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Parasitic Meningoencephalitis in Nurse Sharks (*Ginglymostoma cirratum*)

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ABSTRACT: Based on microscopic examination of the brains of seven wild-caught nurse sharks (*Ginglymostoma cirratum*), we observed a severe meningoencephalitis associated with numerous parasitic granulomas. The parasites were larval nematodes with morphologic characteristics of the Superfamily Dracunculoidea. Although meningeal larval aggregates were associated with chronic inflammation, additional parasitic nodules found on the endocardial surface and perimandibular region did not provoke an inflammatory response. Neither the route of infection nor life cycle were determined.

Key words: Elasmobranch, Dracunculoidea, meningoencephalitis, nematode larvae, nurse shark, *Ginglymostoma cirratum*.

Wild-caught elasmobranchs are often parasitized; however, there are relatively few reports of nematode infection, especially in the nervous system (Cheung, 1992). We here describe aggregates of larval nematodes associated with meningoencephalitis in wild-caught nurse sharks (*Ginglymostoma cirratum*) from the Florida Keys, Florida (USA) (24°33′N, 81°46′W). During a 3-yr period (1987 to 1990), six nurse sharks were captured for the purpose of studying normal elasmobranch microanatomy. The sharks ranged in standard length from 1.5 to 2 m. None of the sharks was noted displaying abnormal behavior.

No lesions were observed grossly. Samples of formalin-fixed, paraffin-embedded tissues from multiple areas of the brain and most organs were sectioned at 4–5 μm, stained with hematoxylin and eosin, and examined microscopically. Histologically, the cerebral meninges were multifocally expanded ≤20 times their normal thickness by dense aggregates of coiled nematode larvae and inflammatory cells (Fig. 1). Inflammation consisted of plasma cells, lymphocytes, and acidophilic granulocytes within a loose fibrovascular stroma. The inflammatory response was most severe surrounding larval aggregates. Meninges also contained occasional lymphoid nodules and granulomas of epithelioid macrophages not centered around discernable nematode larvae. Meningeal vessels and lymphatics were frequently thickened, ectatic, and cuffed by chronic inflammatory cells. The superficial layers of the cerebral neuropil had moderate gliosis and numerous, multifocal perivascular cuffs of primarily mononuclear cells surrounded by congested vessels.

In all cases, parasitic nodules were located in the superficial layers of the thickened meninges, supported by fibrous connective tissue and surrounded by inflammatory cells. The largest parasitic nodule measured 1,600 μm × 800 μm and four other nodules averaging 800 μm × 600 μm also were present in the same cerebral section. Most nodules were not encapsulated, but well-demarcated by a perimeter of thickened fibrous stroma and inflammation. Within nodules the parasites were separated by inflammation and a fine fibrous tissue meshwork surrounding individual larvae (Fig. 2). Extra-parasitic, globular, eosinophilic material, similar to that noted within larval digestive tracts, occasionally surrounded the nematodes. Larvae were not found within blood vessels.

The diameter of the anterior end of the larvae measured 8 μm, the mid-body 20 μm, and the posterior tapered into a thin tail, 4 μm in diameter. The coiled larvae

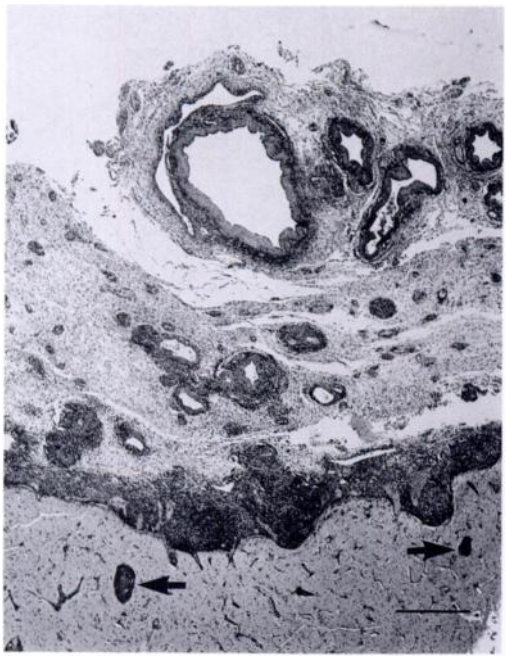

FIGURE 1. Thickened, inflamed meninges with ectatic vessels of a nurse shark. Superficial vessels in the cerebral neuropil are made prominent by thick perivascular cuffs of mononuclear inflammatory cells (arrows). H&E. Bar = 450 μm.

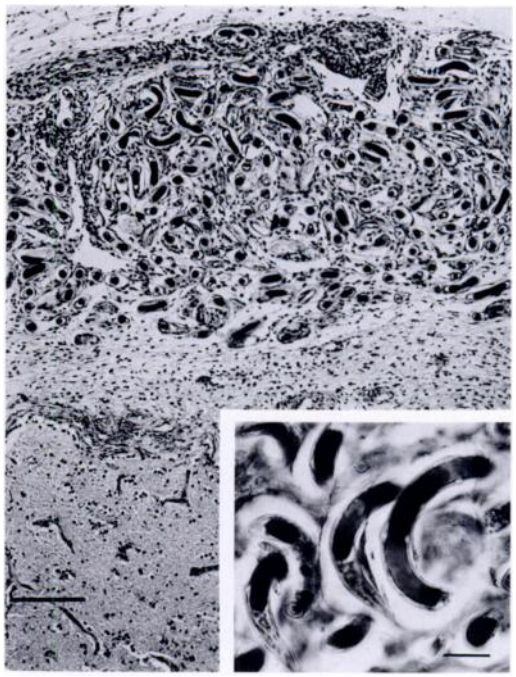

FIGURE 2. Meningeal larval aggregate in a nurse shark. Bar = 180 μm. Inset of nematode larvae depicting their darkly staining digestive tract and tapered tail. H&E. Bar = 40 μm.

measured between 200 μm and 280 μm in length. Larvae had a thin (1 μm) finely striated cuticle and a distinct digestive tract made prominent by an intensely eosinophilic staining material. In larval cross sections, there were five to six plump basophilic cells lining the interior of the body cavity and three or four thin, flattened cells surrounded the cylindrical, eosinophilic-staining digestive tract. The identity of the larvae was most likely Superfamily Dracunculoidea of the Order Spirurida (R. Lichtenfels, pers. comm.). A representative microslide of the nematode larvae has been deposited in the U.S. National Parasite Collection, Beltsville, Maryland as USNM Helminth Collection Number 82487.

The meninges of one heavily parasitized shark contained nematode larval nodules as previously described, and a parasitic granuloma incorporating morphologically different larval nematodes. The single nodule, measuring 480 μm × 640 μm, demarcated by peripheral chronic inflammatory cells and central unencapsulated, spicular, eosinophilic material, contained numerous nematode larvae resembling microfilaria (Fig. 3). Larvae measured 6 to 10 μm in width and more than 120 μm in length. These nematodes had rounded cephalic extremities and tapered caudal regions. The parasites did not possess a discernable digestive tract and lacked the distinct eosinophilia characteristic of the larvae within other meningeal nodules. Larvae had a thin cuticle with numerous, densely-packed cells defining their body cavities. Most microfilaria-like larvae were slightly curved and surrounded by macrophages, lymphocytes and eosinophilic granulocytes. Adjacent to this granuloma was an apparently degenerate adult nematode body wall, measuring 100 μm in width, with the interior cavity containing five fragments of the previously described

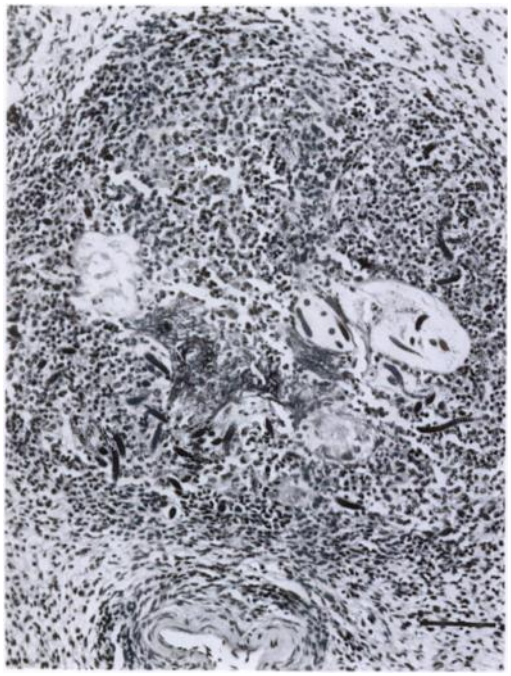

FIGURE 3. Focal parasitic granuloma involving microfilaria-like nematode larvae in the meninges of a nurse shark. H&E. Bar = 83 μm.

FIGURE 4. Apparently degenerate adult nematode containing fragments of larvae. H&E. Bar = 14 μm.

larvae (Fig. 4). These may represent a microfilaroid type of first-stage larvae similar to that described in a report of *Ichthyofilaria canadensis*, another member of the Dracunculoidea superfamily (Appy and Anderson, 1984).

Parasitic nodules of the type described in the meninges were found in other tissues from sharks in this group. In one shark, a nodule measuring 1,800 μm × 800 μm was attached to the endocardium, bulging into the ventricular sinus (Fig. 5). In another case, four nodules were located in the connective tissue adjacent to mandibular cartilage. Morphologic characteristics of these nematode larvae were similar to those previously described. In contrast to the meningeal larval aggregates, however, there was little to no inflammation associated with nodules in either the heart or mandible.

An additional younger nurse shark, measuring 0.7 m in length, was caught in the Florida Keys, Florida (24°33′N, 81°46′W) for a separate study in May 1987, and died soon after capture. On histologic examination, we observed ventral meningeal aggregates of nematode larvae similar to those described in this study. In the shark captured in May 1987, there was little inflammatory reaction associated with the larval mass. Neither adults nor microfilaria-like larvae were noted.

Further identification of these parasites was difficult due to the lack of characteristic features of the larvae and a limited amount of material available to study. No adults were found during necropsy nor following histologic evaluation of these sharks. Other dracunculoids reported in sharks are the genera *Granulinema* (Moravec and Little, 1988), *Lockenloia* (Adamson and

Caira, 1991), and *Phlyctainophora* (Steiner, 1921; Adamson et al., 1987). None is reported as occurring in the brain or meninges. This is the first report of nematode larvae in the meninges of nurse sharks with morphologic characteristics of the Superfamily Dracunculoidea.

Reports of elasmobranchs infected by larval dracunculoid nematodes are rare, although larval granulomas have been found in ovaries of black-tip sharks (*Carcharhinus limbatus*) (Rosa-Molinar et al., 1983) and the uterus of a sandbar shark (*Carcharhinus plumbeus*) (Benz et al., 1987). The larval nematodes found in granulation tissue from a sandbar shark appear morphologically similar to those encountered in the meninges of these nurse sharks. As in these cases the intestinal lumen of the larvae stained intensely, however a tooth-like projection was noted at the anterior extremity, a feature not detected in the larvae found in the nurse sharks.

It is unclear whether the meningeal location of the larval nematodes seen in these cases is a normal or aberrant migration pattern. Based on Adamson and Caira's (1991) description of fragments of an adult dracunculoid nematode identified as *Lockenloia sanguinis* found in the heart of a nurse shark, we suggest that larvae could reach the brain from a cardiac location. The larval nematode nodule adherent to the endocardium of one of the sharks in this study is evidence that such a route is likely, even though larvae were not observed in vessels.

Also unclear is whether the parasitic nodules are the sole cause of the severe, chronic meningitis, as similar larval nodules in other tissue locations provoked a less intense inflammatory response. The marked meningitis may have predisposed these sharks to capture, and as each was similarly parasitized, this condition may not be uncommon, at least not in some shark populations. Questions persist as to the taxonomy and life cycle of this nematode, the location of the adults, and why larval aggregates failed to provoke an intense inflammatory response in locations other than the brain.

FIGURE 5. Parasitic nodule adherent to the endocardium of one nurse shark. H&E. Bar = 200 μm.

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