THE JOURNAL OF PARASITOLOGY
Vol. 52, No. 5, October 1966, p. 899–902

EARTHWORM TRANSMISSION OF *HETERAKIS* AND *HISTOMONAS* TO TURKEYS AND CHICKENS*

Everett E. Lund, Everett E. Wehr, and David J. Ellis
Beltsville Parasitological Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, USDA, Beltsville, Maryland

ABSTRACT: *Lumbricus terrestris*, *Allolobophora caliginosa*, and *Eisenia foetida* are vectors in the transmission of *Heterakis gallinarum* and *Histomonas* to chickens and turkeys. The infective stage for *Heterakis* was found to be the second-stage (first post-hatching stage) larvae. Earthworms from infected premises that were maintained 2 to 3 days in each of three or more changes of clean soil or 7 days on moist filter paper transmitted as many cecal worms and produced as many *Histomonas* infections as did worms of the same species and source fed directly from the contaminated soil. The worms' castings produced no infections. Subjecting earthworms to either 1% formalin or 1.5% nitric acid prevented the transmission of *Heterakis* and *Histomonas*. *Heterakis* eggs were never found in dissected earthworms or in washings of parts of earthworms. Living larvae emerged from the tissues into the coelomic cavities of dissected worms, and into the medium in which intact worms were immersed.

As early as 1907, Curtice reported fatal blackhead in turkeys produced by feeding earthworms from a poultry yard. He noted that turkeys fed earthworms from soil not traversed by poultry did not acquire the disease. He, of course, knew nothing of the role of the cecal worm, *Heterakis gallinarum* (Shrank, 1788) Madsen, 1949, in the transmission of blackhead. Curtice concluded, "The earthworms in this instance were probably carriers of infected soil and were not necessarily a second host of the parasite." Ackert (1917) infected chickens with *Heterakis* (probably *H. gallinarum*) by feeding them earthworms, but if *Histomonas* infections occurred, they did not come to his attention. He suggested that the heterakid eggs "might be carried on the slimy surface of the earthworm or in its engulfed food," but he did not rule out the possibility of other means of transmission. Madsen (1962) reported that some chickens acquired *Heterakis* and some developed blackhead after having been fed earthworms from pheasant yards. He commented as follows: "Before being fed to the chicks, the worms were thoroughly washed and allowed to empty their intestines as much as possible. Nevertheless a certain amount of contents must have remained, as the group contracted infections with *Heterakis gallinarum* and blackhead."

Received for publication 14 March 1966.

* Portions of this study were presented at the 38th Annual Meeting of the American Society of Parasitologists, November 1963. An abstract of that presentation has been published (Lund et al., 1963).

In our laboratories, the earthworm had been under suspicion for years as playing a part in the transmission of blackhead (Lund, 1960). The senior author later observed that turkeys given earthworms sometimes developed clinical blackhead 24 to 48 hr sooner than could be expected as a result of feeding them embryonated *Heterakis* eggs. This prompted a more thorough investigation of the role the earthworm actually plays in the transmission of the parasites.

MATERIALS AND METHODS

All earthworms used for the initial studies here reported were collected from a yard that had been used for several years exclusively for rearing ring-necked pheasants, *Phasianus colchicus* L. 1758. *Lumbricus terrestris* L. 1758 and *Allolobophora caliginosa* Rosa were used for three major experiments. Also, miscellaneous other studies were conducted using these two species and *Eisenia foetida* Savigny, collected at times from sources other than the pheasant yard.

The birds used in the three major studies were 5-week-old Small Beltsville White turkey poults that had been reared and maintained in wire-floored batteries or cages under conditions that precluded the possibility of accidental infections with the parasites being studied. Chickens used for the miscellaneous tests were New Hampshires of similar age, reared and maintained in a similar manner.

For the first test, approximately 200 *Allolobophora caliginosa* of comparable size were divided into three groups, one of which was returned to the same soil from the pheasant yard in which all worms had been brought to the laboratory. Worms of the second group were rinsed in tap water and transferred to clean soil that had been thoroughly air dried and restored to normal moisture content by sprinkling with water; these worms were trans-

ferred through three changes of this clean soil at intervals of approximately 2 days, the entire process lasting 7 days. Worms of the third group were maintained for 1 week between layers of moist filter paper, and given a few flakes of rolled oats each day to prevent shrinkage and to induce evacuation of the intestinal contents.

The worms surviving in each group after 7 days were washed in three changes of tap water and fed to poults. Each of 20 poults received three *Allolobophora* from the soil of the pheasant yard. Several worms failed to survive the three changes in clean soil, and therefore 13 poults received only two earthworms each. Fifteen poults each received two earthworms from the filter paper. The castings voided by these worms were suspended in physiological saline and given orally to 21 poults.

Two tests were subsequently run in which a single *Lumbricus terrestris* was given to each poult. In both tests, the procedures were similar to those described above except that, in the first, some of the worms were immersed 41 hr in a 1% formalin solution and washed 30 hr in tap water before they were fed to the poults. In the second test some of the worms were immersed 21.5 hr in 1.5% nitric acid solution and then washed 5 hr in tap water before being fed to the poults. These solutions are not harmful to *Heterakis* eggs but kill hatched larvae.

All birds on the above tests were observed twice daily, and microscopic examinations of their cecal droppings were made at appropriate intervals to detect the presence of *Histomonas*. Birds that died were necropsied as soon as practicable and their ceca and livers were examined for *Histomonas*. All *Heterakis* were collected. Most of the birds that survived were killed 14 to 23 days after the earthworms had been fed, but a few were not killed until the 30th to the 40th day to permit the cecal worms to mature and produce fertile eggs.

Miscellaneous other tests and observations were made to ascertain whether *Heterakis* and *Histomonas* could be established in chickens by feeding them earthworms from the pheasant yard, and to determine the infective stage of *Heterakis* in the earthworms. Because of the varied nature of these tests, information regarding them is presented briefly with their results.

RESULTS

The results of the three major tests are presented in Tables I, II, and III. Table I shows that 55% of the 20 poults that each received three *Allolobophora* which had been kept in the soil from the pheasant yard were found to have *Histomonas*. In comparison, 46% of the poults that had each received two *Allolobophora* which had been transferred through three changes of clean soil during a 7-day period were found to have *Histomonas*, and 47% of the birds that each received two *Allolobophora* kept 7 days on moist filter paper had the protozoon. There were no conspicuous differences among the numbers of *Heterakis* recovered from the birds of the three groups, either before appreciable loss of worms had been sustained as a result of histomoniasis (prior to the 16th day of infection) or subsequently. The castings alone transmitted neither parasite.

The results of the first test employing *Lumbricus terrestris* are shown in Table II. Six poults that each received one worm of this species collected in November developed blackhead and four (66.6%) of them died, even though the worms had been transferred through three changes of clean soil at intervals of 2 to 3 days and were thoroughly washed before being fed to the birds. Despite the extensive cecal involvement present, 499 *Heterakis* were recovered from the six poults, an average of 83 per bird. Earthworms collected from the same source the following spring and merely washed before being fed were less than one-half as effective (37.5%) in producing infections with *Histomonas* and mortality therefrom as were the former. Moreover, the birds yielded only about one-seventh

TABLE I. *Results when young turkeys were fed* Allolobophora caliginosa *from pheasant yard soil.*

Item	No. worms per bird	Per cent *Histomonas* infection	Per cent mortality	Day of death		Average no. cecal worms	
				Range	Average	To 16 days	All birds
20 turkeys fed vectors kept in soil from pheasant yard[1]	3	55	35	15–22	17	104	17
13 turkeys fed vectors passed through three changes of clean soil[1]	2	46	23	12–17	15	163	41
15 turkeys fed vectors kept 7 days on moist filter paper[1,2]	2	47	33	12–20	15	107	27

[1] All worms were washed in three changes of tap water immediately prior to feedings.
[2] Castings from these worms were fed to 21 poults and the results were negative.

TABLE II. *Results when young turkeys were each fed one* Lumbricus terrestris *procured at different seasons from pheasant yard soil.*

Item	Per cent *Histomonas* infection	Per cent mortality	Average day of death	Average no. cecal worms
6 turkeys fed vector after three changes of clean soil and three washes in tap water (fall)	100	66.7	15.3	83.2
8 turkeys fed vector washed three times in tap water (spring)	37.5	25	17	12.4
8 turkeys fed vector immersed 41 hr in 1% formalin and 30 hr in H_2O (spring)	0	0	–	0

as many heterakids as did poults that were fed worms collected the previous fall. Birds fed *Lumbricus* collected in the spring, treated 41 hr with 1% formalin and then washed 30 hr in tap water, acquired neither *Histomonas* nor *Heterakis*.

Table III presents the results of a similar study employing *Lumbricus*, some of which were placed in a 1.5% nitric acid solution. Again it may be seen that earthworms transferred through clean soil, this time 11 to 12 days, transmitted both *Heterakis* and *Histomonas* as readily as did the worms taken directly from the contaminated soil. Worms treated 21.5 hr with nitric acid solution and subsequently washed five hr in tap water transmitted neither parasite.

The various other studies conducted simultaneously with those already described gave the following results:

Allolobophora and *Lumbricus* from the pheasant yard and *Eisenia foetida* from a poultry yard transmitted both *Heterakis* and *Histomonas* when fed to 5-week-old chickens. In each instance transmission occurred regardless of whether or not the worms used were transferred through changes of clean soil. Treatment of worms with either 1% formalin or dilute nitric acid prevented transmission, just as it did with the worms fed to poults. In general, the chickens yielded fewer *Heterakis* at necropsy than did the turkeys fed similar worms from the same source.

Several *Lumbricus* and a few *Allolobophora* were dissected and various organs and groups of segments were washed thoroughly or soaked in physiological saline for periods up to 48 hr. In no instance were *Heterakis* eggs detected on microscopic examination of the washings, but nematode larvae, usually of several kinds, were invariably present. Frequently, some of these were second-stage larvae (first post-hatching stage) of *Heterakis*. Very few larvae were detected in the coelomic cavities of freshly opened worms, but they sometimes appeared there in great numbers when the opened worms were kept for 30 min or more in saline. Usually the larvae were most abundant in the intestinal region, but a few were found nearer the anterior end of the worms.

Earthworms collected from soil not known to have been traversed by poultry were also found to harbor nematode larvae, but usually neither *Heterakis* nor *Histomonas* infections developed in either chickens or turkeys fed such worms. Worms from soil several feet outside of poultry yards often transmitted both parasites, as occasionally did earthworms collected from areas visited by game birds.

TABLE III. *Results when young turkeys were each fed one* Lumbricus terrestris *from pheasant yard soil.*

Item	Per cent *Histomonas* infection	Per cent mortality	Average day of death	Average no. *Heterakis*
30 turkeys fed vector directly from soil of pheasant yard	26.7	10	17	7
22 turkeys fed vector kept 11 to 12 days in clean soil	27.3	13.6	15	15
40 turkeys fed vector immersed 21.5 hr in 1.5% HNO_3 and 5 hr in H_2O	0	0	–	0

DISCUSSION

From the above results it is apparent that earthworms of at least three genera can transmit *Heterakis* and *Histomonas* to turkeys and chickens by serving as true vectors for the cecal worm. While these studies do not preclude the possibility of these annelids carrying embryonated eggs of *Heterakis*, it is evident that the retention of unhatched eggs is only of secondary importance, otherwise such eggs would have been found in the washings of the earthworms or of their parts. Moreover, such eggs would have resisted the action of the 1% formalin solution and the 1.5% nitric acid solution (Tyzzer and Fabyan, 1922; Lund and Burtner, 1958), unless they had been made permeable to these reagents by some action of the earthworms. In that event, they should still have been detectable in the washings. Apparently few if any embryonated eggs or larvae remained viable in the castings.

Inasmuch as earthworms collected in November transmitted far more *Heterakis* than those of the same species collected from the same source in early spring, it appears that there was a substantial reduction in viable larvae during the period of hibernation of the earthworm. But it is also apparent that this loss was not complete. Also one must infer that during a summer's feeding an earthworm acquires a considerable accumulation of larvae.

Obviously, the earthworm plays an important role in the transmission of both *Heterakis* and *Histomonas* in several important ways. It provides a mode for the acquisition of *Heterakis* and also serves as a concentrating mechanism. Because the earthworm is capable of migrating both laterally and vertically in the soil, it provides a means for the spread of infective stages, and for the preservation of these stages during periods of inclement weather, such as cold and drought. To some extent the earthworm also shields the heterakid larvae from the ravages of fungal activity and from predation by other invertebrates in the soil.

With the recognition of the role played by earthworms as vectors of *Heterakis* we are now in a position to reevaluate much of the data accumulated during a period when the survival of the *Heterakis* egg seemed to provide the best explanation for many of the phenomena reported in the past. Not the least among these has been the question of why so many of the heterakids are in the same stage of development when recovered from naturally infected birds, and why so frequently the age of the worms indicated their acquisition within 24 to 48 hr after a rain. Earthworm transmission also provides a logical basis for the use of deep stone yards as a means of eliminating blackhead in turkey flocks. It seems also to explain why this disease is sometimes more prevalent in the fall than in the spring.

LITERATURE CITED

ACKERT, J. E. 1917. A means of transmitting the fowl nematode, *Heterakis papillosa*, Bloch. Science **46**: 394.

CURTICE, C. 1907. Further experiments in connection with the blackhead disease in turkeys. R. I. Agr. Exp. Sta. Bull. **124**: 67–105.

LUND, E. E. 1960. Factors influencing the survival of *Heterakis* and *Histomonas* on soil. J. Parasit. **46** (Suppl.): 38.

———, AND R. H. BURTNER, JR. 1958. Effect of four embryonation media on the embryonation and infectivity to chickens of *Histomonas*-bearing eggs of *Heterakis*. J. Parasit. **44**: 197–200.

———, E. E. WEHR, AND D. J. ELLIS. 1963. Role of earthworms in transmission of *Heterakis* and *Histomonas* to turkeys and chickens. J. Parasit. **49** (Suppl.): 50.

MADSEN, H. 1962. On the interaction between *Heterakis gallinarum*, *Ascaridia galli*, "blackhead" and the chicken. J. Helm. **36**: 107–142.

TYZZER, E. E., AND M. FABYAN. 1922. A further inquiry into the source of the virus in blackhead of turkeys, together with observations on the administration of ipecac and sulphur. J. Exp. Med. **35**: 791–812.