Braz J Vet Pathol, 2020, 13(3), 602 – 608
DOI: 10.24070/bjvp.1983-0246.v13i3p602-608

BJVP
Brazilian Journal of Veterinary Pathology
ISSN 1983-0246

Case Report

Metastatic osteoblastic osteosarcoma in a captive scarlet macaw (*Ara macao*)

Larissa G. A. Moreira[1], Thaynara P. Carvalho[1], Ayisa R. Oliveira[1], Daniel O. dos Santos[1], Nayara F. Paula[1], Herlandes P. Tinoco[2], Carlyle M. Coelho [2], Renato L. Santos[1]

[1]Escola de Veterinária, Universidade Federal de Minas Gerais. Av. Antônio Carlos, 6627. 31270-901 Belo Horizonte, MG, Brazil.
[2]Jardim Zoológico - Fundação de Parques Municipais e Zoobotânica de Belo Horizonte, 31365-450, Belo Horizonte, MG, Brazil.
[*]**Corresponding author:** RLS. Departamento de Clínica e Cirurgia Veterinária, Escola de Veterinária, Universidade Federal de Minas Gerais, Av. Presidente Antônio Carlos, 6627 – CEP 30161-970, Belo Horizonte, MG, Brazil. Phone: 55-31-3409-2239. Fax: 55-31-3409-2230. E-mail: rsantos@vet.ufmg.br

Submitted June, 1st 2020, Accepted July, 30th 2020

Abstract

Osteosarcoma is a malignant primary tumor of the bone, which is considered rare in birds. This report describes an osteoblastic osteosarcoma in a scarlet macaw (*Ara macao*), which was apathetic with progressive weight loss, and had a tumor in the distal portion of the femur and proximal tibiotarsus with ulcerated pododermatitis in the contralateral limb. Euthanasia was elected due to poor diagnosis after radiographic and cytological examination. Histopathology and immunohistochemistry were performed and confirmed the diagnosis of an osteoblastic osteosarcoma with hepatic and pulmonary metastases.

Key words: zoological, pathology, wild animals, bone neoplasia, avian.

Introduction

Osteosarcoma is a malignant tumor primary of the bone characterized by proliferation of malignant osteoblasts with production of osteoid or immature bone (26). Although it is a rare neoplasm in birds, it is the most frequent malignant primary tumor of the bone in avian species (7, 22), and it tends to occur on the extremities, usually adjacent to joints (2). Therapeutic protocols for this neoplasm are poorly described in birds. In some reported cases, amputation of affect limb has been described. However, in most reported cases of avian osteosarcoma were left untreated and the patient subjected to euthanized (1, 3, 7, 11, 13, 24). Despite the fact that it has been described in many different avian species (3, 7, 9, 11, 13, 17, 21, 24), there are few reports of osteosarcoma in psittacines, which include a blue-fronted Amazon parrot (*Amazona aestiva*) (8) and budgerigars (1, 21).

Scarlet macaw (*Ara macao*) is a psittacine naturally occurring mostly in Central America and in the northern part of South America, inhabiting tropical forests. This specie is currently classified as *least concern*, based on IUCN Red List of Threatened Species criteria (12). However, increasing studies about the Psittacidae family is of great interest since the Brazilian fauna includes several of its members, with some endangered species as well as some popular pet species (10).

This report aims to describe a case of osteoblastic osteosarcoma in a captive scarlet macaw (*Ara macao*).

Case Report

An adult scarlet macaw (*Ara macao*) was found apathetic in its enclosure at the Belo Horizonte Zoological Garden (Belo Horizonte, Brazil) after a period of approximately two weeks of progressive weight loss. Physical examination revealed a tumor in the distal portion of the femur and proximal tibiotarsus of the left limb, and ulcerated pododermatitis in the contralateral limb. A mediolateral radiograph of the left limb was taken,

 All rights reserved 2007.

Braz J Vet Pathol, 2020, 13(3), 602 – 608
DOI: 10.24070/bjvp.1983-0246.v13i3p602-608

demonstrating that the mass was composed of non-mineralized tissue with low radio-opacity, and evident osteolysis in the affected femur and tibiotarsus (Fig. 1). A cytologic examination was performed through fine-needle aspiration of the mass, which revealed polygonal to fusiform cells with intense anisocytosis and cell pleomorphism, often characterized by abundant cytoplasm, and with intense anisokaryosis, including atypical nuclei and one mitosis figure per 10 higher magnification fields (400x) with a little extracellular matrix (Fig. 2), which was compatible with a presumptive diagnosis of sarcoma. Euthanasia was elected due to poor prognosis, and a thorough necropsy was performed.



Figure 1. Mediolateral radiograph of the left limb with a large area of soft tissue with low radio-opacity around the distal femur and proximal tibiotarsus, with marked osteolysis of the preexisting adjacent bone (arrow).

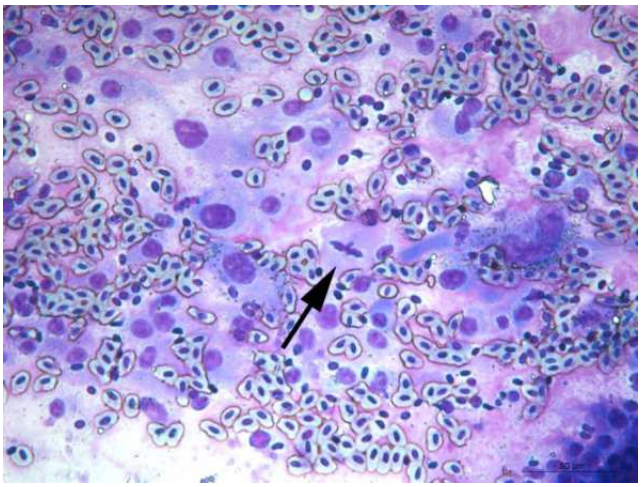


Figure 2. Cytologic aspirate with an abundant population of fusiform cells with intense anisocytosis and pleomorphism. Cells have an abundant cytoplasm, with intense anisokaryosis, including atypical and bizarre nuclei, and one mitotic figure in this particular microscopic field (arrow). Panotico Fast, 400X.

Grossly, a tumor with 4cm x 4cm x 3cm was observed in distal epiphysis of the left femur and adjacent proximal tibiotarsus (Fig. 3A), with irregular and soft surface and a firmer consistency in the deeper parts of the mass. On cut surface, it was multilobulated, white and poorly demarcated, extending to the adjacent muscle and into the medullary canal of the femur and tibiotarsus (Fig. 3B). The liver had two soft and whitish millimetric foci in the left lobe (Fig. 3C), the lungs presented multifocal to coalescent areas depressed and dark red (Fig. 3D), and the spleen was slightly enlarged and pale. Samples of these organs and of the tumor lesion were collected, fixed in 10% buffered formalin, submitted for routine histological processing and stained with hematoxylin and eosin (HE) for microcopy analysis. Selected sections were also stained with Masson's trichrome, alcian blue, and picrosirius red.

Histologically, the neoplastic tissue was non-encapsulated, poorly demarcated, lytic and infiltrative, consisting of mesenchymal cells arranged in bundles with extracellular deposition of collagenous matrix morphologically compatible with non-mineralized osteoid (Fig. 4). Neoplastic cells had scanty and slightly basophilic cytoplasm. Nuclei were round to oval, sometimes bizarre, with granular chromatin and single to multiple large and prominent nucleoli. Nuclear pleomorphism with intense karyomegaly was also observed. There were eight mitotic figures per 10 higher magnification fields (400x), with moderate numbers of bi and multinucleate cells. Both lungs and the liver (Fig. 5) presented multifocal areas of losses of their normal architecture with focally extensive and well delimitated areas containing cells with the same characteristics of the tumor previously described. Additionally, the spleen had loss of its architecture with an infiltrative proliferation characterized by a sheet of round cells with scarce basophilic cytoplasm, large round nuclei,

sometimes cleaved, with granular chromatin and prominent large central nucleoli. There were marked anisokaryosis and anisocytosis with eventual karyomegaly, and five mitosis figures per 10 higher magnification fields (400x), which was diagnosed as a round cell malignant tumor, presumptively a lymphoma.

Immunohistochemistry of the primary bone tumor was performed using vimentin mouse monoclonal antibody (RV202; Santa Cruz biotechnology) at 1:100 dilution. Positive controls included previously tested avian tissues, and for negative controls the primary antibody was replaced with buffer. Neoplastic cells consistently expressed vimentin (Fig. 6).

Due to the observation of both osteosarcoma and a round cell tumor, a PCR for detection of avian leukosis virus (J subgroup) was performed using paraffin-embedded samples of the spleen and the primary bone tumor. DNA extraction from paraffinized tissues was performed as described (23). Template DNA concentration and quality was assessed by spectrophotometry (NanoVue; GE, Healthcare, UK). Appropriate positive and negative controls were included. Amplification was performed using primers targeting sequences of HPRS-10, an envelope J subgroup gene of the avian leukosis virus (GGATGAGGTGACTAAGAAAG and CGAACCAAAGGTAACACACG) using previously described parameters (25). PCR results were negative in both tissues.

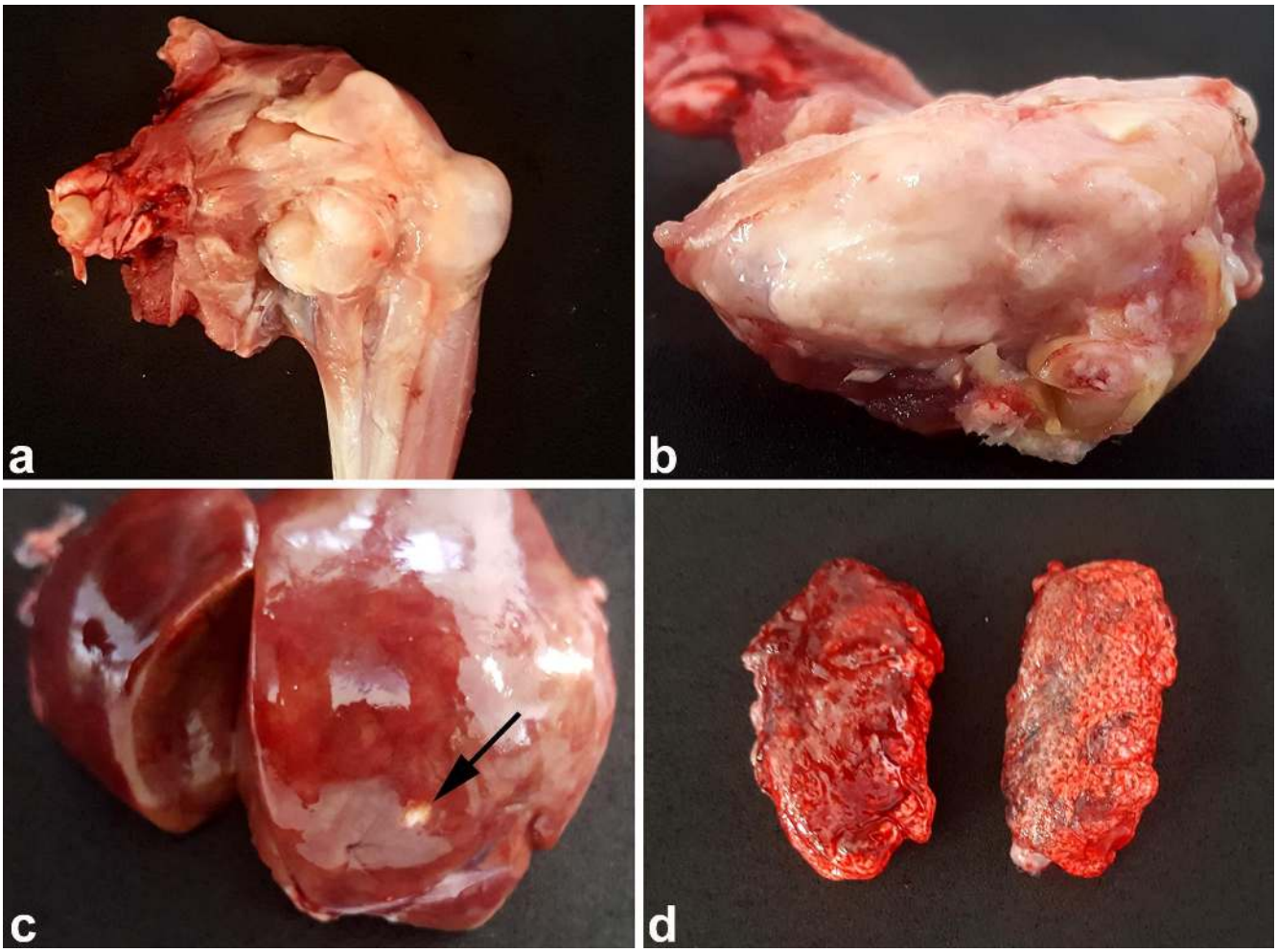


Figure 3. Scarlet macaw (*Ara* macao) with osteosarcoma. **A.** Left limb, distal portion of the femur and proximal tibiotarsus, with an irregular multilobulated, white and soft tumor. **B.** cross section of the neoplasm. **C.** Metastasis in liver characterized by a soft and whitish millimetric nodule in the left lobe (arrow). **D.** Congested lungs with no macroscopic evidence of metastasis.

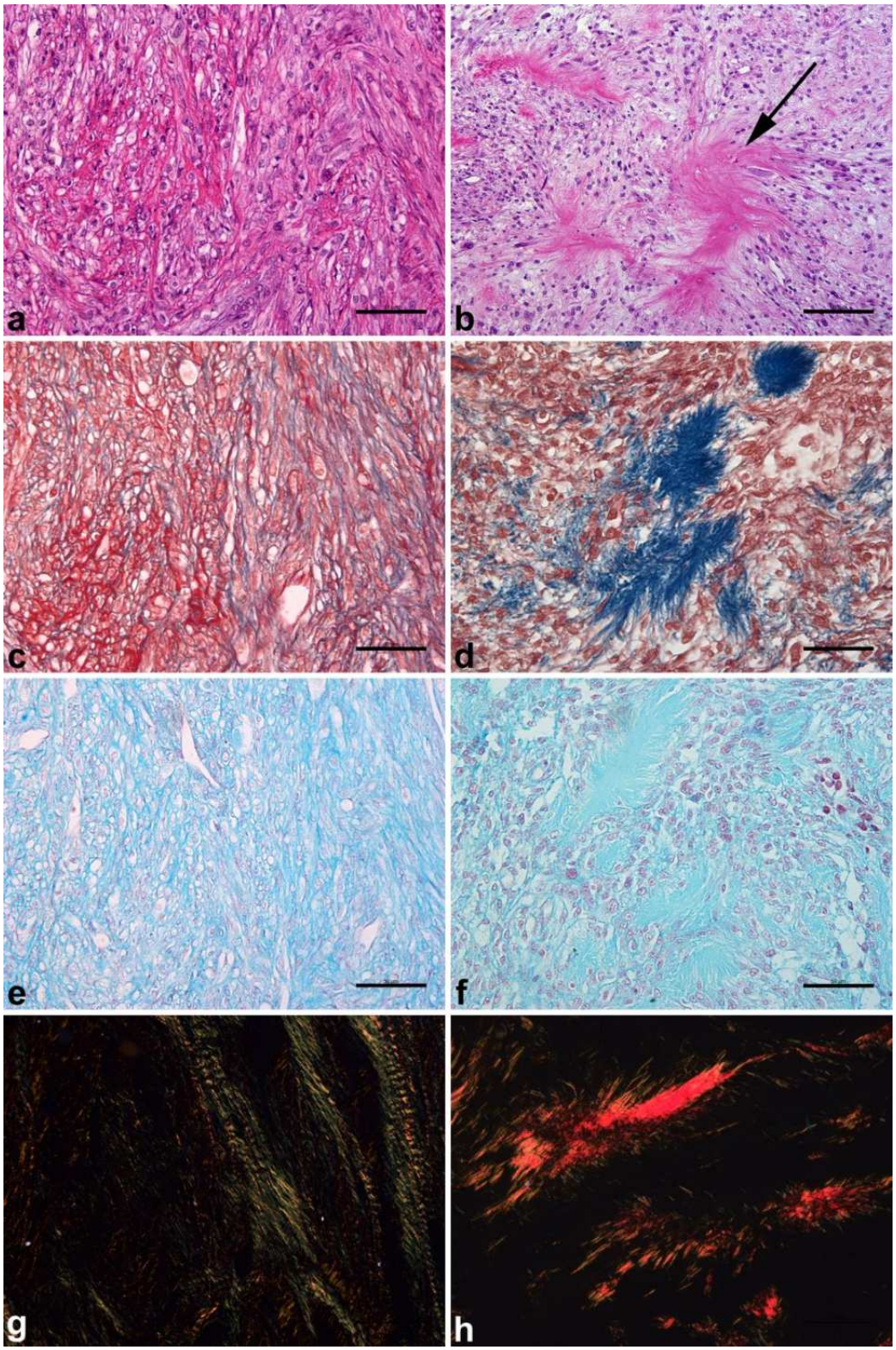


Figure 4. Osteoblastic osteosarcoma in a scarlet macaw (*Ara macao*). **A.** Mesenchymal cells with scanty and slightly basophilic cytoplasm arranged in bundles. Hematoxylin and eosin, bar = 50 µm. **B.** Extracellular deposition of collagenous matrix morphologically compatible with non-mineralized osteoid (arrow). Hematoxylin and eosin, bar = 50 µm. Masson's trichrome (C, D), Alcian blue (E, F) and polarized picrosirius red (G, H) evidenced the characteristic of the extracellular matrix of the tumor, collagenous (intense blue in Masson's trichrome), poor in glycosaminoglycans (slightly blue in alcian blue stain) and rich in collagen type I (red and thick fibers at polarized picrosirius red), bar = 50 µm.

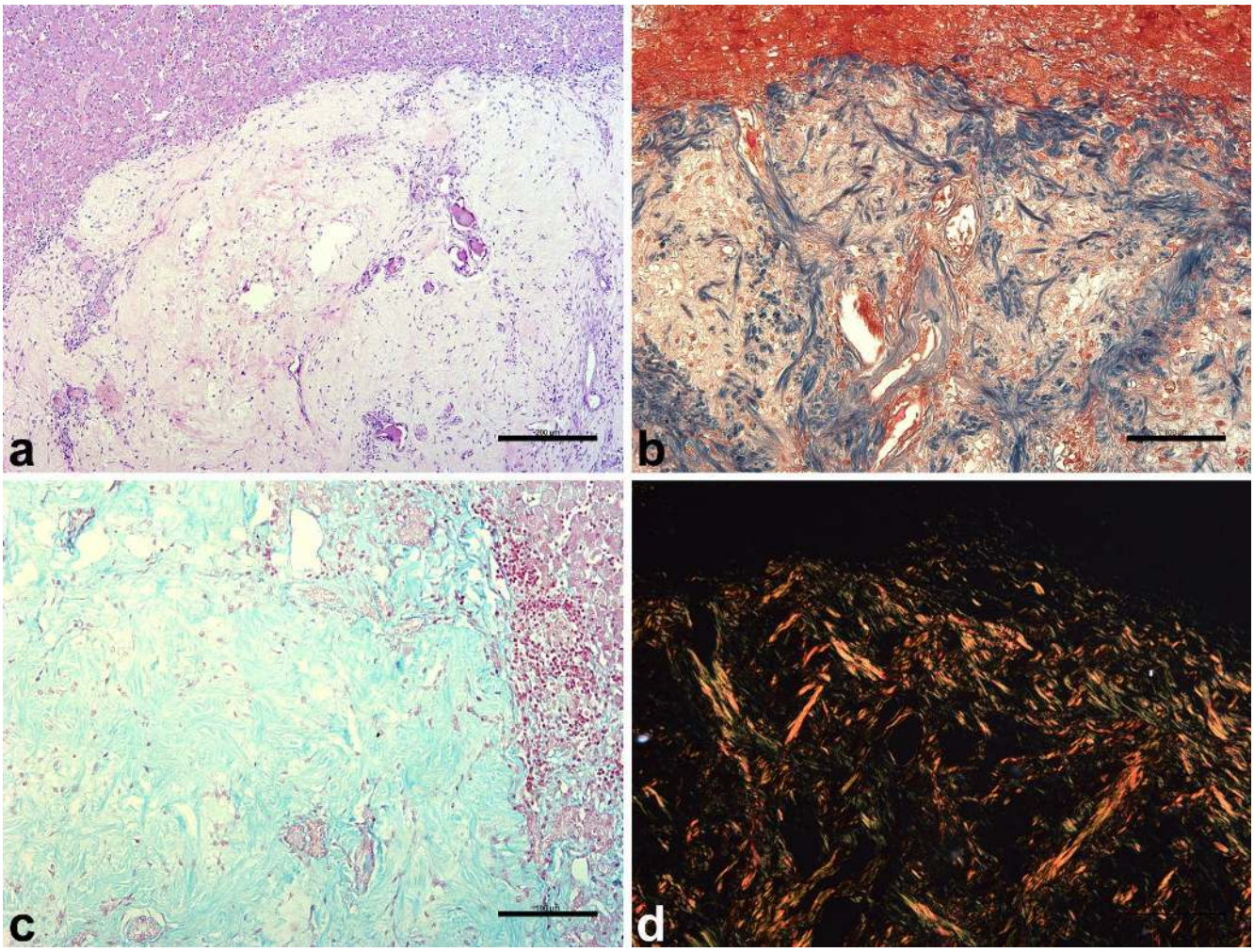


Figure 5. Hepatic metastasis of the bone osteosarcoma of the scarlet macaw (*Ara macao*). **A-D.** Expansive tumor with abundance of extracellular matrix with histochemical features similar to the primary tumor, Hematoxylin and eosin (A), Masson's trichrome (B), Alcian blue (C) and polarized picrosirius red (D), bar = 100 µm.

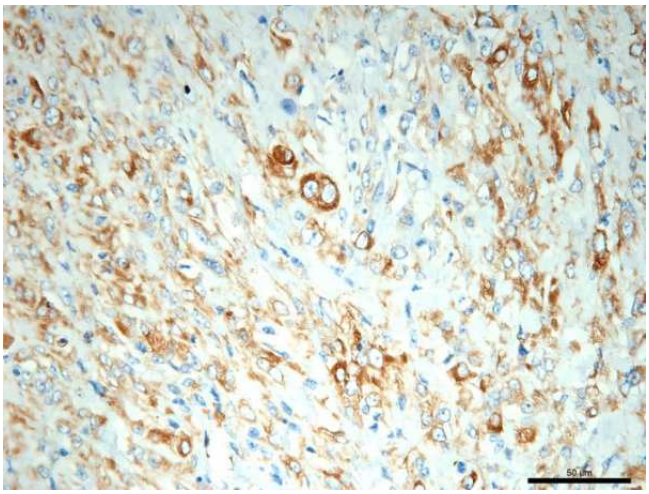


Figure 6. Osteoblastic osteosarcoma in a scarlet macaw (*Ara macao*). Strong and diffuse intracytoplasmic immunoreactivity of neoplastic cells for vimentin. DAB chromogen; counterstained with hematoxylin, bar = 50 µm.

Discussion

To the best of our knowledge, this is the first report of an osteoblastic osteosarcoma in a scarlet macaw (*Ara macao*). Osteosarcoma is considered a rare tumor in avian species. In domestic mammals, particularly in the dog, osteosarcomas are found mostly in the metaphysis of long bones of the appendicular skeleton, although it may also primarily affect other bones (ribs, bones of the head and vertebrae) as well as soft tissues (6). Reported cases in birds affect mostly proximal or distal extremities of long bones such as femur, humerus, and tibiotarsus, whereas unusual locations for osteosarcomas include the mandible, bones of the head, orbit and intraocular tissues (17, 19, 24). Similarly, osteosarcomas develops more often in the appendicular skeleton of dogs and humans, usually affecting only one bone (14) and, in this case, the neoplasm affected primarily the distal epiphysis of the femur, also involving the proximal tibiotarsus and compromising both cortical and medullary. In this case,

cytological evaluation was important for defining malignancy and supporting clinical decision making associated to the poor clinical condition of the animal.

Since the appendicular skeleton is most commonly affected, lameness is usually the earliest clinical manifestation in most cases in dogs and cats (26) as well as in birds (5, 16). In this case, there was obvious loss of function of the affected limb, with an ulcerated pododermatitis of the contralateral limb, likely due to overload. There is not much information available about radiographic features of primary bone neoplasms in birds, but it is known that, in domestic animals, osteosarcomas may induce lytic, mixed, or productive lesions, and the osteoblastic type is often associated with lytic lesions (26). In this case, the presence of a mass with soft nearly radiolucent tissue associated with marked osteolysis of the adjacent bone is compatible with the diagnosis of osteoblastic osteosarcoma.

Although metastases in avian osteosarcomas are uncommon (22), this scarlet macaw had hepatic and pulmonary metastases. Similar to this case, another osteoblastic osteosarcoma metastasis has been described in a chicken (7), where the liver had multiple nodules replacing the normal parenchyma with histology that resembling this case.

The macaw in this case also had a malignant round cell neoplasm in the spleen. Lymphoma is the most common lymphoid neoplasia in psittacine and passerine species (4). Although these tumors are largely attributed to viral infections, little is actually known about their etiology. In poultry, the development of sarcomas and lymphomas is often associated with retroviruses belonging to the avian leukosis/sarcoma group, which results in important economic losses (15, 18). However, in this case there was no amplification of avian leukosis viral DNA from samples of the spleen and osseous neoplasm. However, the gene target was from subgroup J, one of the six subgroups described which although have a broad host range, may not be the subgroup associated with the disease of this case. Importantly, aging is another predisposing factor to be considered in this case due to the increased lifespan of captive animals and, therefore, increased cumulative genetic damage due to environmental carcinogenic agents (20), which may lead to spontaneous tumors in the absence of viral infections.

In conclusion, this is the first reported case of osteoblastic osteosarcoma in a scarlet macaw. This animal also had a malignant round cell tumor, which was an accidental finding in this case, and its association with viral infection could neither be confirmed nor ruled out.

Acknowledgements

Work in RLS lab is supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil), FAPEMIG (Fundação de Amparo a Pesquisa do Estado de Minas Gerais, Brazil), and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Brazil). RLS has a fellowship from CNPq (Brazil).

References

1. Arnall L. Anesthesia and surgery in cage and aviary birds. Vet Rec. 1961;7399:188-91.
2. Bauck L. Neoplasms. In: Rosskopf WJ, Woerpel RW, editors. Diseases of cage and aviary birds. 3rd edition. Baltimore: Williams & Wilkins; 1996. p.480-9.
3. Churgin SM, Steinbeinberg H, Ravi M, Hartuo BK. Sternal osteosarcoma in a blue crane (*Anthropoides paradiseus*). J Zoo Wildl Med. 2013;44(4):1075-8.
4. Coleman CW. Lymphoid neoplasia in pet birds: a review. J Avian Med Surg. 1995;9(1):3-7.
5. De Luca Bossa LM, Mennonna G, Meomartino L, Paciello O, Ciccarelli F, De Biase D, Raia P, Raia V, Fioretti A, Dipineto L. Polyostotic chondroblastic osteosarcoma in a kestrel (*Falco tinnunculus*). J Avian Med Surg. 2015;29(4):336-9.
6. Dernell WS, Straw RC, Withrow SJ. Tumors of the skeletal system. In: Withrow SJ, MacEwen EG, editors. Small animal clinical oncology. 3rd ed. Philadelphia: Saunders; 2001. p.378-417.
7. Dittmer KE, French AF, Thompson DJ, Buckle KN, Thompson KG. Primary bone tumors in birds: a review and description of two new cases. Avian Dis. 2012;56(2):422-6.
8. Doolen M. Adriamycin chemotherapy in a bluefronted Amazon with osteosarcoma. Proc Annu Conf Assoc Avian Vet. 1994;89-91.
9. Duarte-Benvenuto A, Costa PCS, Jerdy H, Carvalho ECQ, Catão-Dias JL, Díaz-Delgado J. Spontaneous primary right tibiotarsal osteosarcoma with pulmonary metastasis in a free-ranging Cory's shearwater (*Calonectris diomedea*). J Wildl Dis. 2020;56(1):243-6.
10. Grespan A, Raso TF. Psittaciformes (Araras, papagaios, periquitos, calopsitas e cacatuas). In: Cubas ZS, Silva JCR, Catão-Dias JL, editors. Tratado de Animais Selvagens Medicina Veterinária. 2nd ed. Roca: São Paulo; 2014. p.550-89.
11. Hartup BK, Steinberg H. Osteosarcoma in an American robin (*Turdus migratorius).* Avian Dis. 1996;40(4):93-4.
12. IUCN (International Union for Conservation of Nature) BirdLife International. 2016. *Ara macao*. The IUCN Red List of Threatened Species 2016: e.T22685563A93079992. https://dx.doi.org/10.2305/IUCN.UK.2016-3.RLTS.T22685563A93079992.en. Downloaded on 16 May 2020.
13. Mawdesley-Thomas LE, Solden DH. Osteogenic sarcoma in a domestic white goose (*Anser anser*). Avian Dis. 1967;11(3):365-70.

14. Morello E, Martano M, Buracco P. Biology, diagnosis and treatment of canine appendicular osteosarcoma: Similarities and differences with human osteosarcoma. Vet J. 2011;189:268-77.
15. Murphy B. Retroviridae. In: MacLachlan NJ, Dubovi EJ, editors. Fenner's Veterinary Virology. 5th ed. Amsterdam: Elsevier/Academic Pres; 2017. p.216-97.
16. Lamb S, Wojcieszyn J, Sitinas N. Osteosarcoma of the tibiotarsus with possible pulmonary metastasis in a ringnecked dove (*Streptopelia risoria*). J Avian Med Surg. 2014;28(1):50-6.
17. Latimer KS. Oncology. In: Ritchie BW, Harrison GJ, Harrison LR, editors. Avian medicine: principles and application. Lake Worth: Wingers; 1994. p.640-72.
18. Nair V, Fadly AM. Neoplastic diseases: leukosis/sarcoma group. In: Swayne DE, Glisson JR, McDougald LR, Nolan LK, Suarez DL, Nair N, editors. Diseases of poultry. 13th ed. New York: John Wiley & Sons; 2013. p.566-76.
19. Reavill DR. Tumors of pet birds. Vet Clin North Am Exot Anim Pract. 2004;7(3):537-60.
20. Reavill DR, Dorrestein GM. Pathology of aging psittacines. Vet Clin North Am Exot Anim Pract. 2010;13(1):135-50.
21. Sánchez-Godoy F, Ledesma-Ramírez I, Morales-Salinas E. A retrospective study of neoplasms in ornamental and pet birds diagnosed at the Hospital de Aves of the Universidad Nacional Autónoma de México (2007-2014). Braz J Vet Pathol. 2020;13(1):1-11.
22. Schmidt RE, Reavill DR, Phalen DN. Musculoskeletal system. In: Schmidt RE, Reavill DR, Phalen DN, editors. Pathology of pet and aviary birds. 2nd ed. New Jersey: John Wiley & Sons; 2015. p.199-220.
23. Shi S, Datar R, Liu C, Wu L, Zhang Z, Cote RJ, Taylor CR. DNA extraction from archival formalin-fixed, paraffin-embedded tissues: heat-induced retrieval in alkaline solution. Histochem Cell Biol. 2004;122(3),211-8.
24. Sladakovic I, Sangster CR, Allan GS, Portas TJ, Howlett CR, Blas-Machado U. Calvarial osteosarcoma with cerebral compression in a free ranging powerful owl (*Ninox strenua*). J Zoo Wildl Med. 2017;48(1):208-12.
25. Smith LM, Brown SR, Howes K, McLeod S, Arshad SS, Barron GS, Venugopal K, McKay JC, Payne LN. Development and application of polymerase chain reaction (PCR) tests for the detection of subgroup J avian leukosis virus. Virus Res. 1998;54:87-98.
26. Thompsom KG, Dittmer KE. Tumors of the bone. In: Meuten DJ, editor. Tumor in domestic animals. 5th ed. New Jersey: John Wiley & Sons; 2017. p.356-424.