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Rhabdoid melanoma in a harpy eagle (*Harpia harpyja*)

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ABSTRACT

A 28-year-old male harpy eagle (*Harpia harpyja*) with a history of anorexia, hyporexia, lethargy, and progressive weight loss was found dead and submitted for post-mortem examination. Gross findings include dark brown discolouration of testes and lungs; the testes were bilaterally enlarged, glistening brown-grey to blackish in appearance, firm, smooth, and multilobulated. The lungs contained a mass with similar features to the testicles, irregularly shaped with multiple nodules. Histology of testis showed round, polygonal and pleomorphic cells, containing melanin pigments and a typical eosinophilic vacuole in their cytoplasm and with severe pleomorphism. An immunohistochemistry panel with Melan-A, vimentin, CK AE1/AE/3, MUM-1 and CD-68 were performed, yielding a positive reaction for Melan-A and vimentin. The morphology of the tumour cells, the presence of melanin pigment and the immunoreactivity for Melan-A and vimentin by the cells led to a diagnosis of rhabdoid melanoma. This is the first case of this pathology in the testis with lung metastasis in a harpy eagle.

Introduction

The prevalence of neoplasms in birds, in general, appears to be low (Madsen et al., 2017). Specifically, in birds of prey, their presentation is not clear (Wendell et al., 2002; Soler-Tovar and Brieva 2007), but they can have a great impact. Forbes et al. (2000) reported its occurrence in 68% of captive raptors and in 32% of free-living.

Melanocytes are dendritic cells derived from neuroectodermal melanoblasts (Mauldin and Peters-Kennedy, 2015) that have migrated during embryogenesis to the epidermis, dermis and other sites; for example, the eye, inner ear, and meninges (Phillips and Lembcke, 2013). According to Smith et al. (2002), they are found within the basal layer of the epidermis interspersed between basal keratinocytes. Through the process of melanogenesis, these cells produce a pigment called melanin. The colour of this pigment is dark and so it absorbs UV-B light and blocks it from passing through the skin layer into the hypodermis, protecting it from the harmful effects of solar radiation. Conversion of normal melanocytes that are nonpigmented and isolated from other melanocytes into pigmented and clustered neoplastic melanocytes is a multistep process, with initiation as the first event, followed by promotion, transformation and metastasis (Smith et al., 2002; Madhunapantula and Robertson, 2012; Kuzu et al., 2015).

Melanocytic neoplasms reported in the veterinary literature include melanocytoma and melanoma (malignant) (Goldschmidt and Goldschmidt, 2017) and are used to describe benign and malignant melanocytic proliferations, respectively. Melanomas are tumours that originate from malignant transformation of normal melanocytes (Goldschmidt and Goldschmidt, 2017; Phillips and Lembcke, 2013) and are characterised by their aggressive and highly metastatic nature (Madhunapantula and Robertson, 2011; Kuzu et al., 2015).

Among avian species, melanoma is extremely rare in free-living and captive birds (Costagliola et al., 2011) and, according to Kufuor-Mensah and Watson (1992) and Barlow and Girling (2004), extensive information on melanocytic neoplastic pathologies in birds of prey is not available. Rhabdoid melanoma is a rare variant of melanoma (Magro et al., 2006). In this study, we describe the gross, microscopic and immunohistochemical appearance of melanoma affecting the testicles and lungs of a harpy eagle (*Harpia harpyja*), a near-threatened species according to the International Union for Conservation of Nature (IUCN; Birdlife International, 2017). To our knowledge, this is the first case of this pathology in this neotropical raptor with a continental distribution in forests of Central and South America (Miranda et al., 2019; Sutton et al., 2021), considered one of the most powerful birds of prey in the world, and one of the largest (Ferguson-Lees and Christie, 2001), whose longevity is estimated to be 35 years (Lerner et al., 2009).

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Case report

Case history

An approximately 28-year-old intact male harpy eagle (*H. harpyja*) (3,650 kg body weight) with human imprinted from eaglet was presented to the Avian Pathology Laboratory of the Universidad Nacional de Colombia, Bogotá, for necropsy due to sudden death. The animal caretaker reported that the harpy eagle had not consumed food for four days before death and showed ataxia, hyporexia, moderate weakness, bristling feathers and abnormal behaviour, such as isolating itself and not seeking shelter during adverse weather conditions or stress. The eagle had been in captivity for 4-years before it was rescued from illegal trafficking. The bird had been housed from the beginning in a cage 15 m long × 15 m wide × 2.30 m high, with another eagle with signs of inadequate health condition defined by severe infestation by lice and feather mites and evidence of respiratory effort. The cage had natural substrate made up of native vegetation, cut branches and logs for perching. The diet was half an adult rabbit (2.5 kg) offered every 3 days for each bird. Water came from the well at the aviary. Due to behavioural alterations by human imprinted, the birds could not be released into their natural ecosystem, therefore, they were specimens used in captive conservation programs and to educate the public about the effects of illegal wildlife trafficking and the importance of their conversation.

Necropsy findings

Physical examination findings included a low body condition score of 3/9 with evidence of diarrhoea in cloacal feathers and severe lice infestation. Gross post-mortem examination revealed that the thoracic air sacs were thickened, cloudy, and had severe black-coloured nodule lesions with numerous fibrous adhesions to the liver, intestines, testes and lungs. The testes and lungs were diffusely affected. There was evidence of severe changes in the colour of the lungs with the presence of an amorphous dark mass of approximately 1.0 × 0.5 × 0.4 cm, involving 2/3 of both lungs, irregularly shaped with multiple nodules and weighing 66 g. The right and left testes were enlarged, firm and smooth, covered with a material of whitish colouration, forming a mass of approximately 5 × 4 cm in diameter; the testicles were 2/3 covered with this mass. The entire parenchyma of the testes had a glistening grey and white appearance and was moderately bulging and moderately irregular in appearance with a multilobulated appearance and with necrotic areas within the parenchyma. In the digestive system, the oral cavity had a moderate bloody mucus content. The liver presented a hard consistency when cut, with perihepatitis, rounded edges and dark colouration. The kidneys had a hard-cut consistency, with a slight increase in size and the ureters contained mild urates. The pericardium was thickened and covered with a yellowish material and the pericardial cavity was filled with fluid and fibrin content. In the haemolymphatic system, there were evident changes only in the spleen, where a thickened capsule of white-

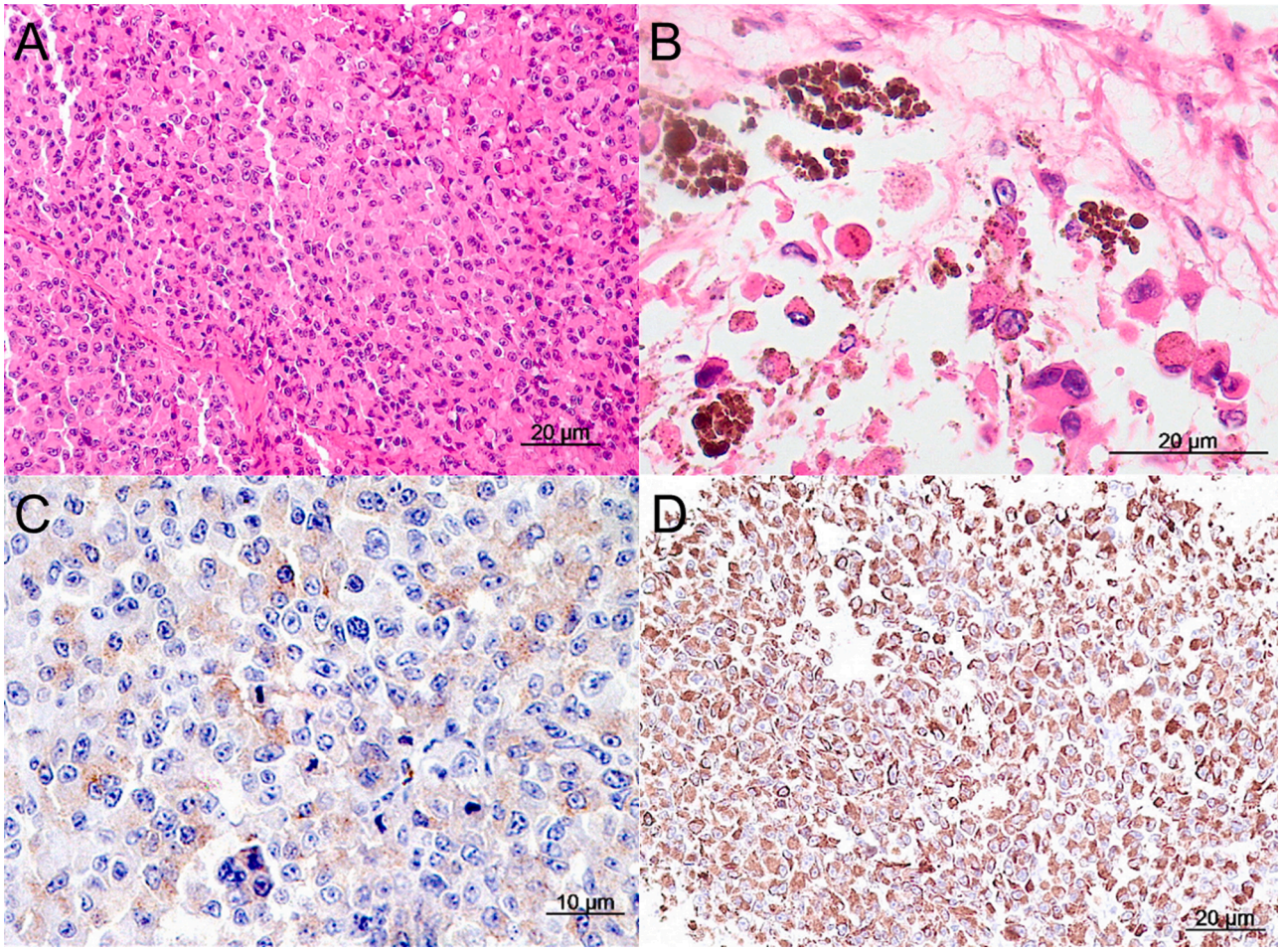

Fig. 1. Histology and immunohistochemistry of the rhabdoid melanoma. A. Testicular parenchyma. Melanocytes extensively replaced the testes; they were composed of large round-to-polygonal cells, vesicular chromatin and abundant eosinophilic cytoplasm and perinuclear eosinophilic hyaline globules, which peripherally displaced the nucleus. HE. B. Testicular parenchyma. A large number of cells showed brownish intracytoplasmic granular material, considered to be melanin. HE. C. Lung. A nodular aggregate of neoplastic melanocytes in the lung parenchyma, with immunoreactivity faint for Melan-A. IHC. D. Testicular parenchyma. Immunohistochemistry for vimentin showing strong and diffuse positivity of neoplastic cells.

yellowish colour was observed. All other organs, including the brain, bones and adrenals, appeared normal.

Histopathological findings

Samples from the brain, liver, intestine, heart, sciatic nerve, spleen, trachea, heart, lung, testis, testicular mass, and lung mass were collected and fixed immediately in 10% buffered neutral formalin. Subsequently, these samples were embedded in paraffin, cut to 5 μm thickness and stained with haematoxylin and eosin (H&E) for histologic examination.

Histologically, the testicles revealed a proliferation of neoplastic cells with invasive growth, unencapsulated and highly cellular, characterised mainly by large and dense pleomorphic cellular nests, composed of markedly anaplastic round-to-polyhedral neoplastic cell populations (Fig. 1A). Round-to-oval polygonal cells with eosinophilic cytoplasm contained variable amounts of small, brown-to-black granules compatible with melanin (Fig. 1B). Some cells showed rhabdoid features, containing eosinophilic inclusions and a peripherally-located nucleus. The tumour had severe anisocytosis and cellular pleomorphism. Nuclei were round-to-oval, clavate and irregular in shape, variably sized and containing vacuolated and finely stippled chromatin and 1–3 prominent nucleoli, with marked anisokaryosis and nuclear pleomorphism. Testicle parenchyma was completely replaced by the tumour and the seminiferous tubules were not observed. The mitotic figures were seen at a frequency of 5–7 cells per high-power field (100X). The lungs had numerous nodules of neoplastic cells infiltrating the parenchyma.

Immunohistochemistry findings

An immunohistochemistry (IHC) panel was performed, including Melan-A (Clone A103, 1:100, Dako, US), vimentin (Clone V9, 1:200, Dako, US), CK (Clones AE1/AE3, 1:100, Dako, US), MUM-1 (Clone EAU32, 1:100, Dako, US), and CD-68 (Clone KP1, 1:100, Dako, US). Neoplastic cells were positive for Melan-A (Fig. 1C) and vimentin (Fig. 1D), and negative for CK AE1/AE/3, MUM-1 and CD-68. According to the arrangement of the neoplastic cells, melanin pigment, histopathology, and immunoreactivity for Melan-A and vimentin, the case was diagnosed as a rhabdoid melanoma of the testicles with possible metastasis to lungs.

Discussion

In mammals, the histological criteria of malignancy are based on cytologic features, mitotic index, and the amount of melanin pigment present (Schultheiss, 2006). Melanomas in wild raptor birds and in captivity have been found to affect multiple organ systems and to be markedly malignant, featuring widespread distribution in tissues (adrenal gland, pancreas, lungs, skeletal muscle, and liver) (Barlow and Girling, 2004; Kufuor-Mensah and Watson, 1992) with aggressive local invasiveness and cellular pleomorphism (Barlow and Girling, 2004).

In all birds, the primary site or exact tissue of origin is not always known given the aggressive nature of this neoplasm (Williams et al., 2012). The degree of multiorgan involvement results in diagnoses at an advanced stage and with metastatic lesions (Kajigaya et al., 2010), as was the case in the present report. A few reports have cited liver (Smith et al., 2002), adrenal or cutaneous sites such as the face and the beak as primary sites of origin (Barlow and Girling, 2004; Costagliola et al., 2011; Smith, Goldschmidt, and McManus, 2002). In this case, there were no adrenal or cutaneous lesions. Therefore, and according to the level of severe lesion in the testicles, it is believed that these could be the origin of the neoplasm.

Melanoma may rarely display rhabdoid features, most often in metastatic lesions (Gardner and Smoller, 2015). Melanoma in our case was uncommon, not only by the species affected but also because this pathology, which affected the testicles bilaterally, has not been described before in no bird group. In addition, some cells showed rhabdoid features, composed of sheets of polygonal cells with round nuclei, vesicular chromatin, abundant eosinophilic cytoplasm, and perinuclear eosinophilic hyaline globules that peripherally displaced the nucleus (Chang et al., 1994; Kaneko et al., 2015). In this case, no conventional malignant melanoma component was detected. According to the avian veterinary literature (Irizarry-Rovira et al., 2007; Barlow and Girling, 2004; Guthrie et al., 2010; Williams et al., 2012; Duncan et al., 2014) and the findings in this case with neoplastic infiltrates in the lungs, it appears that this organ is commonly affected by melanoma. Granules from previous descriptions of avian melanoma are reported as black or dark brown to golden yellow (Campbell, 1951). In the H&E-stained preparations, the granules from the harpy eagle melanocytes were brown-to-black. In contrast, granules in melanomas from mammals are described as green-black, black, or blue-black in stained and unstained preparations (Smith et al., 2002).

Most literature on melanoma in human and veterinary medicine suggests exposure to ultraviolet radiation, carcinogens, viruses, and genetic factors, either alone and in synergy, can create an environment that promotes oncogenesis to melanoma (Modiano et al., 1999; Morris and Dobson, 2001). Melanomas in domestic animals usually involve the oral cavity, the mucocutaneous junction, and skin as primary origins (Goldschmidt and Goldschmidt, 2017). This last site may suggest induction of some avian melanomas by solar radiation, as this would be one of the few sites not well covered by feathers (Reid et al., 1993; Rambaud et al., 2003, Duncan et al., 2014). However, to date, there are no established risk factors to predict the origin of potential melanomas (Foster, 2016). The underlying cause of neoplastic transformation in the harpy eagle we describe is unclear because exposure to ultraviolet radiation and genetic predisposition are unlikely, based on the location of the tumours and the rarity of melanoma in avian species.

The few publications concerning melanoma suggest that prevalence in all birds appears to be low. This could be because the birds typically die and are not discovered, or they succumb to injuries, degenerative changes and harsh environmental conditions before they reach ages where the incidence of neoplasia becomes significant. Despite the above, there also seem to be physiological, genetic, and phenotypic risk factors that alter the occurrence in different species of birds. For example, melanomas occur with low prevalence in chicken (Campbell, 1969; Reece, 1996). Williams et al. (2012) reported that 7 broiler chicken carcasses (0.000194%) were diagnosed with multiple melanomas in 3.6 million chickens slaughtered. Other reports have found a relatively high prevalence of melanocytic tumours in penguins (*Eudyptes chrysolophus, Eudyptes chrysocome* and *Spheniscus humboldti*) (Kufuor-Mensah and Watson, 1992; Shindu, 1998; Rambaud et al., 2003). According to Duncan et al., (2014), the presentation of the pathology ranges from 2–15% in captive conditions and could be attributed to the aging nature of the penguin population as well as to the absence of protective feathers in some body areas.

It is well known that factors that increase the longevity of birds, such as captivity, extend life expectancy but may predispose to stress, and generate physiological, behavioural and genetic pressures. These are risk factors that increase the likelihood of neoplasms (Reavill and Dorrestein, 2010; Nemeth et al., 2016). Melanomas are not the exception. Most reports in wild avian species indicate that these factors are important in the presentation of the disease. Avian melanomas seem to be found more commonly in wild birds from 8 to 33 years of age (Reid et al., 1993; Rambaud et al., 2003; Stern and Lamm, 2009; Duncan et al., 2014), and exceptionally, in broiler chickens from 6 to 8 weeks of age (Williams et al., 2012). Considering that the harpy eagle is a long-lived raptor whose longevity is estimated to be 35 years (Lerner et al., 2009), this species could be predisposed to the presentation of neoplasms not only in captive conditions but also in free living.

Evaluating the health and diagnosing of melanoma in wild birds populations poses several challenges, the more so when the origin and extension of the neoplasm are not easily visible, as in the present case. In

addition, access to live, deceased animals and sample collection can be complicated by many obstacles, such as thick jungle or tissue loss through environmental decomposition, predation or post-mortem scavenging (Pesavento et al., 2018). Although in this case it was not possible to diagnose antemortem, many tools for diagnosis are available for melanoma detection in both mammals and birds. Histologic examination of the mass with H&E and/or Fontana Masson stain may allow for a definitive diagnosis if neoplastic cells are well differentiated and have fine brown granular intracytoplasmic pigment (melanin) (Stern and Lamm, 2009). However, when the pigment is poorly present or absent, immunohistochemistry may be required (Sandusky, 1985; Costagliola et al., 2011).

The immunophenotype of rhabdoid melanoma is highly variable (Gardner and Smoller, 2015). In domestic mammals, commonly used immunohistochemical evaluation melanocytic markers include Melan-A, PNL2, vimentin, S100 protein, neuron-specific enolase and tyrosinase (Ramos-Vara et al., 2000; Koenig et al., 2001; Ramos-Vara et al., 2002; Smith et al., 2002; Choi and Kusewitt, 2003). In avian species, immunohistochemical evaluation of melanomas is often less than satisfactory (Williams et al., 2012) owing to the species-specific nature of protein targets and consequently the antibodies required for detection (McAloose and Newton, 2009). Added to this, it is the scarcity of reported cases and the Melan-A and vimentin immunoreactivity features of such tumours, which are not well characterised (Stern and Lamm, 2009). Melan-A is a cytoplasmic protein, a product of the *MART1* gene (Goldschmidt and Goldschmidt, 2017), of specific melanocytic differentiation with high sensitivity (75–92%) (Ohsie et al., 2008; Ramos-Vara and Miller, 2011). Antibodies to Melan-A have been used in the diagnosis of melanomas from domestic and wild animals by detecting a protein largely restricted to melanocytes (Irizarry-Rovira et al., 2007; Williams et al., 2012). On the other hand, vimentin is a protein also known as fibroblast intermediate filament. In vivo, it is not usually present in normal epithelial cells (Robinson-Bennett and Han, 2006); however, it has been linked to a wide variety of pathophysiological conditions (Danielsson et al., 2018). In human and veterinary medicine, many studies have identified vimentin as a key component of cell invasion and metastasis in melanoma (Hendrix et al., 1992; Chu et al., 1996; Li et al., 2010; Williams et al., 2012). In this sense, it might act as a clinical predictor for melanoma and predict a high risk of metastasis. These markers (Melan-A and vimentin) should be interpreted in conjunction with the histological appearance of the neoplasm. In the present case, neoplastic cells have multifocal immunolabeling for Melan-A and strong positive staining for vimentin, demonstrating the positivity of the neoplastic cells that appeared nonpigmented by H&E. To rule out other tumours, mainly round-cell tumours (histiocytic sarcoma and malignant plasmacytoma), several immunohistochemistry tests were performed, including MUM-1 and CD 68, respectively. To rule out undifferentiated malignant tumours of epithelial origin, the marker CK AE1/AE3 was tried. The histological findings and the results of immunohistochemistry markers allowed a final diagnosis of rhabdoid melanoma in testicles metastasising to lungs.

Immunoreactivity was faint for Melan-A and strong for vimentin. This suggests that melanocyte immunoreactivity varies between different species of birds and can be distributed in a differential way among avians, as not all contain the same epitopes as are recognised by antibodies raised against mammalian proteins. For example, the lack of immunoreactivity to Melan-A marker has been previously reported in zebra finch (*Taeniopygia guttata*) (Irizarry-Rovira et al., 2007), umbrella cockatoo (*Cacatua alba*) (Stern and Lamm, 2009), broiler chickens (*Gallus gallus domesticus*) (Williams et al., 2012), penguins (*Eudyptes chrysolophus, Eudyptes chrysocome, Spheniscus humboldti*) (Duncan et al., 2014), but was positive in a seagull (*Larus fuscus*) (Costagliola et al., 2011). The case of vimentin has already been reported with strong positivity by Williams *et al.* (2012).

In humans and domestic mammals, primary melanoma of the testis is extremely rare and even the existence of such an entity is questioned. Theoretically, according to histogenesis, melanoblasts cannot be demonstrated in organs of mesodermal origin. Since the testis is an organ of mesodermal origin, it is unlikely that a melanoma can arise from this organ or any of the other visceral structures. On the other hand, non-neoplastic melanoblasts resulting from the migration of melanin-producing cells from the neural crest to mesodermal derivatives during embryologic development can explain the presence of melanocytes in the testis and support the possibility of a primary melanoma developing at this rare site. We report this case to emphasize the need for awareness of the possibility of the testis being the primary site in a bird with melanoma and to underline the necessity of considering a metastatic melanoma from unknown primary sites (Katiyar et al., 2007; Contreras et al., 2009; Aslam et al., 2010).

Oncogenic phenomena and their implications for wildlife management and conservation remain undeveloped (Hamede et al., 2020). Melanomas originate from resident melanocytic cells in tissues, which in turn are derived from a group of embryonic cells of neural crest, particularly pigmented melanocytes come from the trunk neural crest, but in birds, the cranial neural crest (mesencephalic neural crest cells) may give rise to melanocytes as well (Baker et al., 1997; White and Zon, 2008; Vandamme and Berx, 2019). Melanocytes can undergo a neoplastic transformation promoted by stimulating factors, such as genetic mutations amplifications or deletions, and disrupting gap-junctional intercellular communication, which stimulate proliferation, amplification and survival of neoplastic cells (Karachaliou et al., 2015; Smith, Goldschmidt, and McManus, 2002; Trosko, 2001). Assess the contribution of multiple factors, such as patterns of the emergence of neoplasms in wild birds in captive conditions is imperative because growing numbers of wild avian species are existing at the interface between humans and the environment (Madsen et al., 2017; Pesavento et al. 2018).

However, many programs do not account the multifactorial phenomena of oncogenesis, which impair both health and reproductive success over wild animals in captivity (Pesavento et al., 2018; Hamede et al., 2020). A strategy in conservation programs for wild birds in captivity is making the bird's captive sentinels for wildlife bird health. This perspective could help to elucidate possible founder effects that predispose wild and captive populations to cancers. In this regard, captive animals could provide new knowledge for understanding patterns of carcinogenesis and helping to mitigate risks of cancer emergence in the animals in conditions of wildlife and captivity.

Conclusion

The present report expands the list of avian species in which melanoma has been reported. Furthermore, it offers significant information if one considers that for the tropics, and worldwide, there are insufficient data on wild bird melanocytic neoplasm diseases, specifically in raptors. Our approach to the diagnosis of melanoma in a harpy eagle by the use of immunohistochemistry with commercially available antibodies could help other investigators in the diagnosis of this rare tumour in birds. We recommend considering this pathology as a differential diagnosis for masses of unknown origin that affect the testicles and lungs of birds. To our knowledge, this is the first known report of rhabdoid melanoma in testicular tissues in a harpy eagle with lung metastasis.

Etical animal research

The client signed a release form to consent to post-mortem and sample collection from his/her animal for diagnostic and learning purposes. The study was approved by the Bioethics and Animal Welfare Committee, School of Veterinary Medicine, Universidad Nacional de Colombia.

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CRediT authorship contribution statement

César Augusto Pinzón-Osorio: Investigation, Formal analysis, Writing - original draft. **Jersson Ávila-Coy:** Formal analysis. **Arlen P. Gomez:** Formal analysis, Writing - review & editing. **Diana Marcela Álvarez-Mira:** Formal analysis, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No conflict of interest has been declared.

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