Case Report

Intracranial mature teratoma in a pet rabbit

Sara Degl'Innocenti[1] Claudia Salvadori[2] Andrea Pirone[2] Carlo Cantile[2*]

Abstract

A mature intracranial teratoma was identified in a 5-month-old pet rabbit showing a sudden onset of obtundation, recumbency, flaccid tetraplegia, and hyporeflexia. A space-occupying suprasellar, irregular cystic mass extending from the thalamus to the brainstem was observed at necropsy. Histologically, the tumor was composed of irregular, multilocular cysts lined by a thin wall and interposed tissue where a heterogeneous collection of structures including adipose and myxomatous tissues, cartilage, glandular and ductal structures, skeletal muscle, nervous tissue, nerve fascicles, sebaceous glands, and exocrine pancreatic cells were recognized. Immunohistochemistry showed labeling of the diverse tissues with cytokeratin, vimentin, smooth muscle actin, GFAP, NSE, and S-100.

Keywords: central nervous system, extragonadal germ cell tumor, immunohistochemistry, rabbit, teratoma

[1]*School of Veterinary Sciences, University of Bristol, Bristol BS40 5DU, UK*
[2]*Department of Veterinary Sciences, University of Pisa, Viale delle Piagge, 2, Pisa, Italy*
***Correspondence**: carlo.cantile@unipi.it* (C. Cantile)

Introduction

Teratomas are a subset of non-germinomatous germ cell tumors that are composed of tissues derived from at least two, but usually from all three germ cell layers (endoderm, ectoderm, mesoderm) and that are foreign to the organ in which they arise (Chénier *et al*., 1998). Gonadal and extragonadal teratomas have been described in both humans and animals (Gonzalez-Crussi, 1982; Ferraz *et al*., 2014; Headley *et al*., 2016). Extragonadal teratomas have features similar to gonadal teratomas and most often occur in the midline of the body (Gonzalez-Crussi, 1982; Ferraz *et al*., 2014).

Central nervous system (CNS) teratomas are very rare neoplasms in animals. Generally affecting young animals, intracranial teratomas have been described rats (Maekawa *et al*., 1989; Reindel *et al*., 1996), and birds (Jones, 1964; Homer and Riggs, 1991; Hooper, 2008; López and Múrcia, 2008). Single cases have been reported in a kitten (Chénier *et al*., 1998), dog (Patnaik and Nafe, 1980), alpaca (Hill and Mirams, 2008), and pampas deer (*Ozotoceros bezoarticus*) (Headley *et al*., 2016). In other animals, such as horse, ox, rabbit, and guinea pig, intracranial teratomas have been described in old references cited by Bishop (1978), unavailable for review, in which the diagnosis may not represent true teratomas. Intracranial teratomas mainly arise ventrally in paramidline or midline sites, prevalently in the pineal region, also involving the optic chiasm, the third ventricle, the hypothalamus, and the thalamus (Headley *et al*., 2016), or extending rostrally from the pyriform lobe and caudally to the pons (Patnaik and Nafe, 1980). Moreover, cases with involvement of the cerebellum (Reindel *et al*., 1996; López and Múrcia, 2008), left temporal dura mater (Hill and Mirams, 2008), cerebral hemisphere (Hooper, 2008), and parietal bone (Homer and Riggs, 1991) are reported. A teratoma associated with an intracranial dermoid cyst in a kitten (Chénier *et al*., 1998) and a multicentric teratoma in a lesser kestrel (*Falco naumanni*) (López and Múrcia, 2008) were also described. Dissemination of teratoma via the cerebrospinal fluid has been reported in a rat (Reindel *et al*., 1996).

Histologically, mature and immature teratomas have been distinguished according to the differentiation level of tissues (Lakhoo, 2010; Ferraz *et al*., 2014). Mature teratomas consist of mature elements such as skin, cartilage, or glands, whereas immature teratomas, also called teratocarcinomas, are composed of immature neuroepithelial and mesenchymal tissues (Patnaik and Nafe, 1980; Lakhoo, 2010).

Herein, we describe the neurologic and neuropathological findings of a congenital intracranial mature teratoma in a pet rabbit.

Case report

A 5-month-old mixed-breed black fur female rabbit was presented with a sudden onset of obtundation, lateral recumbency, and flaccid tetraplegia. The rabbit was regularly vaccinated (Nobivac® Myxo-RHD) and dewormed and no history of toxin exposure or drug treatment was reported by the owner. Physical examination revealed an adequate body score condition with normal breathing and heart rate, but the animal was completely unresponsive to external stimuli. Neurological examination showed hyporeflexia with bilateral absence of menace response and fixed dilated pupils. Neuroanatomic localization was consistent with an intracranial lesion involving forebrain and brainstem. The respiratory function progressively worsened, and the rabbit was intubated and manually ventilated for nearly an hour, but due to the poor prognosis, the owner elected euthanasia.

A complete necropsy was performed, and gross lesions were restricted to the brain, except for multifocal lung hemorrhages and severe diffuse multiorgan congestion. On the ventral and lateral aspect of the brain, a suprasellar space-occupying mass extending from the thalamus to the brainstem was observed. On transverse sections, a 20x16 mm, irregular cystic and solid mass, associated with marked compression of surrounding neuroparenchyma, mild ventricles dilation, and perilesional edema was detected. Moreover, multifocal black pigmentation of the leptomeninges, involving both the convexity of the cerebral hemispheres and cerebellum, was observed (Fig. 1). Representative samples of major organs and brain were fixed in 10% neutral-buffered formalin and routinely processed for histology. Five-micron sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Goldner's trichrome. Immunohistochemistry was performed using mouse monoclonal antibodies against cytokeratin (1:200; clones AE1/AE3, Dako), vimentin (1:300; clone V9, Dako UK Ltd., Ely, UK), smooth muscle actin (SMA, 1:500; clone 1A4, Dako), neuron-specific enolase (NSE, 1:100; clone BBS/NC/VI-H14, Dako), and rabbit polyclonal antibody against glial fibrillary acidic protein (GFAP, 1:1000; Dako), and S-100 (1:500; Dako). The EnVision Plus System-HRP (3,3'-diaminobenzidine, Dako) was used to detect antibody binding. Negative controls were obtained by omitting the primary antibody. Normal nervous tissue was used as internal positive control for neural cell markers and samples of rabbit intestine were used as positive control for the other markers.

Histologically, the neoplastic mass was almost completely well-demarcated and not encapsulated and was composed of irregular, sometimes multilocular cysts lined by a thin wall and interposed tissue. A heterogeneous collection of structures including adipose tissue, fibrillary and myxomatous connective tissue, glandular and ductal structures, skeletal muscle (Fig. 2), cartilage, nerve fascicles with myelinated fibers, and small and medium-sized blood vessels (Fig. 3) were recognized within the interposed tissue. A large portion of the mass was composed of mature nervous tissue with NSE-labeled recognizable neurons (Fig. 4), and GFAP-labeled glial cells (Fig. 5). Sebaceous and serous glands, and irregularly arranged acinar cells with strongly eosinophilic cytoplasm resembling exocrine pancreatic cells were also present. Two types of cysts were identified: cysts lined by squamous stratified epithelium, sometimes forming small papillae, and containing lamellar keratin in the lumen; and more numerous cysts lined by cuboidal ciliated pseudostratified epithelium with an empty lumen or containing amorphous weakly PAS-positive material or necrotic cellular debris. Numerous melanocytes and

melanophages, accompanied by leakage of melanin pigment were evident in the cyst walls and within interposed connective tissue, which was highlighted by Goldner's trichrome stain. In the leptomeninges, the pigment ranged in quantity from isolated granules to compacted brown to black deposits. The adjacent neural parenchyma was distorted and compressed showing marked microvacuolation and axonal degeneration. Histological examination of sample tissues of other organs was unremarkable, except for congestion of liver, kidneys, and lungs in which multifocal alveolar hemorrhage was also observed.

By immunohistochemistry, cyst wall epithelium was immunoreactive for cytokeratin (Fig. 6), and vimentin immunolabeled adipocytes, endothelial and mesenchymal cells (Fig. 7). SMA immunolabeled cells were evident in the cyst walls, in the interposed tissue, and around glandular structures. S-100 immunoreactivity was found in chondrocytes, adipose tissue, and nerve fascicles within the teratoma, as well as in leptomeningeal melanocytes.

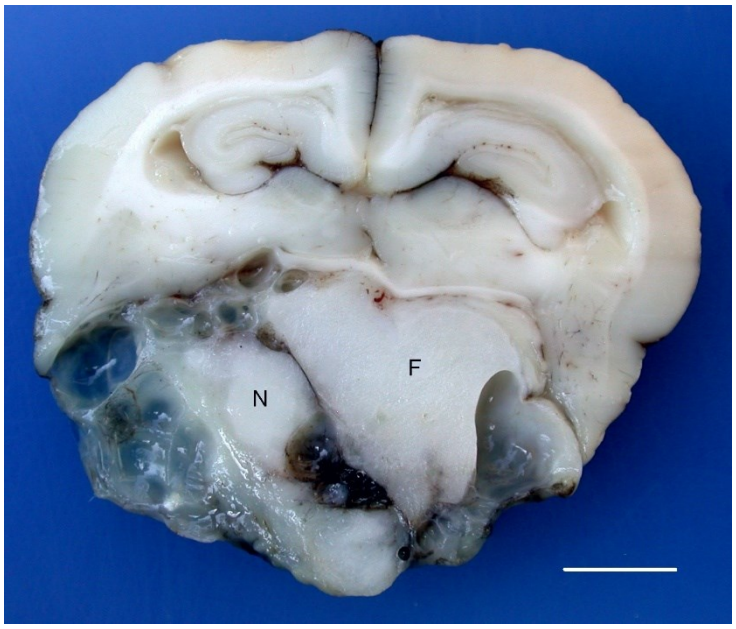

Figure 1 Transverse section of fixed brain at the level of the hippocampus. The large teratoma firmly adheres to and markedly compresses the ventral aspect of the brain. The tumor is composed of numerous cysts and solid areas of nervous (N), adipose (F), and mesenchymal tissues. Also note pigmentation of lining structures both in the tumor and in the brain (leptomeninges). Bar = 5 mm.

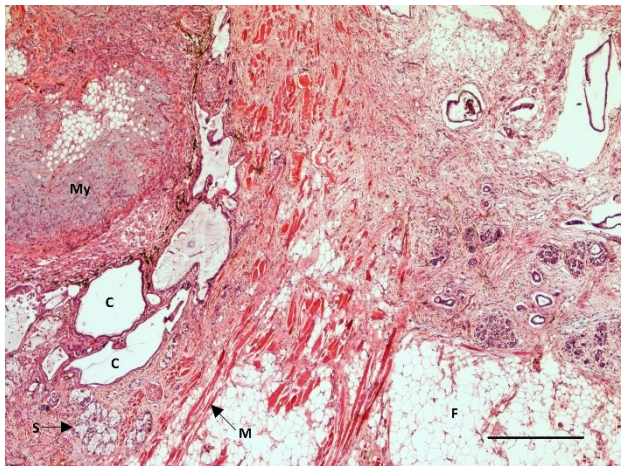

Figure 2 Section of teratoma composed of diverse mature tissues including multiple cysts (C), adipose tissue (F), bundles of muscle fibers (M), nests of sebaceous gland cells (S), and myxoid tissue (My). Melanin pigment is scattered within the connective tissue. (Hematoxylin and eosin, 40x; bar = 1000 µm).

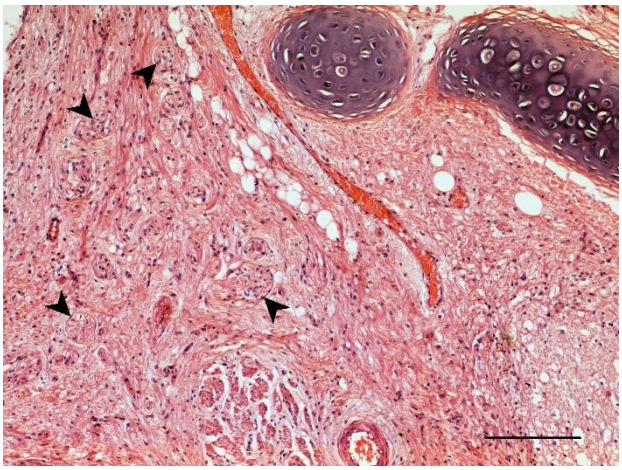

Figure 3 Mature cartilage, vascular structures, and nerve fascicles (arrowheads) are evident within the teratoma. (Hematoxylin and eosin, 100x; bar = 400 µm).

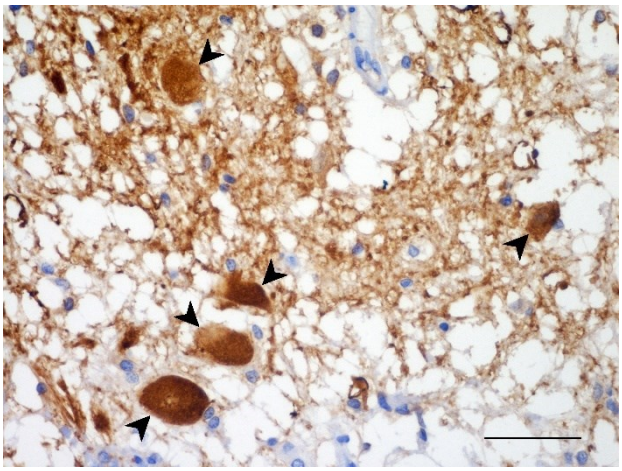

Figure 4 Mature neural cells (arrowheads) and processes scattered within the teratoma are labeled with NSE. (NSE immunohistochemistry, 400x; bar = 100 µm).

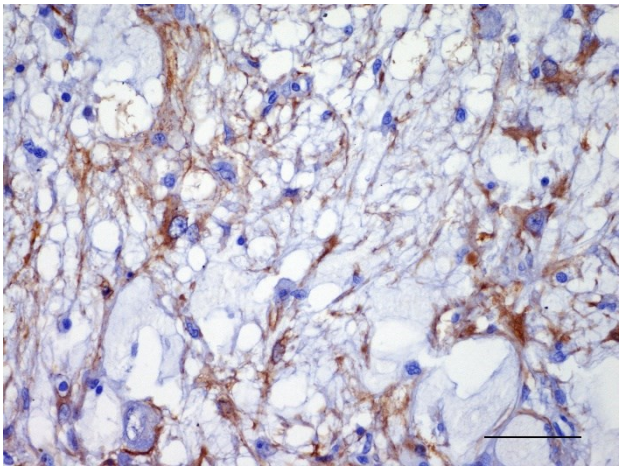

Figure 5 Glial fibrillary acidic protein labeled cells, interpreted as mature astrocytes, are intermingled within the teratomatous elements. (GFAP immunohistochemistry, 400x; bar = 100 µm).

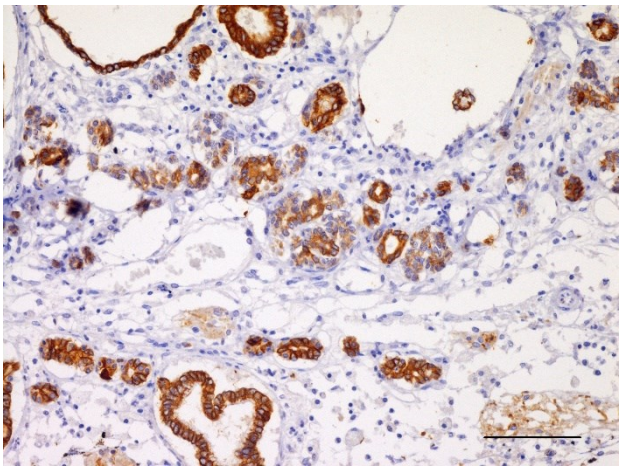

Figure 6 The cyst lining epithelium and the ductal epithelium is strongly immunolabeled with cytokeratin. (Cytokeratin immunohistochemistry, 100x; bar = 400 μm).

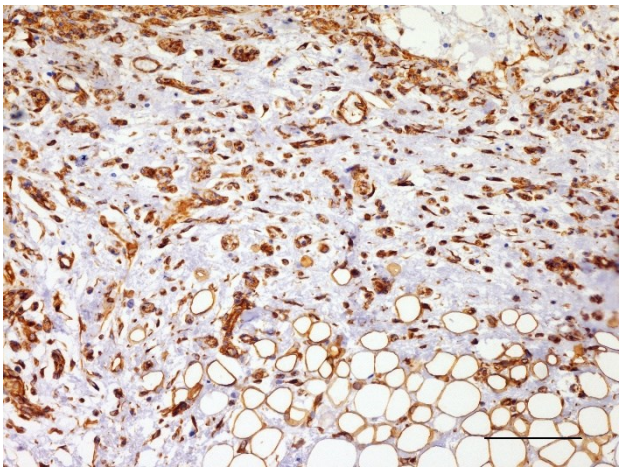

Figure 7 Adipocytes, endothelial cells, and scattered fibroblasts are labeled with vimentin. (Vimentin immunohistochemistry, 200x; bar = 200 μm).

Discussion

Macroscopic differential diagnoses included hamartoma, that can be defined as a disorganized overgrowth of tissues in their normal location, and choristoma. In this latter condition, the tissue derives from germ cell layers foreign to the involved anatomic structure. The histopathological and immunohistochemical findings in our case were consistent with a mature cystic teratoma since mature tissue elements originating from all three germinal layers were observed. Our findings are similar to those previously described in a teratoma originating in the midline of the preoptic area and the tuber cinereum of the hypothalamus in a juvenile New Zealand White rabbit (Bishop, 1978). In that case, solid areas and multiple cysts measuring up to 3.5 mm in diameter were observed and several types of tissue originating from all three germ cell layers, such as neural cells, respiratory and gastric mucosa, mucous and mucoserous glands, collagenous connective tissue, cartilage, and fat were described.

Although the progressive growth of teratoma, no neurological signs were evident during the first five months of life of the rabbit of our report. The increase of intracranial pressure eventually led to the development of neurological deficits that culminated in respiratory failure, extravasation hemorrhage of the lungs, and diffuse congestion, due to compression of brainstem respiratory and blood pressure centers. The localization and morphological features of the teratoma of our rabbit resembled human intracranial teratoma. Indeed, human primary intracranial teratomas are known to arise in the pineal and suprasellar region but also in the hypothalamus and, less frequently, in the region of the third ventricle and posterior cranial fossa. Teratomas with cystic and solid areas are commonly recognized (Gonzalez-Crussi, 1982).

Etiology and pathogenesis of intracranial teratomas are not completely clarified. Midline is a location with great potential for misplacement of multipotential germ cells. Two theories could explain the pathogenesis of germ cell tumors in the CNS. The germ cell theory hypothesizes that these tumors arise from primordial germ cells which have migrated aberrantly during embryogenesis, surviving to the physiologic dissolution, and later undergo malignant transformation. Conversely, the embryonic theory suggests that these tumors develop as a consequence of misfolding or misplacement of embryonic cells that could be carried at the base of the cerebrum by ectoderm migration (i.e. Rathke pouch) during embryogenesis of the head (Patnaik and Nafe, 1980; Chénier *et al*., 1998; Ferraz *et al*., 2014). In humans, teratoma does not represent a recognizable genetic

syndrome but could be related to chromosome 21 trisomy, Klinefelter syndrome or associated with Klippel-Feil syndrome that is due to congenital fusion of the cervical vertebrae (Gonzalez-Crussi, 1982; Ferraz *et al*., 2014). In veterinary medicine, more investigations could be worth to elucidate the etiology of this intracranial tumor as a potential model for the human counterpart.

The meningeal pigmentation found in this case could be correlated with the dark eyes, skin and black fur of the rabbit, as known in humans, where the color of eyes, skin, and leptomeninges are related (Aaron and Lerner, 1955). Meningeal melanin has also been described in cows and sheep in which the pigmentation intensity is greater than in other species (Innes and Saunders, 1962).

In conclusion, intracranial teratoma, although rare, should be considered in case of sudden onset of intracranial neurological signs in young rabbits.

Conflict of interest*:* authors declare that they have no conflicts of interest.

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