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Surgical Management and Histopathological Evaluation of a Mammary Tumor in a Buffalo (*Bubalus bubalis*)

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ABSTRACT

The study aimed to define the histopathological features of mammary gland tumours in buffaloes (*Bubalus bubalis*) in the form of a case series and to highlight the importance of a microscopic study in the correct diagnosis and treatment of mammary masses in large ruminants.

Ten adult buffalo females introduced with hard, slowly growing masses of the mammary glands of some three months. Each of the cases was surgically handled, histologically processed representative tissues were examined under the microscope. Clinical follow-up during the period of the postoperative showed no lesion recurrence in all treated animals.

Microscopic analysis showed a significant impairment of the normal structure of the mammary glands. The mammary ducts were distended, irregular and cystic at times. The ductal epithelium was hyperplastic, pleomorphic, enlarged, and lost polarity with occasional loss of polarity. Fibrovascular proliferation, congested blood vessels and multifocal inflammatory cells infiltrations in the interductal stroma were seen. There were eosinophilic secretory material and exfoliated epithelial cells in the lumina of the ducts. Mitotic figures were not frequent.

According to these histological characteristics, the lesion was categorised as a low grade mammary ductal origin adenocarcinoma.

Conclusively, mammary tumours are not common among buffaloes; however, they must be included in the list of diagnostic possibilities of chronic mammary masses. The histopathological investigation is crucial in the accurate diagnosis, classification and proper clinical treatment of tumours. Reporting of these cases will be a good source of information to enhance knowledge, early diagnosis and management plan of the mammary lesions in large ruminants.

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Introduction

The mammary gland is one of the most specialised exocrine glands that produce and secrete milk and is composed of a highly developed structure and is under constant remodelling due to age, hormonal status and lactation cycles. In addition to inflammatory and degenerative diseases, this organ can also be neoplastic, which can be either epithelial or mesenchymal (Meuten, 2017). Although mammary tumours are common in companion animals, they are very rare in large ruminants like buffaloes (*Bubalus bubalis*) (Zachary, 2017). The low reporting of buffaloes is normally due to precautionary elimination of the diseased animals or mistaking of the masses as chronic mastitis (Radostits et al., 2007). Despite their low incidence in large ruminants such as buffaloes, neoplastic diseases are also of clinical significance in poultry, in which Marek's disease virus in layers is linked to lymphoid neoplasia, which may impair health and productivity (Najem et al., 2024).

Clinically, mammary neoplasia is clinically important in the food-producing animals because it is negatively associated with milk production and the health of the udder, as well as the general well-being of the animals (Divers & Peek, 2018). On the occasion of such masses, surgery is one of the most common modes of management with an aim of finding a way of excising the neoplastic tissue, avoid future complications, and reestablish the normal functioning of the animal. Nevertheless, clinical distinction between neoplasia and inflammatory conditions can be complicated, and the complex approach is required, which includes surgery and intensive pathological examination.

The gold standard of proper diagnosis and tumour classification is histopathological evaluation (Goldschmidt et al., 2017). It is the sole accurate way of measuring biological behaviour and will prove the surgical margins to be clear and this is paramount in deciding the prognosis. The purpose of the report is to present the surgical treatment and histopathologic features of a tumour of the mammary gland in a buffalo, along with clinical information to the insufficient literature and with an accent on the combination of surgery and microscopic examination as the key to successful diagnosis and treatment (Sorenmo et al., 2019).

Materials and Methods

Study Design and Sample Size

The current study was a case series study which was descriptive and histopathological. Ten buffaloes (*Bubalus bubalis*) were used where samples of the mammary gland tumours were sampled after full surgical resection of the lesions. Routine processing of tissue samples of every case was done to have an evaluation of the histopathology.

Case History and Clinical Examination

Masses of mammary glands were firmly and gradually increased and introduced to ten mature female buffaloes. The owners reported that the lesions had been there about three months before and gradual in onset and without significant pain or systemic clinical.

The owner had reported the lesion about three months earlier and it had gradually increased over time without causing any major pain or general symptoms.

Physical assessment indicated that there was a circumscribed hard swelling that was limited to the mammary tissue. No abnormalities on the size of regional lymph nodes or systemic abnormalities were observed (Radostits et al., 2007). Surgical excision was done under due restraint and local anaesthesia because of the progressive nature of the lesion. The removed tissue was sent to the histopathological examination to verify the diagnosis and inform the further clinical processing (Meiten, 2017).

Surgical Intervention and Management

The presence of all the affected animals was subjected to complete surgical removal of the mammary masses, which acted as both a curative measure to improve the functionality of the udder and a diagnostic measure to promote a conclusive histopathological assessment.

Pre-operative Preparation and Anesthesia

The fitness of the animal to undergo surgery was also tested by a comprehensive clinical examination carried out before the surgery. Surgical site was prepared with hair clipping and hair shaving and dual stage aseptic scrub with 10% povidone-iodine and 70% isopropyl alcohol. Desensitisation of the area was done by using 2 percent Lidocaine hydrochloride, applied as a ring block or linear pattern around the base of the mass as a local infiltration anaesthesia. Mild sedation was also added in certain instances in order to control the temper of the animal (Abduljaleel 2024; Abduljaleel et al., 2025).

Surgical Technique (Excisional Mastectomy)

A skin incision was done over the part of the mass that was most prominently located with a margin of healthy tissue (2-3 cm) to reduce chances of recurrence. Both blunt and sharp dissection were done carefully so as to isolate the neoplastic tissue out of the underlying mammary parenchyma as well as fascia.

Hemostasis was given special attention: major feeder vessels were defined, and then ligated with an absorbable suture material (Vicryl) and minor bleeders were treated with either electrocautery or pressure. After removing all the mass completely, the surgical cavity was washed using sterile normal saline to clear the residual debris and blood clots.

Wound Closure and Post-operative Care

The subcutaneous tissue was closed in a simple continuous pattern to eliminate dead space, while the skin was apposed using a simple interrupted or horizontal mattress pattern with non-absorbable silk or nylon. Post-operative management included a course of broad-spectrum antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) to manage pain and prevent secondary infections. Sutures were typically removed 10–14 days post-surgery, following evidence of complete primary intention healing (Ibrahim et al., 2025).

Tissue Collection and Fixation

Representative tissue samples were collected immediately after surgical excision. Specimens were fixed in 10% neutral buffered formalin for 24–48 hours to preserve cellular morphology and tissue architecture (Zachary, 2017).

Histological Processing

Fixed samples were dehydrated through ascending ethanol concentrations, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned at 4–5 μm thickness using a rotary microtome (Goldschmidt et al., 2017).

Staining and Microscopic Evaluation

Sections were mounted on glass slides and stained with hematoxylin and eosin (H&E) (Mohsin et al., 2025; Khaleefah & Najem, 2020). To determine tissue architecture, morphology of epithelial and stromal cells, and pathological changes, microscopic analysis at low (10.40 \times) and high (40 \times) magnifications was carried out (Meuten, 2017).

The assessments of all the histological slides of the ten cases were done on a collective basis so as to observe common patterns and reach a consistent pathological diagnosis.

Results

Histopathological Findings

In the buffaloes the observation under the microscope of the mammary gland masses, showed an extreme distortion of architectural structures and a considerable change of cells, which were the morphological characteristics of a complex neoplastic process. As demonstrated in the figures below, these results point at the appearance of a pathological state in the shift of a normal glandular tissue both the epithelial and mesenchymal elements.

Structural Alterations and Ductular Changes

The basic structure of the mammary gland has been completely impaired, which is accompanied by the prominent dilation and widening of the mammary ducts. This ductal ectasia

usually advanced to cystic structures, in which the normal lobular structure was substituted by irregular and enlarged structures. This growth (as illustrated in Figure 1 and Figure 4) was also accompanied by a strong stromal reaction, with the inter-lobular trabeculae experiencing massive fibrous expansion. The occurrence of eosinophilic proteinaceous secretory material and exfoliated epithelial cells in these dilated lumina was found similar in all samples, which had been specifically illustrated in Figure 3 and Figure 4.

Epithelial Hyperplasia and Cellular Atypicality

There were aggressive growth patterns observed in the ductal epithelium through elevated hyperplasia and stratification. At increased magnifications, including Figure 2, cells were characterized by strong pleomorphism and crowding with all of the enlarged, hyperchromatic nucleus and a significant loss of cellular polarity apparent. Although the number of mitotic figures was low, the level of cellular atypia was high enough to warrant a neoplastic transformation. These are characteristics of epithelial malignancy that are key in the diagnosis of low grade mammary adenocarcinoma of ductal origin.

Stromal Proliferation and Chronic Inflammation

The enlargement of the interductal stroma following fibrovascular proliferation was also a major characteristic of the observed pathology. Figure 5 illustrates this high stromal cellularity, showing evident nuclear pleomorphism and anisokaryosis within the proliferating fibro-connective tissue. Furthermore, the presence of multifocal infiltration by lymphocytes and macrophages—noted in the results and visualized in Figure 4 suggests a concurrent state of chronic mastitis. The combination of these proliferating fibrous elements and glandular changes supports a diagnosis of Mammary Adenofibroma in certain regions of the tissue

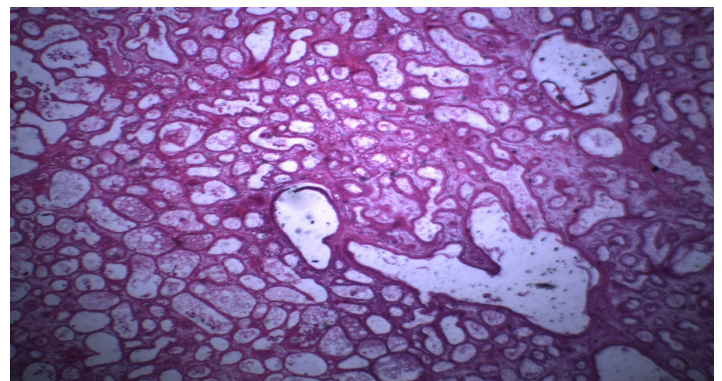


Figure 1: Mammary gland tissue of a buffalo showing marked dilation and expansion of mammary ducts with surrounding stromal proliferation (H&E, 10 \times).

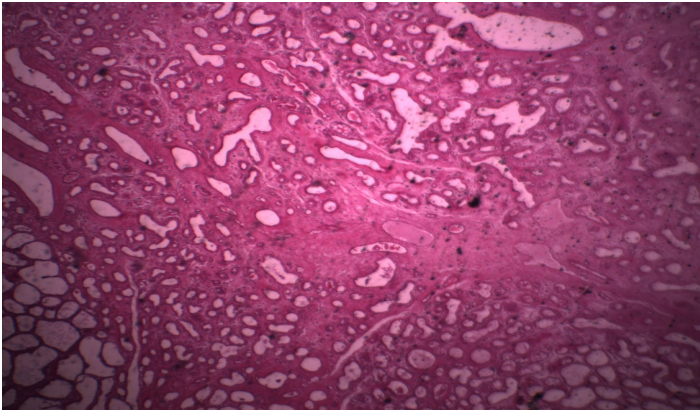


Figure 2: High-power view demonstrating epithelial hyperplasia, cellular pleomorphism, hyperchromatic nuclei, and loss of normal ductal architecture (H&E, 40 \times).

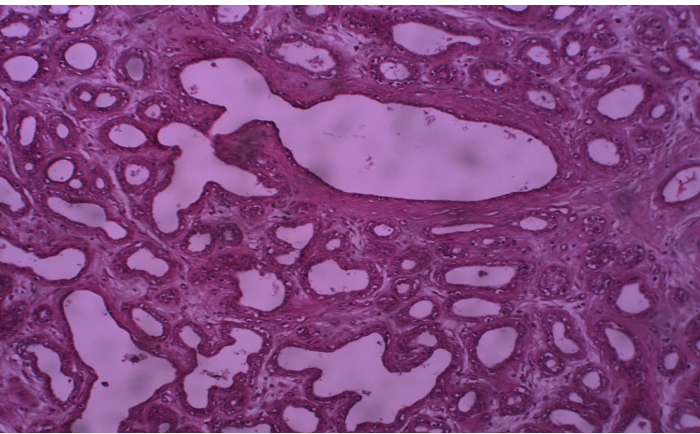


Figure 3: Dilated mammary duct containing eosinophilic secretory material and exfoliated epithelial cells, with fibro-vascular stromal response (H&E, 10 \times).

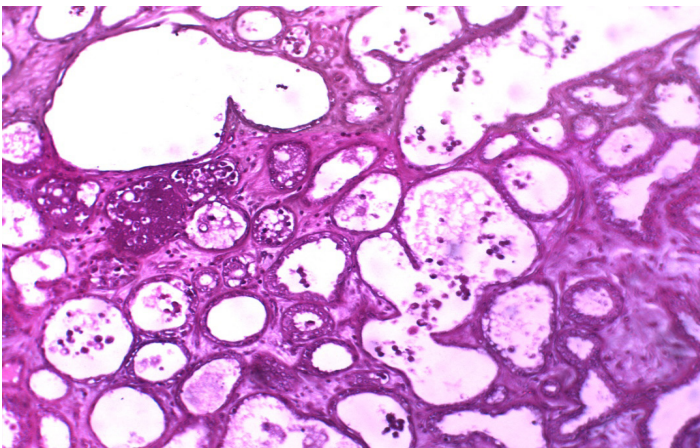


Figure 4 : Microscopic examination of the mammary gland tissue reveals marked alveolar cystic degeneration and atrophy associated with extensive fibrous expansion of the inter-lobular trabeculae and prominent ductal ectasia containing proteinaceous secretions alongside focal mononuclear inflammatory cell infiltration suggesting a diagnosis of Mammary Adenofibroma with Chronic Mastitis (H&E, 10 \times).

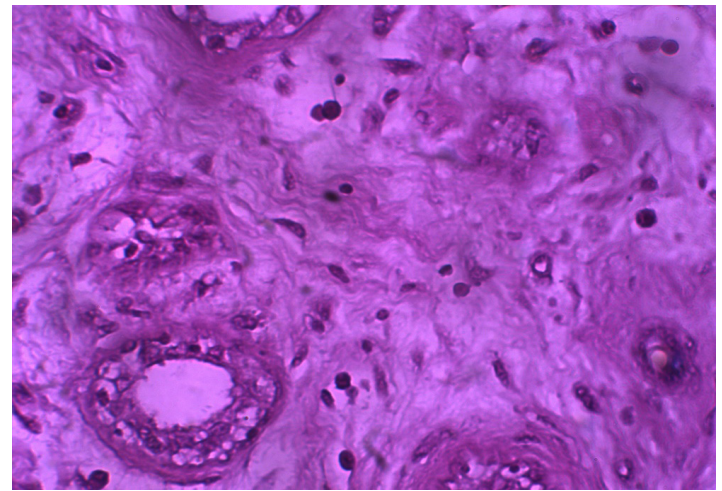


Figure 5: Microscopic examination at high magnification reveals significant stromal cellularity with evident nuclear pleomorphism and anisokaryosis within the proliferating fibro-connective tissue alongside degenerative changes in the adjacent glandular epithelium suggesting a diagnosis of Mammary Adenofibroma (H&E, 40 \times).

Discussion

The occurrence of mammary tumors in buffaloes (*Bubalus bubalis*) is infrequently reported in veterinary literature compared to the high prevalence documented in companion animals or other cattle species. The scarcity is in many cases due to early culling of affected animals or to a commonly misdiagnosed clinical finding of such masses as chronic mastitis. The histopathological observations of the case such as the presence of significant ductal dilation, epithelial hyperplasia with the presence of severe cellular atypia, stromal proliferation, and inflammatory infiltration are consistent with the existing descriptions of tumors of the mammary epithelial cells in domestic animals (Meuten, 2017). The clinical interest of these neoplasms in food-producing animals is considerable since they have a deleterious effect on milk production, the wellbeing of the udder, and the welfare of the animal. Such mammary masses should also be diagnosed with the help of the differential diagnosis with chronic mastitis, mammary hyperplasia, galactocele, and abscess (Goldschmidt et al., 2017). Although chronic mastitis can be characterised with ductal dilation and inflammatory infiltrates, it does not have the deep pleomorphism of the epithelial cell and the gross structure disorganisation typical of neoplastic changes (de Freitas Silva 2024). Moreover, mammary hyperplasia is usually hormone-related and is marked by a homogenous proliferation of the epithelial cells without the cellular atypia or loss of polarity detected in such samples (Fedets et al., 2026). The gold standard to a specific diagnosis is histopathological testing because it is the only test that is conclusive to determine

biological behavior and distinguish between neoplasia and inflammatory diseases. The reviewed cases were all pronounced as low-grade mammary adenocarcinomas of ductal origin (Sorenmo et al., 2019). These are generally low grade tumors with minimal invasive potential and this is backed up by the infrequent mitotic activity as evidenced in the tissues sections. This low grade nature along with the successful total excision of the surgical excision could be the possible cause of the good clinical results and non-recurrence during the post operative follow up. Nevertheless, one must note that it cannot exclude the possibility of local recurrence or metastasis without the help of a long-term monitoring or a complex immunohistochemistry examination (Papparella et al., 2022; Silva et al., 2026). The uniform histopathological characteristic in all the cases confirms the categorization of the lesions as a homogenous pathological entity (Radostits et al., 2007; Divers and Peek, 2018). Finally, the reporting of such rare cases would enhance veterinary consciousness and encourage the routine histopathological evaluation of mammary masses that is needed in enhancing our knowledge on tumor prevalence, biological behavior, and the prognosis of buffaloes.

Conclusion

This is a series of cases where the histopathology of mammary gland tumors has been recorded in ten buffaloes. In all cases, a histopathological examination revealed that the diagnosis is low-grade ductal adenocarcinoma. Wholesome surgical excision was an effective method of treatment since no recurrence was seen after the surgery. These results demonstrate that histopathology should be relevant in diagnostics and provide surgical treatment as an anti-optical variant of low-grade mammary tumor in buffaloes.

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