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A RARE CASE OF OCULAR FIBROSARCOMA IN *Bubalus Bubalis* (WATER BUFFALO): CYTO AND HISTOPATHOLOGY

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ABSTRACT

Though primary tumors of the conjunctiva are not uncommon, ocular fibrous tumors are quite rare in most species, including buffaloes. A buffalo with an ocular growth at the bulbar conjunctiva was presented to the Veterinary Clinical Complex, SVPUAT, Meerut. Fine needle aspiration cytology (FNAC) of the ocular growth was performed, followed by surgical removal. Furthermore, the formalin-fixed sample was processed for histopathology and special staining (Masson's trichrome) for confirmatory diagnosis. Based on cytopathological as well as histological features, the growth was diagnosed as fibrosarcoma. To the best of our knowledge, no report of fibrosarcoma is available at this location in buffaloes. The current case is presented with the aim to describe the clinical and histological features of an unusual case of ocular (conjunctival) fibrosarcoma.

Keywords: *Bubalus bubalis*, buffaloes, bulbar conjunctiva, eye tumour, fibrosarcoma

INTRODUCTION

The eye is a vital sense organ in vertebrate animals, relied upon for survival and interaction with their surroundings. Ocular pathology, the study of pathological processes affecting the eye bulb and its adnexal tissues, is a relatively recent field in veterinary medicine, with the first papers dating back to the early twentieth century (Gelatt, 2008).

The conjunctiva is a vascularized mucous membrane that can be divided into three parts: bulbar conjunctiva, conjunctival fornices, and palpebral conjunctiva. Martins and Barros (2014) reported that in bovines, the eyelid, cornea, and third eyelid were the anatomical locations most frequently affected by ocular and periocular diseases, in decreasing order of incidence. Neoplastic lesions comprised 85.0% of all diagnostic lesions, with squamous cell carcinoma (SCC) accounting for 80.3% of all diagnoses. We did not come across any report of conjunctival fibrosarcoma in buffaloes. Therefore, the current case study is an attempt to present a rare case of ocular fibrosarcoma in domestic buffalo (*Bubalus*

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bubalis).

MATERIALS AND METHODS

A 7-year-old female buffalo was presented to the Department of Veterinary Surgery and Radiology with a smooth, blackish-white, pedunculated growth measuring approximately 4 cm x 3 cm x 1.5 cm, protruding from the upper lateral bulbar conjunctiva, covering almost the entire left orbit. The growth had persisted as a small mass for the last four months but had drastically increased in size in the last two weeks. The animal was otherwise healthy with no significant familial history. Fine needle aspiration cytology (FNAC) was performed, and the FNAC sample was screened after staining with Leishman stain.

After a proper fasting period of 24 to 36 h, the animal was sedated using Xylazine injection at a dosage of 0.1 mg/kg body weight administered intramuscularly. Following sedation, the animal was placed in lateral recumbency with the affected eye positioned upwards. Regional anaesthesia was induced through Auriculopalpebral and Peterson nerve blocks using 2% lignocaine hydrochloride solution (Figure 1).

The neoplastic growth on the conjunctiva was excised with the assistance of a 24-gauge needle and subsequently removed. After excision, the eye underwent flushing with normal saline. Postoperatively, antibiotic enrofloxacin were administered parenteral at a rate of 5 mg/kg body weight, and analgesic meloxicam was given intramuscularly at a rate of 0.5 mg/kg body weight daily for five consecutive days. Additionally, eye drops containing Gentamicin and flubiprofen were recommended for administration, three times a day, over a period of 7 to 10 days.

The mass was removed and sent to the Department of Veterinary Pathology for diagnosis where it was first cut into suitable pieces, and scraping samples were taken from the cut surface for cyto-pathological diagnosis. After that, the pieces were fixed in 10% neutral buffered formalin for 72 h followed by overnight washing under running tap water. The tissue was dehydrated in graded alcohol and acetone followed by clearing in benzene and paraffin embedding. The tissue was cut into 4µm thick sections and processed for Masson's trichrome and routine haematoxylin and eosin staining as described by Luna in 1968.

RESULTS AND DISCUSSIONS

Grossly, the mass appeared very firm and multinodular, with a superficial blackish tinge, with a mild grating sound during the process. The cut surface exhibited a glistening white appearance (Figure 2). FNAC indicated poorly exfoliated cells; however, scraping yielded a sufficient number of cells, which appeared large and spindle-shaped with elongated to plump nuclei, along with single to multiple nucleoli and reticular chromatin. Anisocytosis and anisokaryosis were markedly present, with a dominance of fibroblasts and an absence of inflammatory cells (Figure 3).

Histologically, the tumor's periphery was rich in melanocytes (Figure 5). The epithelium remained intact, with subepithelial closely packed spindle cells arranged in an irregularly whorled pattern (storiform/matted), displaying marked atypia. Nuclei appeared plump and elongated, with coarse chromatin and multiple nucleoli. Vigorous mitotic activity, along with significant anisocytosis and anisokaryosis, was observed at the tumor's periphery (Figure 5). Moving towards the center,

relatively lesser anaplasia was noted, characterized by multiple whorls of spindle-shaped tumor cells arranged in multidirectional interwoven or herringbone fascicular patterns (Figure 4 and 6). The tumor demonstrated local aggressiveness, although no distant metastasis was detected. Additionally, there were no unusual extracellular stromal deposits, significant vascular patterns, or inflammatory infiltrates.

Masson's trichrome staining revealed a blue coloration in the fibrous tissue, aiding in the differentiation between fibrosarcoma and leiomyosarcoma, as both tumors share several common cyto/histo-morphological features (Figure 4).

Initially, considering the tumor's location, ocular surface squamous neoplasia, spindle cell carcinoma, keloid, and pyogenic granuloma were among the differentials considered. However, pterygium was ruled out due to the tumor's unique gross morphology, consistency, and cytology. The progressive unusual growth history and lack of inflammatory cells raised suspicion towards a neoplasm. While fibrosarcoma is not rare, its occurrence at this location is highly unusual. Some previous researchers have described ocular fibrous tissue neoplasms in human patients (Nair *et al*., 2018), with epibulbar or conjunctival fibromas believed to originate from Tenon's fascia in human pathology (Nair *et al*., 2018). The presence of melanocytes at the periphery may be acquired from conjunctival epithelial cells, as documented in human pathology (Horita *et al*., 2019), which explains the blackening of the growth surface.

Central mature fibrocytes result from enhanced differentiation of fibrosarcomas, producing cells with fewer criteria for malignancy. Squamous cell carcinoma is so commonly reported that the term 'Cancer eye' is used synonymously for this tumor (Nithya *et al*., 2022; Islam *et al*., 2017). Prasad and Samatha (2013) conducted a 24-month prevalence study on ocular tumors in buffaloes, reporting the highest occurrence of squamous cell carcinomas (50%), followed by neurofibromas (25%), papillomas (12.5%), and lymphomas (12.5%). However, Sharma *et al*. (2020) diagnosed 68.8% of ocular tumors in buffaloes as leiomyoma, followed by fibro-leiomyoma and squamous cell carcinoma. Priyanka *et al*. (2021) reported the highest incidence of ocular tumors in the 6 to 10-year age group, while Nithya *et al*. (2022) observed it in the 3 to 6-year age group.

Chronic irritation, ultraviolet radiation, genetic susceptibility, breeds with light pigmentation, age (adults), viruses (Epsilonpapillomavirus), chemicals, and persistent ulcers are some predisposing factors of ocular tumors in bovines (Priyanka *et al*., 2021; Srivastav *et al*., 2022). Typical features of fibrosarcoma, as described by previous researchers (Deyhimi *et al*., 2019; Shokrpoor *et al*., 2023), such as high cellularity, anisocytosis, hyperchromatia, overlapping of nuclei at places, variable cell differentiation from poor to well-differentiated, infiltrative growth pattern, presence of a variable amount of collagen between infiltrating cells, mitotic activity, aggressive clinical behavior, and blue Masson's trichrome staining, led to the final diagnosis of fibrosarcoma.

CONCLUSION

Based on the examination of the gross appearance, histopathology, and special staining, a diagnosis of conjunctival fibrosarcoma was confirmed. This tumor is exceptionally rare, with only a handful of reports documented, primarily in species other than buffalo.

Figure 1. Gross picture (*in situ*) showing multi nodular growth protruding out from bulbar conjunctiva covering almost entire eye orbit.

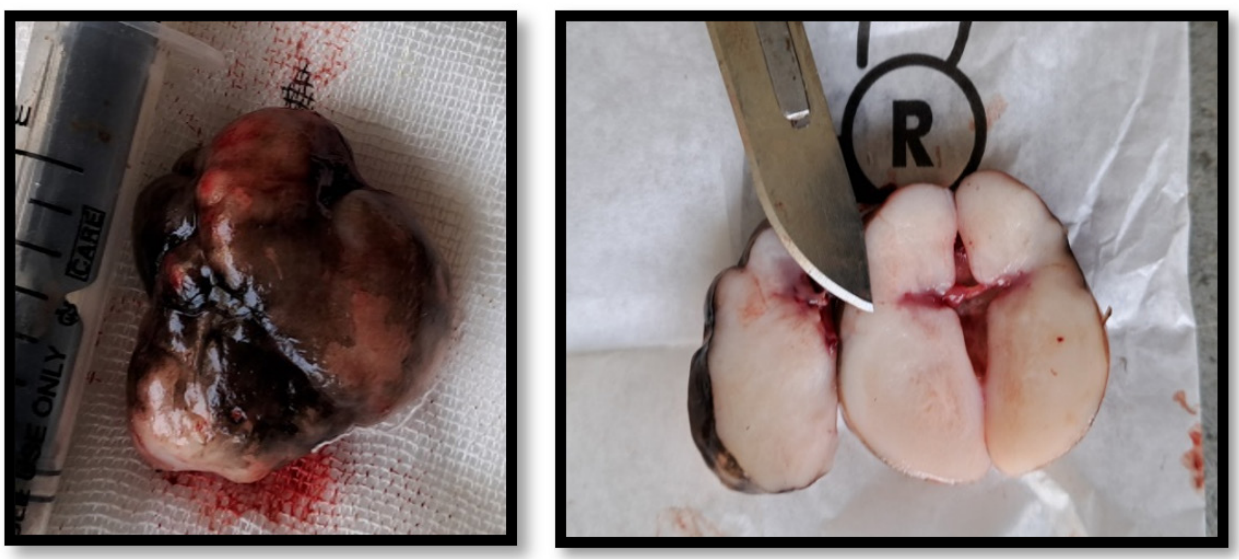

Figure 2. Gross picture and cut surface of tumor mass recovered after surgery.

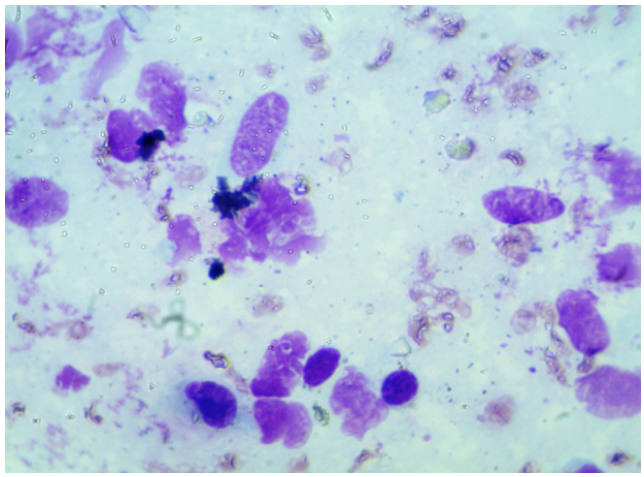

Figure 3. Cytology of this ocular tumor showing spindle shape cells with plumpy prominent nucleus and prominent nucleoli in few of nucleus (Leishman Stain; 1000X).

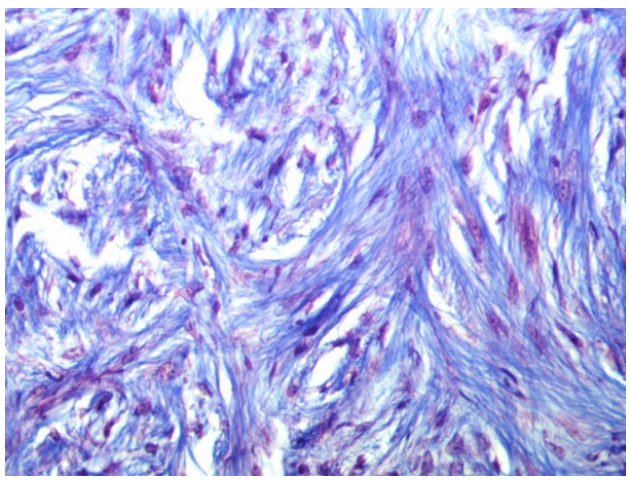

Figure 4. Microscopic picture showing high cellularity, multidirectional swirling of fibers depicting prominent herringbone pattern of fibers (Masson's trichrome Stain; 400X).

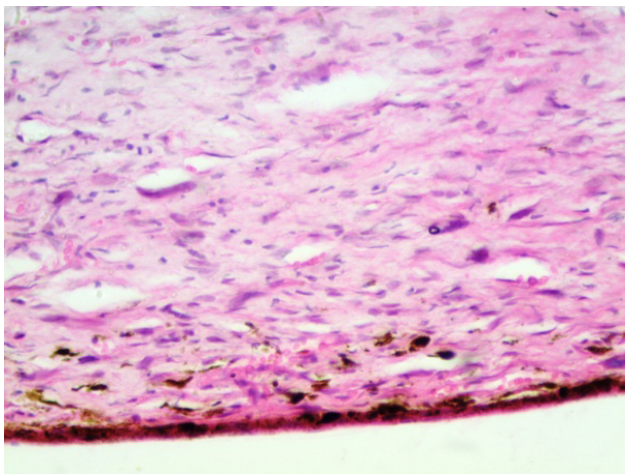

Figure 5. Microscopic picture of marginal area of tumor showing high cellularity, marked anisocytosis of connective tissue fibers and prominent marginal melanocytes (H and E Stain; 400X).

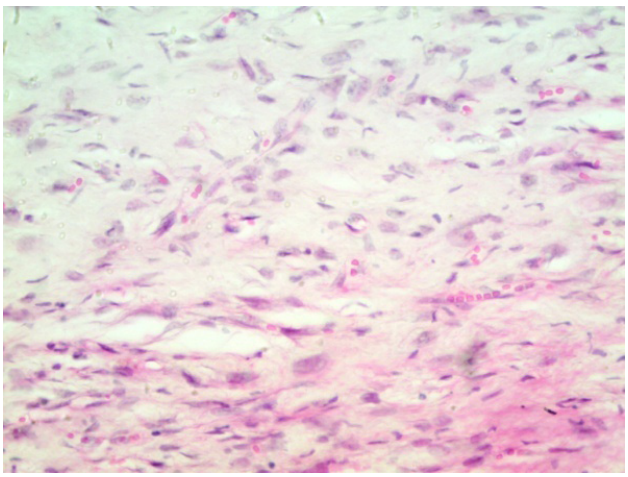

Figure 6. Microscopic picture of central mass of tumor showing high cellularity, marked anisocytosis, prominent nucleus & nucleoli, multidirectional swirling of muscle fibers (H and E Stain; 400X).

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