entire mass of worms may be lifted out at a single operation. If this material is desired for histological study, very delicate forceps must be used, otherwise many worms will be crushed. From these infarcts the worms should be placed directly into salt solution in a shallow glass dish. This salt solution should have a fairly high density (12 grams NaCl to a liter of water). The ordinary physiological salt solution is not sufficiently dense, the result being that great numbers of nematodes will swell and burst if placed into such a medium. Lungworms were kept alive in the above salt solution for nine days when it was changed daily and thoroughly aerated.

Killing and fixing. Specimens intended for whole mounts or histological studies should be killed at once by dropping them into hot salt solution or Carnoy's fluid. The salt solution as a killing agent is in some ways superior to Carnoy's, Zenker's or other killing fluids. Living worms possess a cuticle that is very permeable to gases and fluids. Practically all parasitic nematodes, if placed in tap water, will swell and burst in a very short time, showing there is a rapid interchange of fluids through the body-walls. Even dead alcoholic material, trachinae for example, will exhibit active, life-like movements when placed upon a slide under a microscope. These movements will continue as long as evaporation takes place or if some other fluid of lesser density be added. Undoubtedly the activity of such a dead worm can be explained only on the basis that there is a rapid and violent interchange of liquids of different osmotic pressures through the walls of the parasite. However, when such a nematode is killed and fixed by

the usual methods, it is almost impossible to obtain a ready diffusion of stains or balsam in subsequent stages. Apparently some chemical reaction takes place between the killing fluid and the substance comprising the body wall of the worm, rendering the same impervious. Comparative studies with different killing agents have shown that better results are obtained in whole mounts if the nematodes are killed in hot water or salt solution only. The killing solution should be hot but not boiling. A temperature of 90° C. gave uniformly the best results. The worms may be dropped into such hot solution singly or in bulk, when most forms will straighten out at once.

After they are killed the specimens should be transferred to a formol-glycerin-acetic fixing solution,[1] in which they can remain indefinitely. It has been determined that, so far as parasitic nemas are concerned, the details are sharper in whole mounts when the specimens are fixed in this solution than is the case when other fixatives are used. Best results were obtained when the specimens were allowed to remain in the above solution for several days before mounting. The swelling power of the acetic acid counteracts the shrinkage caused by the formalin, so that the tissues show little distortion in the final mounts.

Staining. The stains which gave the best results with nematodes were Delafield's iron hematoxylin counter-stained with eosin, and Schneider's acid carmine. The last stain gave excellent results but was somewhat exacting in its preparation and use. For this reason the former combination of stains was chiefly used, and was especially useful in histological work. Practically all the steps, preliminary to mounting, were carried out in small stender dishes with ground glass covers to exclude the dust. The procedure for using the above stains is briefly as follows:

1 Remove worms from fixing solution and place in hematoxylin for 24 hours.
2. Destain with acid alcohol until specimens are of a light brown color.
3 Counterstain with eosin for 24 hours.
4 Destain with 95 per cent alcohol.
5 Place nematode on a clean slide and cover with a cover-glass. Wrap a few turns of white thread around the slide and cover glass to keep the latter in place and to compress the nematode.
6 Place slide into carbol-xylol for 24 hours.
7 Place in xylol for 24 hours.
8 Remove cover-glass and cover nematodes with dilute balsam; replace cover, and add more balsam as it dries out from under the cover-glass.

Very satisfactory whole mounts were obtained by the use of the above method, and with the exception of the last step little difficulty was experienced in the process. Occasionally a refractory specimen was found that was difficult to infiltrate with balsam. In such a case

[1] 10 per cent formalin solution..... 90 c. c.
Glycerin ..... 9 c. c.
Glacial acetic acid..... 5 c. c.

it was removed from the slide and placed into a stender dish containing balsam of sufficient dilution so that infiltration did take place. For this purpose it was found adequate to have four dilutions of ordinary commercial balsam, namely 1:4, 1:3, 1:2 and 1:1. The last figure in each case represents the proportion of xylol that was employed. In the use of this method the nematodes were placed directly into the weakest balsam, in which they were allowed to remain for 24 hours. They were then transferred to each of the denser grades in which, in turn, they remained for the same length of time.

The method outlined above possesses advantages over others commonly recommended, in that it was more economical in the use of materials, and yielded uniformly better results. The differentiator and string siphon methods, as generally employed for mounting nematodes, used much greater quantities of expensive reagents and yielded results that were little if any better than the above. In the case of microscopic nemas, such as the lungworm larvae of this discussion, all the necessary steps of killing, fixing, staining and infiltrating with balsam were carried out in half-ounce vials, 24 hours being allowed for each step in the process. Transfers of liquids were effected with pipette, the vial being shaken each time and then allowed to stand until the nemas separated out by sedimentation. The excess of liquid was then readily removed with a pipette.

Specimens showed least distortion when only small steps were taken in transferring to the different dilutions. For example, when the transfer was made from alcohol to carbol-xylol, the steps in effecting this change were approximately as follows: 3:1, 2:1, 1:1, 0:1. The last figures in these dilutions represent the amounts of carbol-xylol that were used. All the steps in the above method were carried out in a single vial, in which also the balsam infiltration was effected. By this means the nemas were ready to mount on a slide when the process was completed.

Propagation. Little difficulty was encountered in growing young worms, taken directly from the lungs, when the right environmental conditions were provided. It was found that these larvae are very susceptible to moisture and temperature changes, to bacteria and decomposition products, and to proper aerobic conditions. For this series of experiments small, earthenware pots were used, and gave uniformly the best results. The optimum soil types were mentioned at length in the preliminary report, and will therefore be omitted from the present discussion.

Separation from the soil. To separate the young nemas from the soil, a simple sieve-funnel apparatus was devised which proved very satisfactory. The sieve was of copper and improvised from a piece of fly-screen wire, nine inches square. This was turned up at the edges to form a trap two inches deep. The funnel was of glass, ten inches in diameter, and to its bottom was attached a piece of rubber tubing kept closed with a pinch-cock. When ready for use the apparatus was assembled by supporting the funnel firmly in an iron stand, and then filling it with water to within an inch of the top. Into the sieve were

next placed a thin layer of sterile absorbent cotton, and on this a layer of soil one inch deep. Finally, the sieve was lowered slowly into the funnel, in which it remained suspended for 24 hours. By this method very clean separations were effected, most of the nemas, contained in the sample of soil, working their way through the cotton and becoming aggregated at the pinch-cock.

LIFE HISTORY

Detailed experiments dealing with the habits and life-history of these parasites are given in the preliminary report already mentioned. In this paper only such factors will be considered as help to evaluate the whole problem. The following summary is based upon the examination of several hundred affected hog lungs, and incidental experiments conducted for over three years.

The development of the infarcts of typical infested lungs, falls naturally into three groups. These, for convenience, may be designated as the diffuse, the firm, and the infiltrated types. Freshly infested areas are pink in color, and invariably of a swollen and spongy texture. This condition has been found due to the active migrations of young larvae through the lung substance. As these larvae develop, they migrate toward the posterior tips of the lungs, where they finally lodge in the terminations of the smaller bronchioles. Here they grow rapidly in size, living upon the blood and lymph exudate of the host. In about six weeks these larvae become sexually mature. The worms and the mucous that surrounds them effectively clog up the openings in which they occur so that air can not penetrate to the remoter areas of the lung. As a result the tissue becomes edematous and assumes a characteristic gray color. Such areas are always sharply defined from the surrounding pink tissues of the lung. As the infestation progresses, there is a gradual effusion of watery fluids from the capillaries into the alveoli of these spaces. Parasitic waste products also accumulate, and as a result the affected areas become necrotic, hard and watery to the touch and of a purple color. Another factor often observed in the course of these investigations was the common occurrence of bacterial infections associated with these parasites. Of course not every verminiferous lung showed bacterial infection, but as a rule, where infection did exist parasitic infarcts could also be found. For example, out of a group of 100 pneumonic lungs, 83 were found to be infested with worms also. Probably these worms do not carry infection themselves, but the watery tumors which they produce undoubtedly make excellent culture media for any bacteria that chance to find their way into the lungs.

The transfer of these parasites from host to host can be effected in a variety of ways. The commonest method is for the larvae to find their way from the lungs into the digestive tract, from which in turn they are excreted with the feces. By this means the hog lot becomes heavily infested in a very short time. The larvae, remaining active and continuing their development in the soil, become infective in about five days. They were readily found in the sweepings of hog houses, and in

hog wallows. These facts indicate the need of exercising greater care along preventive and sanitary regulations in swine husbandry.

Returning now to their development, it was found that these nemas persist indefinitely in the soil. For the first four weeks, growth is very rapid and is characterized by frequent moults, rapid differentiation, and profound changes in metabolism. Some cultures containing these worms were subject to repeated dessication, to mild antiseptics such as one per cent phenol, five per cent copper sulphate, and top dressings of lime and wood ashes, all with apparently little effect. The accompanying graph shows the growth attained by these parasites within laboratory cultures for different intervals of time. The lungworm larvae, upon which these measurements were based, were grown in small earthenware pots of sterile soil. Twenty of these small pots containing humus soil were sterilized at one time, and divided into two equal groups. Ten sexually mature lungworms were then placed into each pot, making a total of 100 worms for each group. Into the pots of the first group were placed only the largest, adult female lungworms, namely, those over 4 cm. in length. Into the other ten pots were placed the smallest lungworms that could be found. These were all under 3 cm. in length, the average being about 2.5 cm. Clean grass seed was then planted in each pot. These soil cultures were kept in a greenhouse, watered with distilled water, and examined at weekly intervals.

In every pot two kinds of larvae could always be found, namely, a stout, sluggish form and an active, slender form. At about eight weeks of age these slender larvae showed a small bursa and spicules at the posterior end. There is no question, therefore, that they were males, and by elimination, it can be assumed that the thick larvae were females. The measurements given in the graph (fig. 1) are expressed in millimeters, and represent averages derived by selecting at random ten larvae of both kinds from every sample of soil.

It was a relatively simple matter to trace the development of these nemas within the soil, but the same could not be said of their development within the body of the host. The occurrence of these forms within the entire digestive tracts of several pigs that were dissected, would indicate contaminated food and water as prime factors in dissemination. This probability becomes further strengthened by the successful infestation through feeding described in the preliminary report. Different larval stages have also been found throughout the respiratory tract, and in the sinuses of the head. As sweepings of hog houses contained these nemas in abundance, it is quite probable that these larvae can be breathed in by the host.

Another method of ingress that suggested itself was the possibility that these parasites may burrow directly through the skin. To test this theory two young rats were placed into infested water in a tall glass jar so adjusted that only their heads protruded. Five-weeks-old larvae were used for this experiment and the rats were left in contact with them for ten hours. After this the rats were killed and washed thoroughly in running tap water, when the skin was carefully removed. Examination of scrapings from the dermis of both rats yielded negative results, as did also the liver, blood, spleen and other organs that

were examined. It may be inferred, therefore, that infestation does not take place readily, if at all, by this method.

Experimental evidence would indicate that the larvae which find their way into a pig do not migrate directly to the lungs. Apparently, a definite cycle exists, during which the lungworm larvae persist in dif-

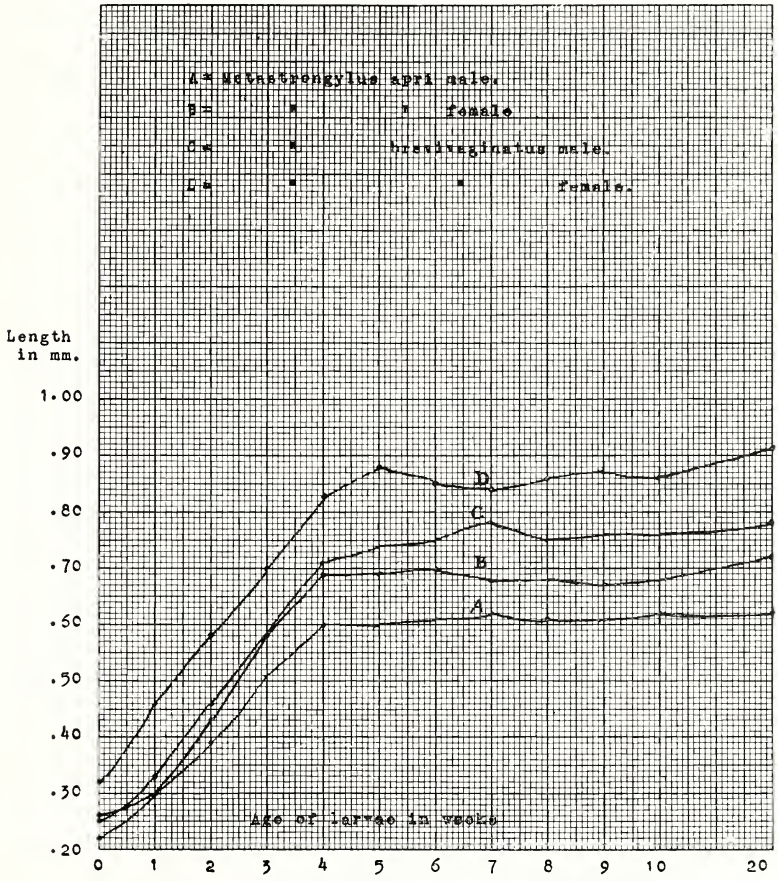

Fig. 1. Graph showing the rate of growth of the two species of lungworm larvae in laboratory soil cultures.

ferent organs for varying lengths of time. The contention for this cycle is based on the following series of experiments, which indicate that such a course is highly probable if not entirely correct:

Experiment 1.—Two rats were restricted for a whole week to a diet of heavily infested lung tissue. Daily examination of feces was made, but only dead larvae were found. The rats showed no ill effects from their diet, and when killed, 12 hours after the last feeding, no infestation of any kind was observed. Some lungworm larvae were found in the intestine, but these were inactive and seemed to have undergone digestion. No living larvae were found in the different

organs that were examined. Infested lung tissue was then fed to two pigs with the same negative result. It can be assumed, therefore, that worms and larvae taken directly from the lungs are non-infective.

Experiment 2.—In this experiment four rats were used, and into the hind leg of each was injected, subcutaneously, a 1 c.c. salt solution suspension of living lungworm larvae. The larvae injected into the first two rats were taken from the lungs of hogs, while those injected into the second pair of rats had been grown in soil cultures in the laboratory and were five weeks of age. After 20 days all four rats were killed and examined. The first pair of rats showed only a few disintegrated nemas at the site of injection. The second pair of rats contained several larvae in the liver and lungs, and in one of these rats two gravid females were also found, tightly coiled in the intestine. From these results it may be inferred that pigs are also parasitized through abrasions in the skin, or, more likely, through the renal and anal openings. The wallowing habits of the host would indicate such a mode of ingress a highly probable one.

Experiment 3.—Five albino rats weighing about 150 grams each, were used for this experiment, and were starved for two days prior to the initial test. On October 8, 1922, several hundred lungworm larvae were fed to each rat on pieces of bread. The first rat was killed and examined after ten hours, when many active larvae were found throughout the whole digestive tract. No infestation was observed in any of the other organs. A second rat was killed 20 hours after this first feeding. Upon dissection, the larvae were found localized in the small intestine, only a few occurring in the stomach and colon. Pieces of tissue from the liver, lungs and spleen, compressed between two slides and examined under the microscope, yielded negative results. The same procedure was followed with the three remaining rats, which, however, were given two additional feedings of larvae. The results of all of these tests are indicated in Table 1. The larvae used to perform this experiment were taken from cultures grown in the laboratory and were four and five weeks of age.

TABLE 1. THE COURSE OF MIGRATING LUNGWORM LARVAE IN ALBINO RATS

Rat	No. 1	No. 2	No. 3	No. 4	No. 5
Age of larvae	4 weeks	4 weeks	4 weeks 5 weeks 5 weeks	4 weeks 5 weeks 5 weeks	4 weeks 5 weeks 5 weeks
Date of Feeding	Oct. 8, 1922	Oct. 8	Oct. 8 11 14	Oct. 8 11 14	Oct. 8 11 14
Rats Killed	After 10 hours	After 20 hours	Oct. 15	Oct. 23	Nov. 4
Intestine	+	+	+	−	−
Liver	−	−	−	+	+
Lungs	−	−	−	+	+
Spleen	−	−	−	+	+
Blood	−	−	−	−	−
Feces	*	*	*	+	*

\+ Living larvae found.
− No living larvae found.
* Dead larvae found.

An analysis of the facts thus far considered reveals several points of interest. Undoubtedly the important morphological changes that these parasites undergo in the first few weeks after they leave the maternal uterus, accounts for the fact that they are non-infective until they attain a degree of development in the soil. It is apparent from the experiments given that infestation of a host is not a direct process. A definite cycle is traversed, during which the larvae persist for varying lengths of time in other organs. Thus for the first 24 hours they can be found only in the intestine. Later they occur in the liver and spleen, and only after some 15 days do they make their appearance in the lungs of the host. The intestine that harbors these larvae always presents a red, inflamed appearance, due doubtless to the burrowing action of the parasites, as they can be found imbedded in numbers in the musoca. From the intestine they very likely find their way into the blood, by which in turn they are carried to the liver and other organs. This seems to be the most probable course even though the experiment cited above does not entirely uphold this view.

Possibly the reason no larvae were found in the blood was due to faulty technique. In performing this test, the blood was obtained by inserting the needle of a syringe into the heart of an anaesthetized rat. About 1 c.c. of blood was thus withdrawn, laked with acetic acid, and examined in a syracuse watch glass under the microscope. It is quite possible that the larvae may have been overlooked by this method, especially if they were present only in small numbers.

STUDIES IN CONTROL.

The inaccessibility of these lungworms to any of the common anthelmintics, at once complicates the problem of their control. Unfortunately no systematic studies of this nature have been attempted, due, no doubt, to the common impression that these parasites cause little injury to the host. Such literature as is available is of a semi-popular nature where inference rather than experiment is made the basis of discussion. Most attempts at direct control may be divided into two groups, namely, intertracheal injections and the use of various fumigants, all designed to kill by direct contact. The annual report of the Bureau of Animal Industry for 1900 recommends the following, presumably scientific, treatment for lungworms in calves and sheep:

"Success is more certain with fumigations, as they penetrate directly to the worms, stupefy them, and induce fits of coughing that cause expulsion. They are practiced in buildings from which all forage is previously removed, and which are well closed. Into these the diseased animals are introduced, and on a red-hot shovel are placed rags, horns, feathers, hair, old pieces of leather, empy-reumatic oil, tar, juniper berries, asafetida, etc. The intensity, duration, and number of these fumigations are graduated as the sheep become accustomed to them. At first, once a day may suffice, and then the intensity should be moderate and the duration about ten minutes; afterwards two, and finally three, may be given during the day, each lasting for twenty minutes. Kowalewsky says he has obtained very good results from similar fumigations. Fumigations with chlorine, sulphur, and sulphuret of mercury or cinnabar have been recommended, but they are dangerous."

"(Stephen recommends as follows: Put about forty lambs at a time into air-tight house, and place tar, sulphur, and turpentine in a pot of burning coals, suspended by a chain from the ceiling and brought as near to the heads of the animals as possible. The fumes are to be allowed to fill the house, and more ingredients are added as required, the lambs being kept in the place for twenty-five minutes each time, and the process to be repeated on three occasions.)"

Assuredly, a lamb that can withstand such drastic treatment should have little difficulty in withstanding a few lungworms. Even the devotees of the above methods admit that they are dangerous in unpracticed hands. In view of the resistance possessed by these parasites, it is more than probable that any poison effective in their control would be apt also to injure the lung parenchyma beyond repair. So far as pigs are concerned, intertracheal injections are not practical and could be accomplished only with great effort. In view of the many difficulties presented and the questionable results, this method of approach was not seriously considered. However, it is just to admit that a chemical highly specific for lungworms might exist. For pigs at least, such a substance should be administered by way of the blood and not by direct contact with the parasites.

From the consistently negative results reported by most workers along the lines of treatment suggested above, it was deemed advisable to attack this problem from an entirely different standpoint, namely, that of immunity. In practically all forms of verminiferous infestation, young animals show a much greater susceptibility than do the older animals. This is noticeably true in the case of lungworms. Young pigs are the greatest sufferers, and harbor these parasites in abundance. Mature hogs, on the other hand, are practically immune, although most of them will show in the lungs, old, infiltrated infarcts, the sites of former infestations.

These facts could be readily explained on the assumption that they are due to an active immunity acquired by the host through the absorption of toxins secreted by the parasites. These waste products passing into the blood stream stimulate the production of antibodies which in turn become toxic to the worms. In this way a gradual resistance could be built up, rendering the host immune from further attacks. Apparently this is what actually takes place, for the natural elimination of worms, from the lungs that once harbored them, can not be explained readily in any other way.

The above line of reasoning was made the basis of a series of experiments, in which it was attempted to immunize the host against the specific lungworm proteins. To do this, various salt-solution, alcohol, and ether extracts of dried lungworms were prepared, and their effects noted upon the blood of the pig and of other animals in vitro and in vivo. The results obtained are by no means conclusive, yet they do suggest latent possibilities that might be developed if an exhaustive study were made of this subject.

Studies with salt solution extracts of lungworms. For the purpose of this and subsequent experiments, dried lungworm powder was used, which was prepared in the following manner: The tumors containing

the worms were first washed in distilled water and emptied into a clean glass dish in which they were cut open and removed. The worms were then placed into a fine sieve, washed thoroughly with distilled water, and spread out in a Petri plate in which they were left to dry over night in an incubator at 35° C. In the morning the worms were scraped from the bottom of the plate with a glass slide and the resulting dry powder was stored in a dessicator until ready for use. Extracts from these worms were then prepared by triturating half a gram of the powder in 100 c.c. of physiological normal salt solution for 20 minutes. Dissolution of the powder was conveniently effected with the aid of glass beads in a large Erlenmeyer flask. When completed, the solution was rendered sterile by passing through a Berkfeldt filter.

The worm extracts thus prepared were injected subcutaneously into four pigs. Five injections were given at intervals of five days. Six days after the last injection, 50 c.c. of blood was withdrawn from the tail of each pig, which was defibrinated and centrifuged to obtain the serum. Two samples each of the defibrinated blood and serum were then poured out into sterile Petri plates, and into each some living, partly grown larvae or mature lungworms were introduced. The results of this experiment are given in tabular form in Table 2. The assumption was that if any antibodies were elaborated in the blood of the pigs against the specific protein of the lungworms, this fact should be evidenced by the death of such lungworms as came into contact with the blood. Assuming that this contention is correct, then the results were negative insofar as neither the mature lungworms nor larvae were killed when placed into the above samples of blood. None of the pigs showed any ill effects from the injections they received, and from the results it is evident that salt solution extracts of lungworms cause no appreciable reactions in the blood of the host.

TABLE 2. The reactions of Lungworms and Larvae to blood of pigs injected with Salt-Solution Extracts of Lungworm Powder

Pig	No. .0763	No. .0764	No. .0765	No. .0766
Injections				
1st.	10cc.	10cc.	10cc.	10cc.
2nd.	20cc.	20cc.	20cc.	20cc.
3rd.	40cc.	40cc.	40cc.	40cc.
4th.	50cc.	50cc.	50cc.	50cc.
5th.	50cc.	50cc.	50cc.	50cc.
Reactions of parasites				
To defibrinated blood				
Larvae	—	—	—	—
Adults	—	—	—	—
To blood serum				
Larvae	—	—	—	—
Adults	—	—	—	—

Explanation:

1. Injections were made into the peritoneum with a hypodermic syringe.
2. The lungworm larvae were three weeks of age.
3. Ten adult worms were placed into each Petri plate; five males and five females.
4. Physiological salt solution was used in the proportion of 100cc. to .5 gm. of lungworm powder, in making up this series of worm extracts.

The next question that naturally arose was, "What is the specific response that these parasites produce?" for it is inconceivable that pathogenic changes could go on without some corresponding adjustment in the blood of the host. To answer this question, a series of blood tests were conducted with different extracts derived from these worms.

These studies developed the fact that salt solution extracts of lungworms are non-toxic, non-hemolitic, and do not prevent the clotting of blood. Albino rats injected with these extracts likewise showed no ill effects, even when they received as high as one cubic centimeter of solution per 100 grams of body weight. Lungworms when placed into the blood of such treated rats remained alive for over five days, showing that no specific antibodies were produced.

Studies with alcohol extracts of lungworms. Alcohol extracts of lungworms were prepared in the same way as the above, except that absolute alcohol was used as a solvent. The light amber colored fluid thus obtained was evaporated to a gray consistency after filtering, and this was administered as a suspension, in minimum quantities of sterile salt solution. This suspension was also used in a series of tests to determine its effect upon the blood of various animals.

Inter-peritoneal injections of this suspension were given to two pigs. Five injections were given five days apart, and subsequent procedure was the same as that given in Table 2. The response of this pair of pigs was strikingly different from those injected with salt solution extracts of lungworms. After every injection there were marked symptoms of pain and labored breathing, which subsided only after several hours. Five days after the last injection of 50 c.c. of the suspension, both pigs were bled by cutting of a piece of the tail. Fifty cubic centimeters of blood were thus obtained from each pig, and this was centrifuged and poured out into sterile petri plates. Living worms and larvae were introduced into this serum as in the previous experiment. Under the microscope the larvae showed active signs of irritation, becoming noticeably less active within an hour, and ceasing to move entirely after ten hours. The mature worms taken from the lungs likewise showed irritation, but continued to live for three days. Worms in the blood serum of normal pigs and of pigs injected with salt solution extracts remained alive for seven days, beyond which time it was found impossible to keep the serum from decomposing.

Experiments conducted with rats and guinea-pigs showed that alcohol extracts of lungworm powder were highly toxic, and produced prompt reactive symptoms. Where the salt solution extracts could be administered in practically unlimited amounts, even small injections of the alcoholic suspensions into rats gave prompt reactions, characterized by labored breathing, lassitude, and marked congestion of the lungs. In one rat prompt anaphylaxis and death occurred, when .1 c.c. of the suspension was given ten days after an injection of .5 c.c. of the same suspension.

These alcoholic suspensions were also found to be thermostable, highly haemolitic, and non-specific, as the same results were obtained

with the blood of several different animals. Tests were conducted with pig, guinea pig, rat and human blood. Complete haemolysis was effected in all of these cases both with boiled and unboiled suspensions, when equal quantities of washed blood corpuscles and suspension were left in contact for thirty minutes. Salt solution extracts, on the other hand, produced very slight haemolysis, even when used in excessive amounts and allowed to act on the blood corpuscles over night. The red corpuscles of all the above animals were not dissolved by the alcoholic extracts of lungworms, if several volumes of normal pig or rat serum was added. Small quantities of serum inhibited haemolysis, but did not prevent it entirely.

Additional tests, conducted with three rats and one guinea pig, gave essentially the same results. Four injections of the alcoholic extract were given subcutaneously into the hind legs of each of these animals at five-day intervals. The amounts of suspensions given were .2, .3, .4, .5 c.c. respectively. Two days after the last injection the three rats were anaesthetized, and bled under aseptic conditions. The defibrinated blood was poured out into small stender dishes, two samples being prepared for each rat. Into one sample adult lungworms were placed, and into the other, three-weeks-old larvae that were grown in the laboratory. Check experiments were conducted at the same time with blood taken from untreated rats.

It was found that the adult lungworms were not affected, and remained alive in all of the samples of blood, even when left in them for four days. The larvae, however, were inactivated and apparently dead in the blood of the treated rats after ten hours. In the samples of normal blood the larvae remained alive for three days before they were discarded. When the serum of treated rats was tested against a clear alcoholic extract of lungworm powder, an abundant precipitate was thrown down, showing that the reaction occurring in the blood of the rat is in the nature of a precipitin formation. This same reaction was obtained with guinea-pig serum, which was taken from the above guinea pig, killed seven days after the last injection. In this last, only five-weeks-old lungworm larvae were used, and the same results were obtained as with the blood of the rats.

It is rather difficult to interpret the contradictory facts of the above experiments. The theory that the writer would advance is that the parasites themselves acquire immunity against the specific antibodies produced by the host. On this basis the negative results with the adult worms, and the positive results with the larvae, would be intelligible. Of course, it is possible that the larvae were not sufficiently developed to resist the normal bacteriocidal power of the blood; however, the fact that these same larvae persisted indefinitely in samples of blood from untreated rats, and successfully withstood immersion in the different poisons already mentioned, makes this last premise a doubtful one.

Experiments with ether extracts of lungworms. The ether extracts were prepared in exactly the same way as the others, except that

the lungworm powder was extracted with ether. For this purpose 50 c.c. of ether were used to every half gram of dry powder. After filtering and evaporating the ether, a light yellow, waxy product was obtained. Of this substance suspensions were made with various dilutions of salt solution. These suspensions, in turn, were used to perform the following series of tests.

In one experiment the residue from 50 c.c. of the ether extract was suspended in 10 c.c. of normal salt solution. Two centimeters of this suspension were injected into the peritoneum of a 220 gm. rat with no deleterious results. Another similar injection administered ten days later, failed to produce anaphylaxis or any other visible effect. Attempts to hemolyse pig, rat and human blood corpuscles with these suspensions were likewise without results. Thus 10 c.c. of the suspension failed to haemolyse .5 c.c. of the washed rat and human corpuscles when left in contact for ten hours, and only slight haemolysis was effected after two days. Neither the larvae nor worms were affected when placed into the blood of such treated rats. Additional experiments were discontinued, when it became evident that the toxic substance was not associated with these extracts.

Conclusion. The results of the above studies are by no means conclusive. However, it is believed that they do contribute some new points of view in a rather neglected field of parasitology. It is likewise regretted that due to lack of facilities, the above studies could not be carried to completion. The next logical procedure would be to feed larval nematodes to normal and injected pigs, and to compare the degree of infestation on posting. The experiments performed indicate strongly that some type of immunity might be developed in pigs by injecting alcoholic extracts of lungworm powder into them. They show, moreover, that lungworm larvae are infestive only after a period of development in the soil. Selective anti-bodies undoubtedly were elaborated which were specific for these larvae, if not for the adult lungworms. Therefore, young pigs might be rendered immune to infestation by the larvae if they were given injections of the alcoholic extracts of the worms.

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