

## **OBSERVATIONS ON THE SEASONAL PREVALENCE, PATHOLOGY AND TRANSMISSION OF *Dracunculus insignis* (NEMATODA: DRACUNCULOIDEA) IN THE RACCOON (*Procyon lotor* (L.) IN ONTARIO 1**

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**OBSERVATIONS ON THE SEASONAL PREVALENCE,  
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*Dracunculus insignis*  
(NEMATODA: DRACUNCULOIDEA) IN THE RACCOON  
(*Procyon lotor* (L.) IN ONTARIO<sup>1</sup>**

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**Abstract:** Lesions due to *Dracunculus insignis* in the legs of raccoons (*Procyon lotor*) in southern Ontario occur seasonally as most larvigerous females emerge in the spring and early summer (April-June). The pathology of dracunculiasis in the raccoon is described and the transmission of the parasite in the wild is discussed with respect to seasonality and local agricultural practices. Crayfish, fishes and frogs (including tadpoles) were given infective third-stage larvae of *D. insignis* to test their suitability as paratenic hosts. Most of the larvae fed to adult *Rana pipiens* and *R. clamitans* were recovered from the somatic musculature. Larvae had increased in size and were highly infective to raccoons.

#### INTRODUCTION

Dracunculiasis in the raccoon (*Procyon lotor* (L.)) in southern Ontario is a seasonal phenomenon. Recently it has been demonstrated that the prepatent period for the guinea worm, *Dracunculus insignis* (Leidy, 1858) Chandler, 1942, in the raccoon is usually about one year (354 (309-410) days),<sup>8</sup> so one might assume that transmission is confined to a few weeks of the year.

Fyvie<sup>7</sup> suggested that wild mammals become infected by the accidental ingestion of infected copepods, as in the transmission of *D. medinensis* to man. However, it appears doubtful that the high prevalence of guinea worm in the raccoon in Ontario<sup>5</sup> could be sustained solely by this mode of transmission because of the improbability of raccoons drinking large enough quantities of water containing infected copepods.

The present paper describes the seasonal prevalence and pathology of *D.*

*insignis* in raccoon, the behavior of experimentally infected copepods, and the experimental involvement of various animals as possible paratenic hosts.

#### MATERIALS AND METHODS

Raccoons were obtained either from fur trappers or by live-trapping over a period of 9 months (April-December) during 1970 and 1971 from the Lake Huron district of Ontario and examined as previously described.<sup>4</sup> Raccoons were not available from January-March but it is assumed, from experimental evidence,<sup>8</sup> that female worms were migrating to the legs during these months.

Tissues for histopathology were preserved in 10% buffered formalin, sectioned at 5-8  $\mu$ m, and stained with hematoxylin and eosin. Calcified worms were preserved *in situ* in buffered formalin.

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*Cyclops vernalis* and *C. bicuspidatus thomasi* were infected with the first-stage larvae ( $L_1$ ) of *D. insignis*.<sup>6</sup> Mortality of  $L_1$  larvae during ingestion by the copepods was determined as follows: 30 larvae and 20 copepods were placed in each of 10 petri dishes (diam. 30 mm) containing pond water. As a control, 30 larvae were placed in a similar dish which did not contain copepods. The dishes were examined every 30 min for 3 h and the dead larvae removed.

Infected copepods contained, after appropriate incubation periods, third-stage ( $L_3$ ) infective larvae.<sup>6</sup> Copepods were killed in a pepsin digest<sup>6</sup> and the larvae administered orally, either by pipette, by stomach tube, or by natural ingestion, to: *Orchonectes propinquus* (crayfish); *Rana pipiens* and *R. clamitans* (frogs and tadpoles); juvenile *Catostomus commersonnii* (white sucker); juvenile *Salmo gairdnerii* (rainbow trout); *Notropis cornutus* (common shiner); *Ameiurus nebulosus* (brown bullhead); *Hyborhynchus notatus* (bluntnose minnow); *Noturus flavus* (stone cat) and *N. miurus* (madtom). These were collected near the University of Guelph campus or were obtained from University stocks. For convenience, only small fishes were selected. Prior to experimentation, potential hosts were examined randomly for natural infection with guinea worm but were negative for larvae.

Adult frogs were maintained at 22-23 C in glass aquaria containing pond water to a depth of 2 cm. Crayfish, tadpoles, and fish were similarly maintained in aquaria filled with pond water. A continuous supply of air was provided and the water changed frequently. Fish were fed commercially prepared fish food and aquatic vegetation was kept in the aquaria containing the tadpoles.

At necropsy the visceral organs, skeletal muscles and skin of the animals to which  $L_3$  larvae had been given were cut into small pieces, placed on disposable tissues (Kimwipes)<sup>7</sup> in pepsin solution (166 ml distilled water, 1.33 ml

HC1, 1.0 g powdered pepsin) and incubated for 3-4 h at 37 C in a Baermann apparatus. A 10 ml aliquot of the solution, drained from the bottom of each funnel, was examined for larvae. When larvae were recovered some were fixed in hot glycerin-alcohol, cleared in glycerin and measured, while others were administered to uninfected raccoons which had been born and maintained in captivity.<sup>6</sup>

## RESULTS

Larvigerous females were found in the legs of 25 of 34 (73.5%) and 35 of 42 (83.3%) raccoons examined in the spring (April-June) of 1970 and 1971, respectively. In contrast, larvigerous females were found in the legs of only 19 of 78 (24.4%) infected raccoons examined during the fall months of 1970 and 1971 (combined). In the fall samples, most of the female nematodes were found in the subcutaneous tissue of the thorax and abdomen. These females measured 20-50 mm long and were either immature or, if fertilized, contained only ova. In the spring of 1971, only 3 of 42 (7.1%) infected raccoons carried similar small female worms in the trunk region. Male worms were not found in two of these animals and the absence of a vaginal plug indicated that the females had not been fertilized; the third raccoon contained some larvigerous females in addition to smaller unfertilized worms.

The condition of female worms obtained from raccoons killed during April-June of 1970 and 1971 was recorded and increasing numbers of dead females were observed as the season progressed (Fig. 1). Worms containing larvae were found in the legs of only 1 of 10 raccoons examined in July of 1970 and 1971 (combined); no worms were found in the remaining nine animals. Dead worms may become calcified and calcified females were often found in the legs but only rarely in the trunk.

<sup>7</sup> Kimberly-Clark of Canada Ltd.

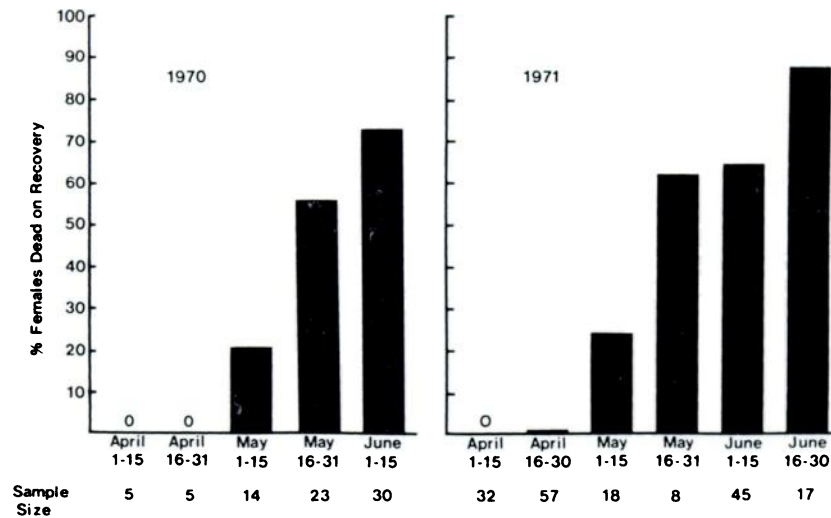


FIGURE 1. Percentage of female *D. insignis* dead on recovery from raccoons during April-June.

#### Clinical Signs

The behavior of an experimentally infected raccoon with a total of 25 larvigerous female worms (involving all legs) was compared with the behavior of two uninfected litter mates. The infected animal often was inactive for prolonged periods (30-60 min) and only moved slowly and with obvious difficulty and distress. It frequently raised a leg for short intervals and occasionally scratched and rubbed the skin overlying female worms. A road-killed raccoon found in June, 1975 had extensive swelling in all legs and skin damage due to rubbing. The animal harboured 22 larvigerous females and 5 calcified females. All of the raccoons obtained by trapping which were carrying naturally acquired guinea worm infections showed temporary discomfort during the formation of the open lesions. These lesions usually occurred on the upper or lateral surfaces of the carpal or tarsal regions. Initially the affected areas became swollen (Fig. 2) and inflamed. A small round ulcer appeared as the head of the female worm moved into the dermal layers. Pus formation occurred after the release of L<sub>1</sub> larvae. The lesions became enlarged and local loss

of hair was observed in animals which indulged in prolonged rubbing and/or scratching. Once the female worms were exhausted the raccoons rapidly recovered and showed no after effects.



FIGURE 2. Swollen hind foot (on left) of a raccoon experimentally infected with *D. insignis*.

#### Pathology

Gross and microscopic lesions were not observed in subcutaneous tissue adjacent to migrating male and female worms. Lesions were formed only when

larvigerous females reached the extremities. Grossly, the skin overlying the worms was erythematous, with local loss of hair. The denuded areas were haemorrhagic and had round, shallow ulcerations, approximately 25 mm in diameter. Ulcerations contained a purulent exudate and bacteria. Larvae could be recovered from the exudate taken from the ulcer. With the exception of the ulcers, the lesion appeared to be largely traumatic; presumably from scratching and rubbing by the host. In a few instances the only evidence of infection was a small round hole in the skin overlying the worms.

Microscopically, the affected tissues showed localized oedema with various amounts of fluid and, rarely, small haemorrhagic areas. The connective tissue capsule surrounding larvigerous females in the legs was interspersed with inflammatory cells. Sections of tissues surrounding near-emergent females showed inflammatory cells on superficial surfaces of muscles and, occasionally, degenerate muscle fibers. A purulent exudate, with a preponderance of eosinophils and neutrophils (Fig. 3),

perivascularitis and, less frequently, vasculitis were typical. The dermis surrounding the ulcers was thickened (Fig. 4) and characterized by acanthosis, hyperkeratosis and parakeratosis. The epidermis and dermis at the site of the ulcer were obliterated.

Recently dead, exhausted females were surrounded by granulation tissue. Neutrophils, mononuclear leucocytes and giant cells were the predominant cells present. Approximately 14-21 days after female worms had passed all their larvae the only visible signs of infection were yellow patches of necrotic debris in subcutaneous tissue. Eventually, small, superficial scars were the only evidence of infection (Fig. 5).

#### Intermediate Hosts and Larval Mortality

Experimentally infected copepods became lethargic and were generally found on the sides or bottom of the glass containers among organic debris. In contrast, uninfected copepods moved actively in mid-water. Copepods collected from ponds in areas frequented by infected raccoons and mink were negative for larvae.



FIGURE 3. Eosinophils surrounding female *D. insignis* located in the leg of a raccoon. This worm has moved out of tissue capsule. X 95. E, eosinophils, N, nematode.

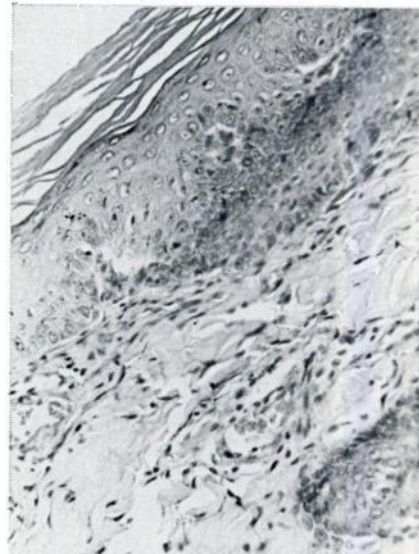


FIGURE 4. Thickened dermis in the leg of a raccoon infected with *D. insignis*. X 215



FIGURE 5. Hind feet of a raccoon showing ulcer 18 days after female *D. insignis* had ceased passing larvae.

In the dishes containing experimental copepods, 40-60% of the  $L_1$  larvae were found dead over the 3 h interval. Many dead larvae showed evidence of mechanical damage. All but three larvae in the control dish were alive after 3 h. When copepods were examined 7 days after exposure most larvae were alive and developing, with only a few dead  $L_1$  larvae. Infected copepods contained 1 to 23 larvae; more than 15 was rare and 4 to 5 was usual.

#### Attempted Involvement of Potential Paratenic Hosts

No larvae were recovered from 2 crayfish each given 20  $L_1$  larvae and examined the following day, nor from 6 crayfish each given 10  $L_1$  larvae and examined after 5 days. Ten other crayfish

taken from the same area were negative for larvae.

No larvae were recovered from 45 tadpoles collected from four stagnant ponds abounding with *R. pipiens* and *R. clamitans*, although infected raccoons had been caught in the vicinity.

A few larvae were recovered from 2 tadpoles given  $L_1$  larvae (Table 1), but it is difficult to administer larvae orally to tadpoles as they tend to regurgitate. Five tadpoles were given  $L_1$  larvae in a drop of water and examined at necropsy 21 days later. Four were not infected. The fifth contained 10  $L_1$  larvae but this was, presumably, a natural infection.

Many  $L_1$  larvae were recovered from 4 adult frogs given  $L_1$  larvae (Table 1). The larvae were not encysted and moved about freely in various tissues. An initial observation showed the distribution of larvae was: 3 (3.2%) larvae in the body cavity; 10 (10.6%) in the wall of the digestive tract; 81 (86.2%) in the skeletal musculature. A more detailed study of the location of larvae in two additional frogs revealed that almost 50% of the larvae recovered from the frog killed 8 days after infection were in the wall of the digestive tract while in the frog killed after 37 days, over 50% of the larvae were in the musculature of the front legs and pectoral girdle (Table 2).

A sample of 40  $L_1$  larvae from copepods measured 434-605  $\mu\text{m}$  (mean 554  $\mu\text{m}$ ). A sample of 40  $L_1$  larvae recovered after 37 days, from *R. pipiens*

TABLE 1. Numbers of infective larvae of *D. insignis* recovered after attempted infection of tadpoles and frogs.

	No.	No. larvae (per animal)	Time necropsy (days)	No. recovered	% recovered
Tadpoles					
<i>R. pipiens</i>	3	105	12	0	0.0
<i>R. pipiens</i>	1	230	32	8	3.5
<i>R. pipiens</i>	1	15	33	1	6.7
Frogs					
<i>R. pipiens</i>	1	208	7	94	45.2
<i>R. pipiens</i>	1	207	8	178	85.9
<i>R. pipiens</i>	1	300	37	260	86.7
<i>R. clamitans</i>	1	450-500	37	400	80.0-90.0

TABLE 2. Distribution of infective larvae of *D. insignis* in tissues of two experimentally infected frogs.

Host	<i>Rana pipiens</i>		<i>Rana clamitans</i>	
No. larvae given	207		450	
Time necropsy (days)	8		37	
Location	No. recovered	% recovered	No. recovered	% recovered
Skin	2	1.1	0	0.0
Body cavity	17	9.6	0	0.0
Muscles of forelimb and pectoral girdle	9	5.1	220	55.0
Muscles of hindlimb	6	3.4	20	5.0
Muscles of back	9	5.1	124	31.0
Abdominal muscles	49	27.5	35	8.8
Wall of digestive tract	86	48.3	1	0.3
Total	178	85.9	400	80.0-90.0

TABLE 3. Infective larvae of *D. insignis* recovered from attempted infections in fishes.

Species	No.	No. larvae (per fish)	Time necropsy (days)	No. recovered
White sucker	1	175	6	1
White sucker	1	100	11	2
White sucker	2	200	21	0
White sucker	1	200	41	0
Rainbow trout	2	60	2 & 7	0
Rainbow trout	1	180	7	2
Common shiner	1	50	9	0
Common shiner	2	80	10	0
Common shiner	1	10	11	0
Common shiner	4*	200	2	0
Common shiner	1*	10	6	0
Common shiner	7*	200+	14	0
Common shiner	4*	200+	20	0
Brown bullhead	2	100	7	0
Brown bullhead	1	110	26	0
Brown bullhead	2	200	27 & 35	0
Bluntnose minnow	1	60	9	0
Stone cat	5	200	20	0
Madtom	4	100	2, 9, 10 & 10	0

\* Ingestion of infected copepods in shallow dish.

+ Group exposures.



(infected with larvae from the same female) measured 570-698  $\mu\text{m}$  (mean 629  $\mu\text{m}$ ). The difference in length (student's t-test ( $P=0.05$ )) was significant. Two hundred and fifty larvae recovered from experimentally infected frogs were given to a raccoon. The animal was killed 167 days later and 13 male worms, 12 larvigerous females and 15 fertilized ovigerous females were recovered (16%). This was the greatest (percentage) recovery of all experimental infections.<sup>9</sup>

The recovery of larvae from fish was poor and is summarized in Table 3.

#### DISCUSSION

The results indicate that most infections of *D. insignis* in raccoons in southern Ontario become patent in late spring or early summer. As the pre-patent period is about one year, transmission must be highly seasonal. The reason for the high prevalence of patent infections in spring is possibly associated with seasonal changes in feeding habits of raccoons, which in turn reflect local agricultural practices. From spring to mid-August raccoons in the Guelph area feed around creeks and ponds and, presumably, become infected at this time. Later in the year they tend to move into corn fields to feed, visiting aquatic locations less often. Some worms found in the legs of raccoons in the fall were probably from infections acquired in early summer of the same year, as experiments have shown that fertilized females can appear in the legs after only 77 days and by 120 days may contain larvae.<sup>6</sup> Chandler,<sup>2</sup> working in east Texas, also concluded that transmission was markedly seasonal with most infected raccoons occurring in December (6 of 9) and, according to trappers, January and February. None of 6 examined was infected in May and September.

Mink (*Mustela vison*) inhabit areas around pools and creeks throughout the year, thus the occurrence of female *D. insignis* in the legs is not as seasonal as in raccoons. Females were found in the legs of 7 of 9 (78%) infected mink in spring and 37 of 68 (54.4%) in fall.<sup>3</sup> This difference between mink and raccoon probably reflects the differences in their use of habitats and food throughout the year.

Another factor which must contribute to the seasonal nature of transmission in southern Ontario is the annual fluctuation in water temperature. In ponds near the University of Guelph, the water temperatures (17 C) in 1971 were such that larvae could start developing in copepods in mid-May. By mid-June the water temperature was 20 C and infective larvae could be available by this time. Animals infected in mid-June of any one year would develop patent infections about one year later.<sup>6</sup> This would explain why most female worms were passing their larvae in the last part of May and first part of June.

Dead or encapsulated males of *D. insignis* were never found in the present study. Muller<sup>9</sup> observed that males of *D. medinensis* died 90-120 days after infection and became encapsulated. Males of *D. insignis* are probably resorbed as are most exhausted females. A detailed study of the pathogenesis of *D. insignis* in wildlife hosts would provide valuable information applicable to human dracunculiasis. Muller<sup>9</sup> described the symptoms of late human guinea worm infections which may include an intense burning sensation and itching at the time of blister formation. Raccoons were observed to scratch at areas of the skin where female worms were located which could be due to a similar irritation. However, providing there are no secondary complications, *D. medinensis* causes little pain or incapacity in man and the lesion heals rapidly following removal of the worm.<sup>9</sup> Lesions caused by *D. insignis* also heal rapidly after the larvae are passed and, in experimentally infected raccoons, obvious signs of acute distress were seen rarely.

The altered behavior of infected copepods may account for their absence in plankton samples taken from water frequented by raccoons and mink infected with *D. insignis*, and otter infected with *D. lutrae*. Similar inactivity has been observed in copepods infected with *D. medinensis*.<sup>10,11</sup> Regardless of temperature, few copepods infected with *D. insignis* lived longer than 50 days.<sup>3</sup> This supports Muller<sup>9</sup> who had similar results with copepods infected with *D. medinensis*. In contrast, Platzer and Adams<sup>12</sup> found that copepods infected with *Philonema oncorhynchi* behaved and swam



normally and some were alive after 210 days.

Fyvie<sup>7</sup> suggested that wild mammals become infected, while drinking, by accidental ingestion of copepods carrying infective larvae. This may occur in some instances but, because of the altered behavior of infected copepods, a paratenic host may be an ecological necessity as an "accumulator" for the maintained high prevalence and intensity of both *D. insignis* and *D. lutrae*. The large number of L<sub>3</sub> larvae recovered from experimentally infected frogs in the present study is ample evidence that frogs could serve in this role. Brackett<sup>1</sup> observed that tadpoles would eat infected copepods and L<sub>3</sub> larvae recovered 2 weeks later were used to establish *D. ophidensis* in snakes. The L<sub>3</sub> larvae of *D. insignis*

probably remain viable in frogs for extended periods so that comparatively large numbers of larvae could be ingested with a single frog. This may account for the heavy infections sometimes observed in wild raccoons. Also, the presence of infected paratenic hosts would extend the transmission period into late summer and fall as infected copepods are short-lived. Another possibility, yet to be investigated, is that mammals are involved in paratenesis as hamsters have been used successfully to transport L<sub>3</sub> *D. medinensis* larvae from west Africa to England.

The growth of L<sub>3</sub> larvae in frogs and their apparently increased infectivity is of interest as it is generally accepted that there is never any "development" of a parasite in a paratenic host.<sup>8</sup>

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