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C.H. Santana
https://orcid.org/0000-0003-2053-6278
D.O. Santos
https://orcid.org/0000-0002-3083-1572
L.R. Souza
https://orcid.org/0000-0003-1685-5348
S.P. Pimentel
https://orcid.org/0000-0001-5613-8373
A.R. Oliveira
https://orcid.org/0000-0002-9031-792X
L.G.A. Moreira
https://orcid.org/0000-0002-6996-0283
L.V.A. Otoni
https://orcid.org/0000-0003-0828-678X
C.L.R. Silva
https://orcid.org/0000-0001-5708-0206
R.L. Santos
https://orcid.org/0000-0002-4830-0470

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Brain teratoma in a free-ranging mallard (*Anas platyrhynchos*) - case report

[*Teratoma cerebral em um pato-real (*Anas platyrhynchos*) de vida livre - relato de caso*]

C.H. *Santana*[1], D.O. *Santos*[1], L.R. *Souza*[1], S.P. *Pimentel*[1], A.R. *Oliveira*[1], L.G.A. *Moreira*[1], L.V.A. *Otoni*[2], C.L.R. *Silva*[2], R.L. *Santos*[1]*

[1]Escola de Veterinária - Universidade Federal de Minas Gerais - Belo Horizonte, MG
[2]Self-Employed Veterinarians - Belo Horizonte, MG

ABSTRACT

Teratoma is a rare neoplasia with differentiation in two or three germ cell lines. Intracranial teratoma in birds has rarely been reported, especially affecting the brain. This is the first report of a brain teratoma in a mallard with neurological clinical signs. The neoplasm was characterized as a mature brain teratoma, extending from the cerebellum to the brainstem, and with one nodule in the cortex.

Keywords: bird, wildlife diseases, brain neoplasm, *Anas platyrhynchos*

RESUMO

O teratoma é uma neoplasia rara, com diferenciação em duas ou três linhagens de células germinativas. Teratomas intracranianos, em aves, são raramente relatados, principalmente, com localização cerebral. Este é o primeiro relato de teratoma cerebral em um pato-real com sinais clínicos neurológicos. A neoplasia foi caracterizada como um teratoma cerebral maduro, estendendo do cerebelo ao tronco encefálico e com um nódulo no córtex.

Palavras-chave: ave, doenças silvestres, neoplasia cerebral, Anas platyrhynchos

INTRODUCTION

Teratoma is a rare neoplasia with cell differentiation from at least two germ cell lines: ectodermal, endodermal, and mesodermal. Teratoma can be classified as mature, with well-differentiated structures, and immature, with undifferentiated mesodermal and primitive neuroectodermal tissues. The mature variant corresponds to a benign behavior of the tumor, whereas immature variants are malignant tumors (Higgins *et al*., 2016). Teratomas have been described in birds, predominantly, in the coelomic cavity (Helmboldt *et al*., 1974; Gupta, 1976; Reece & Lister, 1993; Bolte & Burkhardt, 2000; Mutinelli & Vascellari, 2002; Palmieri *et al*., 2012). Although there are some reported cases of teratomas located within the cranium (Schelling, 1994; Lopez & Murcia, 2008) or in the brain (Jones, 1964; Homer & Riggs, 1991; Hooper, 2008; Lopes & Murcia, 2008), this condition has not been previously reported in wild free-ranging mallards, particularly in Brazil. The purpose of this report is to describe a case of teratoma in the brain of a mallard, with cerebellar, brainstem and cortical location.

CASE REPORT

A free-ranging mallard (*Anas platyrhynchos*) was found alive, with incoordination, difficulty standing, and anorexia. During the following two days, neurological signs worsened and progressed to death. Necropsy was performed and samples of the kidney, lungs, liver, ovaries, intestine, and brain were sent to the Veterinary Pathology sector at the Universidade Federal de Minas Gerais (UFMG) for histopathology. Tissue samples were fixed by immersion in 10% buffered formalin. After gross evaluation, tissue fragments were processed for paraffin-embedding and 3 µm-sections were obtained for histopathology, for hematoxylin and

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*Autor para correspondência (*corresponding author*)
E-mail: rsantos@vet.ufmg.br

eosin (HE), periodic acid–Schiff (PAS), safranin and Fontana-Masson stains, and immunohistochemistry. Briefly, 3 µm-thick sections were deparaffinized in xylene, and hydrated in decreasing concentrations of ethanol.

Antigen retrieval was performed using a pressure cooker for 10min with a high pH Tris-EDTA solution (Dako, USA) followed by incubation with 3% hydrogen peroxide, and 3% skim milk solutions for one hour each at room temperature. Sections were incubated with anti-pancytokeratin (monoclonal, mouse, AE1/AE3 clone) and anti-vimentin (monoclonal, mouse, RV202 clone) primary antibodies (Santa Cruz Biotechnology, USA), at 1:100 dilution for 16 hours at 4°C. The reaction was developed with Novolink Polymer Detection System (Leica, UK) according to the manufacturer's instructions, revealed with DAB (Dako, USA) for 5min, and counterstained with hematoxylin. Previously tested avian tissues were used as positive controls. Negative controls included sections in which primary antibody were replaced by PBS.

Grossly, there were two poorly delimited, expansive, soft, mottled brown to tan, 1.0 x 2.0 x 1.0 cm and 1.0 x 0.5 x 0.5 cm nodules in the cerebellum extending to the brainstem and in the cortex, respectively (Figure 1A). The kidney, lungs, liver, ovaries, and intestine had no gross lesions. Histologically, lesions were restricted to the brain. In the cerebellum extending to the brainstem and in the cerebral cortex, normal tissues were focally extensively replaced by a poorly demarcated, non-encapsulated, and expansive neoplasm (Figure 1B).

Neoplastic cells differentiated into tissue components derived from different embryonic germinative layers: (i) mesodermal cells - structures similar to primitive glomeruli and renal tubules (Figure 1C), cartilage (Figure 1D) as well as mesenchymal tissue; (ii) ectodermal cells - keratinized stratified epithelium (Figure 1E), nervous tissue, and tubular glands with intraluminal mucoid secretion positively stained by PAS and safranin; (iii) endodermal cell line - ciliated pseudostratified epithelium resembling respiratory epithelia and intestinal epithelium, with caliciform cells positively stained by PAS and safranin. Cells with intracytoplasmic black granular material were also observed within the neoplasm. These cells stained positively by Fontana-Masson, and therefore they were interpreted as melanocytes (Figure 1F). Based on these morphologic features, the neoplasm was diagnosed as a mature brain teratoma. Cytokeratin expression was demonstrated in ciliated pseudostratified epithelium, keratinized stratified epithelium, tubular glands, and intestinal epithelium characterizing the epithelial origin of these cells (Figure 1G). Vimentin expression was observed in cartilage and neoplastic stroma (Figure 1H).

DISCUSSION

This neoplasm was classified as a mature teratoma due to the presence of well differentiated neoplastic cells derived from mesodermal, endodermal, and ectodermal germinative layers. Although primitive glomeruli have been observed in this neoplasm, most of the structures were well-differentiated. Therefore, the classification as a mature teratoma applies in this case (Higgins *et al*., 2016). There are few reports describing intracranial teratomas in chicken (Jones, 1964), fantail pigeon (Hooper, 2008), lesser kestrel (Lopez & Murcia, 2008), great blue heron (Schelling, 1994), and domestic ducks (Homer & Riggs, 1991).

To the best of our knowledge this is the first report of intracranial teratoma in a free-ranging mallard. In some of those reported cases, teratoma was located within the brain, affecting the pons (Homer & Riggs, 1991), cerebrum (Hooper, 2008), and cerebellum (Jones, 1964). This mallard had a teratoma extending from the cerebellum to the brainstem, with a smaller nodule in the cortex, replacing extensive areas of the preexisting neural tissue.

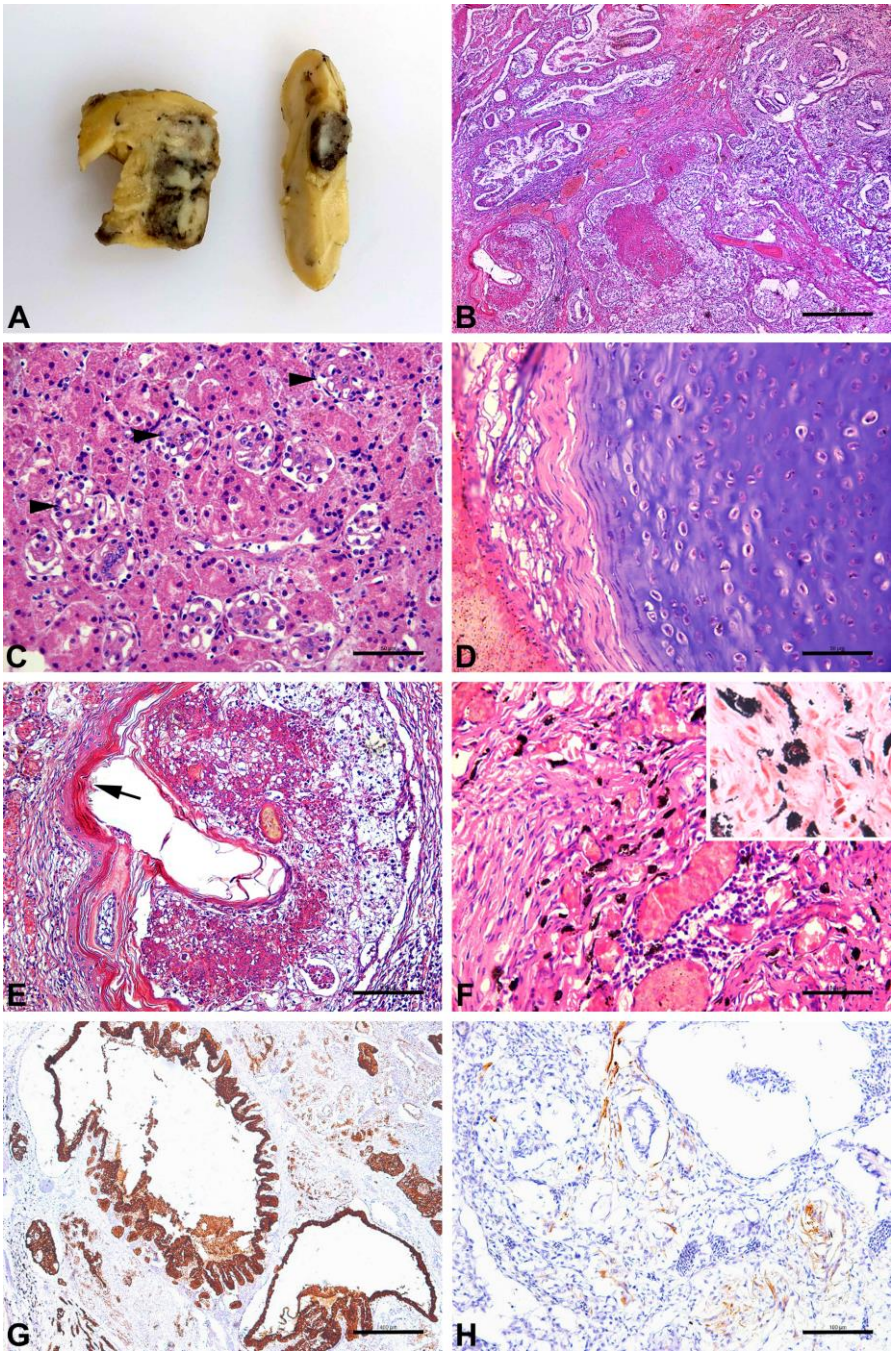

Figure 1. Mature teratoma, brain, free-ranging mallard (*Anas platyrhynchos*). (A) Brain with two poorly demarcated, expansive, soft and mottled brown to tan nodules in the cerebellum, extending to the brainstem (left) and into the cortex (right). (B) Neoplasm with various tissue components (HE, bar = 400 µm). Mesodermal-derived neoplastic cells: (C) Primitive glomeruli (arrow heads) and renal tubules (HE, bar = 50 µm) and (D) cartilage (HE, bar = 50 µm). Ectodermal derived with (E) keratinized stratified squamous epithelium (arrow) (HE, bar = 100 µm). (F) Melanocytes (HE, bar = 50 µm) positively stained by Fontana-Masson (inset). (G) Cytokeratin-expressing neoplastic cells (DAB, counterstained with hematoxylin, bar = 400 µm). (H) Vimentin-expressing neoplastic cells (DAB, counterstained with hematoxylin, bar = 100 µm).

Reported clinical signs associated to intracranial teratomas in birds include head tilt, circling, facial nerve paralysis, head swelling, periocular swelling with exophthalmia, vestibular syndrome, ataxia, and depression (Jones, 1964; Homer & Riggs, 1991; Hooper, 2008; Schelling, 1994; Lopez & Murcia, 2008). The mallard in this case had incoordination and difficulty standing, which are sings mainly due to cerebellar and brainstem neuronal loss. Special stains allowed a better characterization of neoplastic components in this case. For instance, the cytoplasm of caliciform cells as well as the intraluminal glandular material were PAS positive, demonstrating mucopolysaccharide-rich secretions associated with this type of tissue. Therefore, these ancillary staining protocols further demonstrated that neoplastic cells in this teratoma were well-differentiated.

Immunohistochemistry with anti-pancitokeratin antibody demonstrated the epithelial origin of different neoplastic tissue components. Immunohistochemistry have been previously used in birds for diagnosing teratomas (Hooper, 2008; Palmieri *et al*., 2012) since it allows unequivocal characterization of neoplastic tissue components from different embryologic origins as demonstrated in this report. In conclusion, based on histopathology, histochemistry, and immunohistochemistry, a diagnosis of cerebellar, brainstem, and cerebral teratoma was established. To the best of our knowledge, this is the first reported case of brain teratoma in a free-ranging mallard.

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