Case Report

First Report of Immunohistochemically Confirmed Pleomorphic Rhabdomyosarcoma in a Cockatiel (*Nymphicus hollandicus*) in Iran

Morteza Nikzad[1], Yaser Kianfar[1], Sara Shokrpoor[2], Saeed Farzad-Mohajeri[3], Iman Shojaei[4], Mohammad Amin Saeedi[3], Niusha Jafarirad[3], Seyed Mostafa Peighambari[1*]

1. Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
2. Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
4. Department of Clinical Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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ABSTRACT

Sarcomas are malignant neoplasms arising from mesenchymal tissues, including bone, muscle, fat, and connective tissues. Rhabdomyosarcoma (RMS) is an aggressive form that originates from skeletal muscle cells. The present study a case of RMS in a cockatiel (*Nymphicus hollandicus*). The tumor was characterized by its rapid growth and significant clinical signs, which included the development of a hard mass in the bird's head region. Radiographic examination revealed a well-defined and dense structure, and fine-needle aspiration (FNA) provided preliminary evidence of a sarcomatous lesion. Surgical removal was performed to alleviate discomfort and confirm the diagnosis through histopathological and immunohistochemical examination. The neoplastic cells exhibited pleomorphism with an orientated, spiral growth pattern and prominent nucleoli. Based on our findings, pleomorphic RMS was confirmed. Fortunately, the bird is still alive two months post-surgery, with no signs of tumor recurrence. Rapid growth and tendency to spread to other parts highlight the aggressive nature of this tumor and the necessity for prompt and accurate diagnosis. Continued research and collaboration in the study of avian tumors are essential for advancing our understanding and improving clinical outcomes for affected birds.

Keywords: Tumor, Pleomorphic rhabdomyosarcoma (RMS), Histopathology, Immunohistochemistry, Cockatiel

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*** Corresponding Author:**
Seyed Mostafa Peighambari, Professor.
Address: *Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*
Phone: *+98 (21) 61117150*
E-mail: *mpeigham@ut.ac.ir*

Case History

Sarcomas are a diverse group of malignant tumors that arise from mesenchymal tissues, including bone, muscle, fat, and connective tissues. In veterinary medicine, sarcomas are relatively common and can affect a wide range of species. They are characterized by their ability to invade surrounding tissues, and they often have a high recurrence rate after surgical removal (Cormier & Pollock, 2004; Dezfoulian et al., 2012; Cardoso de Almeida Moreira et al., 2023). In the poultry industry, avian leukosis and sarcoma historically caused significant mortality, production declines, and economic losses. However, these diseases are now relatively well-controlled (Nemeth et al., 2016; Fandiño et al., 2023). Among the various types of sarcomas, rhabdomyosarcoma (RMS) is a particularly aggressive form that originates from skeletal muscle cells. This type of sarcoma can occur in both humans and animals, including birds. RMS is known for its rapid growth and tendency to metastasize to other parts of the body (Zarrabi et al., 2023). In avian species, RMS has been reported in several cases, like pigeon and caracara, highlighting the need for further research and understanding of this tumor (Fernández-Bellonet et al., 2003; Maluenda et al., 2010). The present study reported a case of RMS in a cockatiel (*Nymphicus hollandicus*) located caudal to the right eye. To our knowledge, this is the first immunohistochemically confirmed case of RMS in a cockatiel in Iran, with the patient surviving after a successful surgical excision.

Case Presentation

A 2-year-old cockatiel was presented to the Birds Clinic of the University of Tehran. The owner's primary concern at presentation was the observation of a mass in the cranium (Figure 1). The mass had been noticed two weeks earlier and had progressively enlarged over time. The owner reported that the mass initially appeared small but grew steadily. Further examination revealed that the tumor was not only increasing in size but also causing discomfort to the bird; however, the patient was still able to consume food and water. Considering the rapid growth and the location of the tumor, concerns were raised regarding its nature and the possible implications for the bird's health.

Diagnostic testing

Due to the location of the mass, radiographic examination (iRay Technology, Venu 1717x, China) with orthogonal projections of the skull was performed to evaluate any bone involvement and the size of the mass. Three milliliters of blood were collected from the basilic vein for the evaluation of hematological and biochemical parameters. In the next step, fine-needle aspiration (FNA) was performed to evaluate the nature of the mass and establish a preliminary diagnosis. The samples were fixed and stained using Giemsa stain.

To excise the mass from cranium, the case was administered inhalation anesthesia utilizing isoflurane. The induction phase employed 5% isoflurane over a period of 1-2 minutes, after which the anesthetic concentration was adjusted to 3.5%. Oxygen was delivered at a flow rate of 2 liters per minute. As prophylactic antibiotic coverage, 5 mg/kg enrofloxacin 10% was administered intramuscularly (Carpenter & Harms, 2022). Analgesia was achieved using 15 mg/kg intramuscular tramadol (100 mg/2 mL AMP) (Aliansyah et al., 2021), and 0.1 mg/kg meloxicam 2% was administered subcutaneously to mitigate inflammation (Carpenter & Harms, 2022). For the mass removal, a skin incision was made using a No. 15 scalpel, and after dissecting the surrounding tissues, the mass was removed from the surface of the skull. During the separation of the mass from the surrounding tissues, we noticed the mass had invaded the right eye, which led to the removal of its right eye. To close the surgical site, the subcutaneous tissue was sutured with 4-0 PGA absorbable sutures in a simple continuous pattern, and the skin was closed with 4-0 PGA absorbable sutures in a simple interrupted pattern. After the surgery, the size of the mass was measured and cut into pieces thinner than 6 mm to allow for formalin penetration. Samples were submitted in 10% neutral buffered formalin in a non-breakable plastic container and referred to the pathology laboratory for histopathological and immunohistochemical (IHC) examinations. For histopathology evaluation, samples were embedded in paraffin wax and then sectioned into slices using a microtome, and stained with hematoxylin and eosin (H&E) (Hoque et al., 2024). IHC involves treating tissue sections to expose target proteins (antigens), applying a primary antibody that binds to these proteins, and subsequently utilizing a secondary antibody linked to an enzyme or fluorescent dye. This secondary antibody binds to the primary antibody, enabling the detection of the target proteins via a visible signal produced by the enzyme or dye. In this study, markers, such as vimentin, desmin, smooth muscle actin (SMA), and S-100 protein were utilized in immunohistochemistry to accurately identify specific cell types and structures (Crespo & Shivaprasad, 2021).

Table 1. Hematological and biochemical statistics

Parameter	Result	Reference Range
WBC (10^3/µL)	12	5-10
HCT (10^6/µL)	45	40-49
HET (%)	59	50-80
MON (%)	3	0-2
EOS (%)	1	0-2
LYM (%)	33	20-45
BOND (%)	4	-
Total protein (g/dL)	1.5	2.4-4.1
Albumin (g/dL)	0.6	0.7-1.8
AST (U/L)	160	95-345
ALT (U/L)	11	5-11
ALP (U/L)	110	20-250
GGT (U/L)	4	1-30
Creatinine (mg/dL)	0.4	0.1-0.4
Ca (mg/dL)	9	8-13

Abbreviations: WBC: White blood cell; HCT: Hematocrit; HET: Hemoglobin electrophoresis test; MON: Monocytes; EOS: Eosinophils; LYM: Lymphocytes; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyl transferase; Ca: Calcium.

Note: Reference ranges are obtained from "clinical avian medicine" (Harrison & Lightfoot, 2005).

Figure 1. A cockatiel (*N. hollandicus*) with a mass on its cranium

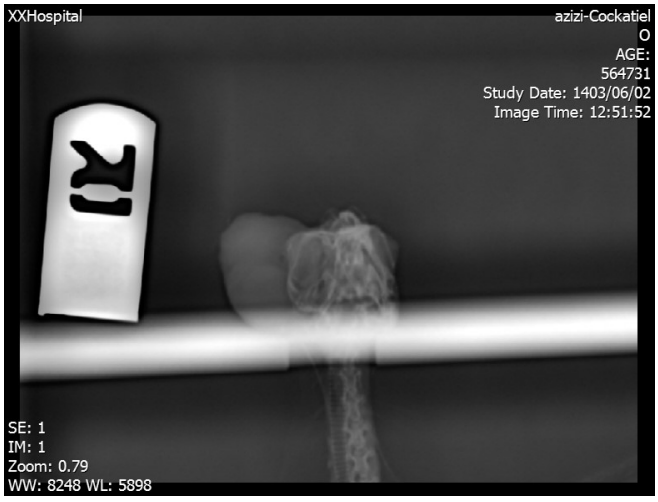

Figure 2. Radiographic evaluation of the lesion showed an amorphous soft tissue opacity mass lesion (approximately 2.5×2.6 cm) in the right side of the skull without any bone involvement

Assessments

The overall radiographic opacity of the skeletal structures appeared within normal limits. Notably, an amorphous soft tissue opacity mass lesion, measuring approximately 2.5×2.6 cm, was identified on the right side of the skull. Importantly, no involvement of the underlying bone was observed. Mass lesion in right side of the skull without bone involvement could be due to benign conditions (e.g. abscess formation, granuloma, etc.) or a neoplastic condition (Figure 2). As indicated in Table 1, the hematological evaluation revealed evidence of an inflammatory condition, while the biochemical profile showed hypoproteinemia with hypoalbuminemia, which may be attributed to enteropathy, diarrhea, an inflammatory condition, or other causes. Both enteropathy and inflammatory conditions could be clinically confirmed. Enteropathy conditions may arise from the use of an inappropriate diet. Moreover, inflammatory responses could also be attributed to the presence of a tumor and the consequent pressure it exerts on the surrounding soft tissues. The excised mass measured approximately 3×2.7 cm. The majority of the mass was located in the frontal region of the cranium and posterior to the bird's eye, while the retro-orbital area was also affected (Figure 3). In this investigation, the FNA sample revealed the presence of individual neoplastic spindle-shaped mesenchymal cells characterized by a high nuclear-to-cytoplasmic (N:C) ratio, prominent nucleoli, granular to coarse chromatin, wispy cytoplasmic borders, and anisokaryosis in a hemodilution background.

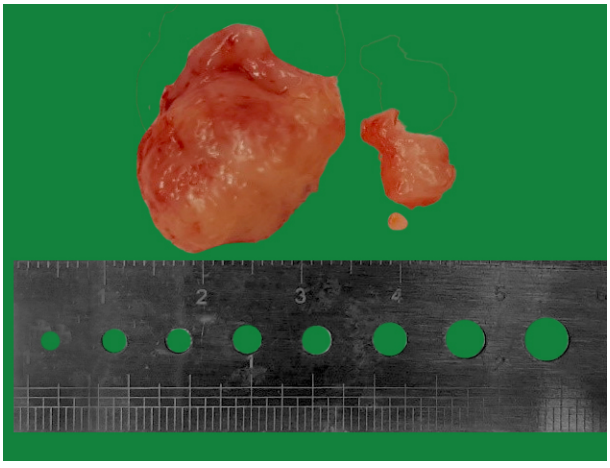

Figure 3. Excised mass size: approximately 3×2.7 cm

Note: The majority of the mass was located in the frontal region of the cranium and posterior to the bird's eye, with the retro-orbital area also affected.

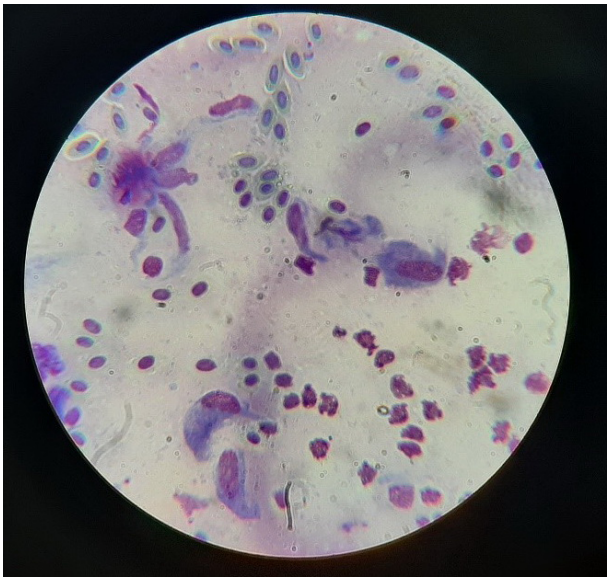

Figure 4. Giemsa-stained FNA sample of the mass

Note: Some individual neoplastic spindle-shaped mesenchymal cells with high N:C ratio, prominent nucleoli, granular to coarse chromatin, wispy cytoplasmic borders, and anisokaryosis are shown in a hemodilution background.

These findings suggest a preliminary diagnosis of sarcoma (Figure 4) (Suster, 2022). In histopathological examinations, the mass was highly cellular and composed of spindle-shaped to fusiform cells, with eosinophilic cytoplasm. Neoplastic cells were arranged in an orientated, spiral-shaped growth pattern, but areas of irregularly orientated cells were also present. Cell borders were indistinct (Figures 5a and 5b). The neoplastic cells had round to oval nuclei containing one to three prominent nucleoli. There were large, atypical, polygonal, pleomorphic, and bizarre cells with abundant eosinophilic cytoplasm (Figure 5c). The mitotic count was 1–2 per high-power field (400×) (Figure 5d). The tumor cells showed strong diffuse cytoplasmic immunopositivity for vimentin (Figure 6a) and desmin (Figure 6b) (Al Obaid, 2020). Immunostaining for SMA (Figure 6c) and S-100 protein (Figure 6d) was negative. The mass was diagnosed as

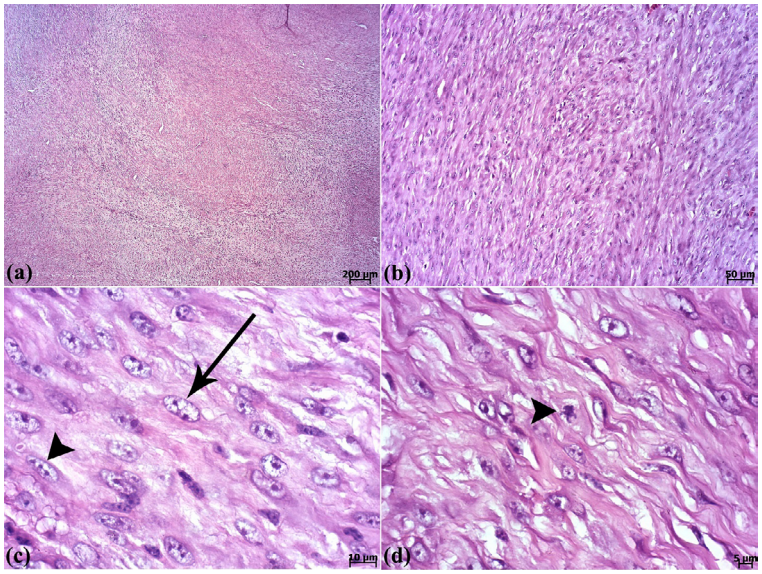

Figure 5. Microscopical findings of RMS

a & b) Highly cellular mass composed of spindle-shaped to fusiform cells, c) The neoplastic cells contained three prominent nucleoli (arrow) and bizarre cells (arrow), d) Mitotic figure (arrowhead) (H&E)

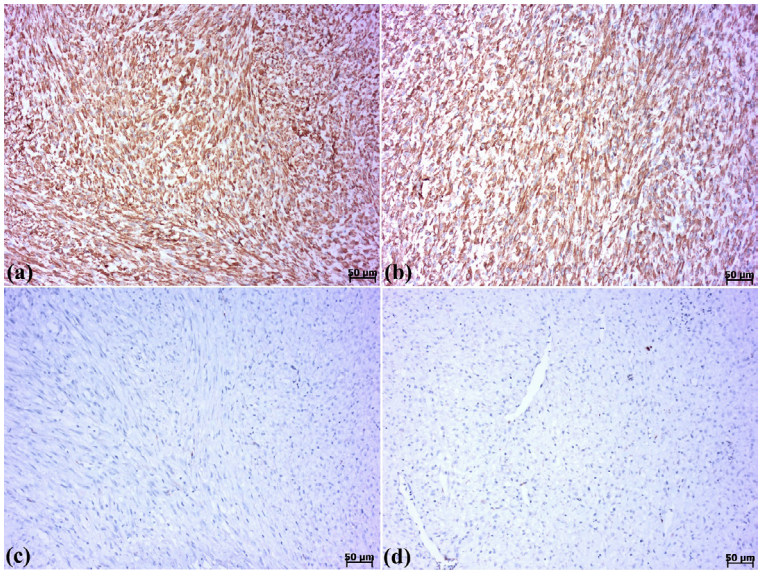

Figure 6. Strong and diffuse reactivity for vimentin (a) and desmin (b); No immunoreactivity for SMA (smooth muscle actin) (c) and S-100 protein (d) in the neoplastic cells (IHC)

pleomorphic RMS based on histopathological and immunohistochemical examinations.

In this case report, we presented the first immunohistochemically confirmed case of pleomorphic RMS in a cockatiel in Iran. The tumor was characterized by its rapid growth and significant clinical signs, which included the development of a hard mass in the bird's cranium. Radiographic examination revealed a well-defined, dense structure, and FNA provided preliminary evidence of a sarcoma. Surgical removal was performed to alleviate discomfort and confirm the diagnosis through histopathological examination. Fortunately, the bird is still alive two months post-surgery, with no signs of tumor recurrence observed thus far.

Our findings are consistent with previous reports that highlight the aggressive nature of RMS in avian species. The morphological features and immunohistochemical profile of the tumor, with strong positivity for vimentin and desmin, align with the characteristics described in other studies. Similar findings have been reported in cases involving a European robin and a yellow-headed caracara, where the tumors also showed strong immunopositivity for vimentin and desmin (Manarolla et al., 2008; Maluenda et al., 2010). The absence of immunostaining for SMA and S-100 protein further supports the diagnosis of RMS, as these markers are typically negative in this tumor. This immunohistochemical profile is consistent with previous studies on avian skeletal muscle tumors (Moll et al. 2006; Bamac & Arun; 2020) and other animals, like dogs (Szaluś-Jordanow et al., 2023). Furthermore, a recent study conducted in 2023 on a 68-year-old woman who suffered from RMS showed similar immunohistochemical profile (Kamel & Hasby, 2024). This case contributes valuable data to the limited literature on RMS in birds. The detailed description of the tumor's histopathological and immunohistochemical features provides a reference for future cases and emphasizes the importance of comprehensive diagnostic workups in avian oncology. Our report underscores the need for veterinary practitioners to consider RMS in the differential diagnosis of rapidly growing masses in birds and to utilize advanced diagnostic techniques to confirm the diagnosis.

Conclusion

In conclusion, this case report documents the first immunohistochemically confirmed occurrence of pleomorphic RMS in a cockatiel in Iran, contributing valuable information to the field of veterinary oncology. The findings highlight the aggressive nature of this tumor and the necessity for prompt and accurate diagnosis. Continued research and collaboration in the study of avian tumors are essential for advancing our understanding and improving clinical outcomes for affected birds.

Ethical Considerations

Compliance with ethical guidelines

All ethical principles were considered in this work according to the principles outlined by the ethical commit-

tee of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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Authors' contributions

Analysis: Morteza Nikzad, Yaser Kianfar, Sara Shokrpoor, Iman Shojaei, Mohammad Amin Saeedi, and Saeed Farzad-Mohajeri, Niusha Jafarirad; Writing the original draft: Morteza Nikzad; Review, editing and supervision: Seyed Mostafa Peighambari; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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