

# Histopathological Study of Canine Mammary Tumours with Different Stain Techniques

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**Abstract**— Mammary gland cancer is the most frequent malignant tumor in human females and bitches and morbidity and mortality due to it continue to increase. Mammary gland carcinomas are quite heterogeneous in terms of morphology and biological behavior. Twenty-three female dogs were confirmed affected by mammary tumors during the period January 2014 - June 2015. In this study were used different stained techniques in mammary gland tumors of bitches to help us in diagnosing of them.

The aim of this study was to compare H-E technique and Masson Trichrome staining technique in mammary gland tumors, in order to differentiate histological types of neoplastic lesions.

**Keywords**— cancer, mammary gland, histological techniques, dog.

## I. INTRODUCTION

Breast cancer is the most frequent malignant tumor in females and its morbidity and mortality due to it continue to increase, despite remarkable progress in the field of early diagnosis and therapy [10]. Mammary gland neoplasm are the most commonly seen tumors in female dog [6, 8, 13]. These neoplasm are the second ones after skin tumors [10]. Nearly 40% - 50% of the mammary tumors that occur in the bitch are malignant [6].

Mammary gland carcinomas are quite heterogeneous in terms of morphology and biological behavior, and they have been the focus of intensive research over the last few decades [6].

A lot of literature has been compiled on the different factors that predispose canines to mammary tumors which are attributed to factors such as age, breed, sex, glandular wise, etc.

Dog has maximum risk of developing mammary tumor (MTs) at 9 - 11 years of age, which is defined as the “cancer age” [9].

Mammary tumors in bitch are classified as “complex” when myoepithelial cells and secretory epithelial cells are present and “simple” when only one type of these cells is present.

Malignant tumors are classified as carcinomas, sarcomas and the combination of them. In practical terms, malignant mammary tumors can be epithelial

(carcinomas), mesenchymal (sarcomas) or mixed (carcinosarcomas) [4].

Based on histopathological study, all mammary tumors were classified in accordance with the WHO Histological Classification of Mammary Tumors of the Dog and Cat [5].

Keeping in view the above, the aim of this study was to compare H-E technique and Masson-Trichrome special staining technique in mammary gland tumors, in order to differentiate histological types of neoplastic lesions.

## II. MATERIALS AND METHODS

Specimens of mammary gland tumors from twenty-three bitches of different age, breed, and glandular wise submitted to Small Animal Clinic at Faculty of Veterinary Medicine, Agricultural University of Tirana, Albania, were included in this study. Study was carried out at the Department of Clinical Subjects, Faculty of Veterinary Medicine, Tirana, Albania.

Tissues samples for light microscopy were fixed in 10% neutral buffered formalin, paraffin embedded, and sectioned at 5 $\mu$ m thickness. The tissues were deparaffinized in xylene, rehydrated in 100%, 95%, and 70% alcohol, and washed in distilled water. Biopsied mammary tissues stained by Hematoxylin - Eosin method (H&E) (Merck – Darmstadt – Germany), [2]. Duplicate sections were colored with Masson - Trichrome to detect to the deposition of collagen fibers. All mammary tumors were classified in accordance with the WHO Histological Classification of Mammary Tumors of the Dog and Cat (Misdorp et al., 2001). Mammary gland tumors were observed under the MOTIC, BA 210 microscope.

## III. RESULT AND DISCUSSIONS

Mammary tumors are the most common tumors in female dog [6, 8, 13]. Despite intense clinical and pathological investigation, little is known about the etiology and prognosis of these tumors. In this sense it becomes a priority to determine prognostic factors that can function as an aid in identifying high-risk ones [1].

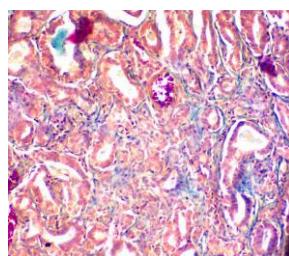
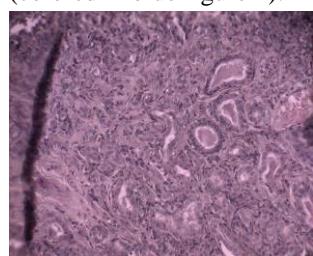
Twenty-three female dogs were confirmed affected by mammary tumors during the period January 2014- June 2015 in the Department of Clinical Subjects, Faculty of Veterinary Medicine. The highest number of tumors is

encountered above the 9-12 years old animals. This interval of risk age is in agreement with other studies [7, 9].

Based on the histopathological findings mammary gland tumors were classified as benign and malignant. Benign tumors were identified as simple adenoma and ductal papilloma.

Simple adenoma of mammary gland observed micro granules separated from fibrotic stroma (figure 1).

Tumor tissue slides were stained with Masson Trichrome, which shows the added amount of deposited collagen (colored in blue figure 2).

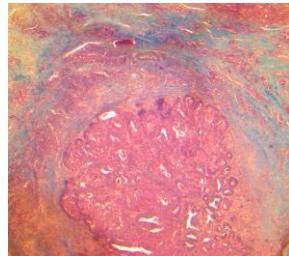


*Fig. 1: Mammary gland. Adenoma. H - E. 20X.*

*Fig. 2: Mammary gland. Adenoma. Masson Trichrome. 20X.*

The second form of benign neoplasm was diagnosed as a ductus papillomae in which there were evidenced cystic spaces and glandular epithelial proliferation in papillary form of growths usually lined in one or more layers over thin to thick connective tissue stroma (Figure 3). Malignant tumors are found as apocrine adenocarcinoma intermediate differentiated (G2) and tubular papillary carcinoma.

Apocrine adenocarcinoma intermediate differentiated (G2): it was observed a lobular lesion which infiltrated the connective tissue, irregular lobule, atypical nuclei and stratified epithelia (X20). Apocrine adenocarcinoma (G2) is formed by cylindrical or cubic cells, which take papillary form and a stroma of connective tissue. In this figure were observed pleomorphism and mitotic figures (figure 4). The slides were staining with Masson Trichrome as well, and it is detected the great amount of collagen in the tumor. It is stained with blue. Tumor cells, in red, differentiate from other cells; it highlights areas in dark blue corresponding to fibrosis (figure 3).



*Fig. 3: Mammary gland. Apocrine adenocarcinoma. H - E. 20X.*

*Fig. 2: Mammary gland. Apocrine adenocarcinoma. Masson Trichrome. 4X.*

Carcinoma (Tubulopapillary): Based on the histological findings of tubulopapillary carcinoma, are noted tubule eschorozes that infiltrated the mammary tissue. It was observed galactofores structures and infiltrations from ducts and irregular solid islands (figure 4).

Histopathological findings of a tumor is essential in order to obtain consistent information on the prognostic factors of mammary cancer and considered it as a basic step for the therapeutic and diagnostic orientation of lesions and concluded that histopathological technique is a more viable alternative for attaining a meaningful and cost-effective prognosis of malignant tumors in dog [12]. The histopathologic examination is considered the gold standard for the diagnosis of canine mammary tumors (CMT).

The incidence of malignant tumors varies widely from 39 to 91% according to different reports [3, 14, 15] whereas, in contrast to Misdorp [5].

#### IV. CONCLUSION

Based on the histological and biological criteria, mammary gland tumors are very heterogeneous in terms of morphology and biological behavior.

The study included all cases with spontaneous mammary tumor in bitch and it is classified them according to the nature and type of their histological characteristics.

Benign tumor was classified by histological type in adenoma, cystic hyperplasia and ductus papilloma. Malignant tumors were adenocarcinoma intermediates grade and carcinoma classification.

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