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. 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. Spontaneous olfactory neuroblastomas have been reported in humans, dogs, cats, a horse and a calf. Mature type C retroviral particles were within tumors from three cats seropositive for feline leukemia virus, implicating a retroviral etiology in this species. Olfactory neuroblastomas are morphologically and histochemically similar to neuroblastomas arising from the adrenal glands and sympathetic nervous system, but differ biologically. Whereas olfactory neuroblastomas occur most frequently in humans 10 to 34 and 51 to 60 years of age, sympathetic neuroblastomas invariably occur in children under 4 years of age. 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. To our knowledge this is the first report of 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 cribriform plate while hospitalized and was euthanatized at the owner's request. 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, Lusol 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, anti-human neurofilament protein (reacts with the 200 kD and 70 kD subunits of neurofilament protein), rabbit polyclonal anti-S-100 protein, mouse MAb anti-cytokeratin, mouse MAb anti-neuron-specific enolase, rabbit polyclonal anti-S-100 protein, mouse MAb anti-fibroblast growth factor receptor 1 (FGFR1), mouse MAb anti-fibroblast growth factor receptor 2 (FGFR2), and rabbit polyclonal anti-fibroblast growth factor receptor 3 (FGFR3).
rabbit MAb anti-chromogranin A, 1:800 dilution (Dako Laboratories, Santa Barbara, CA); mouse MAb anti-synaptophylin, 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 im-
poorly demarcated, infiltrative mass composed of a pleo-
morphic cell population enmeshed in abundant vacuolated
lightly eosinophilic fibrillar matrix (Fig. 1). Undifferentiated
polyonal cells interpreted as neuroblasts had indistinct bor-
ders, scant fibrillar eosinophilic cytoplasm, and round-to-
well-differentiated polyhedral ganglion
densified neurosecretory granules (Fig. 4). Undifferentiated cells had elongated
bodies, and few desmosomes. Neu-
ritic processes had many dense core granules and microfi-

This neoplasm has many histological, ultrastructural, and
immunohistochemical features in common with similar tu-
mors reported in man and animals. Characteristic micro-
sopicroscopic findings in ganglioneuroblastomas of man include
neuroblasts arranged in Flexner and Homer Wright rosettes,
ganglion cells, and a fibrillar matrix that resembles glial tis-
sue. Ultrastructurally these tumors contain abundant tan-
gled unmeyelinarted neuritic processes with 100- to 350-μm
neurosecretory granules.

Sympathetic and olfactory neuroblastomas and gangli-
oneuroblastomas of man and animals are immunopositive
for a variety of neural, neuroendocrine, and glial markers. One olfactory ganglioneuroblastoma in which immuno-
chemistry was performed was positive for neurofilament protein using rabbit antibody reactive to all three polypeptide
components of human neurofilaments. The most consis-
tently positive reaction in human olfactory neuroblastomas is that for neuron-specific enolase, whereas tumors are less
often immunopositive for neurofilament protein. S-100, chromogranin, glial fibrillary acidic protein, and cytoker-
tin. Sympathetic neuroblastomas and ganglioneuroblas-
tomas of man are immunopositive for neurofilament protein,
neuron-specific enolase, the microtubule-associated proteins
MAP-2 and tau-protein, S-100, synaptophysin, and chromo-
granin A. Some ganglioneuroblastomas are additionally im-
munopositive for glial fibrillary acidic protein and myelin
basic protein (MBP).

The likely origin of olfactory neuroblastoma is the basal
cell layer of the olfactory epithelium. This layer is com-
posed of bipotential stem cells that proliferate and differ-
entiate into sustentacular cells and neuroepithelial bipolar
sensory cells throughout the life of the animal. Some olfactory neuroblastomas contain foci of squamous differ-
entiation and rosettes that are immunoreactive for cytoker-
atin, reflecting the bipotentiality of the cell of origin. In contrast to previous reports of olfactory neuroblastoma in
domestic animals and the vast majority of human cases, this
tumor had diffuse ganglion cell differentiation and is best
described as an olfactory ganglioneuroblastoma.

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

Distal symmetrical polyneuropathies have been recognized in genetical, toxic, nutritional, and metabolic disorders in humans\textsuperscript{15} and other animals.\textsuperscript{13} 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.\textsuperscript{13}

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.

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 showed no abnormalities as the predominant signs of neoplasia of the nasal cavity in dogs and cats: seven cases (1973-1986). J Am Vet Med Assoc 195:242-245, 1989

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Peripheral Neuropathy in Twin Calves

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