VERTICAL MIGRATION, DISTRIBUTION, AND SURVIVAL OF INFECTIVE HORSE STRONGYLE LARVAE DEVELOPING IN FECES BURIED IN DIFFERENT SOILS[1]

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INTRODUCTION

Quantitative experiments on vertical migration of infective horse strongyle larvae in soils of different types have been reported recently by the writer (*6*).[3] The investigation reported in this paper was undertaken to determine: (1) Whether infective larvae develop from horse strongyle eggs in fresh feces buried in soils of different types; (2) the degree of vertical migration of the larvae in different types of soil; (3) the quantitative relationship between the number of eggs buried and the number of infective larvae reaching the surface of the soils at certain intervals; and (4) the distribution and total number of infective larvae present in the feces and soil at the expiration of these intervals. Obviously, the last three objectives could be realized only if development of infective larvae occurred in the buried feces. The work was carried out at the Agricultural Research Center, Beltsville, Md., from August 26, 1936, to March 22, 1937.

PROCEDURE AND APPARATUS

For this investigation, sandy clay loam, coarse sand, fine sandy loam, and clay soils were obtained from areas not occupied by horses. For sterilization, soil of each of these four types was separately placed in a lidded, metal-lined box provided at the bottom with a coil of perforated pipe connected to a steam boiler. Steam under a maximum boiler pressure of 25 pounds was admitted for 1 hour into the sterilizer containing the soil. Thermometers inserted in the soil at various points indicated that it was heated to temperatures of 180° to 212° F.; these temperatures are definitely known to be lethal to strongyle eggs and larvae. When examined by means of the Baermann apparatus, samples of soil so treated did not contain living nematode larvae.

Four containers were used for each type of soil. Each container consisted of a watertight, galvanized sheet-metal box 12 inches square and 4 inches deep and a piece of galvanized furnace pipe 10 inches in diameter. The length of the pipe in individual containers exceeded by about 5 inches the desired depth of burial of the feces in the soil subsequently placed in each container. In placing soil in a container, the box was first filled to a depth of about 3½ inches. The pipe was placed centrally on the soil in the box, and its lower edge was pressed down about one-half inch into the soil. The pipe was then filled with soil to within an inch of its upper edge.

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[3] Italic numbers in parentheses refer to Literature Cited, p. 347.

Freshly passed horse feces of two separate lots, collected on different dates, were used for this investigation. Each lot of feces was passed by a single horse. The fecal balls of each lot were thoroughly broken up and thoroughly mixed. Four-g samples from various parts of the fecal mass comprising each lot were taken for egg counts. The egg-count method described by Stoll (*13*) was used. Two 115-g portions of feces of each of the two lots were cultured in glass containers at room temperature for about a week. The cultures were then examined for infective larvae, and the average number recovered from the two cultures of each lot was noted.

An hour or two after their collection, the feces were buried to the desired depth in the soil in the various containers as follows: A weighed quantity of the feces was placed at the bottom of a cylindrical, flat-bottomed wire basket, 3 inches in diameter. The basket was made of galvanized-wire screen having about 64 meshes to the square inch. The depth of the basket was 3 inches greater than the depth to which it was desired to bury the feces. The quantity of feces used filled approximately the bottom 2¾ inches of the basket. During transfer of the feces to the basket, a paper insert prevented contact of the feces with the upper part of the basket. A circular piece of screen about 2⅞ inches in diameter was placed within the basket on the upper surface of the feces. A central excavation about 4 inches in diameter was made in the soil in the cylindrical part of the container to receive the wire basket (fig. 1). It was placed centrally down into the excavation, its upper edge extending slightly above the surface level of the soil in the pipe. The portion of the basket unoccupied by feces and the space around the basket were filled with soil previously removed from the excavation. This soil was gently pressed into place and leveled with the rest of the soil in the pipe. A container filled with soil and having the buried wire basket containing the feces in position is illustrated in figure 1.

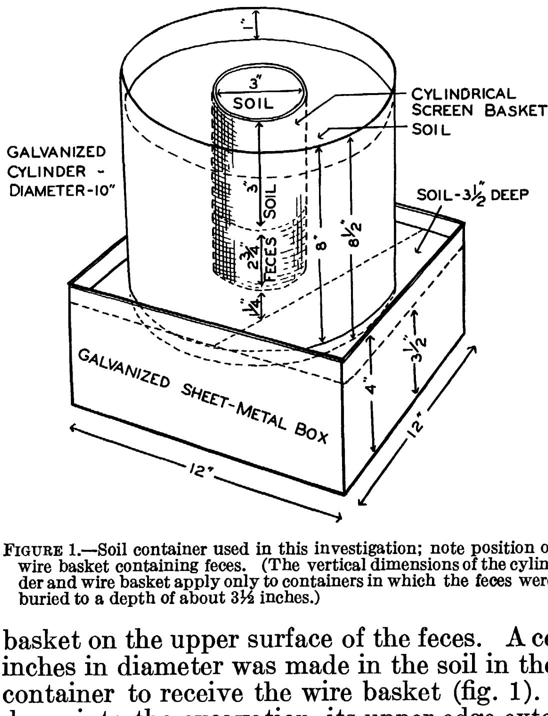

FIGURE 1.—Soil container used in this investigation; note position of wire basket containing feces. (The vertical dimensions of the cylinder and wire basket apply only to containers in which the feces were buried to a depth of about 3½ inches.)

Immediately after the soil was placed in the various containers it was thoroughly moistened with water. When the feces were buried a few days later, the soil was uniformly moist. Water was subsequently added to the surface of the soil in the pipe and in the box, in quantities and at intervals so regulated as to maintain the soil in a moist or damp condition at nearly all times throughout the investigation. On a few occasions the surface layer of soil in various containers became dry. Whenever this was noted, water was promptly added. During the investigation the soil containers were kept in a room of a large building having southern and eastern exposures. From August 26, 1936, to October 15, 1936, outdoor temperatures in the vicinity of the building ranged from a maximum of 91° F. to a minimum of 39°; the mean temperature was 67°. During the remaining period of the investigation, the building was heated. Although the room used was not provided with a radiator, the temperature in it at all times remained above the freezing point. From October 28, 1936, to March 22, 1937, a maximum and minimum recording thermometer was kept in the room. Readings taken at frequent intervals indicated that the usual range of temperature during this time was from about 45° to 65°; a minimum of 38° and a maximum of 76° were recorded.

At various intervals after the feces were buried, a thin layer of soil about one-sixteenth to one-eighth inch in depth was scraped up with a spoon from the entire surface of the soil in the cylindrical part of each container. This soil was transferred to a Petri dish and examined for infective larvae soon after its collection. At intervals the soil thus removed was replaced by moist sterilized soil of the same type so as to maintain approximately the original surface level. Since the object of the examinations was to compare the degree of upward migration of larvae from the feces to the surface of the various soils, the surface of the soil in the box surrounding the pipe was not examined. Subsequent references to surface soil indicate soil from the pipe only.

At the expiration of desired intervals after burial of the feces, the removal of surface soil for examination was discontinued. Shortly thereafter, the contents of eight of the containers, two of each type of soil, were examined according to the following procedure:

A brass tube 1 inch in diameter and of suitable length was inserted in the soil in the pipe at a locus just alongside the outer edge of the wire basket and, hence, about 2 inches from the center of the soil. The tube was forced downward until the bottom of the box was reached. The soil removed when the tube was withdrawn was examined for larvae. A second column of soil was similarly obtained from a locus close to the edge of the pipe and, hence, about 4½ inches from the center of the soil; this soil was also examined for larvae. The soil was then removed from the wire basket down to the level of the wire disk resting on the feces, various levels of the soil being separately removed and separately examined for larvae. The wire basket was then readily withdrawn, and the feces were removed from the basket and examined for larvae. All soil remaining in the pipe was removed down to the level of the surface of the soil in the box. This soil was placed on a large sheet of heavy paper and thoroughly pulverized and mixed. It was then rolled about on the paper for at least 30 minutes in the way that ores are sometimes mixed for sampling. Two samples of the soil, each 16 cubic inches in volume, were removed and examined for larvae. From the average number of larvae recovered in these two samples, the total larval content of the known volume of soil removed from the pipe was computed. The empty pipe was lifted from the soil in the box. By means of the brass tube, a column of soil was removed from the center of the box and a second sample was taken from one corner of the box; these were separately examined for larvae. The soil remaining in the box was removed and subjected to the process of mixing, sampling, and examination, as previously noted. The approximate number of larvae in this soil was computed.

The object of removing, by means of the brass tube, vertical columns of soil from the specified locations was to permit comparison of the number of larvae in equal volumes of soil near the feces and horizontally distant from it. Owing to compaction of the soil as the tube was forced downward and to other factors, the height of the column of soil removed by the tube was always less than the actual depth of soil in the containers. The quantity removed was also influenced by the type of soil, but in a given type, approximately the same quantity was withdrawn by the tube after insertion at comparable loci. Hence, comparisons of the number of larvae recovered in these approximately equal volumes of soil from different horizontal loci gave an index of the degree of lateral migration from the feces in a particular soil.

Examinations of the soil and feces for larvae were made by means of the Baermann isolation apparatus. The depth of soil placed on the screen of an individual Baermann apparatus did not exceed one-half inch. A piece of muslin or silk bolting cloth was placed on the screen to prevent descent of large particles of soil. The soil or feces remained in the Baermann apparatus a minimum of 48 hours before fluid was withdrawn from the bottom of the rubber tube. Successive quantities of the fluid, each about 10 cc in volume, were withdrawn into Syracuse watch glasses. As a rule, these successive withdrawals were continued until the fluid in the last one or two watch glasses examined contained no larvae. Occasionally, when relatively large numbers of larvae were present in the fluid, withdrawals were discontinued when the few larvae found in the contents of the last few watch-glass samples examined were a negligible percentage of the total number of larvae previously recovered. On recoveries of up to about 1,000 larvae, counts were direct. A ruled watch glass or a Scott counting slide was used in making these counts. When larger numbers of larvae were recovered, counts were made by means of a dilution method.

EXPERIMENTAL DATA

STRONGYLE EGG CONTENT OF THE FECES BURIED AND PERCENTAGE OF DEVELOPMENT IN CULTURES

Data on the strongyle egg content and the percentage of development in cultures of the two lots of horse feces used in this investigation are given in table 1. On the respective dates of collection, as shown in this table, 115 g of feces of lot 1 was buried in the soil in each of the four containers of sandy clay loam, and 115 g of feces of lot 2 was buried in the soil in each of the four containers of fine sandy loam, coarse sand, and clay.

As shown by the data of table 1, the strongyle egg content of 115 g of feces of lot 1 was almost twice that of the same quantity of feces of lot 2. No important difference in ability of the eggs to develop and produce infective larvae was indicated by the average percentage of development obtained in the cultures of feces of the two lots.

TABLE 1.—*Strongyle egg content of each of 2 lots of feces and percentage of development in cultures*

Lot No.	Date feces were collected	Egg counts	Eggs per gram of feces (average of all counts)	Eggs in 115 g of feces	Infective larvae from cultures of 115 g of feces (average of 2 cultures)	Development of eggs to infective larvae in cultures (average of 2 cultures)
		Number	*Number*	*Number*	*Number*	*Percent*
1	Aug. 26, 1936	4	1,802	207,230	77,140	37.2
2	Sept. 4, 1936	11	933	107,300	43,800	40.8

RECOVERY OF INFECTIVE LARVAE IN THE SURFACE LAYER OF SOILS

The data on recovery of infective larvae in the surface layer of the four types of soil (tables 2 and 3) show that infective larvae developed from strongyle eggs contained in horse feces buried 3½, 6½, 8½, and 10½ inches in fine sandy loam and coarse sand, and 3, 6, and 8 inches in sandy clay loam, but afford no evidence as to development in feces buried in clay soil, since no larvae were recovered in the series of the examinations of the surface layer of this soil. On the sixth day after burial of the feces to depths of 3 to 3½ inches, some larvae reached the surface of sandy clay loam, coarse sand, and fine sandy loam. The interval between the burial of the feces and the first recovery of infective larvae in the surface layer of these three types of soil tended to increase with increased depth of burial. An inverse relationship between the total number of larvae recovered from the surface of each of the three types of soil, and the depth of burial of the feces is shown by the data.

The rate of migration of infective larvae to the surface of sandy clay loam, coarse sand, and fine sandy loam, after burial of the feces to any of the four depths tested, showed marked irregularity. The rate tended to reach a maximum within about 6 weeks after burial of the feces and to decline irregularly thereafter.

Comparison of the total number of larvae reaching the surface layer of the different soils in about 3½ to 5½ months shows that the greatest number of larvae reached the surface of fine sandy loam soil; this was true for each of the four depths tested. The number of larvae recovered from the surface layer of the other soils during a similar period were of decreasing magnitude, according to the following sequence: Coarse sand, sandy clay loam, and clay. This sequence was applicable to any of the four depths of burial. In the interval mentioned, the surface of fine sandy loam and coarse sand yielded considerable numbers of larvae. The maximum number, equivalent to 8.9 percent of the buried eggs, was from the surface of fine sandy loam after burial of the feces 3½ inches. However, when the depth of burial of feces was 8½ or 10½ inches in either fine sandy loam or coarse sand, the larvae recovered from the surface during the period of observation represented less than 1 percent of the eggs buried. Insignificant numbers of larvae, representing negligible percentages of the eggs buried, reached the surface of sandy clay loam in 101 to 171 days after burial of the feces 3 to 8 inches.

TABLE 2.—*Infective larvae recovered in series of examinations of the surface layer of three types of soil following burial of fresh horse feces containing 107,300 strongyle eggs*

Soil type	Distance from surface of feces to surface of soil	Infective larvae recovered in surface soil removed at indicated number of days after burial of feces																			Total infective larvae recovered	Larvae recovered on surface in relation to eggs buried
		5	6	8	11	14	17	26	33	41	47	54	61	68	75	83	90	104	126	162		
	Inches	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
Fine sandy loam	3½	0	9	115	107	829	303	1, 561	350	1, 315	1, 065	781	806	689	137	374	224	628	269	(1)	9, 562	8. 9
	6½	0	0	10	11	112	43	68	458	747	275	245	582	311	62	239	82	242	165	46	3, 698	3. 5
	8½	0	0	1	1	65	28	76	240	139	71	34	50	38	1	36	0	89	46	5	920	0. 9
	10½	0	0	0	0	0	4	2	28	7	9	2	15	5	1	16	3	16	4	2	114	. 1
Coarse sand	3½	0	7	37	17	537	106	458	305	157	121	75	155	115	339	130	37	165	9	(1)	2, 770	2. 6
	6½	0	0	2	3	72	69	106	76	25	28	19	70	38	10	6	1	15	4	1	545	. 5
	8½	0	0	0	0	64	52	100	100	49	24	23	25	17	9	8	1	21	2	1	496	. 5
	10½	0	0	0	0	7	6	11	11	1	0	0	2	1	0	0	0	0	1	0	40	. 04
Clay	3½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	(1)	0	0
	6½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	0	0	0
	8½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	0	0	0
	10½	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	------	0	0	0	0

[1] As subsequently indicated, the soil was removed from these containers and examined previous to this time.

TABLE 3.—*Infective larvae recovered in a series of examinations of the surface layer of sandy clay loam following burial of fresh horse feces containing 207,230 strongyle eggs*

Distance from surface of feces to surface of soil (inches)	Infective larvae recovered in surface soil removed at indicated number of days after burial of feces																				Total infective larvae recovered	Larvae recovered on surface in relation to eggs buried
	5	6	8	10	13	16	23	31	38	45	52	59	66	75	80	87	94	101	140	171		
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Percent*
3	0	29	18	3	3	24	11	19	11	19	4	5	1	0	0	3	2	0	(1)	(1)	152	0.07
6	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	.0009
8	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	2	.0009
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[1] As subsequently indicated, this soil was removed from the container and examined on the one hundred and tenth day after burial of the feces.

Statistical analysis of the data in tables 2 and 3, made by Fisher's method (*5*), showed that depth of burial independent of soil type, and soil type independent of depth of burial, had a greater effect on number of larvae recovered than did sampling and random variation. No significant difference was found between the effects of soil type and depth of burial.

EFFECT OF SOIL TYPE AND DEPTH OF BURIAL ON DISTRIBUTION AND NUMBER OF LARVAE

The data on the distribution and number of larvae in the feces and soil in eight of the containers (table 4) show that many infective larvae developed from eggs in feces buried 3½ and 8½ inches[4] in clay soil. Development of larvae in feces buried in this soil was not evident from the data presented in table 2. Table 4 also shows that about 3½ to 6 months after the feces had been buried 3½ or 8½ inches in clay or 3 or 8 inches in sandy clay loam, comparatively large proportions of the larvae recovered occurred in the feces and in the soil immediately above the feces. In coarse sand and fine sandy loam, however, there was somewhat more uniform distribution of larvae with respect to the feces and the soil at all levels directly above it. This tendency toward uniformity of vertical distribution of the larvae was most marked in fine sandy loam after burial of the feces to a depth of 3½ inches. These facts are in harmony with the comparative degree of migration to the surface layer previously shown to occur in the respective soils from the respective depths (3 and 8 inches in sandy clay loam; 3½ and 8½ inches in the other soils).

TABLE 4.—*Number and distribution of infective larvae in feces and soil in 8 containers*

Soil type	Distance from surface of feces to surface of soil[1]	Interval from burial of feces to date of examination of soil and feces	Larvae recovered in columns of soil removed at indicated horizontal loci				Larvae in soil removed from indicated levels within wire basket							Larvae recovered from feces	Larvae[2] in remainder of soil in—		Total larvae recovered
			2 inches from center of cylinder	4½ inches from center of cylinder	Center of box	Corner of box	Surface to 1-inch level	1- to 2-inch level	2-inch level to surface of feces	Surface to 2-inch level	2- to 4-inch level	4- to 6-inch level	6-inch level to surface of feces		Cylinder	Box	
	In.	*Days*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Sandy clay loam	3	110	465	0	64	0	31	181	13,729	----	----	-----	-----	8,200	38,534	595	61,799
	8¼	173	413	1	191	0	----	----	------	0	0	8	4,500	6,575	16,850	1,458	29,996
Fine sandy loam	3¾	126	178	0	2	0	411	275	624	----	----	-----	-----	442	1,984	68	3,984
	8½	173	68	56	15	0	----	----	------	39	266	248	1,045	453	5,030	365	7,585
Coarse sand	3½	146	49	1	4	0	40	51	112	----	----	-----	-----	976	1,094	54	2,381
	8	180	69	5	0	0	----	----	------	3	20	23	91	216	436	0	863
Clay	3¾	152	5	0	0	0	0	0	6,035	----	----	-----	-----	4,120	1,874	14	12,048
	8½	187	53	0	6	0	----	----	------	0	0	17	143	1,436	1,524	248	3,427

[1] The distances shown were measured when the soil was excavated from the wire baskets and in most cases differ slightly from those given in tables 2 and 3, which were the original depths of burial. These differences were caused by settling of the soil and compaction of the feces.
[2] Computed from number of larvae recovered in samples.

[4] These and other depths of burial subsequently mentioned are the original depths of burial; as indicated in table 4, unimportant slight variations from these depths were noted when the soil was excavated from the containers.

Data on larvae recovered from two horizontal loci in the cylindrical part of the containers (table 4) show that in about 3½ to 6 months after burial of the feces to depths of 3 to 3½ and 8 to 8½ inches in the four types of soil, larvae were mainly concentrated in the soil horizontally near the location of the feces. However, in fine sandy loam, after burial of the feces to a depth of 8½ inches, a considerable degree of horizontal migration of the larvae occurred. The data suggest that the degree of horizontal migration was affected by the type of soil in which the larvae were present and that in a given soil it tended to increase with increased depth of burial.

The recovery of infective larvae in the soil in the eight boxes indicates a general tendency for a small proportion of the larvae developing in the feces to pass downward in the soil. Except in coarse sand, the proportion of larvae which migrated downward was larger when the feces were buried to the greater of the two depths. The larvae were evidently located mainly in the central part of the box since none were found in the soil removed from one corner of each box.

That the total number of larvae remaining in the fecal and soil content of the eight containers 3½ to 6 months after burial of the feces was influenced by the type of soil in which burial occurred is indicated by the data in table 4. Most striking are the comparatively small numbers of larvae recovered in coarse sand and the comparatively large numbers recovered in sandy clay loam. In each of the soils, except fine sandy loam, the number of larvae recovered after burial of the feces at a depth of 3 to 3½ inches was considerably greater than the number recovered when burial was at a depth of 8 to 8½ inches. An influence of depth of burial on development or survival, or both, is indicated. However, from 34 to 63 days intervened between examinations of the contents of the series of containers in which burial of the feces was 3 to 3½ inches and the series in which it was 8 to 8½ inches. It is possible, therefore, that the results may have been influenced to some degree by death of larvae during these intervals.

RELATION BETWEEN NUMBER OF INFECTIVE LARVAE RECOVERED AND NUMBER OF EGGS BURIED

Table 5 shows the relation between the total number of larvae recovered from two containers of each of the four types of soil and the total number of strongyle eggs in the feces buried in these containers.

The percentage relationship between total number of infective larvae recovered and eggs buried was greatest in sandy clay loam for both depths of burial. The total number of larvae recovered after burial of the feces 3 inches in this soil closely approached the average number obtained in two control cultures of feces of lot 1. For the other three soils, the percentage of eggs recovered as infective larvae decreased in magnitude according to the following sequence: Fine sandy loam, clay, and coarse sand. This sequence applies to both depths of burial. In all four types of soil, the total number of larvae recovered in relation to eggs buried was greater when burial of the feces was at the shallower of the two depths. This is in agreement with the statement previously made concerning the probability that increased depth of burial caused an increase in deleterious effect. Table 5 also shows that in clay or sandy clay loam the migration of infective larvae to the surface after burial of the feces at a depth of 3 to 3½ or 8 to 8½ inches was either entirely prevented or greatly inhibited. However, in fine sandy loam or coarse sand, a majority of all the larvae recovered in the indicated intervals after burial of the feces at a depth of 3½ inches had reached the surface layer.

TABLE 5.—*Relation between number of larvae recovered from 8 containers and number of eggs originally buried*

Soil type	Eggs buried	Distance from surface of feces to surface of soil [1]	Interval from burial of feces to final examination of surface soil	Larvae recovered from surface soil		Interval from burial of feces to removal and examination of feces and soil from containers	Larvae recovered from feces and soil upon removal from containers		Total larvae recovered	Proportion of larvae recovered to eggs buried
				Number [2]	*Percent* [3]		*Number* [4]	*Percent* [3]		
	Number	*Inches*	*Days*			*Days*			*Number*	*Percent*
Sandy clay loam	207, 230	3	101	152	0. 25	110	61, 799	99. 75	61, 951	29. 9
	207, 230	8¼	171	2	. 007	173	29, 996	99. 99	29, 998	14. 5
Fine sandy loam	107, 300	3¾	126	9, 562	70. 6	126	3, 984	29. 4	13, 546	12. 6
	107, 300	8½	162	920	10. 8	173	7, 585	89. 2	8, 505	7. 9
Coarse sand	107, 300	3½	126	2, 770	53. 8	146	2, 381	46. 2	5, 151	4. 8
	107, 300	8	162	496	36. 5	180	863	63. 5	1, 359	1. 3
Clay	107, 300	3¾	126	0	0	152	12, 048	100. 0	12, 048	11. 2
	107, 300	8½	162	0	0	187	3, 427	100. 0	3, 427	3. 2

[1] See footnote 1 of table 4.
[2] As shown in tables 2 and 3.
[3] Represents the percentage of the total number of larvae recovered.
[4] As shown in table 4.

DISCUSSION

Ackert (*1*) and Payne (*9*) have reported that eggs of the human hookworm (*Necator americanus*) hatched in feces buried to various depths beneath different soils and that there was subsequent migration of infective larvae to the soil surface. So far as has been determined by a review of the literature, the only similar study dealing with the effects of burial of feces in soil on the development of eggs of strongyle parasites of domestic animals has been reported by Spindler (*12*). He found that when eggs of *Oesophagostomum dentatum*, the common nodular worm of swine, were buried outdoors at various depths beneath the surface of sandy clay soil, they hatched and the larvae migrated to the surface.

That burial of feces containing horse strongyle eggs in soils likewise did not prevent development of infective larvae in the feces is amply shown by the data of the present paper. The data pertaining to vertical migration of infective horse strongyle larvae in the soils used in this investigation and the results of the writer's (*6*) earlier experiments, in which larvae already in the infective stage were buried in similar soils, agree in indicating a lack of migration to the surface in clay soil and an inverse relationship between depth of burial and number of larvae reaching the surface in other soils. A detailed comparison of the mathematical results of the two investigations is impracticable for obvious reasons. However, both investigations show that lighter soils, such as fine sandy loam, are most favorable for vertical migration of horse strongyle larvae.

The quantitative data supplied by this investigation are in part based on methods and procedures generally used in experimental helminthology. That these and other methods of this investigation, adopted as the most suitable available, do not yield wholly accurate results is recognized. Since the procedures used were carried out n as uniform a manner as possible, the mathematical values obtained are regarded as satisfactory for comparison of the effect of burial at the different depths in the different types of soil on the eggs and resultant larvae. The purpose of the quantitative procedure used was to provide an approximate basis for conclusions for which qualitative data would afford no basis.

The experimental results were obtained under intentionally restricted conditions as to variation in temperature and moisture.

Temperatures in the room where the soils were kept were never so low as freezing, nor excessively high. It appears that these conditions of temperature were more favorable for development of the eggs and migration of the larvae than might frequently be encountered under field conditions. Whether these conditions of temperature were favorable or unfavorable for survival of the larvae is less evident. Augustine (*3*) has studied the influence of temperature on the life span of infective larvae of the human hookworm (*Necator americanus*). Larvae on soil were exposed to temperatures of 0°, 16°, 20° to 31°, 35°, and 40° C.; the larvae kept at 16° survived longest. Payne (*10*) found that the activity of infective larvae of this species increased with increased temperatures up to 35° C. According to Augustine (*2*), environmental conditions, such as tropical temperatures which tend to increase the activity of mature hookworm larvae, will shorten their lives by the more rapid using up of the stored food material. The abundance of food granules has been considered by Payne (*10*) to be an index to the "physiological age" of the larvae. Some other investigators have reported that infective larvae of human hookworms survive longest at room temperature. Horse strongyle larvae are known to be unusually resistant to low temperatures. Alternate freezing and thawing has been found by Ober-Blöbaum (*7*) to be more quickly lethal to these larvae than continuous freezing. The range of temperatures optimum for the survival of infective horse strongyle larvae has not been experimentally determined, so far as the writer is aware.

During this investigation, the soils were kept moist at practically all times, a condition favorable for development of the eggs and larvae and doubtless more conducive to the migration of the infective larvae than would occur ordinarily in a similar period in the field. Whether such a more or less uniform moisture content of the soil was favorable to the survival of the larvae is difficult to determine from such experimental evidence as is available. Augustine (*3*) found the length of life of infective larvae of *Necator americanus* to be longer in moist soils than in water-covered soils, drying soils, or soils subjected to alternate drying and moistening. Payne (*8*) also found a high death rate of human hookworm larvae in waterlogged soils. So far as the writer is aware, the effect of moisture content on the length of life of horse strongyle larvae in soils has not been studied. These larvae may live at least 6 to 8 months in water, according to the reports of various investigators summarized by Enigk (*4*). Horse strongyle larvae, moreover, are very resistant to drying. A fraction of 1 percent of more than 1,000 larvae kept in a dry condition on a glass surface for 4¼ years were viable, according to Enigk (*4*). Raffensperger (*11*) reported that all larvae exposed to drying in an incubator at

24°–26° C. for 6 months were dead; about 10 percent survived exposure for 4 months and 2 days. Alternate drying and wetting reduces the survival of the larvae, according to Ober-Blöbaum (*7*). Taylor (*14*) states that stability of moisture content in the loose soil of tilled land favors the survival of larvae of sheep nematodes as compared with survival under conditions of rapid alternation of moisture and dryness. In the paper referred to, Taylor does not give the experimental basis for this statement.

It thus seems likely that, in this investigation, temperature and moisture were favorable for the development of eggs and larvae and were more favorable for migration of infective larvae than would ordinarily be the case under outdoor conditions. It is less likely that the experimental temperatures and degree of moisture were particularly favorable to survival of the larvae. Conditions similarly affecting survival of the larvae would occur in regions having relatively warm fall and winter seasons with frequent rainfall.

However, general interpretation of the results of this investigation in relation to the control of strongyle parasitism in horses would involve a priori judgments as to the probable effect of many factors not operative in the experiments but capable under field conditions of producing results differing from those obtained in the laboratory. From this investigation, and from another reported earlier by the writer (*6*), fundamental information on the behavior of buried horse strongyle eggs and larvae has been obtained, but the extent to which the laboratory results may be altered under actual field conditions can be determined only by experimental investigation under such conditions. Until such field tests have been completed and the practicability of the measure ascertained, plowing under of infested soils is not advocated for the control of horse strongyles.

SUMMARY AND CONCLUSIONS

Burial of freshly passed horse feces containing strongyle eggs in four types of soil did not prevent development of infective larvae in the feces.

Insignificant numbers of infective larvae, representing 0.0009 to 0.07 percent of the eggs buried, reached the surface of sandy clay loam in 101 to 171 days after burial of the feces to depths of 3, 6, and 8 inches. No infective larvae had reached the surface of this soil 171 days after burial of the feces to a depth of 10 inches or the surface of clay soil in 126 to 162 days after burial of the feces 3½ to 10½ inches. However, the deeper soil layers and the feces still constituted important sources of infective larvae about 4 to 6 months after burial of the feces to depths of 3 to 3½ and 8 to 8½ inches in these soils.

Infective larvae reaching the surface in 126 to 162 days after burial of the feces 3½ to 10½ inches in fine sandy loam and coarse sand represented 0.04 to 8.9 percent of the eggs buried. Less than 1 percent of the eggs buried were represented by infective larvae reaching the surface of these soils from depths of 8½ and 10½ inches. Fine sandy loam was the most favorable soil for vertical migration of the infective larvae. Some infective larvae reached the surface of fine sandy loam, coarse sand, and sandy clay loam on the sixth day after burial of the feces to a depth of 3 or 3½ inches. The ratio of infective larvae reaching the surface to eggs buried was inversely related to depth of burial in these three types of soil.

A considerable proportion of the larvae remained in the feces and the soil immediately above it 110 to 187 days after burial of the feces to depths of 3 to 3½ and 8 to 8½ inches in clay and sandy clay loam. More uniform distribution of the larvae with respect to the feces and the soil at various levels above it occurred in fine sandy loam and coarse sand. The degree of horizontal migration of larvae from the buried feces was, in general, not extensive; it apparently was affected by soil type and depth of burial. A general tendency for a small proportion of the larvae to pass downward in the various soils was shown. In three of the soils, this proportion was larger after burial of the feces to the greater of the two depths.

The total number of infective larvae reaching the surface in 101 to 152 days and the residuum of larvae in the feces and the soil 110 to 152 days after burial of feces 3 to 3½ inches in soils of the four types, accounted for 4.8 to 29.9 percent of the eggs buried. Of the eggs buried, 1.3 to 14.5 percent were represented by infective larvae reaching the surface in 162 to 171 days and remaining in the feces and soil 173 to 187 days after burial in the four types of soil to depths of 8 to 8½ inches. These percentages were highest for both depths when burial was in sandy clay loam, intermediate in clay or fine sandy loam, and lowest in coarse sand.

Statistical analysis of the data on larvae recovered from the surface of the soils showed that migration to the surface was significantly affected by soil type. This factor also appeared to influence the numbers of larvae persisting in the feces and deeper soil layers a few months after the feces were buried. Increased depth of burial in a given soil clearly reduced the degree of migration of infective larvae from the feces to the soil surface. Statistical analysis also showed that the effect of depth of burial, independent of soil type, was significant. Depth of burial also appeared to be a factor in determining the development or persistence of larvae in the feces and deeper soil layers. It is probable that the inverse relationship between depth of burial and amount of migration of larvae to the surface is in some degree related to the effect of depth of burial on larval development, or survival, or both. Deleterious effect on development of larvae and on survival of larvae cannot be differentiated by the data of this investigation.

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