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Source: Journal of Wildlife Diseases, 13(1) : 43-46

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-13.1.43>

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## NEMATODES IN TERRESTRIAL GASTROPODS FROM CENTRAL MAINE<sup>1</sup>

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**Abstract:** Seventeen-hundred terrestrial gastropods collected in central Maine were examined for *Parelaphostrongylus tenuis* and other nematodes. Prevalence of nematodes was 4 to 7% in most gastropod species, but was 19% in *Pallifera* spp. *Cosmoceroides dukae* and *Rhabditis* spp. were the most common nematodes recovered. Only four *P. tenuis* larvae (prevalence 0.1%) were found. Foci important for transmission of *P. tenuis* in Maine have not yet been found.

### INTRODUCTION

The meningeal nematode (*Parelaphostrongylus tenuis*) is a common parasite of white-tailed deer (*Odocoileus virginianus*) in Maine but seldom causes clinical disease.<sup>5,3</sup> In moose (*Alces alces*), however, *P. tenuis* often causes neurological disorders.<sup>2</sup> Maine's moose population has been increasing rapidly since the late 1950's<sup>6</sup> and moose sickness (Cerebrospinal nematodiasis) has long been recognized as an important mortality factor.<sup>10,8</sup>

Because various terrestrial gastropods act as intermediate hosts for *P. tenuis*,<sup>12,3</sup> terrestrial gastropods were collected in central Maine during the summer and fall of 1971. These gastropods were artificially digested to determine if they contained parasitic nematodes, especially *P. tenuis*. Some results of the gastropod studies were reported in a previous paper;<sup>7</sup> this paper describes the examination for nematodes.

### MATERIALS AND METHODS

Gastropods were examined individually for nematodes by a modified Baermann technique.<sup>11</sup> They were placed in the pepsin digesting fluid and then cut and/

or crushed with a scalpel to facilitate entrance of the fluid. Gastropods were then digested at room temperature for at least eight hrs. Fluid remaining in the funnels was then drained into Syracuse watch glasses and examined microscopically for nematodes.

Since this study was concerned with animal parasitic nematodes, and it seemed unlikely that free living or plant parasitic forms would survive in acid-pepsin solution for eight hrs, dead larval nematodes were not included in the results. Living larval nematodes and all positively identified adult nematodes, dead or alive, were included.

Nematodes were transferred to a hanging drop slide with a micropipette. When large numbers of morphologically identical nematodes were recovered from a gastropod, they were counted but only a few were retained. Nematodes were killed and fixed in hot, 70% ethyl alcohol (ETOH) and transferred through a series of ETOH: glycerine: distilled water solutions into a drop of pure glycerine on a glass slide.<sup>13</sup> A cover slip was placed over the nematode and a three percent formalin solution was placed around the coverslip to help preserve the nematode.<sup>17</sup> Finally, the coverslip was sealed with Permount.

<sup>1</sup> A contribution of Federal Aid to Wildlife Restoration, Maine Pittman-Robertson Project W66R.

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Due to the transparent nature of the nematodes, ordinary transmitted bright field microscopy proved unsatisfactory for detailed observations and photomicrography purposes. The overall structure of adult nematodes could best be examined with transmitted oblique illumination using a Zeiss Photomicroscope; larval structure could best be observed with either phase contrast or transmitted polarized light microscopy.

### RESULTS

Seventeen-hundred terrestrial gastropods representing 25 genera were collected and digested. Seven percent of the gastropods digested yielded living larval or adult nematodes (Table 1). The 636

nematodes recovered included 260 *Cosmocercoides dukae*, 196 *Rhabditis* spp., 4 *P. tenuis*, one *Criconema* sp. and 175 unidentified larvae (Table 1).

*C. dukae* was found in seven gastropod genera (Table 1). Of 47 adult *C. dukae* recovered, at least 18 were females. Some had only a few larvae and the female was still alive; others contained many larvae and the female was nearly devoid of internal structure. Internal larvae were readily observable with phase contrast microscopy or transmitted polarized light, which indicated the anisotropic nature of the larvae. Adult *C. dukae* were found in *Anguispira*, *Arion*, *Deroceras*, *Discus*, *Striatura* and *Zonitoides* and the total number recovered per infected gastropod ranged from 1 to 123.

TABLE 1. Gastropods Collected and Numbers of Nematodes Recovered, Maine, 1971.

Genus	Gastropods			Nematodes Found				
	Number Collected	Number Infected	Percent Infected	<i>C.</i> <sup>1</sup> <i>dukae</i>	<i>Rhabditis</i>	Unknown	<i>P.</i> <sup>2</sup> <i>tenuis</i>	<i>Criconema</i>
<i>Pallifera</i>	225	43	19.1	3 <sup>3</sup>	99	74	4	0
<i>Discus</i>	412	27	6.5	212	3	29	0	1
<i>Zonitoides</i>	312	15	4.8	21	88	24	0	0
<i>Deroceras</i>	258	12	4.6	5	2	14	0	0
<i>Striatura</i>	179	9	5.0	4	2	11	0	0
<i>Arion</i>	149	6	4.0	3	0	6	0	0
<i>Helicodiscus</i>	55	3	5.4	0	0	5	0	0
<i>Anguispira</i>	2	1	50.0	12	0	0	0	0
<i>Euconulus</i>	14	1	7.1	0	2	0	0	0
<i>Oxyloma</i>	18	1	5.5	0	0	11	0	0
<i>Philomycus</i>	7	1	14.3	0	0	1	0	0
Others	69	0	0	0	0	0	0	0
Totals	1700	119	7.0	260	196	175	4	1

<sup>1</sup> *Cosmocercoides*

<sup>2</sup> *Parelaphostrongylus*

<sup>3</sup> Does not include larvae within bodies of adults.

*Rhabditis* spp. occurred in six gastropod genera, mainly *Pallifera* or *Zonitoides* (Table 1). All eight adult *Rhabditis* were recovered from *Pallifera* or *Zonitoides*; at least two adults were females. Numbers of *Rhabditis* found ranged from 1 to 31 per infected gastropod.

The four *P. tenuis* found were third-stage larvae. Two *Pallifera dorsalis* contained two meningeal nematodes each; both these slugs also contained living *Rhabditis* larvae.

One adult *Criconema* sp. was recovered from a *Discus cronkhitei*.

#### DISCUSSION

Nematodes are found only occasionally in gastropods according to Ogren.<sup>15</sup> Results of this study generally support Ogren's observations with one exception; 19% of all *Pallifera* collected were carrying nematodes.

*C. dukae* is a common parasite of terrestrial gastropods.<sup>15</sup> This nematode usually lives in the mucus between the shell and mantle in snails and is often visible from the outside in thin-shelled species.<sup>14</sup> Anderson<sup>1</sup> reported a high prevalence of *C. dukae* in *Deroceras*, *Discus* and *Zonitoides* in Ontario and that heavy infestations of individual gastropods occurred. The results of the current study confirmed that *C. dukae* also was a common parasite of terrestrial gastropods in Maine.

*Rhabditis* is a large genus of both free-living and semi-parasitic nematodes.<sup>8</sup> Rhabditid juveniles repeatedly have been found in terrestrial snails, but details of their life cycles are unknown.<sup>9</sup> Since the Rhabditid larvae found in this study were living after eight hrs in the digesting fluid it may indicate that they were semi-parasitic rather than free living forms.

Third stage *P. tenuis* were recovered from only 0.1% of the terrestrial gastropods collected. Parker<sup>10</sup> and Lankester and Anderson<sup>12</sup> also reported low prevalences in terrestrial gastropods collected randomly in Nova Scotia and Algonquin Park, Ontario respectively, however; Lankester and Anderson<sup>12</sup> did find a relatively high prevalence of *P. tenuis* on Navy Island, Ontario. This latter situa-

tion may have been attributable to the high deer and gastropod densities in a relatively small land area.

First stage larvae of *P. tenuis* leave infected deer in the mucous layer of fecal pellets.<sup>12</sup> The larvae must then enter a suitable terrestrial gastropod to become infective.<sup>12</sup> Terrestrial gastropods generally do not move great distances.<sup>4,18</sup> Since densities of deer are low in most of Maine,<sup>5</sup> pellet groups tend to be widely scattered. This may account for the low prevalence of *P. tenuis* in gastropods from central Maine. However, large concentrations of deer pellets are found in deer wintering areas in Maine. Since first stage *P. tenuis* can withstand freezing temperatures,<sup>12</sup> deer yards might be considered important foci for transmission. However, snow usually is present prior to the time deer enter the yards and remains until after they leave. Also, large numbers of deer do not frequent the yards except during the winter when transmission is unlikely to occur. Finally, most of the areas studied in central Maine were selected because they contained deer wintering yards, but the numbers of *P. tenuis* found were extremely low even though gastropods were collected from all major vegetation types present.

Lankester<sup>11</sup> felt that larvae of *P. tenuis* might be dispersed from deer pellet groups by rainfall or floods and gastropods might then become infected without feeding directly on the feces. This seems logical and suggests that deer winter concentration areas may be important dispersal sites for *P. tenuis* larvae even though the deer might not become infected there.

The hypothesis that specific areas might be extremely important to the transmission of *P. tenuis*, due to high gastropod populations combined with extensive deer use, was discussed by Lankester and Anderson.<sup>12</sup> They felt that low, damp forested areas were important foci for the transmission of *P. tenuis* throughout the summer. Damp, forested areas were extensively sampled during the present study, but no *P. tenuis* were found. If foci, important for *P. tenuis* transmission, exist in Maine, they have not yet been found.

### Acknowledgements

The authors are grateful to everyone who aided in completion of this study. Dr. Roy C. Anderson, Department of Zoology, University of Guelph, identified many of the nematodes and critically reviewed the manuscript. Dr. Marvin C. Meyer, Department of Zoology, University of Maine, suggested nematode mounting techniques. Joseph P. Schwarzmann, graduate student, gave photomicrographic assistance.

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Received for publication 8 July 1976