STUDIES ON HOOKWORM LARVAE

V. FURTHER OBSERVATIONS OF INFECTIVE LARVAE IN MIGRATION TOWARDS VEGETABLES

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In the report III (1954) of this series, I reported the experimental results of some investigations carried out during the summer season from July to August 1952-1953. Through these former investigations, I came to understand many points of the migration towards vegetables of the hookworm larvae. However it was unexpected that most of them were easily destroyed by the sun's rays after migration onto vegetables. Such frailness of the hookworm larvae, which climbed up the vegetables under the sunlight, may be a matter of course in their nature that the infective larvae of *Ancylostoma duodenale* perished at all within 24 hours at 40°C (Fujita and Mihara, 1951).

During the summer season, therefore, the hookworm larvae should be destroyed by the sunlight that was more intense than in any other season and the effect of the ultraviolet rays may be harmful to them. Even *Ascaris* ova, which have a strong resisting potentiality against various influences in the natural world, are comparatively shortlived under the scorching sunbeams.

Now I have thought "what will happen to infective larvae after migration onto vegetables under a mild climate of a year" or "would nothing be killed during the spring season?" and then I determined to pursue this question in spring from April to May 1955.

MATERIALS AND METHODS

The experiments were carried out in the same manner as in the former (*vid.* report III, 1954) and I chose again young radishes, *Raphanus sativus* L., as well as infective hookworm larvae, *Ancylostoma caninum* E., as materials. The researches were practised during late April to early May 1955 which is the mildest term of the year in Japan.

EXPERIMENTAL RESULTS

1-a) *Migration of inf. larvae in the daytime* (May 4, 1955): Approximately 4000 larvae were pipetted into the Petri dish so as to be uniformly

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placed on the surface of the sandy soil on which 23 young vegetables grew about 3-4 cm high, and then this Petri dish was kept outdoors for 8 hours from 10 a. m. to 6 p. m. At the end of the allotted time, all the vegetables were cut off near the root and the hookworm larvae that climbed up the vegetables were isolated and counted.

Table 1 shows the results. As shown in the table, the migration of the worms is not so active even under favorable condition, that is cloudy and no wind. Number of the worms that climbed up the vegetables is only 75/4000

Table 1. Migration of inf. hookworm larvae in the daytime (May 4, '55)

Vegetables	Larvae recovered from 23 veg.			Remarks
	Alive*	Dead	Total	
Leaves	0	0	0	
Leaf-stalks	0	0	0	Temp. 16°C**
Upper stems	1	0	1	R.H. 88%***
Middle „	2	0	2	Cloudy and windless all day. No dew.
Lower „	72	0	72	
Total	75	0	75/4000 (1.8%)	

* They are very active or slowly wiggling and even the motionless ones can move actively by mechanical stimulus.

** Temperature was measured at noon.

*** R.H.—Relative Humidity.

(1.8%) and most of them are taken off from the lower stems but not from the leaf-stalks or leaves of vegetables. During daytime, the average temperature was 16°C and this might be somewhat unfavorable for the worms to migrate towards vegetables.

1-b) *Migration of inf. larvae in the daytime* (May 7, 1955): In the same way, two groups of the young vegetables were dealt with as follows:

1st group (26 veg.) ← approximately 4000 larvae

2nd group (22 veg.) ← approximately 2000 larvae

Then these two groups of the vegetables were kept outdoors for 8 hours from 9 a. m. to 5 p. m. on a cloudy and somewhat breezy day.

Table 2 shows the results, indicating that the number of worms that climbed up the vegetables is only 3/4000 (0.08%) in one group and 5/2000 (0.25%) in another group of the vegetables. On hand of these results of the experiment, it was considered that the temperature during daytime was higher than in the former cases, while breezy weather seemed to be unfavorable for the worms to wander towards vegetables.

According to the above two experiments carried out on the migration of the infective larvae, it is obvious that the migration of the infective hookworm

Table 2. Migration of inf. hookworm larvae in a cloudy daytime (May 7, '55)

Vegetables	1st group			2nd group			Remarks
	Larvae recovered from vegetables						
	Alive	Dead	Total	Alive	Dead	Total	
Leaves	0	0	0	0	0	0	Temp. 18.5°C R.H. 94% Cloudy, and a somewhat breezy afternoon.
Leaf-stalks	0	0	0	0	0	0	
Upper stems	0	0	0	0	0	0	
Middle „	0	0	0	1	0	1	
Lower „	3	0	3	4	0	4	
Total	3	0	3/4000 (0.08%)	5	0	5/2000 (0.25%)	

larvae towards vegetables during daytime is not so active even under a mild climate of the year. Especially they are not found on the leaves of the vegetables after all.

2) *Migration of inf. larvae on a windy night* (May 10-11, 1955): In the same way, approximately 4000 larvae were pipetted near the roots of two groups of the vegetables, 3-4 cm high, and then they were kept outdoors for 8 hours from 10 p.m. to 6 a.m. to see how far the larvae would climb up the vegetables during a windy night.

1st group (24 veg.) ← approximately 4000 larvae

2nd group (24 veg.) ← approximately 4000 larvae

Early in the morning, 6 a.m., all the vegetables were cut off and the larvae were isolated from them and counted.

These experiments resulted in the following: According to Table 3 it is found that, even in the night, the larvae do not migrate so actively when it is

Table 3. Migration of inf. hookworm larvae on a fine and windy night (May 10-11, '55)

Vegetables	1st group*			2nd group**			Remarks
	Larvae recovered from vegetables						
	Alive	Dead	Total	Alive	Dead	Total	
Leaves	4	0	4	0	0	0	Temp. 20°C R.H. 74.5% Fine and windy all night.
Leaf-stalks	2	0	2	0	0	0	
Upper stems	5	0	5	0	0	0	
Middle „	9	0	9	9	0	9	
Lower „	56	0	56	36	0	36	
Total	76	0	76/4000 (1.8%)	45	0	45/4000 (1.1%)	

* A few dewdrops developed.

** No dewdrop developed.

Table 4. Death-rate of inf. hookworm larvae in a fine daytime (April 26, '55)

Vegetables	1st group			2nd group			Remarks
	Larvae recovered from vegetables						
	Alive	Dead	Total	Alive	Dead	Total	
Leaves	0	19	19	0	47	47	Temp. 17°C R.H. 83% Fine and breezy all day but some- what cloudy in the morning.
Leaf-stalks	0	7	7	0	4	4	
Upper stems	0	3	3	0	3	3	
Lower „	0	2	2	1	11	12	
Total	0	31/31 100%	31/5000 (0.62%)	1	65/66 98.5%	66/5000 (1.32%)	

Notes: The infective larvae survived on the soil surface are very active forming themselves into so-called petal-like projections.

windy and few or no dewdrops develop and the number of them that climbed up the vegetables is almost corresponding to that in a cloudy daytime.

3) *What happens to the larvae after migration?* (death-rate of inf. larvae after migration): As aforesaid in the introductory remarks, through the former investigations (report III) carried out during the summer season, it was pointed out that most of the infective larvae were killed by the sunbeams after migration onto vegetables while many of them could survive in a cloudy and windless day. Then I was thinking to myself "is nothing killed during spring season?" This question shall be solved by the following investigations.

At the beginning of the experiments, 5000 larvae were pipetted respectively around the roots of two groups of the vegetables and these were kept indoors all night to make the larvae migrate freely towards the vegetables.

1st group (19 veg.) ← approximately 5000 larvae

2nd group (24 veg.) ← approximately 5000 larvae

Table 5. Migration of inf. hookworm larvae after two days and nights (May 11-12, '55)

Vegetables	1st group*			2nd group*			Remarks
	Larvae recovered from every 25 vegetables						
	Alive	Dead	Total	Alive	Dead	Total	
Leaves	73	45	118	104	33	137	In the daytime, Temp. 24°C R.H. 72% At midnight, Temp. 21°C R.H. 84.5% All vegetables were wet with dew.
Leaf-stalks	21	9	30	30	4	34	
Upper stems	1	2	3	2	1	3	
Middle „	2	3	5	2	0	2	
Lower „	2	0	2	1	2	3	
Total	99/158 62.7%	59/158 37.3%	158/4000 (3.9%)	139/179 77.7%	40/179 22.3%	179/4000 (4.5%)	

* During daytime, 1st and 2nd groups of the vegetables were placed respectively under the direct sunlight and in a light shade.

In the following morning they were exposed to the open air until 6 p.m., and then vegetables were cut off and the worms were isolated from the every part of them and counted.

Table 4 indicates the results. Even in the spring season, the death-rate of the worms that climbed up the vegetables is so remarkable that one group of them comes up to 31/31 (100%) and the other to 65/66 (98.5%) respectively as shown in Table 4. Throughout the experiments it was fine, temperature 17°C, relative humidity 83% and breezy all day.

4) *Death-rate of inf. larvae after migration for two days and nights:* In the same manner as in the former, two groups of the vegetables, 3-4 cm high, were dealt with 4000 larvae as follows:

1st group (25 veg.) ← approximately 4000 larvae

2nd group (25 veg.) ← approximately 4000 larvae

These were kept outdoors for two days and nights. During daytime, the 1st

Table 6. Migration of inf. hookworm larvae in relation with development of dewdrops (May 9-10, '55)

Vegetables	1st group (Natural dewdrops)			2nd group (artificial dewdrops)			Remarks
	Larvae recovered from 20 vegetables						
	Alive	Dead	Total	Alive	Dead	Total	
Leaves	224	0	224	0	0	0	May 9 Temp. 20°C R.H. 79% May 10 Temp. 21°C R.H. 80%
Leaf-stalks	169	0	169	0	0	0	
Upper stems	26	0	26	1	0	1	
Middle „	9	0	9	4	0	4	
Lower „	42	0	42	33	0	33	
Total	470	0	470/4000 (11.8%)	38	0	38/4000 (0.9%)	

and the 2nd groups of the vegetables were placed respectively under the direct sunlight and in a light shade. Temperature 24°C, relative humidity 72% at noon of the first day and temperature 21°C, relative humidity 84.5% at midnight of the following day.

On the second day, 9 a.m., all the vegetables were cut off to recover the worms from them. The results are as indicated in Table 5. As indicated in Table 5, the larvae recovered from two groups of the vegetables are 158/4000 (3.9%) in the 1st and 179/4000 (4.5%) in the 2nd groups, while the death-rate of them comes up to 59/158 (37.3%) and 40/179 (22.3%) respectively and the other survivors are found from the vegetables without any depression of their own activities. So that it is considered that, in a field, some living larvae must be found on vegetables in company with dead ones because the larvae that remain on the surface of the soil will be able to climb repeatedly up the vegetables, on which some of the worms cling even after dying the previous day.

5) *Migration of inf. larvae towards vegetables with artificial dewdrops* (indoor experiments): Approximately 4000 larvae were carefully pipetted near the roots of two groups of 20 young vegetables, 3–4 cm high, and the 1st group of them was covered with a glass pot (1000 cc) to make dewdrops naturally develop on them and, on the other hand, on the leaves of the 2nd group of vegetables, numerous water drops were carefully placed through a fine pipette looking just like natural dewdrops.

1st group (20 veg.) ← approximately 4000 larvae
 ↑
 developing natural dewdrops
 2nd group (20 veg.) ← approximately 4000 larvae
 ↑
 developing artificial dewdrops

After 32 hours, each group of the vegetables was cut off and the hookworm larvae were isolated and counted.

According to Table 6, we can see a remarkable disparity between two groups of the vegetables in migration of the hookworm larvae, *viz.*, 470/4000 (11.8%) larvae are recovered from the 1st group of the vegetables, while only 38/4000 (0.9%) larvae are found from the other group developing the artificial dewdrops.

These results show that the water drops placed on the leaves just like natural dewdrops were not attractive at all for the hookworm larvae. They can actively migrate only under condition such as a suitable moisture hanging around.

DISCUSSION

The infective larvae migrated onto vegetables are closely connected with an oral or cutaneous infection of the hookworm. I had already studied, therefore, how they climb up and distribute on blades or stems of the vegetables during the summer season from July to August 1952–1953 (Harada, 1954) and further investigations were carried out on the same problems during the spring season from April to May 1955.

Through these investigations it was found that, also even in spring, the larvae that climbed up the vegetables during daytime were few in number, *viz.*, only 75/4000 (1.8%) of them climbed up 23 vegetables during daytime even under a good condition and they could not be found after all on the leaf-stalks or leaves of the vegetables. It was pointed out, therefore, that migration of the worms towards upper parts of the vegetables during daytime would be impossible because all the surface of the vegetables, especially the leaf-stalks or leaves of them, should be desiccated by the sunbeams regardless of seasons, summer or spring, and also the hookworm larvae could not creep out in the least when they were placed on such dry materials (Harada, 1953).

Even in the night through which the worms were able to migrate actively towards vegetables in the most former cases, they did not climb on the leaves of vegetables at all when it was windy and little or no dew was found on the

vegetables and, on the other hand, it was impossible for them to migrate into the water drops that were placed artificially on the leaves through a pipette just like natural dewdrops.

From these results of the experiments, it is obvious that the dewdrop (or water drop) itself is not attractive to the hookworm larvae but, at least, an atmospheric condition such as dewdrops naturally develop on all the surface of vegetables makes the worms actuate to migrate towards vegetables and, under these circumstances, the vegetables must be covered with so-called water-film through which the worms will be able to climb up the vegetables from the part near the root to the top of them, in other word, when both dewdrop and water-film are continuous, the larvae are able to creep into the former through the route of the latter. Cort, W. W. (1925) has noted also that they can even walk their way up on leaves or twigs when these are covered with a film of water and they were not, however, found migrating up on blades or grass, since on these the film of water is not continuous. I am a advocate of this view. The hookworm larvae climbing up the vegetables would not be able to walk their way upwards since just for a moment they have exhausted their own moisture they carry on the body surface. According to my observations, under these conditions, the larvae were able to climb with difficulty their way up on vegetables to a height of 1-2 cm from the surface of soil.

Though the experiments were practised by fixing especially upon the mildest climate of the year, the death-rate of the hookworm larvae after migration was as high as in the summer season. It was guessed, therefore, that the ultraviolet rays of the sunlight were fairly harmful for the activities of the hookworm larvae. However many of the worms would be able to remain alive for a long time in the soil without migration and migrate repeatedly towards vegetables every night, because the number of them found on vegetables was usually very few even under optimum conditions as compared with the number of larvae pipetted on the soil surface.

These were also proved experimentally. The larvae recovered from the vegetables after two days and nights were found both living and not and it was expected that the former were new migrants during last night. In a field, therefore, the hookworm larvae must be found migrating towards vegetables repeatedly while they survive on the ground, and some of them seem to get killed after migration by the sunbeams or winds during daytime.

SUMMARY

1. Studies were made on the migration towards vegetables of the infective hookworm larvae, *Ancylostoma caninum*, in the same way as in the report III (1954) of this series. The researches were carried out particularly in spring from April to May 1955.

2. The infective larvae that climbed up the vegetables were few in number

even in springtime in agreement with the experimental results obtained during midsummer, July to August.

3. Even under the mild climate, most of the infactive larvae perished after migration onto vegetables before the day passed, while many of them were able to survive on the surface of the soil and climb up again the vegetables during the following night. The migration towards vegetables, therefore, should be done repeatedly every night while they survive in the field. However, even at night, the larvae could not migrate so actively when it was windy and few or no dewdrops developed on the vegetables.

4. On their way up on the vegetables, the dewdrop (or water drop) itself was not attractive to the larvae. They were very willing to migrate only under condition such as a suitable moisture hanging around.

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