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Brain Lesions in a Pacific White-sided Dolphin (*Lagenorhynchus obliquidens*)

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ABSTRACT: A young, male, free-ranging Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) was found disoriented and died after being held in captivity for several months. Malacic lesions in several areas of the brain were associated with helminth eggs. The appearance and location of these eggs suggested they were of the genus *Nasitrema*.

Key words: Brain, pathology, Pacific white-sided dolphin, *Lagenorhynchus obliquidens*, *Nasitrema* sp., case history.

A young, male, free-ranging Pacific white-sided dolphin (*Lagenorhynchus obliquidens*) of undetermined age was found disoriented and circling. It was rescued by personnel from the Vancouver Public Aquarium on 2 March 1983. During captivity (Vancouver Public Aquarium, Box 3232, Vancouver, British Columbia, Canada V6B 3X8), it was placed by itself in a research pool for 5 mo, held for 1.5 mo with two adult female beluga whales (*Delphinapterus leucas*) and was kept with a mature female white-sided dolphin for the next 14 mo. The animal appeared to eat well but never learned to accept food directly from the trainer. Gradual loss of weight was observed over the last few months and 2 days before death the dorsal and caudal aspects of the flukes were rubbed raw. Consumption of food was greatly reduced. On the morning of 17 December 1984 there were numerous bite wounds and scrapes over most of the body surface. The dolphin was moved to a separate tank and within a few hours it had listed to one side and died. A complete postmortem examination was begun within 4 hr of death.

The dolphin was 185 cm long and weighed 69.4 kg. The animal was in poor condition and a concave appearance to the

musculature lateral to the dorsal fin indicated muscle wasting. Except for a small amount of brownish debris in the blowhole and occasional small (approximately 1 mm in diameter) white specks in the auditory canals and tympanic bullae, other gross alterations were absent. Stereomicroscopic examination of the material from the auditory canals revealed only homogeneous debris, presumably an inflammatory exudate.

Tissues from all major organs were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μ m and stained with hematoxylin and eosin. Microscopic examination of the lung revealed focal accumulations of macrophages (some of which contained budding yeasts), fibrin and occasional neutrophils in some peribronchial alveoli. A prominent thrombus consisting of fibrin, neutrophils and necrotic debris was attached to the left atrioventricular valves. Platelet thrombi circulated within several hepatic sinusoids and occasional foci of hepatic necrosis were associated with Kupffer cell aggregates. Sections of skeletal muscle demonstrated diffuse myofiber fragmentation and loss of cross striation within several myofibers. Lipofuscin accumulations were also noted in some muscle fibers.

Swabs from the left auditory canal and blowhole and portions of ulcerated skin, jejunum, ileum, rectum, mesenteric lymph nodes, kidney, liver and spleen were cultured on 5% sheep blood Columbia-based agar and MacConkey agar (Difco, BDH Chemicals, 60 East 4th Avenue, Vancouver, British Columbia, Canada V5T 1E8) for 24 hr at 35 C. All tissues, except liver and spleen, yielded non-lactose fermenting bacteria which were further identified

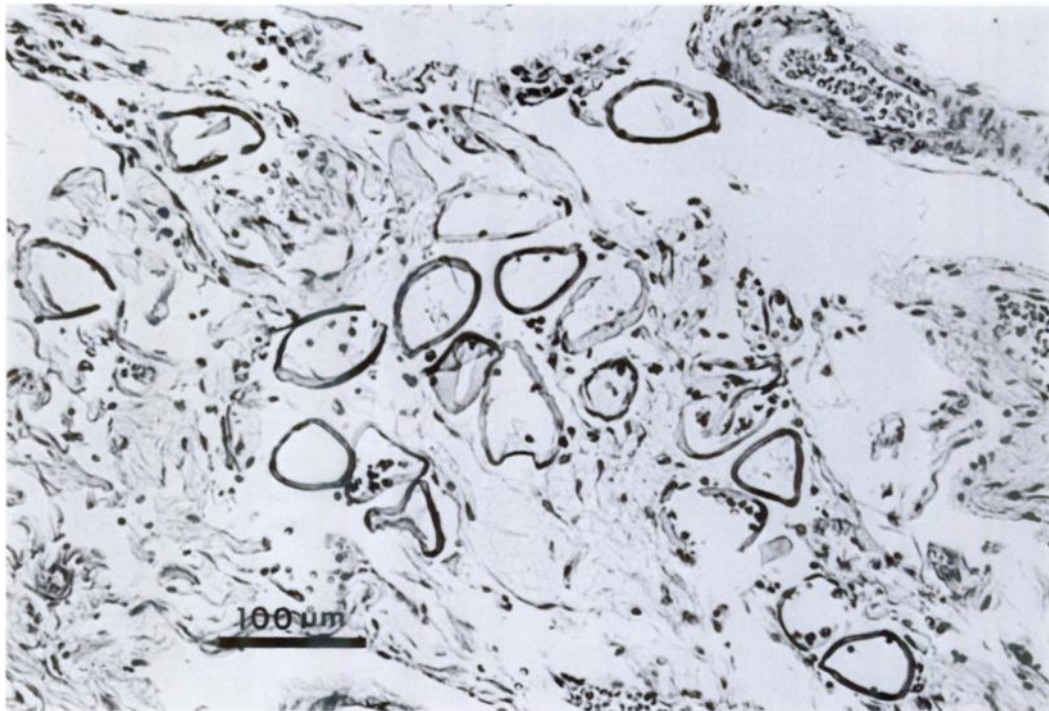


FIGURE 1. Distorted, oval and triangular digenean eggs within thickened, fibrous cerebral meninges of a captive Pacific white-sided dolphin. H&E.

as *Edwardsiella tarda* (Edwards and Ewing, 1972). Moderate numbers of unidentified yeasts also were recovered from sections of the lungs.

Flame atomic absorption spectrophotometry (Instrumentation Laboratory 951, Thermo Jarrell Ash Ltd., 5730 Coopers Avenue, Unit 24, Mississauga, Ontario, Canada L4Z 2E9) for copper, zinc, iron manganese, lead and cadmium; cold vapor atomic absorption spectrophotometry (Instrumentation Laboratory 951, Thermo Jarrell Ash Ltd., 5730 Coopers Avenue, Unit 24, Mississauga, Ontario, Canada L4Z 2E9) for mercury; and fluorometric procedures (Fluorometer Model 110, Sequoia Turner Corporation, 755 Ravendale Drive, Mountain View, California 94043, USA) for selenium analysis failed to add any further significant findings. Using gas liquid chromatography (GLC-5880A, Hewlett-Packard Ltd., 10691 Shellbridge Way, Richmond, British Columbia, Canada V6X 2W8) polychlorinated biphenyls and or-

ganochloride pesticides were found in kidneys, liver and bladder but their concentrations were not considered significant.

Tissues from major organs were harvested, prepared for virus isolation and inoculated onto African green monkey kidney cells (VERO cells, American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852, USA) for 14 days. Two further passages of 7 to 10 days each and electron microscopy (EM10C, Carl Zeiss, D-7082, Oberkochen, Federal Republic of Germany) failed to demonstrate viral infections.

Abnormalities were not detected on external examination of the brain or spinal cord. Coronal sections of the brain demonstrated an area of brownish discoloration $3 \times 1.5 \times 2$ cm in the right parietal convexity cortex extending to the ependymal lining of the ventricle. Similar $1.5 \times 0.5 \times 1$ cm discolorations involved the left medial parietal cortex in the region of the cingulate gyrus and subcortical white mat-

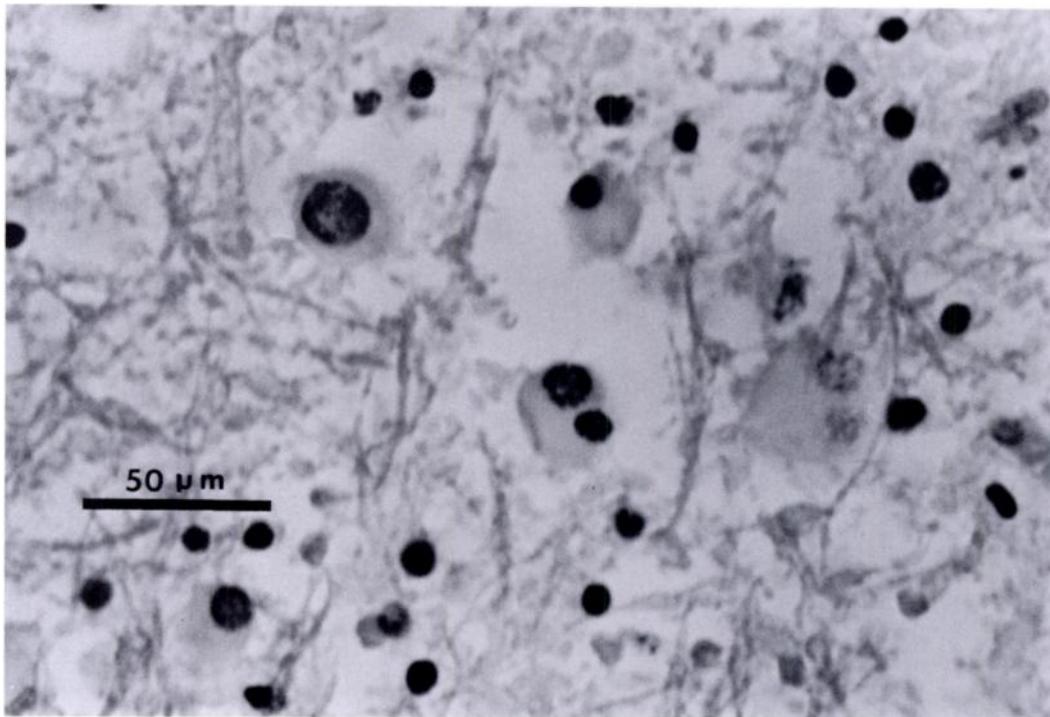


FIGURE 2. Swollen, occasionally binucleate, astrocytes within malacic area of cerebellar white matter from a Pacific white-sided dolphin. H&E.

ter of the right parietal lobe, the cortex and white matter of the left occipital convexity (2 cm in diameter), the left temporal lobe (1 × 1.5 × 1 cm) and the right cerebellar hemisphere (1 × 1.5 cm). Bilateral, patchy, whitish areas of thickened meninges overlaid the lateral aspect of the Sylvian fissures.

Microscopic examination of these areas of discoloration in the brain revealed a marked, irregular thinning of the cortex and intense gliosis extending to the underlying white matter. Focal areas were replaced by large cystic spaces and small collections of hemosiderin-laden macrophages. Numerous eggs of digeneans were associated frequently with these areas of encephalomalacia and within the thickened, fibrous meninges (Fig. 1). Although frequently distorted, the eggs were generally oval or triangular and had a yellowish-brown thick homogeneous shell. All appeared nonviable and were empty or

contained amorphous debris. A structure suggestive of an operculum was observed in some eggs. The eggs were approximately 60 to 80 μm in diameter and elicited only a minimal inflammatory cellular response consisting of a light lymphocyte infiltrate and collections of foamy macrophages. Pigment-laden macrophages and gliosis were often associated with these eggs. Adjacent to, but not directly associated with the eggs were areas of malacia and hemorrhage accompanied by swollen, occasionally binucleate, astrocytes in cerebral and cerebellar white matter (Fig. 2). Examination of several areas of the brain failed to reveal any adult digeneans.

The neuropathological lesions may have rendered this dolphin unable to survive in the wild; captivity probably prolonged its existence. The sunken appearance to the body below the dorsal fin was an indicator of emaciation (Ridgway and Fenner, 1982). The septicemia caused by *Edwardsiella*

tarda is probably a direct result of decreased resistance in this emaciated animal.

Extensive brain damage has been reported due to infection with adults and eggs of the nematode *Contraecum osculatum* in a Pacific white-sided dolphin (Martin et al., 1970). However, the shape and size of the eggs in this case are considered to most closely resemble eggs of digeneans but their specific identity is speculative. Although similar lesions have been reported in dolphins and ascribed to *Campula* sp. and *Zalophetrema* sp., there also were marked microscopic alterations in the liver and pancreas (Ridgway and Dailey, 1972; Dailey and Walker, 1978). It was suggested by these authors that the eggs from *Campula* sp. and *Zalophetrema* sp. arrived in the brain through the circulatory system. Since examination of the liver, pancreas, lungs, stomach and pterygoid sinuses of this dolphin did not demonstrate any adult flukes or the lesions expected with infection by *Campula* sp., *Zalophetrema* sp., *Hunterotrema caballeri*, *Braunina cordiformis* (Woodard et al., 1969; Ridgway and Dailey, 1972), or *Crassicauda* sp. (Harris, 1982), it is likely the eggs were from *Nasitrema* sp. (Howard et al., 1983).

Nine species of *Nasitrema* have been reported and all occur in the air sinuses or inner ear of odontocete cetaceans (Neiland et al., 1970). Members of the family Nasitrematidae have triangular eggs measuring approximately $50 \times 80 \mu\text{m}$ (Neiland et al., 1970). *Zalophetrema* sp. eggs are circular or oval in cross section and may be readily differentiated from the eggs noted in this case. The eggs of *Campula* sp. are very similar to those of *Nasitrema* sp. in tissue section; however, *Campula* sp. occurs in the liver and lesions were not present in this organ in the present case (Howard et al., 1983). Eggs from *Nasitrema* sp. have been recovered also from blowhole swabs of captive Atlantic bottlenosed dolphins (*Tursiops truncatus*) and associated with a bacterial and mycotic sinusitis (Wright et al., 1979).

Digeneans were not observed in the sinuses or ear canals of this animal and there was no indication of any damage in these areas. Little or no direct pathogenic effects are expected in the air sinuses of animals infected with *Nasitrema* sp. (Neiland et al., 1970) and it is assumed that the parasites had died some time before necropsy. Adult *Nasitrema* sp. and their eggs associated with similar pathological alterations in the brain have been reported in a wide variety of stranded cetaceans in southern California (Dailey and Walker, 1978). The brain lesions induced by these parasites were sufficient to interfere with echolocation, balance or orientation and could provide a partial explanation for single strandings of affected animals (Dailey and Walker, 1978). Although the life cycle for *Nasitrema* sp. is presently unknown, the brain lesions probably result from an aberrant migration of the parasite (Howard et al., 1983).

The behavioral abnormalities and disorientation in this animal reflected the lesions in the central nervous system. It is likely that these lesions were induced by infection with *Nasitrema* sp. Aberrant parasitic migration should be considered in the differential diagnosis of behavioral abnormalities, learning problems or disorientation in captive cetaceans.

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