








1 **Case Report**

2
3 **Extragenital mature teratoma in a 6-months-old kitten**

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5 Rayane Chitolina Pupin^{1*}  (<https://orcid.org/0000-0002-9471-3639>), Jhony Ferreira Marcari² 
6 (<https://orcid.org/0009-0006-4407-138X>), Danilo Carloto Gomes¹  (<https://orcid.org/0000-0002-7128-8756>), Gabriela de Souza Silva^{2*} 
7 (<https://orcid.org/0009-0004-4777-1598>), Larissa Correa
8 Hermeto³  (<https://orcid.org/0000-0002-4123-3447>), Igor Ribeiro Santos⁴ 
9 (<https://orcid.org/0000-0002-4247-7478>), Saulo Petinatti Pavarini⁴ 
10 (8980-6294)

11
12 ¹ Laboratório de Anatomia Patológica, Faculdade de Medicina Veterinária e Zootecnia,
13 Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil

14 ² Programa de Residência Multiprofissional, Faculdade de Medicina Veterinária e Zootecnia,
15 Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil

16 ³ Setor de Clínica Cirúrgica de Pequenos Animais, Faculdade de Medicina Veterinária e Zootecnia,
17 Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil

18 ⁴ Setor de Patologia Veterinária, Faculdade de Veterinária, Universidade Federal do Rio Grande do
19 Sul, Porto Alegre, RS, Brazil

20
21 ***Corresponding author:** rayane.pupin@ufms.br

22
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24
25 **Abstract**

26 Germ cell tumors can be classified as teratoma, embryonal carcinoma, yolk sac carcinoma,
27 dysgerminomas and seminomas. Teratomas are composed by well-differentiated cells from at least
28 two of the three embryonic layers, and are more commonly in ovaries or testicles, but can occur
29 extragonadal. This case describes a subcutaneous mature teratoma in a 6-month-old kitten.
30 Macroscopically, the tumor was multilobulated and firm, measuring 11 x 6,5 x 5cm; on cut surface,
31 multiple cystic cavities, solid white areas and hard portions. Histologically, cells derived from the
32 three layers were present – hair follicles and glands, nervous tissue, odontogenic epithelium,
33 respiratory epithelium, and different mesenchymal cells (bone, adipocytes, muscle fibers and fibrous
34 tissue). Immunolabeling for pancytokeratin, vimentin, GFAP and p40 were strong in the different
35 types of cells, and no immature tissue was seen. So, the tumor was classified as a mature extragonadal
36 teratoma. The mass was completely excised and no recurrence was seen.

37 **Keywords:** cat, cutaneous tumors, extragonadal teratoma germ cell tumors.

38

39 **Introduction**

40

41 Germ cell tumors (GCT) are those derived from the cells that give rise to sperm and eggs (5),
42 and are more commonly found in the ovaries or testicles. They are classified as teratoma, embryonal
43 carcinoma, yolk sac carcinoma, dysgerminomas (ovary) and seminomas (testicles) (1).

44 Extragonadal germ cell tumors occur outside the gonads, without a primary gonadal tumor (16).
45 The etiopathogenesis is not clearly understood, but it is believed that a failure occurs during the
46 migration of the germ cells to the gonads (13, 16, 18). Since this migration occurs through the midline
47 of the body, these tumors can occur anywhere – from the brain to the coccyx - but are more common
48 in the mediastinum, retroperitoneum, and brain (16).

49 Teratomas are composed of neoplastic cells from at least two of the three embryonic layers.
50 Most are classified as mature, have benign behavior, and show well-differentiated tissues (1, 2, 19).

51 Immature teratomas can be malignant, potentially metastatic, and show less-differentiated tissues (1,
52 2, 9, 15).

53 In cats, teratomas have been described affecting the ovaries (2, 9, 17), testicles (10), skin and
54 subcutaneous tissues (7, 14, 18, 19), retrobulbar region (20), oral cavity (12) and intracranially (4).
55 They have also been described in dogs (15, 21), cattle (3), horses (8), sheep (13), and birds (6, 11).

56 The aim of this paper is to describe the histological and immunohistochemical aspects of an
57 extragonadal mature teratoma in a 6-month-old kitten affecting skin and subcutaneous tissues of the
58 periauricular region.

59

60 **Case description**

61

62 A 3-month-old kitten was presented for veterinary evaluation due to a periauricular nodule
63 measuring 7 x 5 cm, with progressive growth. The mass was firm, irregular, and non-painful (Fig.
64 1A). The animal underwent a computed tomography (CT) scan, which revealed cystic and
65 mineralized areas within the mass. Cytology was performed, and the diagnosis was squamous cell
66 carcinoma. Surgery was recommended but the owners did not agree to do it. A punch biopsy was
67 performed and sent to histopathological evaluation, and the diagnosis was undifferentiated sarcoma.
68 After three months, the kitten showed reduced nasal sensitivity and decreased menace reflex on the
69 right side. Due to the increase in the size of the mass (Fig. 1B), the owners agreed to perform surgery.
70 Preoperative examinations showed no evidence of metastasis. Given the cytological and
71 histopathological diagnosis, the mass was completely removed and electrochemotherapy was
72 performed perioperatively.

73 Macroscopically, the tumor measured 11 x 6,5 x 5cm, was multilobulated and firm; on cut
74 surface, multiples cystic cavities filled with clear, viscous content were observed, intermixed with
75 solid white areas and hard portions. In one of the sections, hair shafts were visible (Fig. 2).

76 Histologically, the mass contained a heterogenous cellular population, well-differentiated,
77 originating from the three embryonic layers. The predominant component was ectodermal – well
78 differentiated hair follicles, occasionally hyperplastic, surrounded by sebaceous and apocrine glands
79 (Fig. 3A). Multiple foci of nervous tissue, composed of neuropil, neurons, ganglia, and nerves were
80 also present (Fig. 3B-3C). Odontogenic epithelium forming multiple nests (Fig. 3D). From the
81 endoderm, respiratory epithelium was present, sometimes surrounded by cartilage and bronchial
82 glands (Fig. 3A). From the mesoderm, mesenchymal cells of various types were observed –
83 adipocytes (Fig. 3B), bone tissue (Fig. 3C), muscle fibers, and fibrous tissue. Based on these
84 histopathological findings, a diagnosis of extragonadal mature teratoma was made.

85 Serial sections of the tumor were submitted to immunohistochemistry (IHC) for pancytokeratin
86 (panCK), vimentin, p40, and glial fibrillary acidic protein (GFAP). Details of the antibodies, antigen
87 retrievals protocols, and positive controls are provided in Table 1. For all assays, NovoLink HRP-
88 Polymer Detection System (Leica, <https://www.leica-microsystems.com>) was used as detection
89 system, Romulin AEC (Biocare Medical, <https://biocare.net>) as chromogen, and hematoxylin as
90 counterstain. As negative control, sections were incubated with Polymer Negative Control Serum
91 (BioCare Medical).

92 There was strong cytoplasmic staining for pancytokeratin in the squamous stratified epithelium
93 of the hair follicles (Fig. 5A), pseudostratified columnar epithelium of bronchial tree (Fig. 5B),
94 odontogenic epithelium (Fig. 5C), and glandular epithelium (apocrine, sebaceous, and bronchial
95 glands) (Fig. 5A-5B). Strong cytoplasmic staining for vimentin was observed in fusiform
96 mesenchymal cells, adipose tissue (Fig. 5D), cartilage (Fig. 5E), muscle fibers, endothelial cells,
97 nerves (Fig. 5F), osseous tissue (Fig. 5G) and fibrous tissue. There was strong nuclear
98 immunolabeling for GFAP in astrocytes (Fig. 5H-5I) Additionally, positive nuclear
99 immunoexpression of p40 in basal squamous cells of hair follicles (Fig. 6A – arrow), germ cells of
100 sebaceous glands (Fig. 6A – arrow heads), basal cells of the bronchial tree (Fig. 6B) and odontogenic
101 epithelium (Fig. 6C), and myoepithelial cells surrounding tracheobronchial glands.

102 Three months after surgery, there was already growth of defenses around the scar, as expected,
103 the patient lost the right eyelid reflex, but had normal corneal reflex and facial movements.

104

105 **Discussion**

106

107 The diagnosis was based on histopathological findings, which were confirmed by the
108 immunohistochemistry. The neoplasm was classified as a mature teratoma because it contained well-
109 differentiated cells from all the three embryonal layers, with no histological evidence of immature
110 cells. Furthermore, it was a well-demarcated mass without evidence of adjacent tissue invasion (2,
111 20).

112 At the time of the surgery, the kitten was 6-month-old, but by-month-old, the mass had already
113 reached a significant size. Teratomas in cats less than 1 year old have been reported in the gonads (2),
114 brain (4), skin and subcutaneous tissues (7, 14, 18, 19), and oral cavity (12). The occurrence of such
115 tumors in young animals or shortly after puberty supports the hypothesis of fetal development of the
116 neoplasm (1, 3). Although more common in young animals, teratomas have also been described in
117 cats between 2 and 17 years (9, 10, 17, 20), but in most of these cases, the masses were intra-
118 abdominal (affecting ovary or a cryptorchid testicle), which may result in a later diagnosis, as
119 extragonadal tumors are more easily noticed by owners (19).

120 Macroscopically, the tumor in this case resembled those described in dog, cats and cattle – a
121 multilobulated mass with both solid and cystic areas, sometimes containing hair follicles and keratin
122 (2, 3, 7, 9, 14, 18, 20, 21). Microscopic findings in teratomas can vary widely because cells from two
123 or three embryonal layers may be present (1, 7, 18). In this case, well-differentiated cells from all
124 three layers were observed, with ectodermal component being the most predominant (hair follicles,
125 sebaceous and apocrine glands, odontogenic epithelium, neural tissue), followed by mesodermal cells
126 (adipose tissue, fibrous tissue, bone, muscle fibers and cartilage), and only respiratory epithelium
127 from the endoderm was observed.

128 The surgical excision is curative in cases of mature teratoma (18) and even in those with
129 immature components (7). Most teratomas exhibit benign behavior, are well-demarcated, and do not
130 invade adjacent tissues, growing in an expansive manner, which make the clinical signs noticeable
131 only when the mass reaches a sufficient size to cause compression (2, 21). This can occur earlier
132 depending on the location, for example, if the tumor develops in the brain (4). In some cases,
133 especially in ovaries or in livestock teratomas, the tumor may be an incidental finding during
134 reproductive evaluations (3, 8). In this animal, the tumor was not attached to adjacent tissues, and the
135 clinical signs became evident only when the tumor reached a large size. Following removal, there
136 was no recurrence. Due to injury to the facial nerve, the palpebral reflex was absent, but the nictitating
137 membrane performed a protective function. The prescription of sodium hyaluronate 0.1% eye drops
138 thrice a day aimed to protect the cornea. In a similar case, facial nerve injury resulted in the loss of
139 palpebral reflex and the ability to blink (19).

140 In this case, the cytological diagnosis was squamous cell carcinoma. Although cytology is a
141 common diagnostic tool for tumors, it can be inconclusive in cases of teratomas due to the cellular
142 diversity present (14, 19). Histopathological examination is recommended for a definitive diagnosis;
143 however, immature portions can be sometimes missed if only hematoxylin and eosin staining is used,
144 so immunohistochemistry is helpful in identifying immature cells (7, 18). The immunolabeling in this
145 case revealed only mature tissues.

146 Extragenital teratoma should be considered as a differential diagnosis for cutaneous tumors in
147 kittens under 1 year old. Histopathological analysis is usually conclusive, but immunohistochemistry
148 allows for the identification of cellular origin and the detection of immature cells.

149

150 **Data Availability**

151 All the original contributions presented in this study are included in the article/supplementary
152 material. Further inquiries can be directed to the corresponding author.

153

154 **Author Contributions**

155 **Rayane Chitolina Pupin:** Investigation, writing – original draft, writing – review and editing.

156 **Jhony Ferreira Marcari:** investigation, writing – review and editing. **Danilo Carloto Gomes**

157 Investigation, writing – review and editing. **Gabriela de Souza Silva:** investigation, writing – review

158 and editing. **Larissa Correa Hermeto:** investigation, writing – review and editing. **Igor Ribeiro**

159 **Santos:** investigation, resources. **Saulo Petinatti Pavarini:** investigation, resources. All authors have

160 read and approved the final version of the manuscript.

161

162 **Conflict of Interest**

163 The authors declare no competing interests.

164

165 **Generative AI Use Statement**

166 The authors did not use generative artificial intelligence tools or technologies in creating or

167 editing any part of this manuscript.

168

169 **References**

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221 and nonsuppurative inflammation in an old dog. *J Vet Med Sci.* 2004; 66(7): 861-864. doi:
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223

224 **Table**

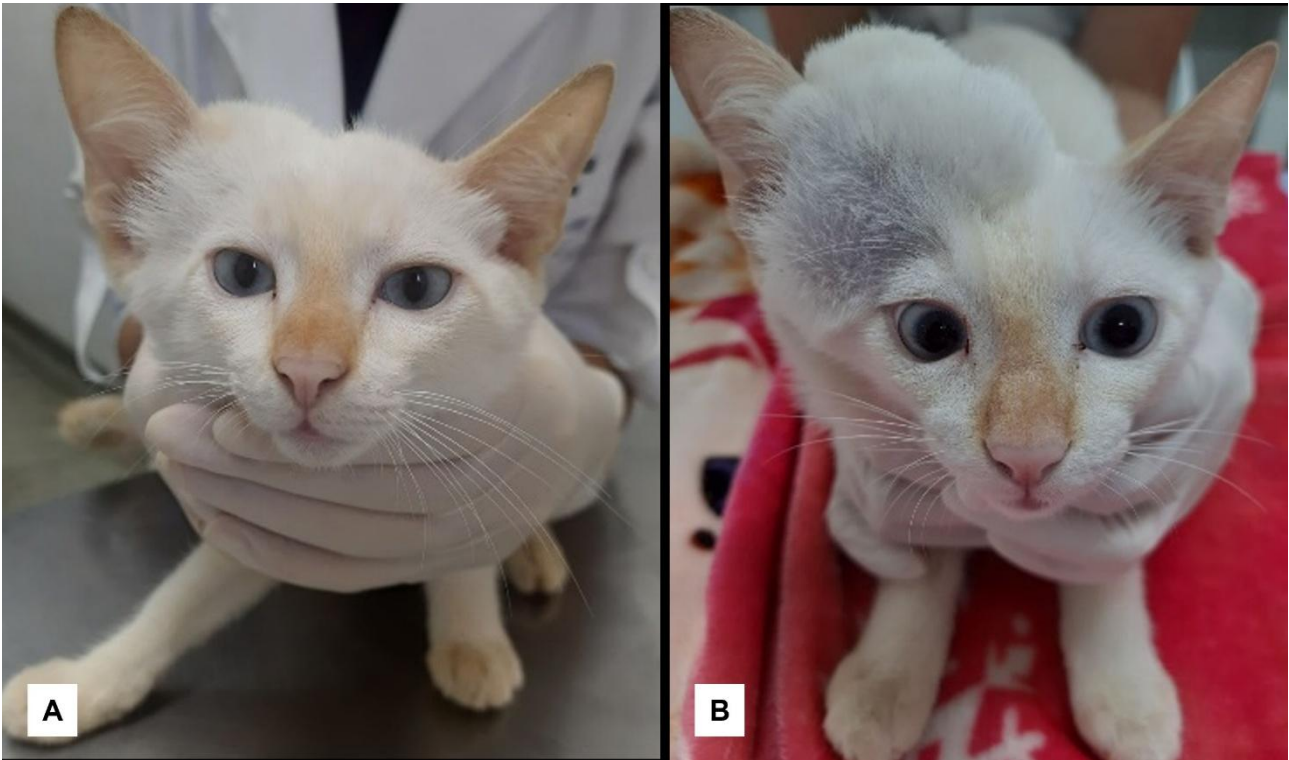
225

226 **Table 1.** Details of the antibodies, antigen retrievals, and positive controls used in
227 immunohistochemistry.

Antibody	Antigen retrieval	Positive control
Pancytokeratin (mouse monoclonal, AE1/AE3, ready-to-use, Dako, www.agilent.com)	Citrate buffer, pH6.0 (40min/96°C)	Canine skin
Vimentin (mouse monoclonal, V9, 1:200 dilution, Invitrogen, www.thermofisher.com)	Citrate buffer, pH6.0 (40min/96°C)	Canine skin
p40 (mouse monoclonal, clone BC28, 1:300 dilution, Biocare Medical, https://biocare.net)	Citrate buffer, pH6.0 (40min/96°C)	Canine skin
Glial fibrillary acidic protein (polyclonal rabbit, 1:500 dilution, Dako, www.agilent.com)	Tris EDTA buffer (10min/100°C)	Canine brain

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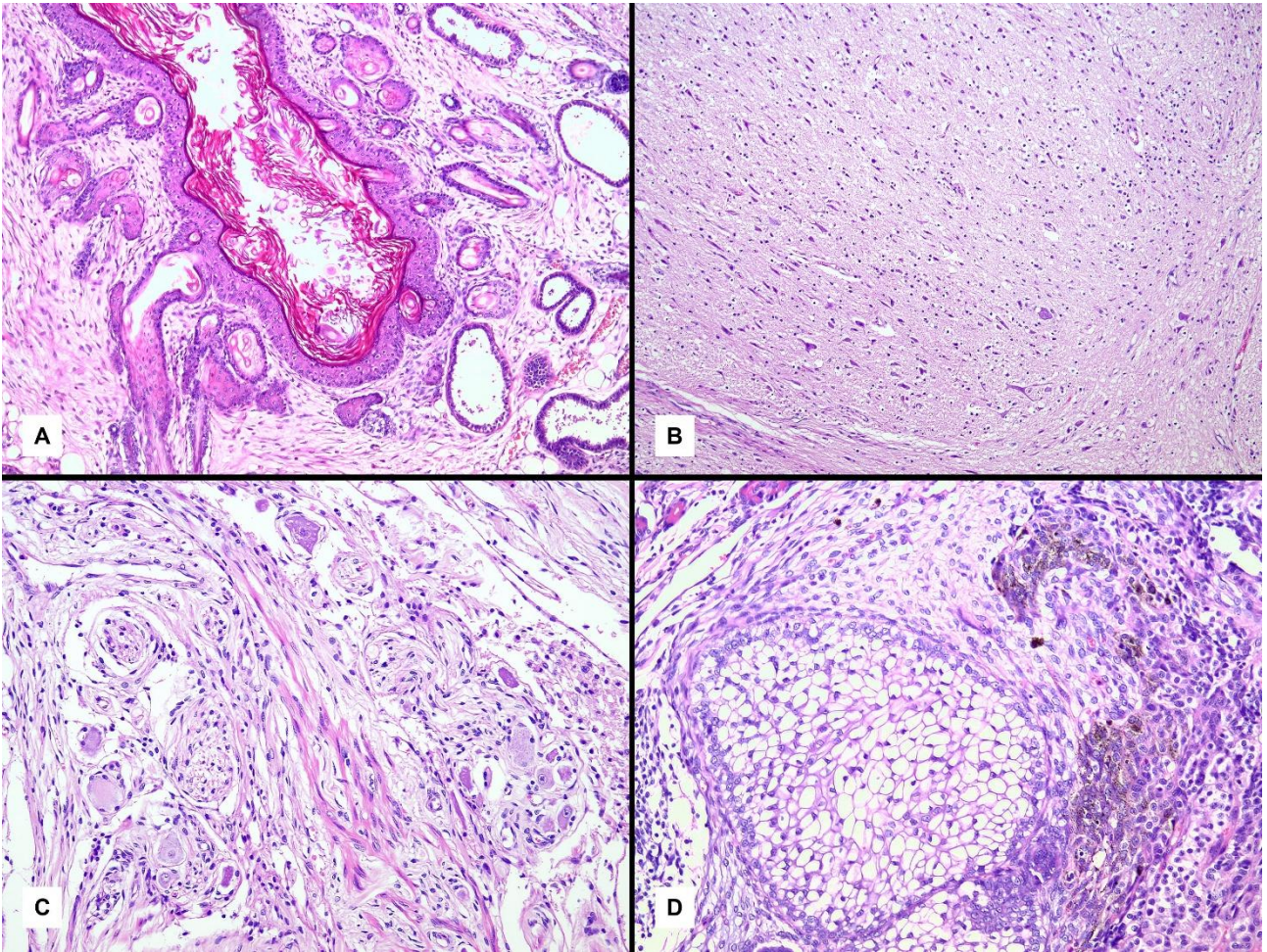
229



233 **Figure 1.** (a) Three-month-old cat, note the tumor located in the right pre-retroauricular region
234 measuring 7 x 5 cm. (b) After 3 months of evolution, a significant increase in mass can be noted.



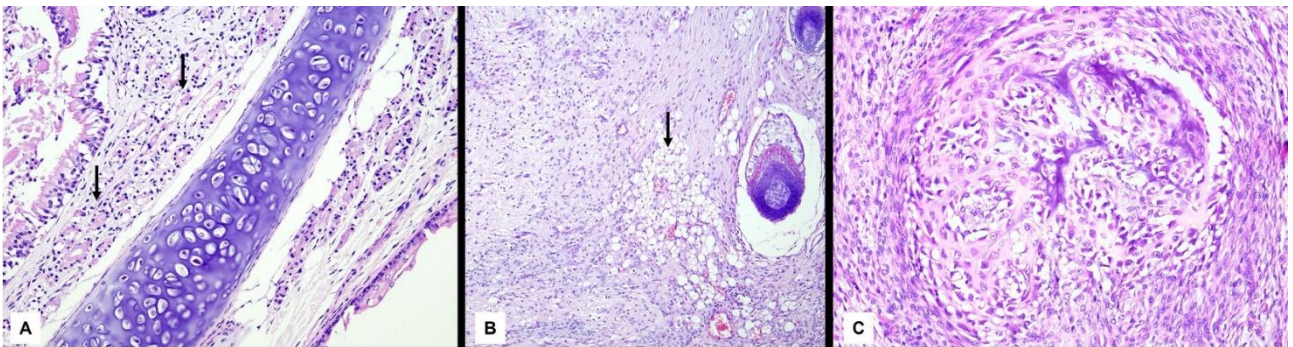
237 **Figure 2.** Extragonadal teratoma in a kitten. (A) The mass is multilobulated, firm, in the periauricular
238 cutaneous tissue. (B) On cut section, there are cystic and solid areas. *Inset:* hair shafts can be seen.



240

241 **Figure 3.** Extragonadal teratoma in a kitten. Histopathological findings – tissues originating from
 242 ectodermal embryonic layer. (A) Squamous cell epithelium forming hair follicles, surrounded by
 243 apocrine and sebaceous glands. (B-C) Well-differentiated nervous tissue, with neuropil, neurons,
 244 ganglia, and nerve fibers. (D) Island of odontogenic epithelium.

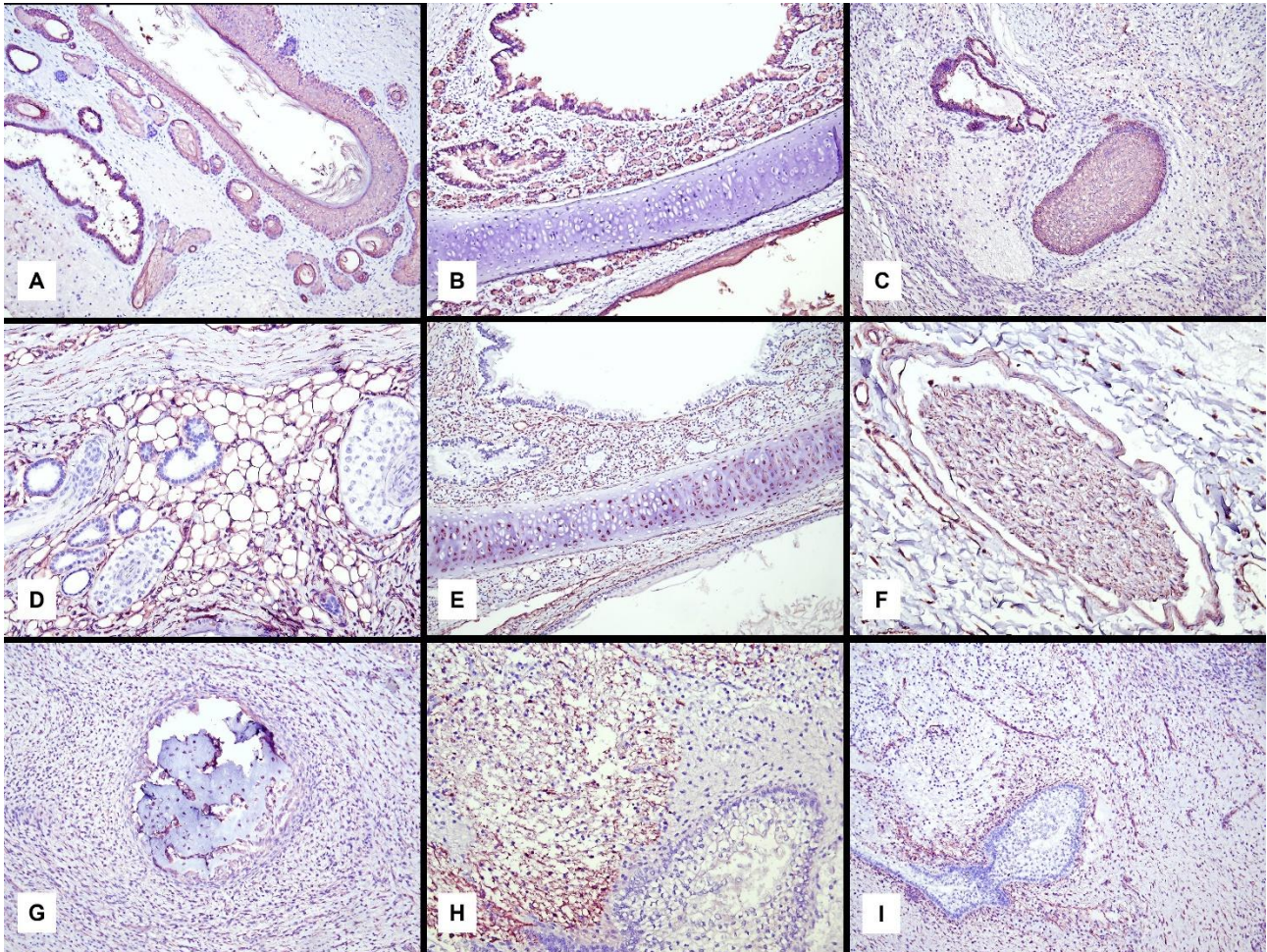
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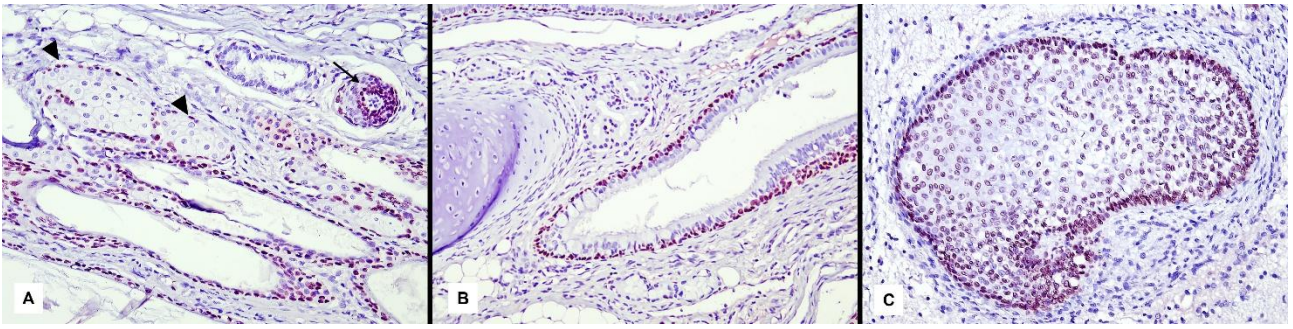
247 **Figure 4.** Extragonadal mature teratoma in a kitten. Histopathological findings. (A) From the

248 mesodermal layer, respiratory epithelium surrounded by bronchial glands (arrow) and cartilage. (B)
249 Adipose tissue (arrow) intermixed with a hair follicle (right side) and nervous tissue (left side). (C)
250 From the endodermal layer, osseous tissue with mineralization (arrow).
251



252
253 **Figure 5.** Mature extragonadal teratoma in a kitten. Immunohistochemical labelling (brown color)
254 (A-C) Strong cytoplasmic pan-cytokeratin immunolabeling in squamous epithelium from hair
255 follicles, apocrine and sebaceous glands (A), bronchial epithelium and bronchial glands (B) and
256 odontogenic epithelium (C). (D-G) Strong cytoplasmic vimentin immunolabeling in adipocytes (D),
257 bronchial cartilage (E), nerve and endothelium cells (F) and osseous tissue (G). (H-I) Strong nuclear
258 GFAP immunolabeling in astrocytes.

259



260

261 **Figure 6.** Mature extragonadal teratoma in a kitten. Strong nuclear p40 immunolabeling (brown
262 color) in (A) basal squamous cells of hair follicles (arrow) and in germ cells of sebaceous glands
263 (arrowheads), (B) basal cells of the bronchial tree, and (C) odontogenic epithelium.

264