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Intrahepatic Larval Nematode Infection in the Northern Spring Peeper, *Pseudacris crucifer crucifer* (Anura: Hylidae), in West Virginia

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ABSTRACT: Larval stages of an unknown nematode were observed encapsulated in the livers of spring peepers, *Pseudacris crucifer crucifer* (Weid-Neuweid), collected from a marsh in western West Virginia (USA) during the spring breeding seasons of 1993 and 1994. Prevalence and mean intensity of infection were 37% (30 of 82 animals) and 2.03 parasites per infected host, respectively. Capsules with white or darkly pigmented walls were observed in infected livers; the former containing viable larvae, and the latter enveloping larvae in various stages of degeneration.

Key words: Nematoda, histopathology, spring peeper, *Pseudacris crucifer crucifer*, West Virginia.

While studying digenetic trematode populations from the spring peeper, *Pseudacris crucifer crucifer* (Weid-Neuweid) in West Virginia, the senior author observed encapsulated nematode larvae in the livers of some host individuals. Several larvae were excised from these encapsulations, and while a definitive species diagnosis cannot be made, these larvae closely resemble the *Agamascaris odontocephalus* described by Steiner (1924).

There are no published reports of larval or adult nematode infections in the spring peeper from West Virginia. Relatively few reports of larval nematode-induced capsules in amphibians are available (Steiner, 1924; von Brand, 1944; Panesar and Beaver, 1979), and detailed descriptions of host tissue response due to such infections in amphibians are virtually non-existent. Our objective was to describe host tissue response to the invasion of this unknown nematode larva.

The collection site for this study was a 0.8-ha permanently flooded marsh at Shoals (Wayne County), West Virginia (USA) (38°19′45″N; 82°28′18″W). A dense cover of buttonbush (*Cephalanthus occidentalis*) lined the perimeter of the marsh, with bladderwort (*Utricularia gibba*) growing extensively throughout the open water. Two sample populations of spring peepers were collected by hand over two breeding seasons, and their livers were examined for encapsulated nematode larvae. The first host sample (n = 50) was collected on the evenings of 17 and 19 April 1993, while the second sample (n = 32) was taken on the evening of 4 April 1994. Upon collection, host individuals were placed in 4-l screw-cap jars (no more than 10 animals per jar) containing moist paper toweling, returned to the laboratory within 1 hr of capture, and stored at 4 C. Within 24 hr of capture, each host was removed from the refrigerated jar, weighed to the nearest 0.1 g, then killed by pithing. Upon necropsy, the liver was removed from each host and examined at 20×. The prevalence of infection was determined, as well as the number of encapsulated nematode larvae per infected liver. Each parasitized liver then was placed in approximately 10 ml of 10% neutral (sodium acetate) buffered formalin (Fisher Scientific, Pittsburgh, Pennsylvania, USA) for tissue fixation and subsequent storage (Sheehan and Hrapchak, 1973). Selected livers were embedded in paraffin and sectioned at 6 μm. Sections were stained in either hematoxylin and eosin, or Masson's trichrome (Poly Scientific, Bay Shore, New York, USA), then mounted in Acrytol® (Surgipath Medical Industries, Inc., Richmond, Illinois, USA). Whole mounts of nematode larvae were prepared by dehydrating in an eth-

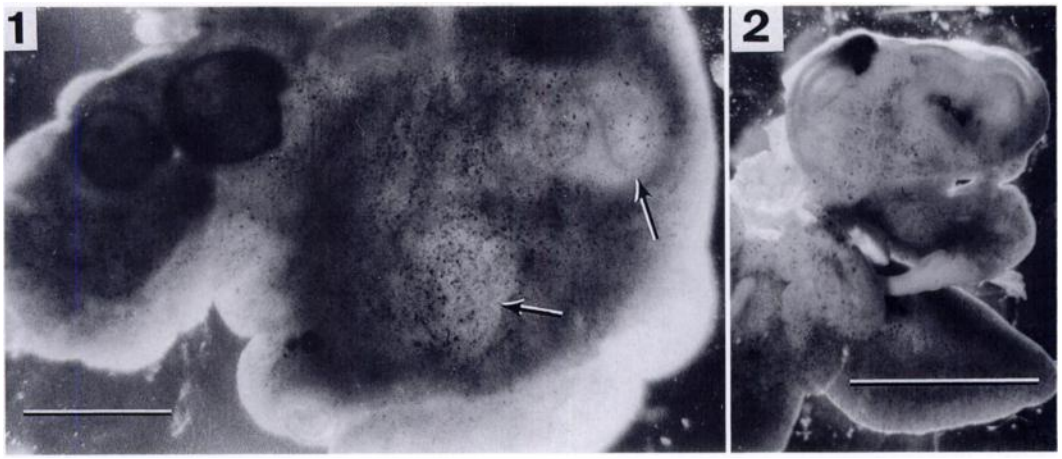

FIGURE 1. Gross view of *Pseudacris crucifer crucifer* liver with five encapsulated larvae. Two larvae at upper left surrounded by dark, capsule walls. Two larvae deeper in the liver parenchyma (arrows) are surrounded by white fibrous walls. Note small larva at bottom middle of photo. Bar = 2 mm.

FIGURE 2. Encapsulated nematode larva occupying entire lobe of host liver. Bar = 5 mm.

anol series, clearing in 100% methyl salicylate, and mounting in Permount® (Fisher Scientific). Tissue sections and whole mounts were photographed with a Zeiss photomicrographic system (Morgan Instruments, Cincinnati, Ohio, USA). Liver was gently pressed between two microscope slides so that encapsulated larvae located deeper in the liver parenchyma could be seen more easily (Fig. 1). Material shown in Figs. 1 and 2 was photographed with a Zeiss stereomicroscopic system having a light source placed below the stage (backlighting). Specimens shown in Figs. 3 and 6 were backlit and photographed with a Zeiss stereomicroscopic system. Liver in Fig. 4 was photographed with an Olympus 55 mm macro lens (Olympus Corporation, Lake Success, New York). Measurements were made with a calibrated ocular micrometer. A whole mount larva voucher specimen was deposited in the U.S. National Parasite Collection under Accession Number USNP 84167. Formalin preserved cysts have been deposited in the Armed Forces Institute of Pathology under AFIP Accession Numbers 2485409 and 2485410. Mean weights of uninfected and infected hosts were compared using Student's t-test for unpaired samples, and the difference was considered significant at $P < 0.05$ (Spence et al., 1983).

The livers of 30 (37%) of 82 spring peepers (23 of 50 in 1993 and seven of 32 in 1994) were infected with one to six ($\bar{X}$ = 2.03, SD = 1.30) encapsulated nematode larvae (Fig. 1). Capsules ranged in size from 0.6 mm to 4.0 mm in length. In several cases, larval-induced capsules approximated the size of the host's heart, occupying nearly an entire liver lobe (Fig. 2). Generally, capsules were either densely white (Fig. 3), or darkly pigmented (Fig. 4) when viewed grossly or under low stereoscopic magnification. The densely white capsule type enveloped a single living larva, while the darkly pigmented type surrounded a larva undergoing disintegration (Fig. 5). The gross picture was not always so sharply delineated, as walls of a few capsules appeared darkened while still encompassing a living larva (Fig. 6). On microscopic examination of tissue sections cut through a white fibrous capsule and affected liver tissue at 60×, we observed an encapsulated larva with a zone of dense fibrous tissue lying between the nematode cuticle and normal hepatic

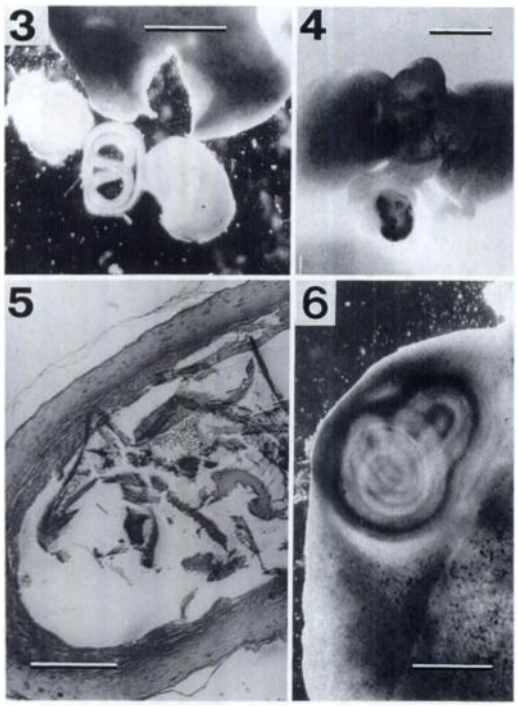

FIGURE 3. Portion of host liver lobe with viable larva teased from fibrotic cyst. Bar = 2 mm.

FIGURE 4. Host liver with dark-walled cyst. Heart appears in upper middle of photo. Bar = 5 mm.

FIGURE 5. Dark non-cellular cyst wall in host liver enveloping degenerating larva. Masson's stain. Bar = 200 μm.

FIGURE 6. Gross view of host liver lobe with initial darkening of capsule wall. Bar = 1 mm.

cells (Fig. 7). The compact appearance of the fibrous zone was due, in part, to the presence of macrophages and collagen deposition by active fibroblasts. This fibrous zone also was devoid of liver sinusoids, bile ducts, erythrocytes, and blood vessels. Accumulations of nuclear debris were seen immediately external to the parasite cuticle in some areas, while other areas external to the cuticle were dominated by fibroblasts (Fig. 7). Much of the inner portion of the parasite capsule was occupied by the nematode larva, but polymorphonuclear leukocytes, macrophages, and lymphocytes were very much in evidence as well (Fig. 7). Pigment-laden Kupffer cells and extreme cytoplasmic vacuolation of hepatocytes were found in hepatic tissues adjacent to the fibrous repair zone.

Sections of darkly pigmented capsules had non-cellular cyst walls with elongate fibrocyte nuclei (Figs. 1 and 5). These capsule walls were similar to those hyaline-like inner capsule walls shown by McAllister et al. (1993) for *Ascarops* sp. larvae in the Mediterranean gecko (*Hemidactylus turcicus*). Internal walls of darkly pigmented capsules in the present study were lined with necrotic debris, and larvae in various stages of degeneration could be seen (Fig. 5).

Weights of uninfected hosts (n = 52, $\bar{X}$ = 1.85 g, SD = 0.35) were slightly higher than weights of infected hosts (n = 30, $\bar{X}$ = 1.80 g, SD = 0.29), but the difference between mean weights was not significant (t = 0.67, df = 80, $P > 0.05$). Variation in larval size (Figs. 1 and 2) was consistent with the finding of Steiner (1924) who reported a considerable size range for *Agamascaris odontocephalus* larvae. The finding of one larva per capsule also corroborated the findings of Steiner (1924).

Infections resulting in such large parasite-induced capsules relative to the size of host organ infected, and a prevalence rate of 37%, might be viewed as possible harbingers of serious parasitic disease. Still, the mean weight of infected hosts was not significantly lower than that of their uninfected counterparts, and many host individuals had signs of successfully walling-off the parasite. As a result, the prognosis for infected hosts appears good, despite the dramatic gross picture of the infection.

We are grateful to Beverly Pofahl for the preparation of tissue sections. Our appreciation is also extended to the West Virginia Division of Natural Resources for granting the requisite host scientific collecting permit (Permit Number 21-1994).

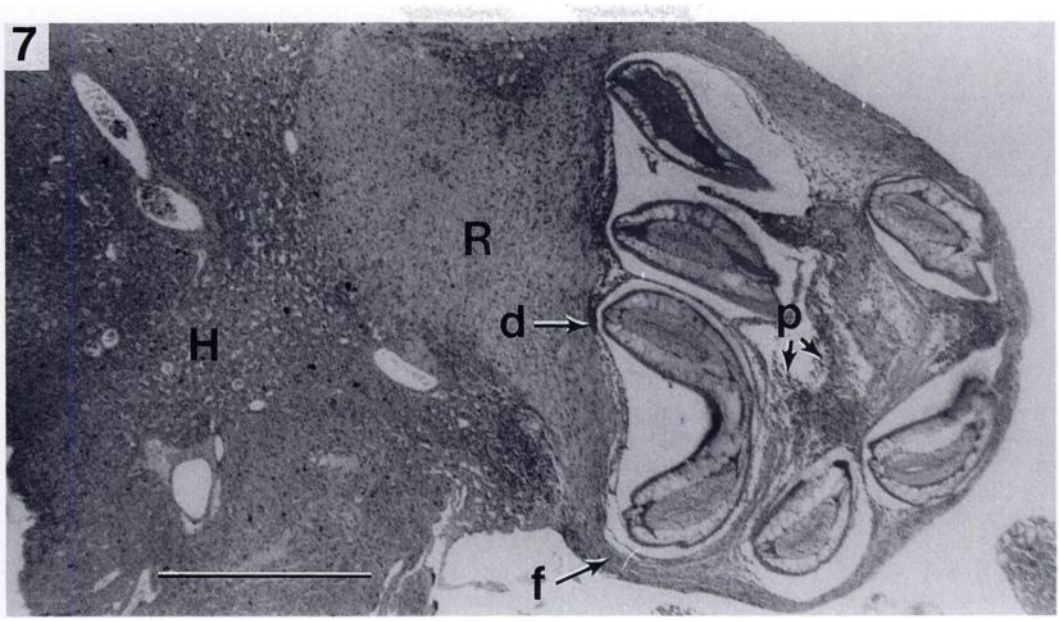

FIGURE 7. Tissue section of host liver with fibrotic capsule enveloping nematode larva (right). Normal liver tissue (H); repair zone (R); nuclear debris (d); area dominated by fibrocytes (f); area of polymorphonuclear leukocytes and lymphocytes (p). H & E. Bar = 1 mm.

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