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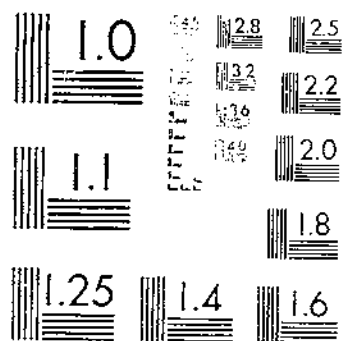
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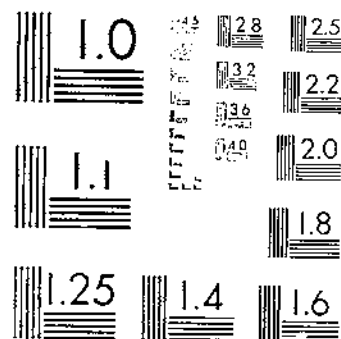
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FIELD AND LABORATORY STUDIES ON THE BEHAVIOR OF THE LARVAE OF THE SWINE
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NATIONAL BUREAU OF STANDARDS-1963-A

UNITED STATES DEPARTMENT OF AGRICULTURE
WASHINGTON, D.C.

FIELD AND LABORATORY STUDIES ON THE
BEHAVIOR OF THE LARVAE OF THE SWINE
KIDNEY WORM, *ST. PHANURUS DENTATUS*¹

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ECONOMIC IMPORTANCE OF KIDNEY WORMS

The swine kidney worm, *Stephanurus dentatus*, has long been recognized as a serious menace to the raising of hogs. The magnitude of the losses it occasions has been stressed not only in the United States but also in other countries, particularly Australia. Shealy and Sanders (10)² consider this nematode one of the most injurious internal parasites of swine in the southern part of the United States and the economic losses attributable to it nearly as large as those caused by ascarids. Nighbert and Connelly (2) estimate that losses due, for the most part, to kidney worms, incident to the dressing of carcasses, amount to \$80,000 annually in one packing house in Georgia. This estimate, however, does not take into account the stunted growth and unthriftiness shown by Schwartz and Price (8) to be a result of infestations with this parasite.

Losses from kidney worms, moreover, are not confined to swine alone, since agamic forms of this parasite are of common occurrence in the livers of cattle in certain parts of the South, according to these authors (9). The presence of these immature worms in cattle livers results, as does their presence in swine livers, in considerable losses owing to the condemnation or necessary trimming of affected parts, according to Schwartz (6, 7). In discussing the swine kidney worm

¹ This investigation was carried out in the field laboratory of the Zoological Division at Moultrie, Ga.
² Italic numbers in parentheses refer to Literature Cited, p. 17.

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he states that this parasite constitutes an actual and potential menace to the swine industry of the southern part of the United States.

Ross and Kauzal (5) report that in certain parts of Australia this nematode is not only one of the commonest parasites of swine, but is also one of great importance from an economic standpoint. The incidence of infection with the kidney worm in certain parts of Queensland is reported by these writers to be as high as 70 percent of all pigs killed. They add:

There is evidence that the number of infections is increasing, as is also the severity of infestations in individual pigs.

SCOPE OF THE INVESTIGATION AND THE APPARATUS USED

In view of the serious losses caused by kidney worms in regions where these parasites are prevalent, it is important that control measures be instituted. These measures must be based on the life history of the parasite, more particularly on its behavior during the preparasitic stages. The results of a study conducted at Moultrie, Ga., on the bionomics of the preparasitic stages, are presented in this bulletin.

In carrying out this investigation the following program was followed: (1) The distribution of larvae in hog pastures and the conditions under which larvae were present were studied by means of the Baermann apparatus; (2) the longevity of the infective larvae, under conditions similar to those in which they were found in nature, was determined; (3) the effects of various environmental conditions upon the eggs and larvae of this parasite were determined; (4) the reactions of the infective larvae to environmental stimuli were noted; (5) the migrations of the infective larvae on soil and on grass were studied both experimentally and under field conditions; and (6) the effect of growing crops on land infested with kidney worms was determined by means of the Baermann apparatus.

The Baermann apparatus used to isolate kidney-worm larvae from soil consisted of a large glass funnel 8 or 9 inches in diameter, with a short rubber tube attached to its stem, a clamp to close the lower end of this tube, and a copper sieve about 5½ inches in diameter, having a 1-mm mesh, which was placed inside the funnel. In order to prevent, so far as possible, small particles of soil from sifting through into the funnel, the sieve was lined with two thicknesses of unbleached muslin.

In routine procedure a battery of several funnels was used. The soil to be examined was thoroughly broken up, and a layer, 2 or 3 cm deep, was placed in the cloth-lined sieves. The sieves were then placed in the funnels, and water at a temperature of about 95° F. was poured over the rims of the funnels until the level of the water was above that of the soil. After a period of from 4 to 6 hours, approximately 1 liter of water was drawn off from each funnel, into a beaker, and allowed to settle for 1 hour. At the end of that time the supernatant fluid was drawn off and the residue examined for *Stephanurus dentatus* larvae. Corn husks and grass, after being cut up with scissors, were also examined for larvae in this manner.

DISTRIBUTION OF KIDNEY-WORM LARVAE ON HOG PASTURES

Soil samples were collected at weekly intervals from pastures where the hogs were known to be infested with kidney worms. These samples were then examined, in the manner previously described, for the presence of infective and preinfective larvae. Owing to the immense amount of work involved in searching for kidney-worm larvae among the hundreds of soil nematodes recovered in each Baermann isolation, quantitative determinations of the numbers of larvae in soil taken from these pastures were made in only two instances.

The greatest concentration of kidney-worm larvae was found on moist soil beneath piles of corn husks, corn cobs, and other debris occurring on the areas on which the hogs were fed. It was observed that the farmers, in the locality investigated, stored corn unhusked in order to protect it from insects. On most of the farms under observation the practice of feeding this unhusked corn on the ground at the same place each day resulted in an accumulation of corn husks and cobs to a depth of almost a foot in some instances. The soil beneath this debris, being protected from the drying action of the sun and wind, in many cases remained moist even over prolonged dry periods. In observing the activities of hogs on these farms, it was noted that the animals usually gathered at the feeding grounds, at irregular intervals during the day, to root through the piles of debris in search of feed. It was further observed that usually one or more of the animals urinated during this period, and that the urine, containing kidney-worm eggs, was soon covered with debris thrown about by other hogs. Under these conditions, animals frequently swallowed infective larvae which had hatched from eggs deposited there on previous days.

Kidney-worm larvae were commonly found also among moist pine needles which covered the ground in the small pine groves usually included in the hog pastures in the locality under observation. It was observed that the hogs often spent the hotter portion of each day in these pine groves, resting and rooting among the pine needles. Consequently the situation in these places was somewhat similar to that on the feeding grounds, although the concentration of larvae was much lighter than on the feeding grounds.

Infective and preinfective *Stephanurus* larvae were recovered in small numbers from moist soil taken from shaded paths, from areas near trees, and from the vicinity of shaded wallows.

No larvae were recovered from either shaded or unshaded dry soil. Furthermore, no larvae were found on moist soil that had been exposed to the sun for a period of more than a few hours.

Infective *Stephanurus* larvae were rarely found on areas covered with forage, and then only on low-lying areas where the soil was continually damp.

Contrary to the opinions of Ross and Kauzal (5) and others concerning the importance of wallows in the dissemination of the swine kidney worm, these areas were found to be of comparatively little importance in this respect. This conclusion seems justified in view of the fact that infective *Stephanurus* larvae were recovered from wallows in only 22 of 472 examinations. Moreover, in 8 cases where infested animals were seen to urinate in wallows, Baermann examinations of mud and water from the entire areas, made twice a week for periods of 6 weeks, gave consistently negative results.

In this connection a comparison was made in two instances of the number of larvae recovered by means of the Baermann apparatus from measured quantities of mud and water taken from wallows, with the number of larvae recovered from known amounts of soil and husks from feeding grounds some distance away from these wallows. In each determination, 3 weekly collections were made of 2 quarts of soil and husks from the feeding ground and 2 gallons of mud and water from the wallow which was to be examined. In the first test a total of 4,567 *Stephanurus* larvae were recovered from the soil and husks taken from the feeding ground. In contrast to this, no larvae were found in the mud and water from the wallow. Similar results were obtained from the second test, 1,498 larvae being found in material taken from the feeding ground and none in that from the wallow.

Since *Stephanurus* larvae were not recovered from mud and water taken from wallows in the examinations described above, a series of tests was carried out to determine the fate of kidney-worm eggs and larvae when exposed experimentally to conditions which exist in wallows. In each of 4 tests, kidney-worm eggs and larvae were placed in a shaded wallow in a vacant hog pasture, and from 3 to 6 weekly examinations were made of mud and water from the entire wallow, by means of the Baermann apparatus. No larvae were recovered in any of the examinations.

These findings indicate that kidney-worm larvae die rather quickly under conditions present in wallows and that the wallow is evidently of minor importance in the spread of the swine kidney worm in the locality where this investigation was conducted.

The results of this series of observations on the distribution of kidney-worm larvae in hog pastures indicate that most kidney-worm infections in hogs are evidently acquired in a comparatively small number of places, namely the feeding grounds and shaded areas in pine woods. This finding has a bearing on control measures.

LONGEVITY OF INFECTIVE KIDNEY-WORM LARVAE

Ross and Kauzal (5) reported that infective larvae of the swine kidney worm lived 154 days in a culture of soil and feces kept in the laboratory. They made no statement, however, as to the time such larvae would live under less favorable conditions outdoors. In order to obtain definite information on this point, the writer carried out two series of experiments. In the first series the larvae were kept in small experimental plots outdoors where variations in temperature occurred but where abundant moisture was supplied at all times. The second series was carried out under pasture conditions where the larvae were subjected to prevailing variations in temperature and moisture. The experiments in which the larvae were kept under favorable conditions as regards moisture, already briefly reported (11), are here reported in detail. Certain other experiments fully reported previously (11) dealt with the length of time larvae lived under experimental conditions when moisture was not supplied.

Kidney-worm larvae that had just reached the infective stage were placed on sterile soil in experimental plots kept under conditions approximating those commonly found in hog pastures of that locality. The experimental plots were laid out by first placing layers of gravel

in the bottoms of metal containers, such as deep pans or tubs, which were then filled with moist, sterile soil. This soil was kept moist by the addition of water through a pipe extending into the layer of gravel in the bottom of each container. Some of these containers were placed in the sun, and others in the shade. The soil in certain of the containers exposed to the sun was covered with a layer of corn husks, and in others a sod of carpet grass was grown. By the addition of large quantities of water the soil in some containers was made to resemble wallows.

The Baermann apparatus was used in examining the soil, daily examinations for larvae being made of soil samples from each plot. In order that the total number of larvae on any experimental area might not be materially reduced by the daily examinations, all the larvae recovered in the Baermann isolations were returned to the places from which they were taken. The next day soil for examination was taken from a different area in the experimental field. Whenever a sample of soil was negative for larvae, additional isolations were made from that plot until positive results were obtained or until it became apparent that all the larvae were dead.

Counts of the number of larvae recovered from the soil of the experimental areas were not made. However, in this connection it was observed that the number of larvae recovered in each isolation decreased progressively until, toward the end of the experiment, only one or two larvae were recovered in each isolation.

In table 1 are shown the periods during which kidney-worm larvae were recovered from the various types of experimental plots, together with the maximum and minimum air temperatures, and the number of cloudy days during those periods.³

TABLE 1.—Periods during which experimental plots contained live *Stephanurus* larvae when adequate moisture was supplied

LARVAE BENEATH CORN HUSKS AND OTHER DEBRIS, ON UNSHADED SOIL

Date of beginning of test	Experimental plots used	Air temperature during longest period that larvae survived		Cloudy days during longest period that larvae survived	Period during which live larvae were recovered		
		Maximum	Minimum		Plot 1	Plot 2	Plot 3
	Number	° F.	° F.	Number	Days	Days	Days
Apr. 4, 1930.....	3	91	49	48	50	60	65
July 26, 1930.....	3	94	62	49	58	64	42
Aug. 9, 1930.....	3	94	62	49	50	73	63
Oct. 8, 1930.....	3	86	30	32	41	55	68
Oct. 31, 1930.....	2	77	30	38	21	53	-----
Nov. 5, 1930.....	1	77	30	38	27	-----	-----
Dec. 12, 1930.....	2	66	32	8	21	25	-----
Jan. 24, 1931.....	1	77	33	31	50	-----	-----
Feb. 10, 1931.....	1	81	45	36	54	-----	-----
Apr. 16, 1931.....	2	91	49	46	75	76	-----

³ The meteorological data were obtained from the U.S. Department of Agriculture Weather Bureau station at Thomasville, Ga., 28 miles southwest of Moultrie.

TABLE 1.—Periods during which experimental plots contained live *Stephanurus* larvae when adequate moisture was supplied—Continued

LARVAE ON SHADED SOIL

Date of beginning of test	Experimental plots used	Air temperature during longest period that larvae survived		Cloudy days during longest period that larvae survived	Period during which live larvae were recovered		
		Maximum	Minimum		Plot 1	Plot 2	Plot 3
	Number	° F.	° F.	Number	Days	Days	Days
Sept. 20, 1929	2	88	44	21	35	28	
Oct. 31, 1929	2	86	24	24	32	29	
Nov. 15, 1929	1	84	24	21	35		
Dec. 10, 1929	1	78	26	8	9		
Jan. 4, 1930	1	77	37	10	10		
Jan. 9, 1930	3	75	32	9	9	12	12
Jan. 22, 1930	3	69	30	1	3	0	0
Feb. 1, 1930	3	74	36	12	12	16	9
Mar. 4, 1930	3	78	39	26	15	30	
Mar. 31, 1930	2	90	47	34	30	55	
Apr. 4, 1930	2	91	49	49	29	64	
Apr. 11, 1930	2	102	58	42	48	66	
July 26, 1930	2	94	62	32	39	43	
Aug. 9, 1930	2	94	62	49	55	63	
Oct. 14, 1930	1	89	35	18	30		
Oct. 31, 1930	1	77	33	16	19		
Nov. 5, 1930	2	77	37	19	15	20	
Nov. 12, 1930	2	73	30	13	15	15	
Nov. 18, 1930	2	73	30	6	8	7	
Nov. 28, 1930	2	58	30	0	1	1	
Dec. 3, 1930	1	70	32	11	15		
Dec. 12, 1930	1	66	32	2	11		
Dec. 24, 1930	1	63	32	8	0		
Jan. 15, 1931	1	50	29	0	1		
Jan. 27, 1931	1	74	30	9	15		
Jan. 30, 1931	1	74	30	6	12		
Mar. 16, 1931	1	84	36	27	38		
Apr. 29, 1931	1	100	41	25	60		

LARVAE ON SOIL, IN SUN

Oct. 31, 1929	3	86	40	0	0	0	
Nov. 15, 1929	2	84	24	0	0	1	
Dec. 10, 1929	2	74	46	1	0	0	
Dec. 11, 1929	2	78	26	7	8	2	
Jan. 4, 1930	2	77	20	12	14	15	
Do	2	55	37	0	0	0	
Jan. 9, 1930	2	75	28	8	10	0	
Do	2	75	54	0	0	0	
Feb. 1, 1930	1	74	43	11	16		
Do	1	72	43	0	7		
Mar. 31, 1930	2	91	47	26	10	37	
Do	2	74	47	0	0	1	
Apr. 11, 1930	3	84	59	3	0	4	
Do	2	82	68	2	2	0	
Aug. 9, 1930	1	94	62	16	14		
Do	1	94	72	2	3		

LARVAE ON SOIL AT BASE OF CARPET GRASS, IN SUN

Dec. 10, 1929	2	78	26	8	9	5	
Dec. 12, 1929	2	78	26	6	7	0	
Jan. 4, 1930	1	78	26	12	15		
Jan. 23, 1930	2	66	30	4	8	18	
Feb. 1, 1930	2	81	31	19	31	20	
Mar. 14, 1930	1	78	60	19	22		
Mar. 31, 1930	2	86	47	21	25	30	
Apr. 4, 1930	1	91	47	40	67		
Apr. 9, 1930	2	94	56	43	20	64	
Oct. 14, 1930	1	80	30	29	44		
Apr. 1, 1931	1	97	63	37	54		

† The larvae were placed on loose, porous soil.

TABLE 1.—Periods during which experimental plots contained live *Stephanurus* larvae when adequate moisture was supplied—ContinuedLARVAE OR EGGS IN MUD WALLOWS, IN SUN¹

Date of beginning of test	Experimental plots used	Air temperature during longest period that larvae survived		Cloudy days during longest period that larvae survived	Period during which live larvae were recovered		
		Maximum	Minimum		Plot 1	Plot 2	Plot 3
	Number	° F.	° F.	Number	Days	Days	Days
Nov. 20, 1929.....	* 1						
Dec. 10, 1929.....	* 1						
Jan. 4, 1930.....	3	75	37			0	2
Jan. 10, 1930.....	3	70	56		2	3	0
Jan. 20, 1930.....	* 2						
Jan. 25, 1930.....	2	66	47		0	2	
Feb. 7, 1930.....	* 3						
Mar. 1, 1930.....	2	60	31		1	4	
Mar. 15, 1930.....	1	74	60		2		
Apr. 6, 1930.....	1	80	49		3		
Apr. 10, 1930.....	2	81	55		0	1	
June 6, 1930 ²	2	83	67		2		0
Do.....	* 2						
June 7, 1930.....	* 1						
Aug. 9, 1930 ³	1	92	72		1		
Do.....	* 1						
Aug. 12, 1930.....	* 1						

¹ Determinations were made on larvae, except when eggs are specified.² Eggs recovered; no larvae.³ Preinfective larvae.⁴ No larvae recovered.

As is shown in table 1, when the larvae were protected from light and desiccation and, to a certain extent, from extreme variations in temperature by a covering of corn husks and other debris, the plots remained positive for larvae during a maximum of 76 days. Areas of moist, shaded soil remained positive during a somewhat shorter time, 66 days being the maximum time in which larvae were recovered under those conditions. In contrast to this, when larvae were placed on moist soil that was exposed to the sun, live larvae were seldom recovered after the first day. However, in one instance two larvae were recovered on the thirty-seventh day after the beginning of the experiment. In this case the soil was very loose and porous and may have afforded some protection to the larvae. It is to be noted also that in this test 26 of the 37 days were cloudy.

As is also shown in the table, when infective larvae were placed in small, artificially constructed wallows they apparently died rather quickly. In one instance larvae were recovered four days after the beginning of the experiment, after which time the plot remained negative. Preinfective larvae failed to reach the infective stage and eggs failed to hatch under these conditions.

As can also be seen from table 1, the periods during which the experimental plots described remained positive for larvae in winter were much shorter than the periods during which they remained positive in the warm months of the year. In every case, when the minimum temperatures dropped below freezing for a sufficient length of time to freeze the ground in the experimental plots, they became negative, the larvae apparently having been killed by the low temperatures.

These observations, which are in accord with the findings of Schwartz and Price (8) and of Ross and Kauzal (5), were confirmed by examinations of infested areas in hog pastures made by the writer during the winter months. As was found to be the case on the experimental plots, the larvae in hog pastures were readily killed by freezing.

In conjunction with these experiments, additional tests were made on the longevity of infective larvae under pasture conditions, in which the larvae were exposed to normal variations of temperature and moisture. These experiments were carried out during the spring and summer of 1931 on a carpet-grass pasture that had been unoccupied by hogs for more than a year. In each of five tests several thousand infective larvae were placed on areas of unshaded soil in this pasture and also among moist pine needles in an area of pine woods adjacent to the pasture. Weekly examinations were made, by means of the Baermann apparatus, of the areas on which the larvae had been placed and of the surrounding soil and debris. Table 2 shows the length of time the larvae lived under these conditions, together with the distances they migrated, the maximum and minimum air temperatures, the rainfall, and the number of cloudy days during each period.

TABLE 2.—Length of time infective *Stephanurus* larvae lived and the distance they migrated under field conditions, 1931

LARVAE ON UNSHADED SOIL OF A CARPET-GRASS PASTURE

Date of beginning of test	Experimental plots used	Air temperature during longest period that larvae survived		Rainfall during longest period that larvae survived	Cloudy days during longest period that larvae survived	Period during which live larvae were recovered		Greatest distance larvae migrated
		Maximum	Minimum			Plot 1	Plot 2	
	Number	°F.	°F.	Inches	Number	Days	Days	Inches
Feb. 27.....	1	74	34	2.13	18	24	—	12
Apr. 21.....	2	95	58	.62	9	14	20	6
May 13.....	2	97	86	.54	12	12	18	10
June 12.....	2	84	67	1.20	6	0	6	0
July 7.....	2	94	70	2.19	0	0	9	0

LARVAE AMONG MOIST PINE NEEDLES SHADED BY PINE TREES

May 14.....	2	97	68	1.00	23	18	29	0
June 12.....	1	103	69	3.40	12	24	—	0
July 7.....	1	79	69	6.03	17	20	—	0

Larvae were recovered from the pasture areas for periods of from 6 to 24 days, and from among pine needles for periods of from 18 to 29 days after the beginning of the experiments. These results indicate that when larvae of the swine kidney worm are exposed to the environmental conditions present in carpet-grass pastures and pine woods, they survive a shorter time than under the more favorable conditions of shade and abundant moisture.

EFFECTS OF VARIOUS ENVIRONMENTAL CONDITIONS UPON KIDNEY-WORM EGGS AND INFECTIVE LARVAE

In order to account for the short time which infective *Stephanurus* larvae lived in unprotected situations as compared with the time they lived under more favorable conditions, a series of experiments was carried out in the laboratory to determine the effect of the various environmental factors upon these larvae. The effects of sunlight, desiccation, and high temperature upon infective larvae are discussed in this order.

EFFECT OF SUNLIGHT ON INFECTIVE LARVAE

As was stated in discussing the results of the survey of hog pastures for larvae, no live kidney-worm larvae were recovered by the writer from either moist or dry soil that had been exposed to sunlight for more than a few hours. Furthermore, as shown in table 1, larvae lived a very short time on experimental plots exposed to the sun, even though abundant moisture was supplied at all times.

In order to account for this, the following experiment on the effect of sunlight upon kidney-worm larvae was carried out: 100 active infective larvae were placed in each of several 5-cc vials filled with water at a temperature of 77° F.; each vial was then suspended in a horizontal position in a 1-liter beaker by means of a wire stuck in the cork of the vial and looped over the rim of the beaker. Each beaker was then filled with water at a temperature of 77°, and the larvae in the vial-beaker set-ups were exposed to sunlight for varying periods over a white background, the water in the beakers being kept at a constant temperature throughout the period of exposure to sunlight. After this exposure, the larvae were kept in the dark for 24 hours at a temperature of from 68° to 77°. At the end of this time they were warmed to 98.6° to determine whether or not they were still alive. In each experiment an equal number of control larvae were exposed in the same manner to the diffuse light from a north window at 77° for the same length of time that the experimental larvae were exposed. All the control larvae survived under these conditions. In contrast to this, it was found in a series of experiments carried out during June and July 1931 that exposure to sunlight for a period of 1 hour on a clear day was sufficient to kill infective kidney-worm larvae. In agreement with the results obtained with the control larvae, an exposure of 9 hours to outdoor conditions on totally cloudy days was found insufficient to kill the larvae.

To test further the effect of sunlight upon these worms, infective larvae were placed on moist, hard-packed soil in Petri dishes. These dishes were then set in the sun. At each 1-minute interval a dish was removed to the laboratory, placed in water in a pan, with the level of the water below that of the soil in the Petri dish, and kept in the dark for 24 hours at a temperature of from 68° to 77° F. At the end of this time both the soil in the Petri dishes and the water in the pans were examined for live larvae.

In a series of 12 tests carried out on clear days during the months of June, July, and August, 15 minutes was the maximum period that larvae were found to survive under these conditions. These findings are supported by the results of field studies on the effect of sunlight upon the eggs and larvae of this parasite. On one of the farms under observation, a brood sow was confined in a farrowing pen from which all vegetation and debris had been removed. The sow habitually urinated on an area of soil close to the north side of a wooden gate. Because of the nature of the soil, this area was continually damp. Examinations were made of the soil twice a week for a period of 4 weeks, the length of time the sow was allowed to remain under these conditions. On three occasions, infective larvae were found on the soil shaded by the gate. In contrast to this, no larvae were recovered from the contaminated areas that were continually exposed to the sun.

These experiments and observations indicate that the larvae of the swine kidney worm are very susceptible to the action of sunlight

and are readily killed on moist soil that is exposed to sunlight. This accounts, in part at least, for the failure to find infective larvae on soil exposed to the sun, and also for the short periods during which larvae survived on unshaded experimental areas. (Table 1 and p. 7.)

EFFECT OF DESICCATION ON EGGS AND INFECTIVE LARVAE

Since, as stated in the results of the survey of kidney-worm-infested hog lots, kidney-worm larvae were never recovered from dry soil, a series of tests was performed to determine the effect of desiccation on the eggs and larvae of this parasite.

To determine the effect of desiccation on eggs, single drops of water, each containing approximately 300 eggs in the early stages of development, were placed in several places on a layer of dry pulverized soil indoors where the temperature varied from 68° to 77° F. At intervals of 15 minutes the soil containing the eggs from a single drop of water was removed, moistened, mixed with charcoal, and cultured for 10 days at room temperature (68° to 80.6°). The viability of the eggs in each test was determined by examinations of soil and charcoal cultures made from eggs not exposed to drying. The results of the 17 tests are given in table 3. These data show that kidney-worm eggs are killed by air-drying in from 7 to 10.75 hours.

TABLE 3.—Effects of air-drying on kidney-worm eggs and infective larvae

Test no.	Time of survival		Test no.	Time of survival		Test no.	Time of survival	
	Eggs	Larvae		Eggs	Larvae		Eggs	Larvae
1.....	Hours	Hours	7.....	Hours	Hours	13.....	Hours	Hours
2.....	10.75	2.50	8.....	8.00	3.50	14.....	10.00
3.....	9.25	3.25	9.....	10.50	2.00	15.....	9.00
4.....	10.00	3.00	10.....	10.50	3.25	16.....	10.25
5.....	8.50	2.75	11.....	10.75	2.50	17.....	9.75
6.....	8.25	3.50	12.....	10.25	1.75		10.50
	7.00	1.00		9.50	3.25			

In order to obtain additional information on this point a study was made, under field conditions, during the spring and summer months of 1931, of the effect of sunlight and desiccation on kidney-worm eggs that had been deposited by infested animals when urinating on dry, unshaded soil. Immediately after these animals had urinated, a portion of the soil on which the urine fell was collected and later taken to the laboratory, kept for varying periods, and then mixed with charcoal and incubated at room temperature. The location of the infested soil was carefully marked, and a second sample taken 24 hours later.

In a study of 62 such areas it was found that 24 hours after the urine had been deposited the soil was caked and dry. Eggs recovered by the salt-floatation technic from these areas appeared to be dead in all cases. When this soil was moistened, mixed with charcoal, and incubated at room temperature, no larvae were recovered from any of the 62 samples, even after an incubation period of 15 days. On the other hand, soil samples collected immediately after the urine was deposited yielded infective larvae, with but two exceptions, after 5 to 8 days' incubation.

Since it was found that *Stephanurus ova* are quickly killed by desiccation, a series of tests was made to determine the effect of air-drying

on the infective larvae of this parasite. In these tests infective larvae, in small drops of tap water, were placed on dry pulverized soil in the shade, where the temperature varied from 68° to 80.6° F. At intervals of 15 minutes, soil samples containing the larvae from a drop of water were removed and placed in the Baermann apparatus to determine whether or not the larvae were still alive. The results of the tests are shown in table 3.

During certain of these tests the larvae were still alive after 3 hours, even though the soil appeared to be dry. On microscopical examination it was found that each particle of soil was surrounded by a thin film of water and that it was only after this water had entirely evaporated that the larvae died. In view of this fact, much of the variation in the survival time, 1 to 3½ hours, can perhaps be attributed to the variation in the rate at which the water evaporated from the soil particles.

The results of these tests and observations of the effect of desiccation on kidney-worm eggs and larvae confirm the findings of Schwartz and Price (8) and of Ross and Kauzal (5) to the effect that these stages of the kidney worm are readily killed by drying. This susceptibility of the eggs and larvae to drying serves to explain the failure to find live kidney-worm eggs and larvae in places where they would be exposed to drying.

EFFECT OF HIGH TEMPERATURE ON INFECTIVE LARVAE

No attempt was made to determine the maximum temperature at which infective larvae will live on soil, but in laboratory tests it was found that the infective larvae are killed almost instantly in water at a temperature of from 123.8° to 124.7° F.

REACTION OF INFECTIVE KIDNEY-WORM LARVAE TO CERTAIN ENVIRONMENTAL STIMULI

REACTION TO LIGHT

In order to determine the reaction of infective *Stephanurus* larvae to light, the following tests were performed.

Several hundred active infective larvae were placed in water in a glass tube which was stoppered at both ends. One half of the tube, placed in a horizontal position, was then inserted in a wooden thermometer case, and the opening around the tube plugged with cotton, leaving half of the tube in constant darkness and the other half exposed to daylight. The tube and thermometer case were then placed in a horizontal position on a table by a window, out of direct sunlight, and the position of the larvae in the tube was determined 4 days later. When a small portion of the covered end of the tube was pulled out, a large number of larvae were seen to have gathered at a point in the tube which had been just inside the mouth of the thermometer case. The majority of these larvae had apparently migrated from both the covered and uncovered portions of the tube to what was probably the point of most favorable light intensity for them.

Further, to test the reaction of infective kidney-worm larvae to light, several hundred active larvae were placed in a Petri dish approximately one fourth full of water, half the dish being covered with black paper. The dish was placed on a table near a north window, and the larvae were distributed in the dish as evenly as possible. When the dish was examined 3 days later, there was, as before, a noticeable grouping of larvae just inside the darker area.

These observations indicate that the infective larvae of the swine kidney worm are, to a certain extent at least, negatively phototropic. This condition, it seems, would enable larvae on soil to escape the destructive effects of sunlight by migrating for short distances into protected situations. In this manner many larvae would be able to survive in relatively dark places, whereas they might be destroyed in lighter areas.

REACTION TO HEAT

When a hot needle was applied to the under side of a watch glass containing infective larvae in water it was found that those in the vicinity of the needle soon became active. When the process was continued for several minutes, the larvae for some distance away gradually migrated toward the point of greatest heat.

The reaction of infective kidney-worm larvae to heat was further tested by a method similar to that used by Mönnig (1). A large number of infective larvae were placed in a small glass tube of water which was stoppered with small corks. A copper wire of small diameter was wound several times around the middle of the tube, and the end of the wire was left projecting a distance of about 4 inches beyond the tube. The tube was placed in a horizontal position on the stage of a microscope, and heat was applied to the end of the wire by means of an alcohol lamp, care being taken not to apply the heat so rapidly that bubbles would be formed and currents set up in the water. As the heat penetrated along the column of water the larvae became quite active. As the water became warmer, many of the larvae for a distance of an inch or more on each side of the wire began to migrate slowly toward the warmest spot. In about an hour many of these larvae had collected near the wire at the point of greatest heat, which indicates that the larvae are positively thermotropic with respect to degrees of heat likely to be encountered under natural conditions.

MIGRATIONS OF INFECTIVE KIDNEY-WORM LARVAE

LATERAL MIGRATION ON MOIST SOIL

In order to determine whether infective *Stephanurus* larvae migrate extensively over the surface of the soil, the following tests were carried out. Infective larvae were placed on narrow strips of shaded soil each of which was bounded on all sides by pieces of dry tin, soldered at the corners and extending 2 inches above and 2 inches below the surface of the soil. Moisture was supplied, as previously described, by means of a pipe extending to the bottoms of the containers in which the tests were performed. The results of the tests are shown in table 4.

TABLE 4.—Extent of lateral migration of infective kidney-worm larvae

Test no.	Distance the larvae migrated	Time	Test no.	Distance the larvae migrated	Time	Test no.	Distance the larvae migrated	Time
	Inches	Days		Inches	Days		Inches	Days
1.....	8	9	3.....	4	12	5.....	2	12
2.....	3	5	4.....	2	30	6.....	3	61

As may be seen from table 4, very little lateral migration occurred under these conditions. In this connection it should be noted, however, that considerable lateral movement of larvae occurred (12 inches in one case) in three of five tests on the longevity of infective larvae under pasture conditions. (Table 2.) In these instances however, heavy rains had occurred during the time the tests were in progress, which accounts, perhaps, to a certain extent at least, for this movement.

VERTICAL MIGRATION THROUGH MOIST SOIL

In order to determine whether or not infective kidney-worm larvae are able to migrate upward through soil, three tests were performed, as follows: Several hundred infective larvae were placed in the bottoms of each of four small tin cans about $2\frac{1}{2}$ inches deep, which were then filled with moist sterile soil. Each can had a number of holes punched in the sides near the top to permit the entrance of moisture to the soil inside the can. These containers were then placed in a tub of sterile dirt with the surface of the soil in the cans slightly above that in the tub. Moisture was supplied to the soil in the tub in the manner previously described. Daily examinations for larvae were made over a period of 3 weeks, both the surface soil in each can and the dirt in the tub being examined. After each examination an amount of sterile soil equal to that used in the examination was returned to each can.

In these tests only a few larvae were found to have reached the surface of the soil in the cans at any time during the periods in which the examinations were made. In the first series of examinations 1 larva was recovered from the surface of the soil in a can on the fourteenth day, and 3 from another can on the fifteenth day; in the second series, 1 larva was found on the tenth day; and in the third series 4 larvae were recovered from the surface of the soil in a can on the eleventh day.

No larvae were recovered from the soil in the tubs, outside the cans, at any time; this indicates that the larvae did not migrate to the soil outside the cans.

Since some vertical migration of infective larvae occurred under experimental conditions, a series of examinations was made of kidney-worm-infested fields, both before and after plowing, to determine whether vertical movement of larvae through soil occurs under these conditions.

Shortly before the fields under observation were plowed, a number of infested spots were carefully located; after plowing, six weekly examinations were made, by means of the Baermann apparatus, of the soil directly over and for some distance around the spots where the larvae were buried by the plow; no larvae were recovered from the soil taken from any of the 95 areas examined. As was the case under experimental conditions, apparently the larvae showed very little tendency to move upward through soil, either by their own efforts or by the capillary movement of water contained in the soil. The depth to which a plow would bury larvae exceeds $2\frac{1}{2}$ inches; and either the percentage of larvae that could migrate upward a distance distinctly greater than $2\frac{1}{2}$ inches was very small, or the distance was too great for any larvae to migrate.

These results confirm the findings of Ross and Kauzal (5), to the effect that extensive vertical migration of kidney-worm larvae through

soil apparently does not occur. The results also indicate that plowing of infested land is a control measure for this parasite.

VERTICAL MIGRATION ON GRASS

Infective kidney-worm larvae were placed on soil in an experimental plot on which was a heavy sod of grass approximately 12 inches in height. Several times each day a number of stems of grass were cut close to the ground and divided into 1-inch lengths, measuring from the bottoms of the stems. The various 1-inch lengths of grass were then washed in separate dishes of warm water which was allowed to settle, following which the supernatant fluid was pipetted off and the residue examined for larvae. The various lengths of these samples of grass were also examined for larvae by means of the Baermann apparatus in the manner previously described.

Before or just after sunrise each morning, when the grass was wet with dew, large numbers of active kidney-worm larvae were recovered from the stems and blades of grass cut at that time, 2 inches being the maximum height at which larvae were found. After the dew had evaporated, however, only a few dead larvae were ever recovered from the grass. On the basis of the number of live larvae recovered before and after the dew had evaporated, and the results of experiments on the reaction of *Stephanurus* larvae to light, it may be concluded that as the grass blades dried the larvae migrated downward to the damp, shaded soil below. Some, however, were killed on the grass, either by the action of light from the sun or by desiccation, as the finding of dead larvae there proved.

The results of these experiments were confirmed on several occasions by the finding of live infective larvae on grass that had been cut from infested pastures before sunrise, whereas grass cut from the same areas after it had dried was always negative. Schwartz¹ found that kidney-worm larvae in charcoal-feces cultures kept in glass containers migrate up the sides of the containers and move in the films of moisture which settle on the walls of the glass vessels. He found that the larvae accumulate on the walls of the vessels in large clusters and wriggle very actively as long as moisture is present.

The upward migration of kidney-worm larvae on grass undoubtedly has a bearing on the dissemination of this parasite. In the locality in which this study was made, heavy dews fall almost every night, and hogs grazing early in the morning undoubtedly pick up many larvae which have migrated up the grass blades. This habit of the larvae, of ascending vegetation, may also explain the frequent occurrence of this parasite in the livers of cattle in this section of the United States.

EFFECT OF GROWING CROPS ON LAND INFESTED WITH KIDNEY-WORM LARVAE

In order to determine whether the growing of a crop on an infested field would destroy the kidney-worm larvae present there, and thus enable the farmer to place clean animals on this land without danger of infestation, the following tests were made.

Infested areas in fields were carefully located immediately before the fields were plowed. After the land had been plowed, weekly examinations of the surface soil plowed over the infested spots were made

¹ Personal communication.

throughout the period of growth of the crop. Furthermore, collections of soil from these areas were made within a few minutes after the land was plowed for the next planting. In this manner examinations were continued, over a period of 18 months, of infested fields on which crops of corn, cotton, peanuts, tobacco, melons, sweetpotatoes, oats, and sugarcane were being grown. No larvae were found at any time; this indicates that plowing infested land and growing a crop on it will free the land of kidney-worm larvae and render it suitable for the raising of pigs free from kidney-worm infestations, if the breeding stock is unparasitized and if extraneous sources of contamination can be avoided.

BASIS FOR CONTROL MEASURES

Suggestions for the control of the swine kidney worm have been made by Shealy and Sanders (10), Schwartz (6, 7), Roberts (4), Ross and Kauzal (5), and others, all of whom emphasized the importance of maintaining sanitary conditions in hog lots as an initial step in the control of this parasite. Roberts (4) and Ross and Kauzal (5) further state that if control is to be accomplished it is important to take advantage of the susceptibility of kidney-worm eggs and larvae to the destructive effects of sunlight and desiccation by raising young pigs on clean, dry, well-drained soil that receives the maximum of sunlight. These writers also emphasize the desirability of plowing and working land on which infested animals have been kept before placing clean animals on it.

On the basis of the experimental data reported in this bulletin and of the results obtained by other workers, the following recommendations are made which embody the essential requirements for control of swine kidney worms.

A field that is used for farrowing purposes should be well drained and should have had at least one crop grown on it since last being used by infested animals. In view of the susceptibility of the eggs and larvae of this parasite to the destructive effects of sunlight and desiccation, farrowing houses, feeding pens, and water barrels should be located close to the fence on an area of dry, well-drained, unshaded soil at least 30 feet wide. This strip of soil should be kept free from vegetation and debris of all kinds. By this arrangement the sows will have to cross the strip of bare soil after leaving the feeding pens, farrowing houses, or water barrels, before reaching the grazing crop used as a source of green feed for the sows and pigs. As a result much of the urine passed by the sows at such times will fall on the bare unshaded soil where the kidney-worm eggs will be killed by sunlight and desiccation. Likewise, in view of the fact that swine habitually make paths along fences, a strip of bare soil 5 feet wide that is kept free from debris and vegetation should extend along the other sides of the farrowing field.

It is desirable that the sows be fed and watered in a pen to which the pigs are not admitted at any time. This pen should be located on the wide strip of bare soil. The sows should remain in the pen for at least 1 hour at each feeding time in order that most of the urine and feces they pass at feeding time may be in a place inaccessible to the pigs.

As soon as the pigs are old enough to eat, a balanced diet should be provided for them in self-feeders located in a creep on the wide strip of bare soil previously mentioned.

After the pigs have been weaned and transferred, by hauling, to clean ground, the old farrowing field should be plowed and at least one crop grown on it before it is again used for clean animals. Nighbert and Connelly (8) also recommended such a procedure.

Additional suggestions for the protection of pigs from kidney worms, based on the life cycle of the parasite, have been given by Schwartz (7). Details of management are also under further investigation on farms in the vicinity of Moultrie, Ga.

SUMMARY

In a survey of kidney-worm-infested hog pastures in the vicinity of Moultrie, Ga., larvae of these worms were found almost entirely on moist, shaded soil where they were protected from light and desiccation. The greatest concentration of larvae was found to occur beneath piles of corn husks, cobs, and other debris, on areas where hogs were fed. Larvae were found less frequently among moist pine needles, on moist, shaded paths, and beneath grass on low-lying pasture areas. On the other hand, live larvae were never recovered even from moist soil when the soil had been exposed to sunlight for more than a few hours. Larvae were not recovered from dry soil at any time during this investigation.

Contrary to the findings of Ross and Kauzal in Australia, kidney-worm larvae were seldom recovered from wallows in the vicinity of Moultrie, Ga.

The maximum length of time infective larvae survived under favorable moisture conditions was found to be as follows: On experimental plots covered with corn husks and other debris, 76 days; on soil covered with growing grass, 67 days; on shaded areas, 66 days; on unshaded soil which was loose and porous, 37 days; in wallows, 4 days; on unshaded areas of bare packed soil, 3 days.

Under actual pasture conditions, live larvae were recovered from carpet-grass pastures for a maximum period of 24 days, and from pine needles in a pine grove for a maximum period of 29 days.

Kidney-worm larvae were found to be susceptible to low temperatures, being killed by freezing, both in experimental areas and under field conditions.

Infective kidney-worm larvae in water were killed by 1 hour's exposure to sunlight at 77° F. When exposed to sunlight on moist, smooth soil, infective larvae were killed in 15 minutes. These results were confirmed by the failure to find larvae on moist soil in hog lots exposed to sunlight.

Kidney-worm larvae and eggs were killed by desiccation on dry soil in the shade under experimental conditions, and evidently died under such conditions in the field.

Infective *Stephanurus* larvae were killed almost immediately by water at a temperature of from 123.8° to 124.7° F.

The results of experiments on the reaction of infective kidney-worm larvae to strong light suggest that these larvae avoid intense light, since they were found to migrate short distances away from strong light into light of slight intensity.

These larvae were also found to be positively thermotropic, being definitely attracted to moderately warm areas, into which they moved from colder areas.

Under experimental conditions infective larvae migrated laterally a maximum distance of 4 inches on moist soil. Under field conditions some lateral movement of larvae over soil was found to occur, 12 inches being the maximum distance recorded. Under experimental conditions a small number of larvae migrated vertically through $2\frac{1}{4}$ inches of moist soil. These findings are in agreement with those of Ross and Kauzal (5), who found that these larvae would migrate vertically only a short distance through soil. From a study of infested fields no evidence was found which would indicate that infective larvae are able to migrate to the surface of soil after being covered to usual plowing depths.

Vertical migration of infective larvae on grass to a distance of approximately 2 inches was found to occur, both under experimental and field conditions when the grass was wet with dew.

In a study of kidney-worm-infested fields, extending over a period of 18 months, it was found that growing a crop on an infested area will apparently free the fields of the larvae present there.

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