

INVESTIGATIONS ON THE CONTROL OF HOOKWORM DISEASE. IX.

ON THE POSITION OF THE INFECTIVE HOOKWORM LARVAE IN THE SOIL.*

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(Received for publication December 13, 1921.)

While it is known that the soil is the natural habitat of the infective hookworm larvae, only a few observations have been made as to their activities and position in it. Baermann (1917, p. 622), in his studies of the conditions of development and existence of hookworm larvae in the soil, found them to be rather evenly distributed throughout the soil to a depth of 24 cm. He did not find them directly on the surface but close to it. In other experiments, where larvae were studied in soil overgrown with vegetation, he found hookworm larvae constantly in the soil, but never on the plants themselves. No information is given on the exact conditions existing at the time his observations were made. The data from a series of experiments performed by Dr. James E. Ackert, to study the conditions under which hookworm eggs develop and hatch, show that by far the greater number of the hookworm larvae which developed were in the upper half inch of the soil. The complete discussion of these experiments will appear in a later number of this series. Looss (1905, English translation given in Looss, 1911, pp. 421-422) observed mature *Ankylostoma* larvae on the surface of charcoal cultures when under a high temperature and in a moist atmosphere. He found formations resembling short fungal hyphae, welded together, which upon examination proved to be hookworm larvae. He further observed that the larvae were not present on the surface when it became drier, but upon addition of water would reappear in 20 to 30 seconds. Smith (1905, p. 192) records the same position of the hookworm larvae on the surface of feces, when exposed to the air for a few days under favorable conditions of moisture. The

* This paper is the ninth of the series on the hookworm investigations of the Department of Medical Zoology, School of Hygiene and Public Health, Johns Hopkins University, carried on with the cooperation of the International Health Board, Rockefeller Foundation.

Georgia State Board of Health (1910) makes the statement that during a rain, or when dew drops collect, hookworm larvae find their way into the water where they remain, if the water does not dry up, until they have an opportunity to penetrate the human body. And when the dew drops dry or the pool of water evaporates the larvae burrow into the moist earth, there remaining until a fresh downpour of rain brings them to the surface again. Dock and Bass (1910) in their book on hookworm disease say that the larvae may crawl up the leaves, stalks or fruit of vegetables. No statement is made of exact observations of larvae doing this. Schultz (1912) found that in cultures from which blades of grass, small sticks, or other objects project, upon which a film of water condenses, hookworm larvae leave the cultures, follow the water film and ascend to the most advantageous point for contact with an eventual host.

Early in the experiments performed in Trinidad, British West Indies, on the migration of hookworm larvae in the moist loam soils (Augustine, 1922) I found them on the surface extending from the larger and more prominent soil particles. The greater number appeared singly like the hairs on young plants, while others were massed together in flame-shaped formations. Upon close examination with a hand lens the larvae seemed to be in constant undulating movement which greatly increased when the slightest stimulus was applied. A portion of the soil upon which several larvae were extended was placed on a glass slide and treated with a few drops of water. Upon microscopical examination both sheathed and unsheathed larvae were found which left the soil and moved with great rapidity to the margins of the preparation. The bit of soil was then broken and spread over the slide. In it several cast-off hookworm sheaths were found. The larvae on the surface of the soil readily transferred themselves to most objects that touched them, but not to insects, such as ants, as well as small Arachnids which passed through and about the infested centers.

Larvae were found on the surface of the soil in all my experiments, as long as their numbers were sufficiently large for observation and the soil was kept moist. In an experiment to test the effect of direct sunlight on the migrations of hookworm larvae (Augustine, 1922, p. 165) they were found extended from the soil particles as long as the soil remained moist, even at a temperature of 101° F. and in direct sunlight. As the surface of the soil became drier they left their more prominent positions and were found in the deeper cracks and crevices, disappearing altogether if the drying continued. If water was

then added the hookworm larvae reappeared in a few seconds in their previous positions. This process was repeated several times a day with the same result, until their numbers became so reduced that the reactions could not be observed. It seems clear from this experiment that sunlight in itself has no effect upon the movements of the larvae, but influences them indirectly by raising the temperature and increasing the rate of evaporation.

In two experiments on the migration of hookworm larvae (Augustine, 1922, p. 168), where water covered the soil, hookworm larvae appeared extended from short sticks, pebbles and larger soil particles which protruded above the water. They were usually motionless, but the application of a slight stimulus, such as blowing over the surface or jarring the pan, would start them to intense activity, especially where they had formed themselves into petal-like clumps. Toothpicks were placed upright in the soil in the infestation centers. A few hours later the larvae were found one third of the distance up the toothpick, but only as far as the moisture had ascended.

During the study of the active migrations of hookworm larvae in grass-covered soil (Augustine, 1922 p. 165) observations were made to discover whether the larvae would climb upon the blades of grass and collect in drops of water, as has been suggested might happen in morning dew. With a hand lens larvae were found at the bases of the stems and on the soil within the centers of infestation. They were also found along the outer dead sheaths of the grass which were moist, but never on the green stalks and blades. The green stalks and blades were cut from and surrounding the infestation centers and washed well in water. This water was then centrifugated and the residue examined with the microscope. No hookworm larvae were found. This test was repeated several times with constantly negative results. The probable reason for the larvae not creeping up the green stalks and into drops of water collected in the axiles of the leaves is that there was no continuous film of moisture present as on the dead stalks and sticks. Their movements seem to be definitely limited by the extent of the moisture film.

In connection with a study carried on by Cort and Payne (1922, p. 149) to determine the sources of infestation with hookworm on a cacao estate an attempt was made to duplicate in the laboratory the conditions existing in this environment, to determine the activities of the infective hookworm larvae under such conditions. In a particular grove of this estate the subsoil was a fine, compact, yellow

clay. The surface soil contained an abundance of humus and was of a loose texture and dark. Scattered over much of the surface was a layer of decaying twigs and cacao leaves which during the rainy season were constantly moistened by intermittent showers. Here and there the surface of the yellow clay was exposed, and in other places the humus was free from the leaves and twigs. Soil pollution and infestation was found on all three types of surfaces. The soils, twigs and leaves taken for the laboratory experiment were selected from places widely separated from polluted areas. The yellow subsoil was first placed to a depth of $1\frac{1}{4}$ inches in a circular pan, 3 feet in diameter. Two thirds of its surface was then covered with the humus, and half of the humus area was carpeted with the leaves and twigs. At the center of each of these surfaces were placed approximately 1000 hookworm larvae, their positions being marked by circles of cord. On the following day a careful examination was made with a hand lens. Numerous larvae were found extended from the soil particles within the infestation centers on the yellow clay and humus soils. The larvae which had been placed upon the leaves were not confined within the center, but were found distributed within a radius of eight inches. They were found on the center margins and prominent veins of the leaves and on decaying bark of twigs, especially where the bark was broken and distinct edges were formed. It is probable that the greater extent of distribution of the larvae on the leaves was not due entirely to their active migration, but was aided by the washing of the water during the process of moistening; for while the water which was added was immediately absorbed by the two soils, it ran off the leaves, from one to another. Larvae were found within the infestation centers of the two soil surfaces at all times until their numbers became greatly depleted. The leaves lost their moisture quickly, and when dry the larvae disappeared to the deeper moisture places, returning to their former position at the next watering.

The position of the hookworm larvae, as found in these and other experiments, is significant since it makes easy the transfer of the larvae from the soil and other infested objects to the human foot.

SUMMARY.

1. Infective hookworm larvae under optimum conditions of moisture and temperature were found to remain on and within the upper surface of the soil.

2. They creep up pieces of wood, decaying vegetation and other objects only as far as the film of moisture extends.

3. Hookworm larvae were not found within the drops of water collected in the axile of the leaves of green plants nor upon the leaves themselves.

4. At centers of soil infestation, where the surface is covered with leaves or twigs, the infective hookworm larvae were found extended from the leaves or twigs when moist, but in the soil beneath when dry.

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