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Malignant Melanoma in a Female Mallard Duck (*Anas platyrhynchos*)

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Abstract

Melanomas are malignant neoplasms originating from melanocytes. They reported in birds. In this case, macroscopic and microscopic (the histopathologic and IHC) findings supported a final diagnosis of cutaneous malignant melanoma in a mallard duck (*Anas platyrhynchos*). A female mallard duck (*Anas platyrhynchos*) was observed with a mass on the ventral portion of the neck. The bird was anesthetized with Diazepam/Ketamine. A skin incision was made on the ventral surface of the mass and blunt dissection was performed to separate the mass. The incision was sutured by a simple interrupted suture pattern. Mass was surgically excised for histopathological evaluation. Histologically, the mass was composed of nests and sheets of anaplastic, epithelioid, multinucleated and polygonal cells containing variable amounts of brown to black granules of melanin. The neoplasm showed immunoreactivity for S-100 and Melan-A in the cytoplasm of the neoplastic cells. Based on the histopathological and IHC findings, this is the first report of malignant melanoma in a mallard duck (*Anas platyrhynchos*).

KEYWORDS: *Anas platyrhynchos*, female, IHC, malignant melanoma, mallard duck

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Case History

Melanomas are malignant neoplasm originating from melanocytes (Nishiya et al., 2016). They have been described in mammalians, reptiles and fishes (Kimberly et al 2015; Rahmati-Holasoo et al., 2015; Nishiya et al., 2016). These tumors were less commonly reported in birds. However, they have been identified in a variety of avian species, including African grey parrot (*Psittacus erithacus erithacus*) (Shrader et al., 2016), thick-billed parrot (*Rhynchopsitta pachyrhyncha*) (Guthrie et al., 2010), zebra finch (*Taeniopygia guttata*) (Irizarry-Rovira et al., 2007), seagull (*Larus fuscus*) (Costagliola et al., 2001), mandarin duck (*Aix galericulata*) (Reid et al., 1993), merlin (*Falco columbarius*) (Barlow and Girling, 2004), rock hopper penguin (*Eudyptes chrysocome*) (Duncan et al., 2014), *macaroni penguin* (*Eudyptes chrysolophus*) (Duncan et al., 2014), and Humboldt penguin (*Spheniscus humboldti*) (Duncan et al., 2014). In this report, we describe cutaneous malignant melanoma in a mallard duck (*Anas platyrhynchos*).

Clinical Presentation

In August 2018, a 2-year-old female mallard duck (*Anas platyrhynchos*), at the Qazvin nature village was observed with a large palpable and firm mass on the ventral portion of the neck (Fig. 1a). Within the previous 1-month period the mass had become distinct and grew larger. No other physical abnormalities were seen. On presentation, the bird was quiet and alert. Appetite and stool appearance were normal. The duck was fed a diet of seeds and plants. Finally, surgical removal of the neoplasm was elected. The bird was anesthetized with Diazepam/Ketamine. A skin incision was made on the ventral surface of the mass and blunt dissection was performed to separate the mass. The incision was sutured by a simple interrupted suture pattern. The mass was removed for histopathological evaluation and the bird recovered uneventfully. Enrofloxacin (10 mg/kg IM, once daily for 5 days) was administered IM.

Diagnostic Testing

On gross examination, the mass was firm, black and 10 × 7/5 × 3 cm in size. Tissue samples of the mass were fixed in 10% neutral buffered formalin and routinely processed, dehydrated and embedded in paraffin wax, sectioned at 5 µm thickness (Rotary Microtome RM2 145; Leica) and stained with hematoxylin-eosin (H&E). Additional sections were probed immunohistochemically for S-100 and Melan-A as described previously (Ramos-Vara et al., 2000). Histopathologically, the mass was mainly composed of infiltrative, pigmented melanocytes that extended from superficial to deep dermal regions and had effaced normal tissue architecture (Fig. 1b-c). In the superficial dermis, the neoplastic melanocytes were arranged in various sizes of nests and lobules (Fig. 1b). The neoplastic cells coalesced in the deep dermis, forming dense sheets separated by collagenous stroma (Fig. 1c).

Microscopic examination of the sections revealed anaplastic, epithelioid, round to oval to polygonal cells, ranging in size from 18 µm to 90 µm in diameter (giant cells), with eosinophilic cytoplasm containing variable amounts of brown to black gran-

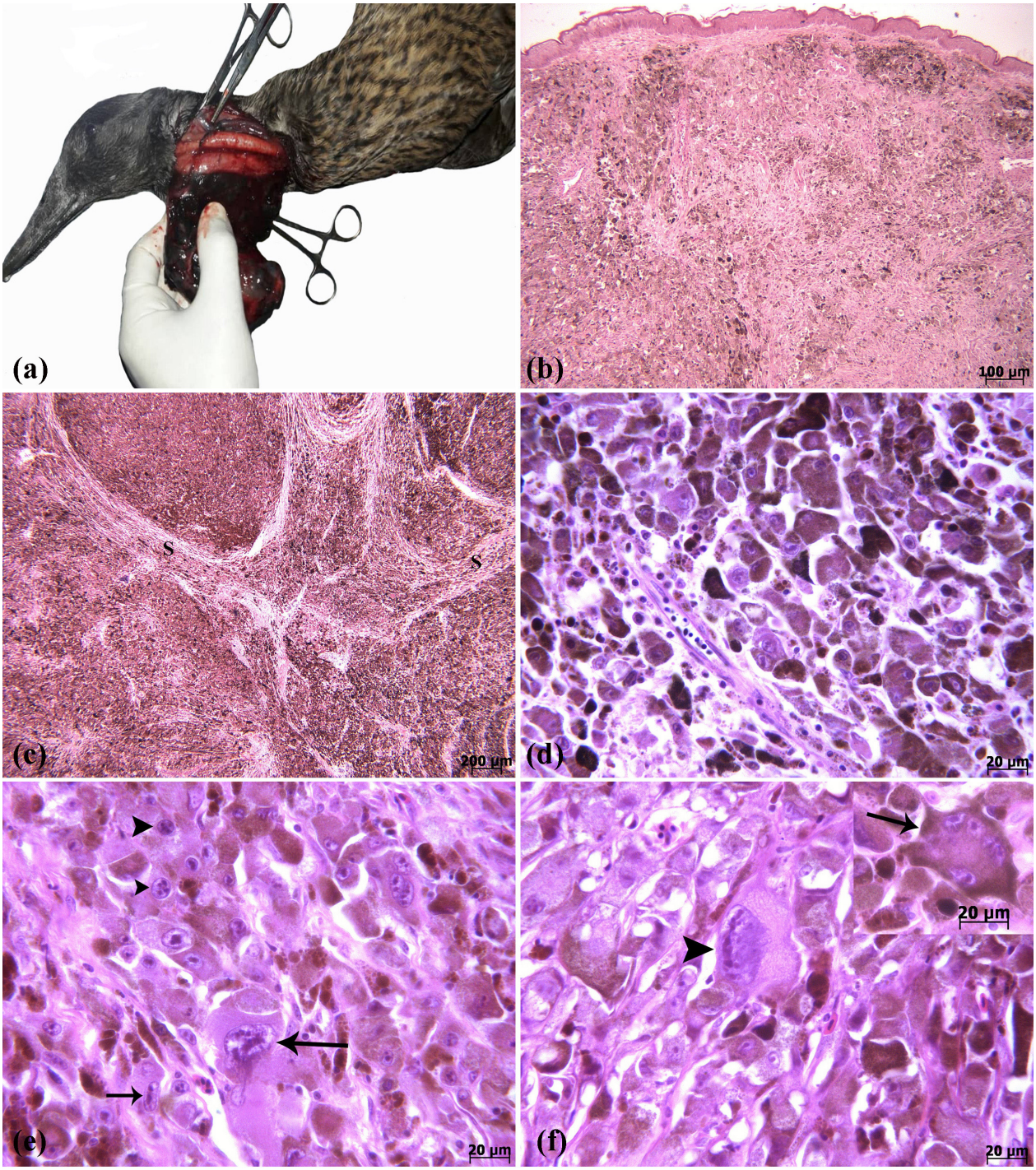

Fig 1. (a) The mass on the ventral portion of the neck. (b-f): Histopathological sections of Melan ma, hmatoxylin and eosin. (b) Variably sized nests of neoplastic cells in the dermis (4x). (c) Dense sheets of the neoplastic melanocytes in the deep dermis. S: stroma (10x) (d) The cytoplasm of cells containing variable amounts of brown to black granules of melanin (40x). (e) Round (large arrowhead) to oval (small arrow) to pleomorphic (large arrow) nuclei with four prominent nucleoli (small arrowhead) (60x). (f). Pleomorphic cells (arrow and arrowhead). Inset: multinucleated giant cell (arrow) (60x).

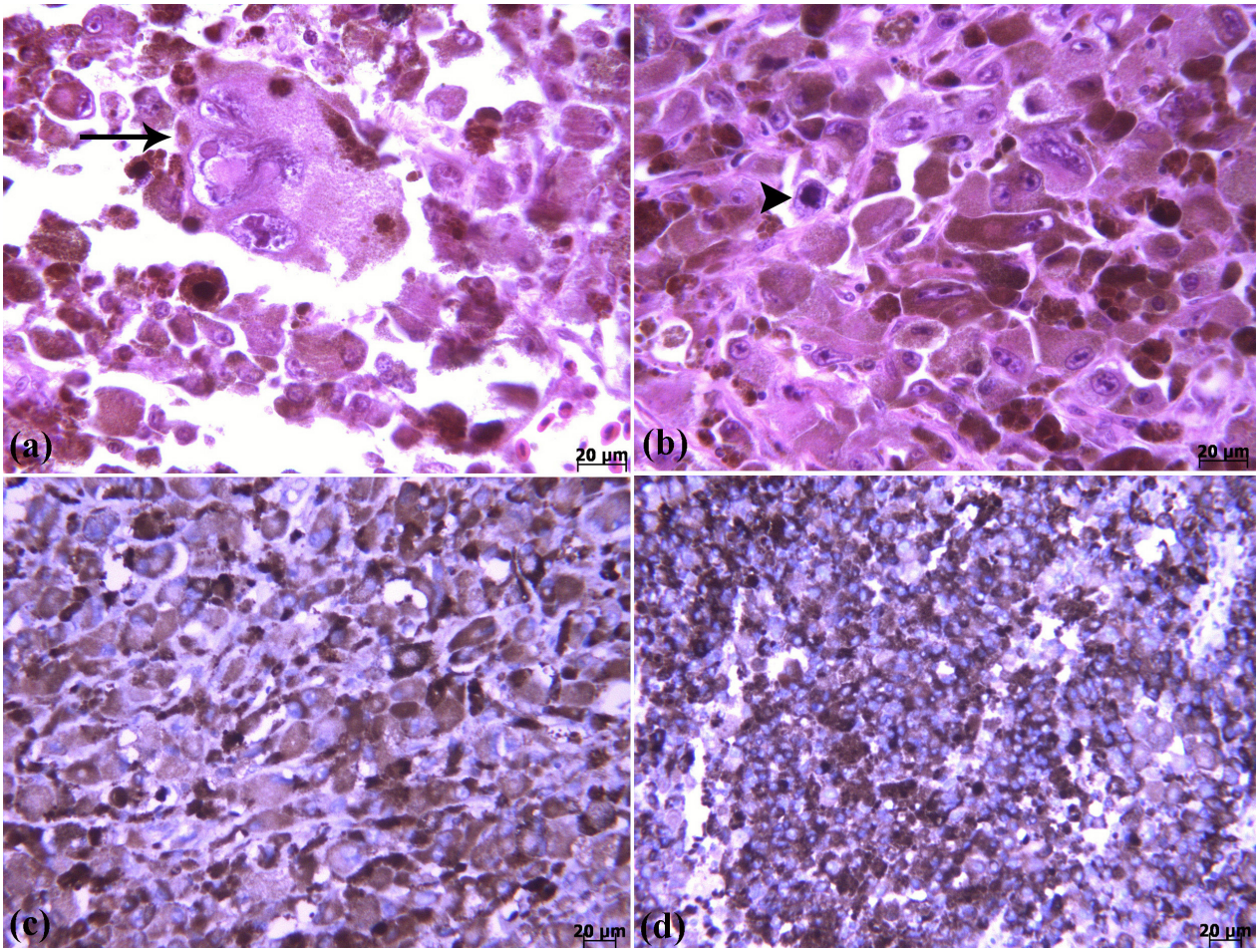

Fig . 2. (a-b): Histopathological sections of Melanoma, hematoxylin and eosin. (a) multinucleated giant cell (arrow) (60x). (b) The mitotic figure (arrowhead) is lesser that 10 in HPF (60x). (c-d): IHC, Immunoreactivity for Melan-A (c) and S-100 (d) in the cytoplasm of the neoplastic cells (40x)

ules of melanin (Fig. 1d). The nuclei were round to oval to pleomorphic, ranging from 8 µm to 45 µm in diameter with one to four prominent nucleoli (Fig. 1e). Markedly multinucleated, pleomorphic and bizarrely shaped cells were also seen. Scattered multinucleated giant cells contained up to 4 nuclei (Fig. 1f, 2a). The number of mitotic figures per 10 high-power fields varied from 3-5 throughout the entire tumor (Fig. 2b). The mass showed more than 80% immunoreactivity for S-100 and Melan-A in the cytoplasm of the neoplastic cells. The histopathologic and IHC findings supported a final diagnosis of cutaneous malignant melanoma. The mallard duck suddenly died and internal tissues of the bird were not available for examination.

Assessments

Avian melanoma may originate from the skin, liver, lung, beak, eyes, and adrenal glands, and is usually reported to be malignant (Reid et al., 1993; Barlow and Girling, 2004; Irizarry-Rovira et al., 2007). Similar to present case, malignant melanomas tend to be fast-growing tumors, and often are pigmented (grey, brown, or black). Melanocytic tumors vary in size from small, pigmented macules, to larger masses which

may be 5 cm or more in diameter (Goldschmidt and Goldschmidt, 2017). Large size of melanoma is considered poor prognostic factor (Smith et al., 2002). In dogs, melanomas that have at least 1 cm diameter are described as malignant neoplasms (Meyrer et al., 2016). Additionally, in mandarin duck, neoplasm over 4 cm in diameter was diagnosed as a malignant melanoma (Reid et al., 1993). Large size of the cutaneous mass in the present study supports malignant behavior of this neoplasia. The marked cellular pleomorphism of the melanoma in our case, which warrants description of an anaplastic malignant melanoma, is similar to other cases of avian malignant melanomas (Irizarry-Rovira et al., 2007; Goldschmidt and Goldschmidt, 2017). Also, the majority of cutaneous malignant melanomas similar to this case have nuclear atypia and multiple nucleoli (Goldschmidt and Goldschmidt, 2017). As in the present report, some avian melanomas may be pleomorphic in histologic sections, contain bizarre neoplastic cells, and/or contain multinucleated cells (Irizarry-Rovira et al., 2007). Nuclear atypia is a common term used by pathologists to help classify neoplastic lesions (i.e., benign versus malignant) (Goldschmidt and Goldschmidt, 2017). In animal, skin melanocytic neoplasms, the most reliable histologic feature for distinguishing malignant from benign is the mitotic index (Goldschmidt and Goldschmidt, 2017). In this case, the number of mitotic figures per 10 high-power fields varied from 3-5. In the World Health Organization's Histologic Classification of Epithelial and Melanocytic Skin Tumors of Domestic Animals (Smith et al., 2002), three or more mitotic figures per 10 high-power fields indicate malignancy. The majority of melanocytic neoplasms are easily recognized by the presence of melanin pigment in combination with histologic features (Goldschmidt and Goldschmidt, 2017). S-100 remains the most sensitive marker for melanocytic lesions, while markers such as Melan-A demonstrate relatively good specificity but not as good as S-100. However, in dogs, Melan-A is highly sensitive and specific for detecting melanocytic neoplasms, whereas S-100 has poor specificity (Smedley et al., 2011). S-100 and Melan-A have not been reliable markers for melanocytic neoplasms in several avian species, including the domestic chicken, zebra finch, and cormorant (*Phalacrocorax carbo*) (Reid et al., 1993; Irizarry-Rovira et al., 2007; Williams et al., 2011). Even more interesting, immunohistochemical findings similar to the melanoma diagnosed in the mallard duck were described in a seagull (*Larus fuscus*) and showed moderate immunoreactivity for S-100 in the cytoplasm of 80% of the neoplastic cells and marked intracytoplasmic positivity for Melan-A in all neoplastic cells (Costagliola et al., 2011). Regardless of the species, malignant melanomas have similar biological behavior in that they frequently recur and have a high rate of metastasis (Reid et al., 1993; Smith et al., 2002). The regrowth of malignant melanomas occurs after surgery. We were unable to find the cause of death. However, it appears regrowth of neoplasia and metastasis after surgery could be reasons for the duck's death. The etiology of spontaneous melanomas remains unknown in most species (Smith et al., 2002). The major risk factors for human melanoma include family history, skin and mucosal pigmentation characteristics, sun exposure, particularly to ultraviolet B-rays (UVB) (Nishiya et al.,

2016). Several etiological factors are supposed to be involved in canine malignant melanomas, including consanguinity, trauma, chemical exposure, hormones, and genetic susceptibility (Nishiya et al., 2016). It is possible that melanomas in birds are the result of spontaneous neoplastic transformation (Shrader et al., 2016). On the basis of the macroscopic and microscopic characteristics, the cause of this neoplasm remains unknown. However, chemical carcinogens or genetic influences appear to be the explanation for this lesion.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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مجله طب دامی ایران، ۱۳۹۷، دوره ۱۳، شماره ۴، ۴۴۴-۴۳۷

ملانوم بدخیم در یک اردک مالارد (*Anas platyrhynchos*) ماده

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چکیده

گزارشات متفاوتی از ملانوم بدخیم با منشاء ملانوسیت ها، در پستانداران، دوزیستان و ماهی ها وجود دارد. تعداد این گزارشات در پرندگان به مراتب کمتر می باشد. در این گزارش، برای اولین بار بررسی های ماکروسکوپی و میکروسکوپی (با رنگ آمیزی معمول هماتوکسیلین-ائوزین و ایمنوهیستوشیمی) ملانوم در اردک مالارد انجام شده است. یک مورد اردک مالارد ماده با یک توده برجسته و سفت در ناحیه زیر گردن ارجاع داده شد. متعاقب معاینات بالینی، در نهایت تصمیم گرفته شد توده با جراحی خارج گردد. پرنده با دیازپام- کتامین بیهوش شد و توده بعد از خارج شدن در فرمالین بافر خنثی ۱۰ درصد فیکس شد و از نمونه های بافتی فیکس شده مقاطع بافتی با رنگ آمیزی معمول هماتوکسیلین- ائوزین و ایمنوهیستوشیمی با S-۱۰۰ و Melan A تهیه شد.در بررسی های میکروسکوپیک مقاطع بافتی،توده مذکور از تجمعات سلول های اپیتلیوئیدی، سلول های نئوپلاستیک چند وجهی و چند هسته ای و سلول های آناپلاستیک تشکیل یافته بود که این سلول ها مقادیر متفاوتی از گرانول های قهوه ای- سیاه ملانین را درسیتوپلاسم داشتند. در بررسی های مقاطع ایمنوهیستوشیمی تهیه شده از نمونه های بافتی، سلول های نئوپلاستیک S-۱۰۰ و Melan A مثبت بودند. با توجه به بررسی های بالینی، کالبدگشایی و میکروسکوپی، برای اولین بار ملانوم در پرنده مالارد تشخیص داده شد.

واژه‌های کلیدی:
ملانوم، اردک مالارد، ماده، ایمنوهیستوشیمی، *Anas platyrhynchos*

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