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# LESIONS ASSOCIATED WITH Philometroides huronensis (NEMATODA: PHILOMETRIDAE) IN THE WHITE SUCKER (Catostomus commersoni)

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Abstract: Lesions associated with Philometroides huronensis in the white sucker (Catostomus commersoni) of southern Ontario occurred during the spring (April-June) and were related to the development and release of first-stage larvae from the gravid nematode. With movement of the subgravid female into the membranous region of the fin, subepidermal tissues were mechanically disrupted and compacted near the nematode. Gravid females were encapsulated by fibrous tissue. Release of first-stage larvae from the gravid nematode was facilitated by a cutaneous opening, disruption of the fibrous capsule and rupture of the nematode. An acute local inflammatory response was associated with this function. Spent nematodes were sequestered and resorbed.

#### **INTRODUCTION**

Information on lesions associated with philometrids (Nematoda: Dracunculoidea) in fishes is sparse.<sup>18</sup> Visceral adhesions and encapsulation of spent nematodes have been reported in infections with Philonema agubernaculum in Atlantic salmon (Salmo salar), 13,14 Philonema oncorhynchi in sockeye salmon (Oncorhynchus nerka),<sup>17</sup> and Thwaitia kotlani in the asp (Aspius aspius).<sup>15</sup> These nematodes complete their development in the body cavity and in Atlantic salmon adhesions resulting from the presence of worms interfere with normal spawning.13 Less is known about the effects on the host of species of philometrids which occur in the body cavity and complete their development in the subcutaneous tissues. Dailey<sup>6</sup> noted little or no tissue damage associated with *Philometroides nodulosa* in the body cavity of the white sucker. He reported a chronic, low-grade inflammatory reaction "characterized by a mild increase of fibroblasts," "increased infiltration of capillaries" and a "paravascular cellular response, limited to the immediate area of the parasite" in the subcutaneous tissues of the head. The infiltration consisted of "mononuclear neutrophils followed by the polymorphonuclear type and lymphocytes." Wierzbicki<sup>23</sup> and Crites<sup>5</sup> reported encapsulated developmental stages of *Philometroides sanguinea* in crucian carp (*Carassius carassius*) and *Philometra* sp. in freshwater drum (*Aplodinotus grunniens*).

Philometroides huronensis<sup>19</sup> is prevalent in the white sucker (Catostomus commersoni) of southern Ontario. Infections acquired during the summer months, mature to the adult stages in the peritoneum around the swimbladder. 20,21 Following copulation, inseminated female nematodes move to the bases of the fins where during the fall and winter they increase significantly in size (3.7 to 76.0 mm). In the spring subgravid nematodes move into the membranous region of the fin and ova develop into first-stage larvae, ready to be released by the gravid worm. Subgravid and gravid nematodes, recovered most frequently

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from the pectoral fins, were not present in the fins or at their bases during the summer period.

In this communication, lesions associated with some of the parasitic stages are described.

#### **MATERIALS AND METHODS**

White suckers caught by trap net in southern Lake Huron and seine in the Humber, Nottawasaga and Talbot rivers (tributaries of Lake Ontario, Georgian Bay and Lake Simco, respectively) during 1973-74 were examined for *P. huronensis* as outlined by Uhazy.<sup>19,21</sup> Fish intended for histopathologic study were maintained in live tanks until examined.

Fins and tissues associated with the swimbladder were fixed in freshly prepared Bouin's fixative or buffered 10% formalin (Coleman buffer tablets pH 7.03). Fixation in Bouin's was for 48 hr followed by storage in 70% ethyl alcohol. Fins were decalcified in formic acid-sodium citrate<sup>7</sup> and embedded in paraplast. Sections were cut at 7  $\mu$ m and stained with Ehrlich's haematoxylin and eosin or Whipf's polychromatic stain.<sup>22</sup>

## RESULTS

Examination of the peritoneum around the swimbladder revealed that male and uninseminated and inseminated female nematodes moved freely along these tissues in the serous cavity formed by the peritoneum. Gross lesions were not observed and microscopic study did not reveal evidence of a host response.

Gross lesions were not associated with subgravid nematodes at the bases of the fins. In the fin, the long, bright red nematode was sharply delineated within the opaque tissue. The nematode occupied the membraneous region of the fin, often crossed over fin rays and occasionally passed between the paired fin ray elements. Because of their width (0.6 to 0.8 mm),<sup>19</sup> the nematodes were generally limited to the wider proximal region of the fin. The presence of the nematode distorted the shape of the fin and the degree of distortion was dependent upon the number in the fin. Microscopic study of transverse sections of fins revealed the nematodes were subcutaneous in location. The dense connective tissue of the dermis and the lattice-like loose connective tissue of the subdermis were disrupted and compacted near the nematode (Fig. 1). The cuticular bosses covering the surface of the nematode, characteristic of Philometroides,19 caused discrete impressions in the connective tissues. There was no evidence of encapsulation in most sections. In one section, however, the posterior end of the nematode was encapsulated. Veins, arteries, lymphatics and musculature within the central zone of the fin rays were not damaged and there was no indication of a cellular infiltrate. Daily observations of a subgravid female in the left pectoral fin of a white sucker indicated the nematode was able to move freelv.

Gross lesions were not evident in association with intact gravid nematodes. Microscopic examination revealed the nematodes were subcutaneous and encapsulated by a concentrically layered connective tissue capsule (Fig. 2). Occasionally, within the capsule there was a diffuse fibrous eosinophilic mass sometimes containing a few host red blood cells. There was some evidence of hemorrhage near the capsule but no evidence of cellular infiltration. The intestine of the nematode contained host red blood cells.

Rupture of the gravid nematode releases first-stage larvae which enter the aquatic environment through a cutaneous opening in the fin. The opening was not readily discernable macroscopically and the region of the fin near the nematode appeared clouded and off-white in color. In fins containing multiple infections, this response was



FIGURE 1. Compressed and disrupted subepidermal connective tissues (CT) associated with a subgravid *P. huronensis* (P) in the membraneous region of a fin.  $H\&E \times 160$ .

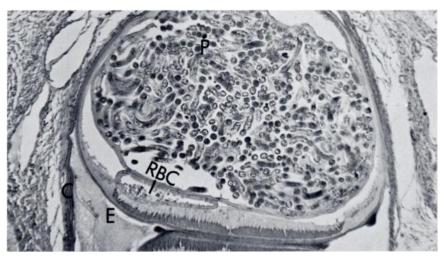


FIGURE 2. Encapsulated (C) gravid *P. Huronensis* (P). Note eosinophilic mass (E) in capsule and host red blood cells (RBC) in intestine of nematode. H&E  $\times$  180.

restricted to the ruptured nematode. Microscopically there was an opening through a well-defined region of necrotic cells (Fig. 3). The normal architecture of the epidermal and dermal cells was retained while their nuclei appeared pyknotic. Both cell layers were thinner and there was sloughing of the epidermis. The remainder of the lesion was characterized by hyperremia, edema, an

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intense cellular infiltration and a complete breakdown of the capsule. The infiltrate consisted mainly of neutrophils, some large eosinophilic granular mononuclear cells with eccentric nuclei and macrophages (Fig. 4). A few red blood cells were present in the exudate. First-stage larvae were found throughout the exudate. Inflammatory cells were attached to the female nematode, the larvae and debris. Exhausted female nematodes and en-

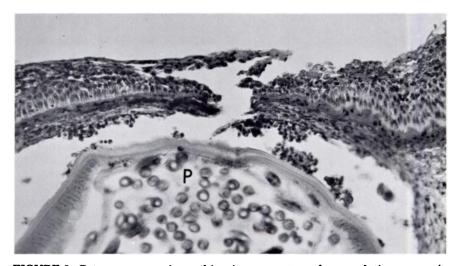


FIGURE 3. Cutaneous opening, thin integument and coagulation necrosis associated with a ruptured gravid *P. huronensis* (P). Whipf's  $\times$  260.

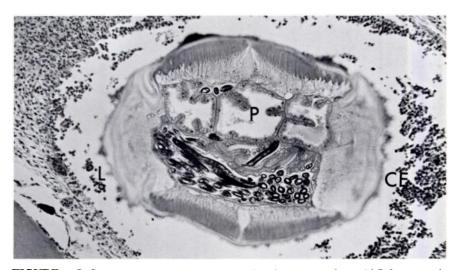


FIGURE 4. Inflammatory response associated with a ruptured gravid *P. huronensis* (P). Cellular exudate (CE) and first-stage larvae (L). H&E  $\times$  160.

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trapped larvae were resorbed by mononuclear cells and macrophages (Fig. 5).

## DISCUSSION

Lesions observed were associated with the subgravid and gravid females of P. huronensis present in the membranous region of the fins and were correlated with the April to June occurrence of these stages in the annual life cycle of the parasite. Tissues associated with movements of (a) infective third-stage larvae out of the gastrointestinal tract into the body cavity and (b) inseminated females from the body cavity to the bases of the fins, were not examined microscopically. Some tissue damage would be expected with these movements. Adams<sup>1</sup> reported small areas of hemorrhage associated with the movement of the infective third-stage larvae of Philonema oncorhynchi across the tissues of the pyloric caecae in sockeye salmon. In the white sucker, no gross lesions were observed and there was no host response associated with adult stages in the peritoneum around the

swimbladder. Dead nematodes apparently were resorbed.

Entrance of the large, subgravid nematodes into the membranous region of the fin and their ability to move in this vicinity represent potentials for extensive fin damage. The magnitude of tissue damage is also influenced by the number of nematodes in the fin. Up to 8 nematodes were recovered from a single fin; although, infections with 1 to 3 nematodes were most frequent.<sup>21</sup> Accompanying the maturatio of firs-stage larvae a progressive development of a connective tissue encapsulation resulted in eventual sequestration of the then gravid nematode. The capsule would provide an obstacle for the release of these larvae into the aquatic environment.

A cutaneous opening in the fin allowed first-stage larvae to escape following their release from the burst gravid nematode. The mechanism for formation of the integumental opening in the fin and destruction of the encapsulation is unknown. The prominent dorsal esophageal gland in the parasite (Fig. 6) may secrete histolytic enzymes which



FIGURE 5. Overwhelmed, exhausted gravid *P. huronensis* (P) and first-stage larvae (L). H&E  $\times$  160.

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FIGURE 6. Anterior end of gravid P. huronensis (P) at level of dorsal esophageal gland (DEG). H&E  $\times$  160.

induce the lesions. The appearance of coagulation necrosis suggests the presence of a locally acting substance. The opening exposes the ripe female nematode to an influx of freshwater and the change in osmotic pressure apparently causes the nematode to rupture. When gravid females of P. huronensis were dissected from fins and held in 0.6% NaCl they did not burst while those in well water soon became turgid, burst and released large numbers of first-stage larvae.<sup>19</sup> Lewis et al.,<sup>11</sup> demonstrated that a sudden change in osmolarity upon entering freshwater caused Philonema oncorhynchi to rupture. The opening in the fin also would predispose the fish to invasion by foreign material (e.g., bacteria).

The inflammatory response was intimately associated with the release of first-stage larvae. Neutrophils and macrophages predominated in the exudate but large eosinophilic granular mononuclear cells with eccentric nuclei also were present. Cells similar in morphology and described as PASpositive granular leukocytes<sup>3</sup> or eosinophils<sup>9</sup> have been described from blood, connective and inflammatory tissues in the white sucker.<sup>10</sup> The identity and function of these cells remain uncertain.

Furuyama<sup>8</sup> and Wierzbicki<sup>23</sup> reported that gravid females of *Philometra fujimotoi* and *P. sanguinea* left the host fins prior to larval release. *P. huronensis*, however, remained in the fins and were eventually resorbed.

Nothing is known about the food sources utilized by dracunculoids.<sup>16</sup> Muller speculated that breakdown products of haemoglobin were present in developing forms of *Dracunculus medinensis*. The presence of host red blood cells in the intestine of a gravid *P. huronensis* indicated these cells were ingested. The effect of this blood loss on overall pathogenesis induced by dracunculoids remains to be resolved.

*P. huronensis* is prevalent in the white sucker and intensities of infection are low, i.e. up to 7 nematodes were recovered from 77% of infected fish. In addition, all age groups of fish are infected, reinfection is common and there is no decline in

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intensity of infection with age of host.<sup>21</sup> The presence of various developmental stages of the parasite and the host response to the burst gravid female worm does not elicit protective immunity. The acute inflammatory response is related to the release of first-stage larvae and foreign proteins associated with the rupture of the gravid worm. This response, similar to those reported for *D. medinen*.

sis in man<sup>16</sup> and *D. insignis* in raccoons,<sup>4</sup> is suggestive of a mammlian hypersensitivity reaction. Reactions of this type have been demonstrated in pleuronectids,<sup>2</sup> but of the specific mediators of anaphylaxis, only low concentrations of histamine have been reported in fishes.<sup>12</sup> Further study of this reaction in fishes is warranted and *P. huronensis* in the white sucker may be a suitable model.

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