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Identification of Benign and Malignant Canine Mammary Gland Tumors and Surgical Intervention via Ultrasonographic Imaging and Hematological Analysis

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ABSTRACT

Tumour is an alarming issue for pet animals, especially for dogs and cats. Mammary gland tumour is the most concerning type for pets after lymphoma and skin tumours. To know the clinical features in the mammary gland, size and stage of the tumour, growth and supply of lymphatic vessels and blood vessels ultrasonography is a useful diagnostic tool. Haematological examination for blood test measure is a classical way to identify the malignancy stage of tumour, which we used for our study. Initially, 10 dogs were proposed to conduct the study but different circumstances like a devastating flood in the greater Sylhet region ultimately delayed our working period. For these reasons, we conducted our study on 8 dogs. Among 8 examined dogs, two were found positive by ultrasonography using B-mode which implies a prevalence of 25% (2 out of 8). Several studies reported a high prevalence of mammary gland tumours in case of dogs. Lower prevalence according to our study, maybe because we examined stray dogs and they acquire strong natural immunity and Canine mammary gland tumours are less prevalent in dogs under the age of 5 years and the average age of dogs examined by our study was 1.875 years. To determine if there is any difference between the ultrasound positive and negative dogs, one sample t-test was done where test positive and test negative is the independent variable and all the blood parameters are the dependent variable and the blood parameters significantly vary (p-value< 0.001) between positive and negative animals. Again, using B-mode was not efficient enough to see the differences between malignant and benign tumours. However, the study result of this project can be used for baseline information of future similar studies. Further study should be conducted with higher age groups and sophisticated diagnostic tools.

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Introduction

Tumor is an alarming issue now days for pet animal basically for the dog and cat. There are several types of tumor found such lymphoma and skin tumors (Novosad, 2003) which are the highest percentage of tumor on pets. After that mammary gland tumor is the most concern tumor for pets. Sweat gland modified for the development of mammary gland with complete ductal network which surrounded by fibro vascular and adipocyte-rich stroma (Sorenmo et al,. 2011). Changes in the normal structure of the mammary gland and its prognosis depends on age, sex, breed, diet, hormone, obesity and posterior mammary gland tumor is more than the anterior mammary gland tumor (A and A, 2016). To know the clinical features that occur in the mammary gland, size and stage of the tumor, growth and supply of lymphatic vessels and blood vessels ultrasonography is an important technique. B mode, Color Doppler, Contrast enhancement ultrasonography and Radiation force impulse elastrography are the conventional techniques of Ultrasonography (Feliciano et al,. 2018). Histopathological examination for biopsy test measure as a classical way to identify tumoral malignancy stage. Whereas ultrasonographical examination consider as a noninvasive, safe, sensitive, portable and economical diagnostic procedure for identifying the biological behaviors and outcome of abnormal growth of tissue (Yan et al,. 2018). Ultrasonographical examination was performed to evaluate the qualitative and quantitative characteristics of a visible tumor mass. Echotexture (homogenous or heterogeneous), margins and invasiveness (present/absent) were examined through B Mode ultrasonography as a qalitative parameters whereas longitudinal width (cm), longitudinal length (cm), width: length ratio; transverse width (cm), transverse height (cm), width: height ratio were analyzed as quantitative parameters. Color Doppler used to visualize the pattern (high resistivity, intermediate or low), characteristics (arterial or turbulent), localization (peripheral, central or diffuse) and vascular color flow (present or absent) of blood vessels as a qualitative parameters (Sahoo, 2020). Contrast enhancement ultrasonography - A contrast agent injected through intravenously and ultrasound examine is performed for the enables of real time evaluation of blood flow and vasculature including microvasculature (Hillaert et al,. 2022). A gray scale map can introduce when waves and transducer interact with each other through virtual touch imaging (VTI) or a velocity value by virtual tissue qualification (VTQ) it's called Acoustic radiation force impulse (AFRI). Acoustic radiation force impulse (AFRI) is a new tissue strain imaging technology that makes use of sound waves to quantify the elastic properties (Gumaa et al,. 2020). The ratio of sensitivity and specificity accordingly 56.3% and 92.9% in case of B mode ultrasound examination whereas malignancy detection through Color Doppler provide 65% sensitivity and 100% specificity. Among four technique of ultrasonographic examination Acoustic radiation force of impulse electrograph considered as an optimistic technique for determining tumor malignancy. Although Contrast enhanced ultrasonography has a great limitation in case of malignancy tumor but have an accuracy for capillarization evaluation of tunor. Histopathological examination of tumor malagnency is a tremendous invention in veterinary medicine. By this technique staining the slides and immunochemistry have done for the confirming the histogensis and subtype of mammary gland tumor (Morton et al,. 2014).

Tumorous sample were collected through surgical intervention and kept it into the menthol solution for fixing the tissue and avoiding metabolism. The biopsies samples were stained with hemotoxylin and eosin after a limited time of interval and proceed for slides preparation for the diagnosis of the stage of malignancy of tumor (Brown et al,. 2012).

Method and materials

Study Area

This study was conducted at Pet and Vet Care, Sylhet and Post-Graduation Laboratory of Surgery and Theriogenology Department, Sylhet Agricultural University (Figure 1).

Figure 1. Post Graduation Laboratory, Department of Surgery and Theriogenology, SAU

Study period and Population

The study was conducted from April 2022 through out September 2022. Stray dogs were selected as the study population. Dogs of Sylhet Agricultural University Campus and surrounding region was selected. 10 dogs were proposed to conduct the study. But different circumstances like devastating flood in the greater Sylhet region while the study period deterred our work which ultimately delayed our working period. This delay led the study towards the breeding season of dogs. For these reasons, we conducted our study on 8 dogs.

Animal preparation

Catching dogs

With breads was used for sedation of dogs. Sedation was confirmed by observing the signs and symptoms. After that Xylazine was injected intramuscularly at a dose rate of 1mg/kg body weight.

General data collection

Immediately after the injection of xylazine, the dog was carried to the hospital and general data like body temperature, body weight, gender, coat color was determined and noted and transferred to Microsoft excel file.

General Anesthesia and blood collection

Venipuncture was done with a 22-gauze cannula and 3 ml of blood sample was collected from each dog and preserved in a vacuum tube with EDTA for further blood analysis. Ketamine was used for general anesthesia at a dose rate of 10 mg/kg intravenously. After that a normal saline was connected with the cannula at a rate of 40 drops per minute until the ultrasonographical examination was completed (Figure 2).

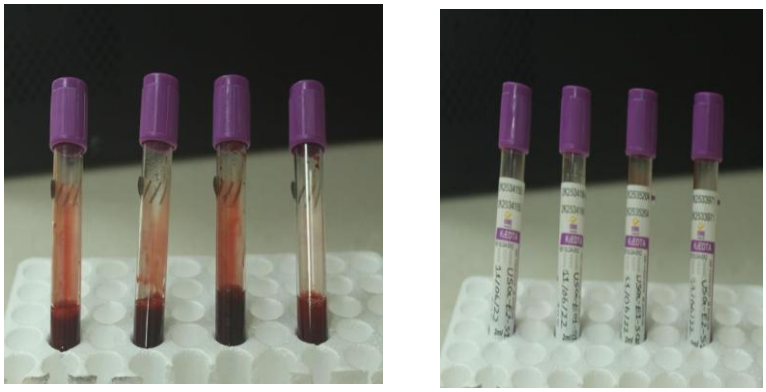

Figure 2. Collection of blood sample

Site preparation and ultrasonographic examination (Figure 1, 2 & 3)

The entire ventral region surrounding the 5 pairs of mammary glands of the dog was clipped and shaved clearly and washed with tincture iodine solution to make sure absence of any kind of pathogens. Then the dog was lied down on the operation table on its spine and ultrasonographic gel (Sono Gel) was applied covering all pairs of mammary glands. Finally, Contec CMS600P2 veterinary ultra sound scanner was used for locating possible tumorous growth. The presence of any tissue mass confirmed by the ultra sound scanner was considered positive.

Figure 3. Site Preparation

Figure 4. Ultrasonographic Examination

Figure 5. Manual measurement

Surgical Intervention

From the positive dogs, tumorous growth was excised by surgery and preserved for further histopathological examination (Figure 5).

Figure 5. Muscular growth was excised by Surgery

Analysis

Firstly, descriptive analysis was done for the general data of every dogs. Frequencies of categorical data determined and descriptive statistics was seen for the continuous variables. Collected blood sample was analyzed by Blood analyzer of central laboratory of Sylhet Agricultural University and data was transferred to a excel file. Finally, blood parameters were analyzed by t-test to find out any significant differences between healthy dogs and dogs with tumors. SPSS version 25.0 was used for all kind of statistical analysis.

Results and Discussion

We conducted our study on 8 stray dogs. Of which all were local breeds and female (Table 2). We chose females as they have functional mammary glands. Table 1 shows the descriptive statistics of general data of continuous variables. The average age of our study dogs was 1.875 years, the average body weight was 14.50 kg, and the average body temperature was 101.15 °F.

Table 1. Descriptive analysis of general data (continuous variable)

Descriptive Statistics	N	Mean		Std. Deviation	Variance
	Statistic	Statistic	Std. Error	Statistic	Statistic
Age (Years)	8	1.875	0.0818	0.2315	0.054
Weight (Kg)	8	14.50	0.189	0.535	0.286
Temp (F)	8	101.150	0.1376	0.3891	0.151

In our study 50% of dogs were black in coat color, two were red, and the other two were red and white (Figure 6).

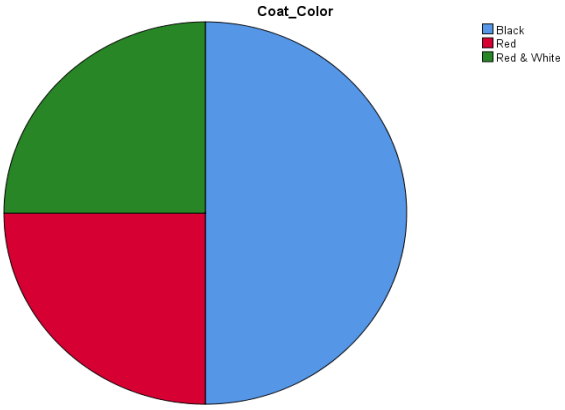

Figure 6. Pie chart of coat color

Table 2. Descriptive statistics of general data (categorical variable) Descriptive statistics of Categorical Variable

	Frequency		Percent	Valid Percent	Cumulative Percent
Valid	Black	4	50.0	50.0	50.0
	Red	2	25.0	25.0	75.0
	Red and White	2	25.0	25.0	100.0
	Total	8	100.0	100.0	
Breed	Local	8	100.0	100.0	100.0
Sex	Female	8	100.0	100.0	100.0

Among 8 examined dogs, two were found positive by ultrasonography using B-mode which implies a prevalence of 25% (2 out of 8). Several studies reported high prevalence of mammary glandtumorsr in case of dogs. One study in Japan (Tagawa et al. 2016) shows a 39% of prevalence, where some others reported a much higher prevalence 50% in Argentina (A and A 2016), 58% in Germany (Stratmann et al. 2008), 86% in Scotland (Simon et al. 2009), and 61% in Turkey (Sontas et al. 2012). One cause of lower prevalence according to our study, may be because we examined stray dogs and they acquire strong natural immunity. One other cause may be the age of dogs. Canine mammary gland tumors are less prevalent in dogs under the age of 5 years (Han, Kim, and Kim 2016) and the average age of dogs examined by our study was 1.875 years. No similar study was reported in Bangladesh. There was one study in India (Sahoo 2020) but they did not report any prevalence. Both of the positive dog has tumor in caudal pair which is similar to most of the study available (A and A 2016; Vannozzi et al. 2018). However, tumor in anterior pair was also reported in some case (Howard and Ferris 1976).

To determine any difference between the ultrasound positive and negative dogs, one sample t-test was done where test positive and test negative is the independent variable and all the blood parameters are the dependent variable. Table 3 shows the descriptive statistics of blood parameters with mean, standard deviation, and standard error mean.

Table 3. Descriptive analysis of blood parameters

One-Sample Statistics					
	N	Mean	Std. Deviation	Std.	Error Mean
WBC	8	13.0012	1.27094	.44935	
LYM%	8	66.1000	7.68542	2.71721	
MON%	8	8.0063	1.33214	.47098	
GRA%	8	22.763	4.9739	1.7585	
RBC	8	6.2113	.79186	.27996	
HGB	8	14.388	2.3697	.8378	
HCT	8	41.1125	4.42055	1.56290	
MCV	8	66.4000	3.84150	1.35818	
MCH	8	23.162	2.3892	.8447	
MCHC	8	35.1000	5.0404	1.7820	
RDWC	8	15.1750	1.58633	.56085	
RDWS	8	38.8625	3.26187	1.15325	
PLT	8	215.00	113.882	40.263	
MPV	8	10.9467	.91043	.32188	
PCT	8	.1597	.06117	.02163	
PDW	8	14.8274	3.20565	1.13337	
PLCR	8	34.5605	7.33062	2.59177	

Results of t-test is shown in Table-4. The table shows that all the blood parameters significantly vary (p-value< 0.001) between positive and negative animals. As the project hypothesized blood parameters increases in case of tumor positive animals compared to the tumor negative animals, this study result supports this project's study result. Several studies supports our study result (Hristov and Binev 2018; Kim et al. 2015; Province 2015; Rasotto et al. 2012; Uçmak et al. 2021). Those study also reported significant change in blood parameter levels in tumor bearing dogs and healthy dogs.

Table 4. One sample t-test statistics for ultrasound diagnosis result

One-Sample t-Test
Test Value = 0

t		df	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
					Lower	Upper
WBC	28.934	7	.000	13.00125	11.9387	14.0638
LYM%	24.326	7	.000	66.10000	59.6748	72.5252
MON%	16.999	7	.000	8.00625	6.8926	9.1199
GRA%	12.944	7	.000	22.7625	18.604	26.921
RBC	22.186	7	.000	6.21125	5.5492	6.8733
HGB	17.173	7	.000	14.3875	12.406	16.369
HCT	26.305	7	.000	41.11250	37.4168	44.8082
MCV	48.889	7	.000	66.40000	63.1884	69.6116
MCH	27.420	7	.000	23.1625	21.165	25.160
MCHC	19.696	7	.000	35.1000	30.8861	39.3138
RDWC	27.057	7	.000	15.17500	13.8488	16.5012
RDWS	33.698	7	.000	38.86250	36.1355	41.5895
PLT	5.340	7	.001	215.000	119.79	310.21
MPV	34.008	7	.000	10.94667	10.1855	11.7078
PCT	7.387	7	.000	.15975	.1086	.2109
PDW	13.083	7	.000	14.82738	12.1474	17.5074
PLCR	13.335	7	.000	34.56048	28.4319	40.6890

One limitation of our study is the number of dogs examined. Further, more studies can be conducted with larger sample size to explore more.

Summary and conclusion

The purpose of this project was to find out the prevalence of canine mammary gland tumor, surgical management if any tumor found by ultrasonography, and preparing histo-pathological slide to compare the changes. One another purpose was to evaluate if there are any differences in blood parameters between healthy and tumor containing dogs. We used B-mode for ultrasonography and found 25% prevalence. After excision noticeable changes was found in histo-pathological slides. We also found significant differences in blood parameters between healthy and unhealthy dogs. In comparison to most study available the prevalence we found was lower, that is may be because of we examined younger age animals. Again, using B-mode was not efficient enough to see the differences between malignant and benign tumors. However, the study result of this project can be used for baseline information of the future similar studies. It is recommended that further more study should be conducted with higher age group and sophisticated diagnostic tools.

Conflict of interest

There is no conflict of research interest.

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