Iniencephaly and Other Neural Tube Defects in a Litter of Ferrets (*Mustela putorius furo*)

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Key words: Ferret; neural tube defect; iniencephaly.

Iniencephaly is a rare form of neural tube defect (NTD) that has been documented in human beings and rats, but not in the European (domestic) ferret (*Mustela putorius furo*). The term iniencephaly, derived from the Greek word "inion," meaning "the nape of the neck," is applied to a syndrome characterized by the following features: 1) deficiency of the occiput, 2) rachischisis (congenital fissure of the spinal column) of variable degree, and 3) cervical retroflexion. In this report, we describe a litter of ferrets with multisystemic congenital malformations including iniencephaly.

A litter of four ferret kits was presented to a veterinary practitioner following a term delivery. Three of the four were stillborn; the fourth died within minutes of delivery. The dam was an albino ferret, approximately 2.5 years old, and had given birth to two previous litters; these kits had no abnormalities. The sire of both litters was a 3-year-old “black-eyed white” ferret, a color-diluted strain with whitish-grey fur and dark eyes. This litter was the second litter born at this breeding operation with similar gross malformations; although the dams were different, the same sire was responsible for both litters. The first litter did not survive and were not available for examination.

All fetuses were grossly abnormal. There was marked variation in nose-to-tail length that corresponded to the degree of dorsal cervical retroflexion in two fetuses and apparent intrauterine growth retardation in a third (Fig. 1). Two fetuses were iniencephalic with anencephaly (absence of cranial vault and cerebral hemispheres) (one shown in Fig. 2); a third had anencephaly and an omphalocele (Fig. 1, far right). Radiographic examination demonstrated marked cervical vertebral malformation with dorsal retroflexion in the two fetuses. Three fetuses had varying degrees of palatoschisis; two of the fetuses had unilateral or bilateral cheiloschisis. The single fetus without a skull defect exhibited hydrocephalus.

Renal abnormalities were observed in several of the kits. One kit (No. 1) had unilateral renal agenesis, while the contralateral kidney was grossly cystic due to extreme hydronephrosis. One iniencephalic kit (No. 2) had bilateral hydronephrosis. Additional gross defects in the two iniencephalic fetuses (Nos. 2 and 3) included low-set eyes, low-set or absent ears, and protruding tongues.

Histologic examination of the three anencephalic fetuses (Nos. 2–4) revealed an absence of cerebral tissue, with numerous prominent congested meningeal vessels (cerebrovasculara). Fusion of multiple cervical and lumbar vertebral bodies was observed in one of the iniencephalic fetuses (No. 2) that had been sectioned longitudinally in toto (Fig. 3).

Iniencephaly has rarely been reported in animal species and only under experimental conditions. This paper represents the first report of spontaneous iniencephaly in domestic animals. There is no information on the genetic causes of this disorder in animals in the current literature. A significant correlation between number of previous litters and congenital malformations has been reported in ferrets, with cranioschisis occurring most often in kits delivered by female ferrets with two previous litters, as seen in this case. Iniencephaly has been experimentally induced in rats with the administration of the antineoplastic agents streptonigrin and vinblastine, as well as with triparanol, a cholesterol reducing agent. Various combinations of rachischisis, anencephaly, and cleft palate have been observed in numerous animal species following administration of Vitamin A (retinoic acid).

NTD in man includes three general categories: spina bifida, anencephaly, and encephalocele (listed in decreasing order of frequency). NTD are occasionally found in conjunction with specific genetic syndromes, but most are thought to be the result of a combination of genetic and environmental factors. The abnormality in all NTD is a failure of closure of the neural groove or a part of its epithelial or bony coverings. In general, events occurring earlier in gestation result in open defects (resulting in the exposure of neuroepithelium to amniotic fluid), whereas later events result in closed defects.
Fig. 2. Iniencephalic kit (No. 2) with craniorachischisis and exposure of the spinal canal.

(covered by skin). Rarely, re-epithelization of an open defect may give the false appearance of a closed defect.

Craniorachischisis is a combination of an open defect of the spinal canal occurring in conjunction with a cranial defect. Iniencephaly may occur as an isolated defect or be found in combination with any of the NTD. NTD generally result from a precipitating event 17–30 days post-ovulation in human beings; when iniencephaly occurs alone, the precipitating event occurs later, approximately 30–60 days after ovulation. In the ferret, which has a normal gestation of 42 days, closure of the neural tube occurs from days 16–17 post-coitus. Thus, it is assumed that the defects seen in this litter of kits arose from fetal insult during this time period.

In human beings, it has been shown that the use of prenatal vitamins containing folic acid immediately preceding pregnancy or during the first 6 weeks of gestation results in a substantial reduction in NTD. On the other hand, NTD have been causally related to gestational administration of salicylates, tetracycline, and sulfonamides. There is an increased recurrence risk of NTD after one child with NTD. It has not been investigated whether this increased risk is limited to the female, to the male, or to their particular mating. The risk is generally attributed to the female, and this is where folic acid therapy has been directed. Interestingly, the malformations seen in this breeding operation were traceable to the same sire; however, the number of cases is too small to interpret this as significant.

NTD, especially craniorachischisis, have an increased association with unrelated congenital anomalies. This association was also observed in the litter of ferret kits. Omphalocele (failure of the gut to return to the abdominal cavity from the umbilical cord) in humans is usually sporadic, though it may be associated with chromosomal or genetic syndromes. Omphalocele is often associated with other malformations of the gastrointestinal system, malformations of the genitourinary system, congenital heart disease, and NTD. One fetus (No. 4) in this group exhibited both omphalocele and anencephaly.

A definitive cause was not determined for the defects seen in this litter. There was no history of these animals receiving xenobiotics other than a commercially available multivita-

min supplement (Probalance®), given to pregnant ferrets. Consanguinity could not be proven between the sire and the two dams that delivered these litters.

Based on the observation that two consecutive litters sired by the same stud were affected, we believe that this syndrome probably represents a genetic condition. Although environmental factors cannot be entirely eliminated, the lack of other abnormal litters within the breeding operation makes environmental factors alone an unlikely cause. The possibility of additional genotypic abnormalities associated with selective breeding for color dilution in the sire should also be considered. To date, no additional kits from this sire or any other mating pair at this facility have shown a similar anomaly.

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References

Brief Communications and Case Reports

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 in 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 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 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, 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-cytokeratin, mouse MAb anti-cytokeratin, and submitted to the Department of Comparative Pathology, Walter Reed Army Institute of Research (Washington, DC) for histopathological examination.