Effects of Birds Ingesting Heterodera rostochiensis Cysts on Viability of Eggs and Larvae[1]

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Abstract: Rate of passage through the digestive systems and effects of ingestion on viability of contents of cysts of *Heterodera rostochiensis* were determined in feeding trials with pigeons, thrushes, starlings, cowbirds, sparrows, and quails. Depending upon species of birds, 12-82% of the cysts ingested passed through the digestive system within 0.5 h. Pigeons required 6 h for complete evacuation. All other birds completely evacuated ingested cysts from their digestive systems within 3 h. Contents of cysts were nonviable if they were retained in the digestive system of starlings for more than 1.5 h, pigeons more than 1 h, or other birds more than 0.5 h. Cyst contents were nonviable if they remained in contact with excreta from cowbirds or quails for 4 h, thrushes for 96 h, or other species for 72 h after passage. Viability of contents of cysts was inversely related to exposure to excreta-filtrate concentration. Larvae failed to emerge from cysts that were exposed to a 25% concentration of excreta filtrate from starlings, 50% concentration from pigeons or thrushes, or 100% concentration of excreta filtrates from each of the other species. Cysts that were subjected to 44 C (avg. body temperature of cowbirds) for more than 3.5 h were nonviable. Cysts that passed through birds and collected with excrement on polyethylene or soil produced no infective larvae on potato. *Key Words:* Spread of nematodes, pigeons, thrushes, starlings, cowbirds, sparrows, quails.

Incipient spread of soil-borne plant pests by wildlife, particularly spread of a quarantined pest such as *Heterodera rostochiensis* Wollenweber, deserves consideration. The importance of farm equipment, harvest containers, and seeds in the spread of cyst-forming nematodes has been well documented (9), but that of animals, especially wildlife, has not.

A 1958 report (5) indicated that cysts of *H. rostochiensis* passed through the digestive systems of farm animals unharmed, but prolonged storage of cysts in dung rendered their contents nonviable. Also, cysts which passed through the digestive system of domestic chickens were not viable (Inagaki, personal communication). A few larvae emerged from cysts of *Heterodera glycines* Ichinohe that passed through swine, but such larvae failed to infect soybean (8). A report of an extensive effort to investigate the role of animals in the spread of *H. rostochiensis* indicated that animals with simple stomachs passed viable cysts, but ruminants and birds passed nonviable cysts (7).

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The recently-reported recovery of viable contents of cysts of *H. glycines* after they passed through blackbirds (3) and the discovery of viable contents of cysts of *H. rostochiensis* in guano imported from Peru to Japan (4) raised the possibility that these nematodes may be disseminated by birds. Consequently, a thorough investigation of the relation of birds to the spread of *H. rostochiensis* was undertaken. A preliminary report was given in 1974 (1).

MATERIALS AND METHODS

Birds used in this study were starling (*Sturnus vulgaris* L.), bobwhite [*Colinus virginianus* (L.)], domestic pigeon (*Columba livia* Gmelin), wood thrush [*Hylocichla mustelina* (Gmelin)], brown-headed cowbird [*Molothrus ater* (Boddaert)], and white-throated sparrow [*Zonotrichia albicollis* (Gmelin)]. All experiments were conducted in a 2.4 x 2.4 x 3.6-m cage covered with 0.6-cm mesh hardware cloth. Sufficient perching or cover (as appropriate) was provided for the birds. Each species was allowed about a week to adjust to the cage before experiments were begun. Eight to 12 specimens of each bird species were used for the experiments.

The birds were fed a 1:1 mixture of turkey starter mash (Agway, Inc., Syracuse, NY) and Big Red® brand dog meal (Agway, Inc., Syracuse, NY) to which a small quantity of cooking oil (Wesson oil®) was added to assure adherence of cysts. Depending on

the species of birds, 2,000-6,000 cysts were added to each 200 cm^3 of food. Grit and water were available to the birds at all times.

Infested food was placed in the cage for 30 min and then replaced, for the remainder of the experiment, with non-infested food. Excrement was collected on 60 x 60-cm polyethylene sheets at 30-min intervals for 4 h (1-h intervals for 6 h with pigeons) and after 24 h. Cysts were extracted from excrement by flotation in a 2,000-ml flask and collected on a 250-μm sieve. Viability of cysts was determined in hatching tests which consisted of a 1-week soak in water and a 3-week exposure to potato root diffusate at 22 C.

To test the viability of cyst contents after exposure to bird excrement, excrement was collected the first 4 h after birds ingested food and divided into four equal parts. One part was immediately processed for cysts and the other parts were processed 24, 48, and 72 h (96 h for thrushes) after collection. Attempts were then made to induce hatching of eggs in the cysts in each sample.

For testing the effects of excrement filtrates on viability of cyst contents, 30 gm of excrement from each bird species were mixed in 200 ml of distilled water and filtered after 30 min. Samples of 20 cysts each were exposed for 1 week in petri dishes to 10 ml of 20, 25, 33, 50, and 100% concentrations of excrement filtrates. Each treatment was replicated 5 times. After exposure to the filtrates, the cysts were rinsed in tap water and then exposed to potato root diffusate to induce hatching.

To study the effects of methods of collecting excrement on viability of cyst contents, excrement from each species was collected on polyethylene sheets or on sterile soil for the first 4 h after the birds ate infested food. The excrement was then divided into four equal parts and, after 6 weeks, was placed into each of four, 7.5-cm clay pots filled with sterile soil. Potatoes (*Solanum tuberosum* cv. 'Katahdin') were planted in each pot and, after 6 weeks, the roots were examined for cysts.

To elucidate the effects of temperature on viability, samples of 20 cysts each were placed in water at 44 C (avg. body temperature of cowbirds). The cysts were removed at 30-min intervals for 4 h and then placed in root diffusate to induce hatching.

RESULTS

Rate of passage: Cysts passed rapidly through the digestive system of all species of birds. The cysts were completely evacuated from the digestive systems of sparrows, starlings, thrushes, and cowbirds within 1.5, 2.0, 2.5, and 2.5 h, respectively, after the birds fed on infested food (Table 1). The larger species (quails and pigeons) required 3 and 6 h, respectively, for complete evacuation of cysts. In no case were cysts recovered from excrement passed 24 h after birds fed from infested food.

Effects of ingestion: Viability of cyst contents was inversely related to the time the cysts remained in the digestive system. Viable larvae emerged from cysts that passed from the digestive systems of all birds within 0.5 h (Table 2). However, no larvae emerged from cysts that remained in the digestive systems of starlings more than 1.5 h, pigeons more than 1 h, or other species more than 0.5 h.

Effects of excrement: Exposure to bird excrement lowered viability of cyst contents. No larvae emerged from cysts that were exposed to excrement of thrushes for 96 h, or other species for 72 h (at most) after passage (Table 3).

Effects of excrement filtrate: Viability of cysts was inversely related to concentration of excrement filtrates. Larval emergence

TABLE 1. Relation of time to passage of cysts of *Heterodera rostochiensis* through selected birds.

Time after feeding (h)	Accumulative percentages of cysts passed[a]					
	Sparrow	Starling	Thrush	Cowbird	Quail	Pigeon
0.5	64	43	82	25	12	26
1.0	86	85	92	69	12	35
1.5	100	96	96	76	34	—
2.0		100	96	88	78	70
2.5			100	100	78	—
3.0					100	83
4.0						87
5.0						96
6.0						100

[a]Average of three tests.

TABLE 2. Viability of cyst contents of *Heterodera rostochiensis* after passage through selected birds.

Time in birds (h)	No. of larvae hatched[y]					
	Sparrow	Starling	Thrush	Cowbird	Quail	Pigeon
0 (check)[z]	271	21	272	92	375	250
0.5	36	54	98	3	46	—
1.0	0	28	0	—	0	10
1.5	0	4	0	0	0	—
2.0	—	0	—	0	0	0
2.5	—	0	0	—	0	—
3.0	—	—	—	—	0	0

[y]Average of three tests. Eggs from cysts which had been in pigeon for 4, 5, or 6 h did not hatch.
[z]Cysts from infested food.

TABLE 3. Viability of cyst contents of *Heterodera rostochiensis* after passing through birds and remaining in excrement for different lengths of time.

Time in excrement (h)	No. of larvae hatched[x]					
	Sparrow	Starling	Thrush	Cowbird	Quail	Pigeon
0 (check)[y]	54	20	357	30	158	243
4	5	—[z]	59	0	0	64
24	42	—	—	0	—	142
48	12	—	68	0	0	13
72	0	0	47	0	—	0
96	—	—	0	—	0	—

[x]Average of three tests.
[y]Cysts from infested food.
[z]Indicates sample not taken.

from cysts that were exposed 1 week to a 25% concentration of excrement filtrate was significantly suppressed in each species except quail (Table 4). No larvae emerged from cysts that were exposed to a 25% concentration of excrement filtrate from starlings, a 50% concentration from thrushes or pigeons, or a 100% concentration from each of the other species.

Effects of collection media: The medium on which excrement was collected had no effect on viability of cysts that passed through birds. Potato was not infected when planted in soil containing cysts that

TABLE 4. Viability of cyst contents of *Heterodera rostochiensis* exposed one week to different concentrations of bird excrement filtrates.

Excrement filtrate (%)	No. of larvae hatched[x]					
	Sparrow	Starling	Thrush	Cowbird	Quail	Pigeon
0 (check)	611 a	9 a	1,011 a	1,314 a	515 a	922 a
20	226 b	8 a	636 ab	324 b	470 ab	581 ab
25	188 bc	0 b	314 bc	256 b	382 abc	194 bc
33	51 bc	0 b	20 c	137 b	297 abcd	5 c
50	97 bc	0 b	0 c	125 b	37 d	0 c
100	0 c	0 b	0 c	0 b	0 c	0 c

[x]Means followed by same letter do not differ ($P = 0.05$) according to Duncan's multiple range test; refers to means in each column. (Each sample included 20 cysts). Means of five replications.

had passed through either of the six species of birds and been collected in excrement on polyethylene or soil.

Effects of heat: Viability of cyst contents was inversely related to length of cyst exposure to 44 C (Table 5). Significantly fewer larvae hatched from cysts that were exposed to 44 C for more than 0.5 h. No larvae hatched from cysts exposed to 44 C for more than 3.5 h.

DISCUSSION

These studies indicate a low probability that birds can spread *H. rostochiensis* by ingesting cysts. Cysts pass through birds too rapidly for long-distance spread. In most cases, if cysts remain in the digestive system long enough (more than 0.5 h) to be transported to other areas, their contents are not viable when they are deposited by the birds. If cysts are deposited within 0.5 h after ingestion and remain in contact with excrement for at least 72 h, their contents are nonviable. Finally, behavioral patterns of birds undoubtedly influence their ability to disseminate *H. rostochiensis*. After feeding, birds rest up to 4 h in wooded or other protective areas (usually adjacent) near their feeding sites (S. E. Weeks, personal communication). Consequently, such behavior reduces the chances of their depositing viable cysts on cultivated land.

The difference between results with *H. rostochiensis* and those reported for *H. glycines* (3) is probably due to two reasons. First, the two nematode species undoubtedly have different biological requirements which could cause them to respond differently to passage through birds. Second, the experimental procedures used in the two studies differed considerably. I examined excrement collected on polyethylene at 0.5-h intervals for passage of cysts. Epps (3) examined excrement collected in water at 24-h intervals. He (personal communication) did not know if the viable cysts encountered in his study passed through the birds within 0.5 h or 24 h after ingestion. Furthermore, collection of excrement in water would dilute any substance in the excrement that was detrimental to cyst contents.

TABLE 5. Viability of cysts of *Heterodera rostochiensis* after exposure to 44 C for different lengths of time.

Length of Exposure (h)	No. of hatched larvae[a]
0 (check)	1,448 a
0.5	2,089 b
1.0	722 c
1.5	186 cd
2.0	291 cd
2.5	21 d
3.0	1 d
3.5	2 d
4.0	0 d

[a]Means of four replications. Means followed by unlike letters differ ($P = 0.05$) according to Duncan's multiple range test.

The numbers of cysts recovered in excrement was low in comparison to the apparent numbers of cysts ingested (pigeons ingested 6,000; 56 were recovered). In tests with quails and pigeons, numerous pieces of cyst wall in the excrement indicated that cysts were broken and the contents digested. The low recovery of cysts in excrement from the other birds indicates that cysts may be digested by other bird species. This low recovery reduces the probability that birds can spread *H. rostochiensis*.

Hatching responses were similar with cysts that passed through birds and with those that were subjected to 44 C. Body temperature measurements of cowbirds used in my experiments averaged 44 C. This finding is in line with other studies on body temperatures of other species of small birds (2, 6). A suppression in hatch of cysts of *H. rostochiensis* that were exposed to 45 C for 0.5 h or longer has also been reported (10). Thus, the low viability of the contents of cysts that pass through birds could be a function of relatively high body temperatures. Cysts of *H. rostochiensis* that were subjected to body temperatures of domestic chickens reacted similarly [Inagaki, personal communication).

Finally, there appears to be a substance or group of substances in bird excrement that is lethal to eggs and larvae of *H. rostochiensis*. This, together with the effects of high temperatures on cyst viability, rate of passage of cysts through birds, and behavior of birds, suggests that migratory birds may be of little significance in the spread of *H. rostochiensis*.

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