Olfactory Ganglioneuroblastoma in a Dog:
A Light, Ultrastructural, and Immunohistochemical Study

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Key words: Dog; olfactory ganglioneuroblastoma; electron microscopy; immunohistochemistry.

Olfactory neuroblastomas are uncommon tumors of the nasal cavity that may arise from olfactory epithelium or cranial neural crest tissue.\(^1\) The considerable morphological heterogeneity of these tumors accounts for their many names, including esthesioneuroblastoma, esthesioneurocytoma, esthesioneuroepithelioma, olfactory esthesioneuroma, olfactory neuroendocrine carcinoma, and olfactory placode tumor.\(^2\)\(^,\)\(^3\)\(^,\)\(^5\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\) Spontaneous olfactory neuroblastomas have been reported in humans,\(^2\)\(^,\)\(^3\)\(^,\)\(^8\)\(^,\)\(^9\)\(^,\)\(^10\) dogs,\(^7\)\(^,\)\(^11\) cats,\(^1\)\(^,\)\(^9\)\(^,\)\(^10\) a horse,\(^6\) and a calf.\(^1\) Mature type C retroviral particles were within tumors from three cats seropositive for feline leukemia virus, implicating a retroviral etiology in this species.\(^9\) Olfactory neuroblastomas are morphologically and histochemically similar to neuroblastomas arising from the adrenal glands and sympathetic nervous system, but differ biologically.\(^2\)\(^,\)\(^5\)\(^,\)\(^8\)\(^,\)\(^10\) Whereas olfactory neuroblastomas occur most frequently in humans 10 to 34 and 51 to 60 years of age,\(^3\)\(^,\)\(^8\) sympathetic neuroblastomas invariably occur in children under 4 years of age.\(^3\)\(^,\)\(^8\) Sympathetic nerve cell tumors commonly display a continuous spectrum from undifferentiated neuroblastomas to well-differentiated ganglioneuromas, whereas ganglion cell differentiation in olfactory neuroblastoma has been described only twice in primary tumors and once in a metastatic focus in human cases.\(^3\)\(^,\)\(^8\)\(^,\)\(^10\) To our knowledge this is the first report of an olfactory neuroblastoma with ganglionic differentiation in a domestic animal.

A 15-year-old spayed female German shepherd mixed-breed dog was presented to the Glenn Dale (MD) Veterinary Clinic with a 7-month history of sneezing and mucoid nasal discharge. Alkaline phosphatase was mildly elevated (380 IU/liter; normal 70–265), and a nasal swab submitted for aerobic culture yielded no growth. The dog had severe epistaxis while hospitalized and was euthanatized at the owner's request. The owners declined a full necropsy, but permitted dissection of the nasal cavity via the hard palate, which revealed a bilateral, friable grey polypoid mass that effaced the caudal turbinates and nasal septum. The cribriform plate was intact. The mass was fixed in 10% neutral buffered formalin and submitted to the Department of Comparative Pathology, Walter Reed Army Institute of Research (Washington, DC) for histopathological examination.

Formalin-fixed tissue was routinely processed, embedded in paraffin, and sectioned at 5 μm. Sections were stained with hematoxylin and eosin, Luxol fast blue, Cresyl echt violet, Bodian, phosphotungstic acid-hematoxylin, and the periodic acid-Schiff reaction. The avidin-biotin complex method was applied to deparaffinized sections using mouse monoclonal antibody (MAb) to neuron specific enolase,\(^1\) anti-human neurofilament protein (reacts with the 200 kD and 70 kD subunits of neurofilament protein),\(^1\) mouse MAb anti-cytokeratin,\(^1\) and submitted to the Department of Comparative Pathology, Armed Forces Institute of Pathology, Washington, DC 20306-6000 (USA).
rabbit MAb anti-chromogranin A, 1:800 dilution (Dako Laboratories, Santa Barbara, CA); mouse MAb anti-synaptophysin, 1:40 dilution (Boeringer-Mannheim, Indianapolis, IN); and rabbit MAb anti-glial fibrillary acidic protein, 1:500 dilution (Vector Laboratories, Burlingame, CA). The secondary antibody was biotinylated mouse or rabbit IgG, and the detection system was Vectastain® Elite ABC kit (Vector Laboratories). The slides were counterstained with Mayer's hematoxylin. Tissue was post-fixed in 1% osmium tetroxide, embedded in Epon, and thin sections were stained with lead citrate and uranyl acetate.

The olfactory mucosa was elevated by an unencapsulated,
A, synaptophysin, and S-100. Rare spindle cells were interconnected by processes that separated cell bodies, and few desmosomes. Neuroblasts arranged in Flexner and Homer Wright rosettes, ganglion cells, and a fibrillar matrix that resembles glial tissue. Ultrastructurally, these tumors contain abundant tangled, undifferentiated, infiltrative mass composed of a pleomorphic cell population enmeshed in abundant vacuolated lightly eosinophilic fibrillar matrix (Fig. 1). Undifferentiated polygonal cells interpreted as neuroblasts had indistinct cell borders, scant fibrillar eosinophilic cytoplasm, and round-to-oval nuclei with one to two nucleoli and up to three mitoses per high-power field. These cells were arranged in nests, cords, palisades, Homer Wright rosettes (pseudorosettes), and Flexner rosettes (Fig. 2). Well-differentiated polyhedral ganglion cells were variably sized up to 50 μm, with abundant pink cytoplasm, Nissl substance, eccentric round vesicular nuclei, and a prominent central nucleolus (Fig. 3). There were multifocal areas of coagulation necrosis and hemorrhage, with infiltration by lymphocytes, plasma cells, and macrophages. Intersecting tangles of unmyelinated argyrophilic neuritic processes were seen in replicate sections silver impregnated by Bodian method and stained with Luxol fast blue. Ganglion cells had many mitochondria, abundant free ribosomes, few peripheral stacks of rough endoplasmic reticulum, and scattered 180- to 400-nm dense core granules (Fig. 4). Undifferentiated cells had elongated indented nuclei, scant cytoplasm with long terminal processes that separated cell bodies, and few desmosomes. Neuritic processes had many dense core granules and microfilaments, and few mitochondria and microtubules.

This neoplasm has many histological, ultrastructural, and immunohistochemical features in common with similar tumors reported in man and animals. Characteristic microscopic findings in ganglioneuroblastomas of man include neuroblasts arranged in Flexner and Homer Wright rosettes, ganglion cells, and a fibrillar matrix that resembles glial tissue. Ultrastructurally, these tumors contain abundant tangled, undifferentiated, infiltrative mass composed of a pleomorphic cell population enmeshed in abundant vacuolated lightly eosinophilic fibrillar matrix (Fig. 1). Undifferentiated polygonal cells interpreted as neuroblasts had indistinct cell borders, scant fibrillar eosinophilic cytoplasm, and round-to-oval nuclei with one to two nucleoli and up to three mitoses per high-power field. These cells were arranged in nests, cords, palisades, Homer Wright rosettes (pseudorosettes), and Flexner rosettes (Fig. 2). Well-differentiated polyhedral ganglion cells were variably sized up to 50 μm, with abundant pink cytoplasm, Nissl substance, eccentric round vesicular nuclei, and a prominent central nucleolus (Fig. 3). There were multifocal areas of coagulation necrosis and hemorrhage, with infiltration by lymphocytes, plasma cells, and macrophages. Intersecting tangles of unmyelinated argyrophilic neuritic processes were seen in replicate sections silver impregnated by Bodian method and stained with Luxol fast blue. Ganglion cells had many mitochondria, abundant free ribosomes, few peripheral stacks of rough endoplasmic reticulum, and scattered 180- to 400-nm dense core granules (Fig. 4). Undifferentiated cells had elongated indented nuclei, scant cytoplasm with long terminal processes that separated cell bodies, and few desmosomes. Neuritic processes had many dense core granules and microfilaments, and few mitochondria and microtubules.

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Peripheral Neuropathy in Twin Calves

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Key words: Peripheral neuropathy; distal axonopathy; dying-back process; calves.

Disorders of the peripheral nerves of domestic animals have been recorded in a number of dog breeds\(^1\)^—\(^7\) and in a few cases of ruminants.\(^8\) However, the majority have been of unknown etiology.\(^1\)^—\(^2\)\(^,\)\(^6\)\(^,\)\(^8\)\(^,\)\(^11\)

Distal symmetrical polyneuropathies have been recognized in genetic, toxic, nutritional, and metabolic disorders in humans\(^1\)^ and other animals.\(^9\) The disease is attributed to the dying-back process, the concept of which is that distal regions of nerve fibers are affected primarily in case of the neuronal cyton’s trophic function impaired because the axon is dependent on the trophic influence of the nerve cell.\(^10\)

This article describes morphological findings in twin calves that were affected with sporadically occurring distal symmetrical polyneuropathies.

Animals used for this investigation were two male 5-month-old (No. 1) and 6-month-old (No. 2) Holsteins that were twins. A male 5-month-old Holstein without clinical neuromuscular disease was used for the control.

Both cases were euthanized. Tissue blocks collected from the skeletal muscles of the whole body, the central and peripheral nervous systems, and visceral organs were fixed in 10% phosphate-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE), and reacted for routine (pH 9.4) adenosine triphosphatase (ATPase) and modified (pH 4.3) ATPase.

The peripheral nerves (\(P.\) brachialis, \(N.\) medianus, \(N.\) ulnaris, \(Nn.\) sacrales, \(N.\) ischiadicus, and \(N.\) tibialis) were immersion fixed in 4% paraformaldehyde in 0.5% phosphate buffer and post-fixed 1% osmium tetroxide. They were dehydrated and embedded in epoxy resin. The semithin sections (1 \(\mu\)m) were stained with toluidine blue. Teased fiber preparations of the peripheral nerves were also made.

For histochemical examination, unfixed muscular tissues collected from calf No. 2 (Table 1) were divided into approximately 0.5 cm cubes and immersed in liquid nitrogen (\(-180\) °C) for 15 seconds. Serial sections were cut transversely at 10 \(\mu\)m with a cryostat microtome at \(-20\) °C, stained with HE, and reacted for routine (pH 9.4) adenosine triphosphatase (ATPase) and modified (pH 4.3) ATPase.

Clinically, calf No. 1 showed abnormal gait with slow walking at 4 months of age. As the condition advanced, calf No. 1 presented progressive muscular weakness of both fore and hind legs, and dysstasia 3 days before necropsy. Calf No. 2 revealed protrusion of fetlock joints in both hind legs at 5 months of age. As the course was steadily progressive, calf No. 2 showed ataxia due to the motor disorder in the legs and inability to stand the day before necropsy (Fig. 1).

According to the owner, these calves were raised by suckling for 3 weeks after birth and given commercial calf-pellets substituted for milk and dried grass during the following days. Other calves reared on the same food were clinically normal. Although their dam was a multiparous cow, these two calves were the first offspring provided with artificial insemination by the sire. This sire was evaluated as excellent and utilized from the sire. This sire was evaluated as excellent and utilized.