
**Conjunctival malignant melanoma in a 10-year old Boerboel dog:
A Case Report**

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Abstract

Conjunctival malignant melanomas are rare but potentially devastating tumours that have the tendency to recur after surgical excision. This case report is on a conjunctival malignant melanoma in a 10-year old Boerboel dog with history of recurrence after two consecutive surgical excisions. The dog was presented at the Veterinary Teaching Hospital, University of Nigeria Nsukka, Nigeria with a large, ulcerated and pigmented growth that almost completely obliterated the left eye. Comprehensive physical examination was done and blood sample was collected from the dog for haematology. The haematological parameters were evaluated following standard procedures. The dog was anaesthetized with a combination of Atropine Sulphate (0.04mg/kg), Xylazine, (0.75mg/10kg) and Ketamine (10mg/kg). The neoplastic mass was surgically excised alongside contiguous conjunctival tissues. The excised mass was sliced and fixed in 10% neutral-buffered formalin and processed for histopathology. Results of the haematological evaluation showed that the red blood cell (RBC) count of the dog was lower than the reference value for the species, while the mean corpuscular volume, total white blood cell (WBC), neutrophil and lymphocyte counts were above the reference values for dogs. Grossly, the excised neoplastic mass weighed 56.8 g, and was spherical, nodular, pigmented and firm. Microscopically, sections of the excised neoplastic tissue showed bizarre melanocytes with each cell possessing one or more prominent nucleoli. There were marked anisocytosis and anisokaryosis, numerous mitotic figures/40× fields, and moderate deposition of melanin pigment. Conjunctival malignant melanoma was diagnosed based on the high mitotic index. Normal vision with the affected left eye was restored by day 10 post-surgery, within which period Penicillin-Streptomycin (1ml/25kg), Paracetamol (15mg/kg) and Vincristine (0.7mg/m²) therapies were administered. Recurrence was again reported 3 months post-surgical excision in spite of treatment with vincristine. Exenteration would have been recommended, but the case was lost to follow-up.

Keywords: Malignant melanoma; Conjunctiva; Boerboel dog; Haematology; Mitotic figures; Conjunctivectomy.

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Introduction

Melanoma is a benign or malignant tumour that develops from melanocytes (Nishiya *et al.*, 2016). Humans and most domesticated animal species, including dogs, cats, and horses, as well as wild terrestrial and marine animals, have been reported to have melanocytic neoplasms (Sweet *et al.*, 2012). Although melanomas can arise in any dog, it has been reported that the prevalence of this neoplastic disease is higher in purebred dogs, especially Standard and Miniature Schnauzers, Doberman Pinschers, Scottish Terriers, Irish and Gordon Setters, and Golden Retrievers; a fact that supports the theory that the occurrence of melanomas in dogs could have a genetic basis (Curtin *et al.*, 2005; Nishiya *et al.*, 2016).

Malignant melanomas in dogs can develop in a variety of anatomical locations, including the mouth, lips, skin, eyes, and digits. The oral cavity and skin are the most typical sites for this disease in dogs, according to the majority of research reports, but there is some debate regarding this (Modiano *et al.*, 1999; Omholt *et al.*, 2011; Turri-Zanoni *et al.*, 2013; Lyu *et al.*, 2015). A survey of 384 melanocytic canine cancers by Spangler and Kass (2006) showed that oral, cutaneous, lips/feet, and eye locations made up 19%, 59%, 19%, and 3%, respectively. Other studies that looked at malignant melanomas found that cutaneous and oral cavity localisation were most prevalent, with respective percentages of 40% – 62% and 27% – 31% (Teixeira *et al.*, 2010; Gillard *et al.*, 2014).

Conjunctival melanomas generally affect the nictitating membrane and the bulbar and/or eyelid conjunctiva; and Rottweiler and Cocker Spaniels dogs had been reported to be more prone to developing them (Conceição *et al.*, 2010; Dubielzig *et al.*, 2010). Conjunctival malignant melanomas are rare but potentially fatal tumours that can invade the local tissues of the eyes and disseminate throughout the body by lymphatic drainage and

haematogenous route, and recur after treatment (Wong *et al.*, 2014). Although it is severe, the evidence for diagnosis and treatment has been limited by the scarcity of cases that have been reported (Ramos-Vara *et al.*, 2000). This report described a rare case of conjunctival malignant melanoma in a Boerboel dog.

Case Report

A 10 year-old male Boerboel dog, weighing 58 kg, was referred to the Veterinary Teaching Hospital, University of Nigeria, Nsukka (VTH, UNN), Enugu State, Nigeria, with a primary complaint of growth on the left eyelid. The dog was housed in a kennel and used as guard dog. Records showed that the dog was up to date on its recommended vaccination and deworming schedule. The dog was usually fed commercial dog food, bone meal and home-made foods. The referral note also stated that dog first presented with a growth on the third eyelid, and this was surgically excised successfully. However, the growth was recorded as having re-occurred and was corrected a second time. On third recurrence, the private veterinary clinic decided to refer the case to the VTH, UNN.

On physical examination, the mucous membranes of the dog were normal and no ectoparasites were found on the body. However, palpable lymph nodes were grossly enlarged. A tissue growth, which was about the size of a hen's egg, was observed on the left lower eyelid. The tissue growth was firm, ulcerated and dark pigmented at the base (Figure 1a). Blood sample was collected through the cephalic vein for full blood count after the physical examination, and the dog was then scheduled for surgery.

Prior to surgery, the dog was fasted for 12 hours and then properly restrained. Hairs around the left eye were clipped. The shaved area was cleaned thoroughly and wiped with

sterile gauze. Surgical instruments were also sterilized. Anaesthesia was achieved by injection of Atropine Sulphate, 0.04mg/kg, intramuscular (IM), followed immediately by Xylazine, 0.75mg/10kg, IM, and Ketamine, 10mg/kg, IM, after 5 minutes of Xylazine administration.

The dog was laid on sternal recumbence and was properly draped. The eyeball was coated with penicillin ointment and packed with sterile gauze to protect it from injury during manipulation and to avoid desiccation. Incision was made on the base of the lower eyelid on its deep surface, and then gently, blunt incisions were used to undermine tissues around the growth. Haemorrhages were arrested using sterile haemostatic forceps. Blood vessels supplying the tissue growth were clamped and incised. The tissue growth was removed and excess tissues were debrided. The eye was unpacked and flushed with normal saline and the wound was closed. The membrane of the lower eyelid and muscles were apposed by simple interrupted suture pattern using size 2.0 vicryl suture materials. The eye was again flushed with normal saline and bandaged. The dog was allowed to recover from anaesthesia before post-operative care. The dog was placed on Penicillin-Streptomycin, 1ml/25kg, (IM), for 7 days; Paracetamol, 15mg/kg, (IM), for 3 days; and Vincristine, 0.7 mg/m² intravenous IV, given at 1 week intervals. At the time of discharge from the hospital, the dog had regained sight completely with the previously occluded left eye (Figure 1b).

The excised tissue growth, including the mass and several millimetres of contiguous conjunctiva, were fixed in 10% neutral-buffered formalin, routinely processed and sectioned at 5 µm thickness and stained with haematoxylin and eosin.

Haematology: The packed cell volume (PCV) and haemoglobin (Hb) concentration of the dog with melanoma were within the reference

interval, but the red blood cell (RBC) count was below the lower reference value for dogs, while the mean corpuscular volume of the dog with melanoma was far higher than the reference value (Table 1). The total white blood cell (TWBC), absolute neutrophil and lymphocyte counts of the dog with melanoma were also above the reference value for dogs (Table 1).

Gross Pathology: The excised neoplastic mass of tissue was spherical, nodular, pigmented, and firm (Figure 2a). It weighed 56.8 g. Its incised surfaces were amorphous, whitish and had large melanin pigments and thin longitudinal striations (Figure 2b).

Histopathology: Microscopic examination of stained sections of the excised tissue growth showed bizarre melanocytes, with each cell possessing one or more prominent nucleoli. There were marked variations in cell size and size of nucleus of the neoplastic melanocytes; there were thus numerous mitotic figures/40× fields and moderate deposition of melanin pigment (Figures 3a & b). Most of the cells were epithelioid, round, and spindles cells with high nuclear-cytoplasmic ratio and nuclear vacuolation. The mitotic figures were in different stages of karyokinesis: prometaphase, metaphase and anaphase chromosomes were identified (Figure 3b). Based on the histological classification of domestic animal tumors according to cytological appearance, number of mitoses, pleomorphism, and presence of anaplastic and poorly differentiated cells (Wong *et al.*, 2014), a diagnosis of primary malignant conjunctival melanoma was made.

Three months after this case was managed, the recurrence of the neoplastic mass was reported by the owner. Exenteration would have been recommended, but the case was lost to further follow-up.



Figure 1: **1a)** Photograph of a Boerboel dog with conjunctival malignant melanoma, showing large, pink coloured and ulcerated tissue growth on the left lower eyelid conjunctiva. Note the black (melanin) pigments at the base of the growth. **1b)** Picture of the dog's left eye (encircled), 10 days post-surgical excision of the tissue growth. Normal vision with the left eye was restored.

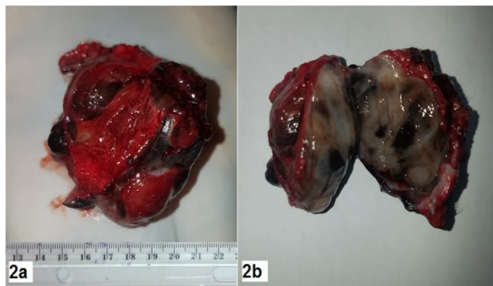


Figure 2: Gross photograph of the excised tissue growth (**2a**) from a dog with conjunctival malignant melanoma, and a longitudinal section through the growth (**2b**) showing large multifocal areas of melanin pigment deposition.

Discussion

Ocular melanomas had been reported to be the most common primary ocular neoplasm of dogs (Diters *et al.*, 1983; Guimarães *et al.*, 2021). They are in general benign, with a few important exceptions where severe aggressive behaviour can be reported (Wang and Kern, 2015). Conjunctival malignant melanomas have been reported to develop from melanocytes found amongst the basal cells of the conjunctival epithelium (Wong *et al.*, 2014). Canine conjunctival melanomas have

been reported to be more likely to occur on the third eyelid (Finn *et al.*, 2008). Previous studies have reported conjunctival melanoma in a cat (Roels and Ducatelle, 1998), horse (Moore *et al.*, 2000), and captive fallow deer (Sakai *et al.*, 2001). The present case in a Boerboel dog exhibited locally aggressive behaviour as shown by recurrence after two surgical excisions and chemotherapy. In one earlier study, local recurrence was reported in 6 out of 11 cases of canine conjunctival melanomas after surgical excision, and metastasis was confirmed in 2 out of the 11 cases (Collins *et al.*, 1994). There was no sign of metastasis in the present case, and this may be due to the routine administration of anti-neoplastic drugs. It had earlier been posited that recurrence in canine conjunctival melanomas is common but metastasis is rare (Schobert *et al.*, 2010).

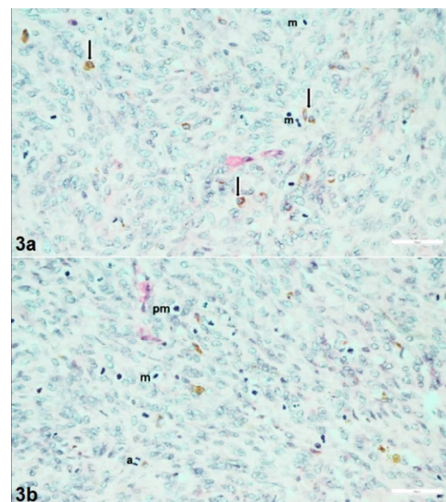


Figure 3: Photomicrograph of sections of the neoplastic tissue obtained from a dog with conjunctival melanoma, showing melanocytes, comprised mainly of epithelioid and spindle cells with 1 – 2 prominent nucleoli, marked nuclear vacuolation, and anisokaryosis. **3a)** Note melanin pigment deposition (arrows) and numerous mitotic figures (m). **3b)** Note karyokinesis showing prometaphase (pm), metaphase (m) and anaphase (a) chromosomes. H & E Stain $\times 400$. Bar = 20 μ m.

Table 1. Haematological parameters of Boerboel dog with conjunctival malignant melanoma, compared with reference values for dogs.

Parameters with units in bracket	Value obtained for the dog with melanoma	Reference interval for dogs*
Packed cell volume (%)	37	35 – 57
Haemoglobin concentration (g/dL)	12.5	11.9 – 18.9
Red blood cell count ($10^6/\mu\text{L}$)	3.5	4.95 – 7.87
Mean corpuscular volume (fl)	105.71	66 – 77
Mean corpuscular haemoglobin concentration (g/dL)	33.78	32 – 36.3
Total white blood cell count ($10^3/\mu\text{L}$)	23.00	5 – 14.1
Lymphocyte counts (%)	30%	8 – 21
Neutrophils counts (%)	70%	58 – 85
Absolute Lymphocyte counts ($10^3/\mu\text{L}$)	6.90	0.4 – 2.9
Absolute Neutrophil counts ($10^3/\mu\text{L}$)	16.10	2.9 – 12

* Source of Reference values: Weiss and Wardrop, 2010; Latimer, 2011.

The gross appearance of the neoplastic mass recorded in this case report is consistent with the earlier reported features of palpebral, bulbar, and third eyelid conjunctival melanocytic neoplasms that manifest as pink, lightly pigmented, or darkly pigmented masses (Labelle and Labelle, 2013). The neoplastic mass in the present case was also ulcerated, which may have provided a portal for microbial infection leading to the occurrence of neutrophilia. Lymphocytosis, on the other hand, may probably be due to an immune response to the neoplastic mass.

Microscopically, the mitotic index (MI) has reportedly been found to be the best indicator of malignancy for conjunctival neoplasms, although a firm cut-off has not been established (Labelle and Labelle, 2013). Identification of 3 – 4 mitoses in 10 high-power fields (HPFs), which has been proposed for mucosal melanocytic neoplasms at other sites, may be useful in the classification of

conjunctival melanocytic neoplasia (Esplin, 2008; Smedley *et al.*, 2011). In dogs, mitotic index is the best prognostic indicator, with more than two mitoses/ten 40 \times fields correlated with a high probability (73%) of tumour-induced death within 2 years (Bostock, 1979). The present case had numerous mitotic figures/40 \times field, which both confirms its malignancy and suggests a poor prognosis. It also showed predominance of epithelioid and spindle cells with one or two prominent nucleoli, consistent with the reported histological features of canine conjunctival neoplasms (Collins *et al.*, 1994).

The lower RBC count and higher MCV value recorded for the dog are suggestive of macrocytic (regenerative) anaemia. Cancer-related anaemias appear to result from a range of factors including chronic inflammation, blood loss, nutritional deficiencies, haemolysis, bone marrow infiltration by malignant cells, low serum

erythropoietin (EPO) levels, and a decrease of bone marrow responsiveness to EPO (Coiffier, 2000). Microcytic hypochromic anaemia was reported together with iron deficiency in melanoma-bearing Minipigs (Čížková *et al.*, 2019), suggesting that haematological responses to melanocytic neoplasms may vary from case to case. Melanoma has historically been considered an immunogenic malignancy as it contains great amount of immune cells (different subsets of T-cells, dendritic cells, macrophages, neutrophils, mast cells, B lymphocytes) and the presence of a dense tumor infiltrating lymphocytes (TILs) is associated with a good prognosis (Neagu, 2012; Mukherji, 2013). However, in the present case, lymphocytosis occurred without infiltration of lymphocytes into the neoplastic mass as seen in cases with good prognosis (Antohe *et al.*, 2019).

Currently, melanocytic neoplasms are treated by surgery (including exenteration and enucleation), cryotherapy, radiation, photodynamic treatment (PDT), laser, and immunotherapy (Guimarães *et al.*, 2021). Melanocytic neoplasms are believed to be generally susceptible to cryotherapy (Jacobiec *et al.*, 1980; Seregard, 1998). Unfortunately, cryotherapy was not used in the present case after surgery due to absence of such facility in the veterinary hospital in the study area. Moreover, there may be exceptions in the susceptibility to cryotherapy as seen in cases of primary conjunctival melanomas of dogs and cats reported by Cook *et al.* (1985) and Collins *et al.* (1994), and in primary malignant conjunctival melanoma reported in a horse by Moore *et al.* (2000), in which tumors recurred after cryotherapy.

Vincristine was used post-surgery in this case reported, because it has been reported to inhibit tumour mitosis by blocking the synthesis of intracellular microtubules (Chun *et al.*, 2007). In an earlier report, Mitomycin C and Interferon alpha-2b were used by Wong *et al.* (2014) after surgery as adjuvant therapy for

conjunctival malignant melanomas, but these specific chemotherapeutic agents (Mitomycin C and Interferon alpha-2b) were not available in the veterinary hospital where this present case was managed.

Furthermore, Penicillin-Streptomycin and Paracetamol were used in the postoperative care to prevent and manage secondary infection and inflammation, as recommended by Wong *et al.* (2014).

Declaration of conflicting interests

The authors declare no potential conflict of interest with respect to the research, authorship and publication of this article.

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