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B-mode and Doppler ultrasound features of mammary neoplasms and their comparison with normal mammary glands in dogs#

Aleena Thomas[1], Sudheesh S. Nair[2], Reji Varghese[2], Anoop S.[3], Soumya Ramankutty[2], V. R. Ambily[4] and K.D. John Martin[5]

Department of Veterinary Surgery and Radiology,
College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 680 651,
Kerala Veterinary and Animal Sciences University, Kerala, India.

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Abstract

Canine mammary neoplasms are naturally occurring non-homogenous group of tumours with many resemblances to human breast cancer. In female dogs mammary tumours are of great clinical relevance due to the high prevalence and mortality rate, which varies according to the histopathological classification and clinical stage. The aim of the study was to compare the ultrasonographic features of the normal mammary gland with benign and malignant mammary neoplasms in dogs, ~~through~~ assessed by B-mode and Doppler mode ultrasonography. Ultrasonographic examination of seven normal mammary glands along with six benign and 12 malignant mammary neoplasms were performed. Among the parameters evaluated by B-Mode ultrasonography, significant differences were found ($p < 0.01$) in the tumour margin and invasiveness of neoplasms, where malignant tumours were invasive with uncircumscribed margin. Significant difference was also found in the presence of posterior acoustic enhancement ($p<0.05$) between benign and malignant mammary neoplasms. Elevated peak systolic velocity of blood flow within the tumour vessels in malignant mammary neoplasm was the only feature assessed with Doppler mode having significant difference.

Keywords: *Canine mammary neoplasm, B-Mode ultrasonography, margin, invasiveness, posterior acoustic enhancement*

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1. *MVSc Scholar*
2. *Assistant Professor*
3. *Professor*
4. *Assistant Professor, Department of Veterinary Clinical Medicine, Ethics and Jurisprudence*
5. *Professor and Head*

**Corresponding author:aleenathomas377@gmail.com, Ph: 8289838054*

Mammary neoplasms are among the most prevalent tumour types in bitches, followed by cutaneous tumours, and accounts for nearly 50 percent of all the tumours reported in dogs (Mohammed *et al.,* 2011). Female dogs have a higher incidence of mammary neoplasia compared to male dogs (Nair *et al.,* 2021). Accurate diagnosis and prognosis are important in the prevention and reduction of morbidity and mortality associated with mammary tumours in dogs. Clinical signs, history, physical and cytological examination are commonly used to make a presumptive diagnosis of mammary tumours.However, histopathological evaluation is necessary to establish a definitive diagnosis. Though mammary mass biopsies provide a definitive diagnosis, they are typically excisional. A non-invasive technique that could give insights into the details of the parenchyma and invasiveness as an indicator of malignancy,will prove very useful for effective surgical management of mammary neoplasms. This distinction prior to surgical excision would allow for a reduction in the number of biopsies, especially in animals with multiple tumours (Nyman *et al*., 2005).

Benign mammary lesions are a diverse group of tissue alterations that include developmental abnormalities, fibrocystic changes, inflammatory lesions, stromal lesions, and neoplasms; only neoplastic lesions are implicated for an increased risk in the development of a subsequent mammary tumour. Malignant neoplasms are characterised by complete loss of architecture and extensive vascularisation (Sorenmo*et al.,* 2003*)*. Under such circumstances, unnecessary invasive procedures could be avoided through pre-surgical, non-invasive demarcation of neoplastic and non-neoplastic benign lesions. The present study was carried out to compare the various B-mode and Doppler ultrasound features of normal mammary glands with benign and malignant mammary neoplasms in dogs, to assess their predictiveness for malignancy.

Materials and methods

Animals

A total of 25 female dogs including seven apparently healthy female dogs, 12 dogs with malignant mammary neoplasia, and six dogs with benign mammary neoplasia presented to the Veterinary Hospitals of Kerala Veterinary and Animal Sciences University at Mannuthy and Kokkalaiover a period of ten months from June 2021 to March 2022, formed the subject of the study.Detailed ultrasonographic evaluation of the mammary glands were carried out before the surgical intervention. All the animals with mammary tumour were subjected to surgical management and tumour excisional biopsy were subjected to histopathological evaluation which confirmed 12 cases as malignant mammary neoplasm and six as benign neoplasm.

Ultrasonographic study

The animals were restrained either in dorsal or lateral recumbency on an examination table after clipping the hair on and around the mammary gland. Ultrasonographic evaluation was conducted in all cases using ultrasound machine, Mylab X8 Exp®, Esaote, Geneva,equipped with a 4-15 MHz linear array transducer,after applying a liberal coating of carboxymethylcellulose gel. Different ultrasonographic features of mammary glands,such as echotexture (homogeneous or heterogeneous), contours (defined or undefined), invasiveness (present or absent),and other findings such as presence of cystic, anechoic, and hyperechoic areas were evaluated.

Statistical analysis

Data was statistically analysed with SPSS version 24.0, where parametric variables were tested using independent sample T test and one way ANOVA. Non-parametric variables were analysed with the Mann-Whitney U test.

Results and discussion

Echotexture

B-mode ultrasonographic evaluation of mammary glands in seven healthy female dogs revealed a characteristic architecture of the mammary gland with three distinct layers (Fig. 1A). The uppermost layer of medium echogenicity represented the skin

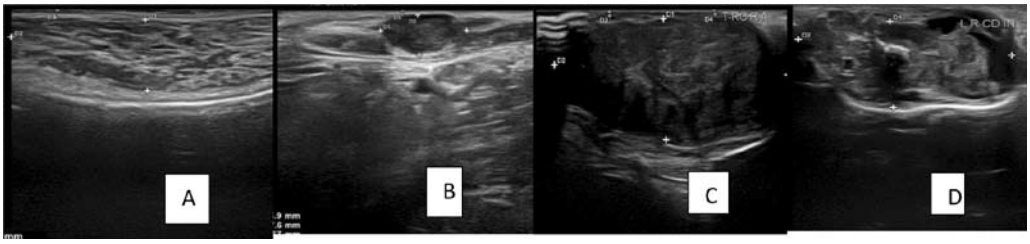

Fig. 1. Sonographic images of mammary gland depicting echotexture. **(A)** Normal healthy mammary gland. **(B)** Homogenous echotexture of a benign mammary neoplasm. **(C)** Heterogenous echotexture of a benign mammary neoplasm. **(D)** Heterogenous echotexture of a malignant mammary neoplasm

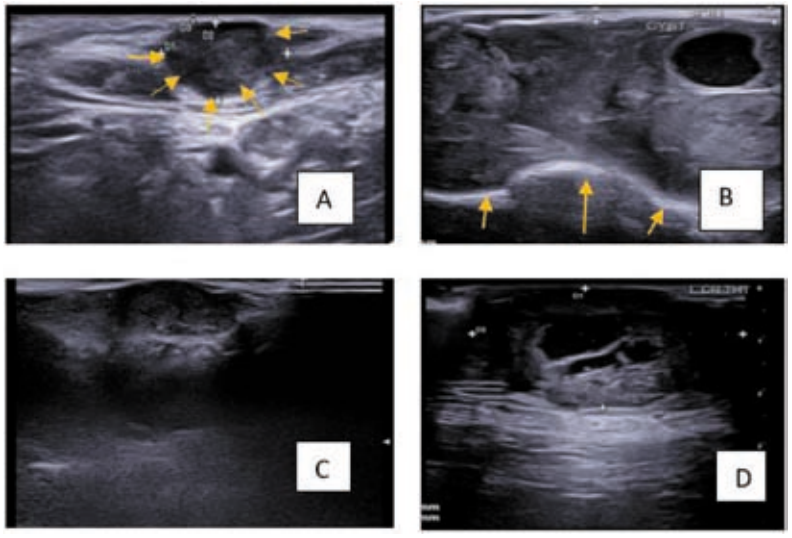

Fig. 2. (A) A benign mammary neoplasm with regular margin (arrows). **(B)** A malignant mammary neoplasm with undefined margins (arrows). **(C)** A benign mammary neoplasm without posterior acoustic enhancement. **(D)** A malignant mammary neoplasm with posterior acoustic enhancement (arrows).

over the gland. Mammary tissue was visible on the ultrasound image as a middle layer of heterogenous echotexture with intermittent hyper-echoic and hypo-echoic areas. The muscular layer was the most profound layer of medium echogenicity which was separated by a thin hyper-echoic layer of abdominal fascia. The findings were in accordance with Trasch*et al*. (2007) and Balaci*et al*. (2015), who reported that mammary parenchyma had coarse-grained structure with medium echogenicity.

In the study, classification of echotexture of the mammary lesion into homogenous or heterogenous was performed as per the reports of Nyland and Mattoon (2015). Homogeneous echotexture was present only in two cases out of the 18 cases of mammary gland neoplasm,as depicted in table 1 (Fig. 1B). Out of the six benign cases, only two cases depicted homogeneous echotexture on B-mode ultrasonography, whereas for the remaining four cases of benign tumour and 12 malignant cases, the echotexture appeared to be heterogenous in nature and no statistical difference was found between benign and malignant canine mammary tumourswith regard to echotexture (Fig. 1C and 1D). The findings of the present study were in agreement with Tagawa *et al*. (2016) and Vannozzi*et al.* (2018) who observed no disparities in ultrasonographic findings of echotexture between benign and malignant mammary lesions. On the contrary, the results of the present study were not in agreement with Bastan*et al*. (2009), Soler *et al*. (2016), and Guedes *et al*. (2020) who reported heterogenous echotexture of mammary lesions with malignancy and homogenous echotexture for benign lesions.

Contours/Margins

The margin or contours of mammary gland in all seven apparently healthy dogs studied were regular and defined (fig. 2B). The findingwas similar to Trasch*et al*. (2007) and Balaci*et al*. (2015). The contours or margin of benign and malignant mammary lesions were either defined or undefined. For all the six cases of benign mammary neoplasm, a defined or distinct margin was evident on B-mode ultrasound imaging. However,all the 12 cases of malignant mammary neoplasms were found to have an undefined margin on B-mode ultrasonography (Fig. 2B). The defined and undefined margins were statistically analysed between the benign and malignant group of mammary neoplasms. There was a significant difference in margin between benign and malignant groups of mammary neoplasm ($p<0.01$). Similar findings were reported by Nyman *et al.* (2006b), Mohammed *et al*. (2011) and Sleeckx*et al*. (2011).

Posterior acoustic enhancement

Posterior acoustic enhancement was not evident in the ultrasonographic evaluation of mammary glands in any of the seven normal, apparently healthy dogs and six cases of benign mammary neoplasms. The presence of posterior acoustic enhancement was detected on ultrasound evaluation of six malignant mammary neoplasms, whereas the artifact was absent in the remaining six cases of malignant mammary neoplasm (Table 1). The presence of the artifact in malignant mammary neoplasms was statistically significant ($p<0.05$) when compared with the benign group of mammary gland neoplasm and hence it can be considered as a specific finding for malignant mammary neoplasms. The findings of the present study were not in accordance with the reports of Soler *et al*. (2016) who identified acoustic enhancement equally evident in both the groups of tumours. Stavros *et al*. (1995) documented the desmoplastic host response to the mammary neoplasia, attenuated sound beam, and thus resulted in acoustic shadowing which was seen behind the tumour and the enhancement could be attributed by necrosis. According to Nyman *et al*. (2006a) presence of posterior acoustic enhancement could be associated with the prevalence of necrotic and cystic regions within the canine mammary neoplasms.

Invasiveness

The parameter invasiveness was not included for the ultrasonographic assessment of mammary gland in all the seven apparently healthy dogs as the normal mammary gland architecture depicted a distinct three-layered structure with defined and regular margins.

A statistically significant difference was observed for the invasive nature of neoplasm between benign and malignant mammary neoplasms ($p< 0.01$), with only one out of five benign mammary neoplasm exhibiting the feature, whereas 11 out of 18 cases of malignant mammary neoplasms displayed invasiveness to adjacent tissue. The findings were in agreement with Nyman *et al*. (2006b) who observed invasiveness more commonly with malignant tumours than benign tumours. Similarly, Sleeckx*et al*. (2011) reported invasive expansion of mammary lesion into surrounding tissue as a prevalent characteristic of malignant tumours.

Doppler ultrasonography

Vascular visualisation by colour flow

The major colour Doppler ultrasound finding of normal mammary glands in seven apparently healthy female dogs was the absence of neovascularisation. Neovascularisation could be identified from the abnormally numerous vascular supply to the mammary gland which is evident in mammary neoplasms.

The presence or absence of vascular visualisation and the pattern of vascular flow for various mammary gland neoplasms studied are enlisted in Table 4.9. Using colour-flow Doppler, the presence of vascular flow was detected in 15 mammary gland lesions and it included four benign and 11 malignant neoplasms. Three cases of neoplasms showed no detectable vascularisation. With regard to the flow distribution in benign neoplasms, vascularisation was detected only in four lesions among the six benign neoplasms (Fig.

Table 1. B-mode ultrasound features of mammary neoplasms (n=18)

Parameters		Benign mammary tumour (n=6)	Malignant mammary tumour (n=12)	p value
Echotexture	Homogenous	2	0	0.61
	Heterogenous	4	12	
Contours/Margin	Defined	6	0	0.00**
	Undefined	0	12	
Posterior acoustic attenuation	Present	0	6	0.039*
	Absent	6	6	
Invasiveness	Present	1	11	0.002**
	Absent	5	1	

*Indicates significance at 5 per cent level of significance

**Indicates significance at 1 per cent level of significance

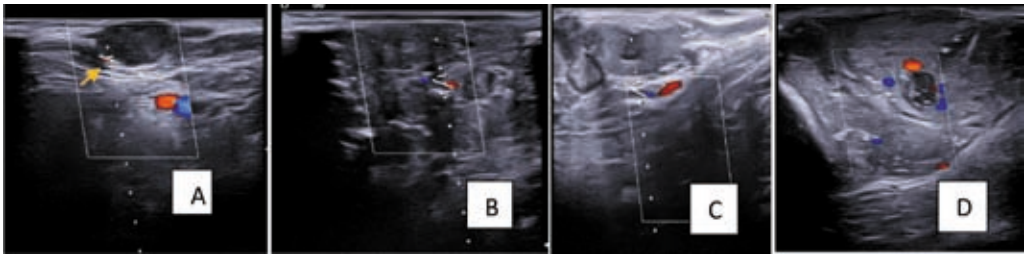

Fig. 3. (A) A benign mammary neoplasm with peripheral flow pattern (arrows). **(B)** A malignant mammary neoplasm with central flow pattern. **(C)** A malignant mammary neoplasm with peripheral flow. **(D)** A malignant mammary neoplasm with mixed pattern of neovascularisation.

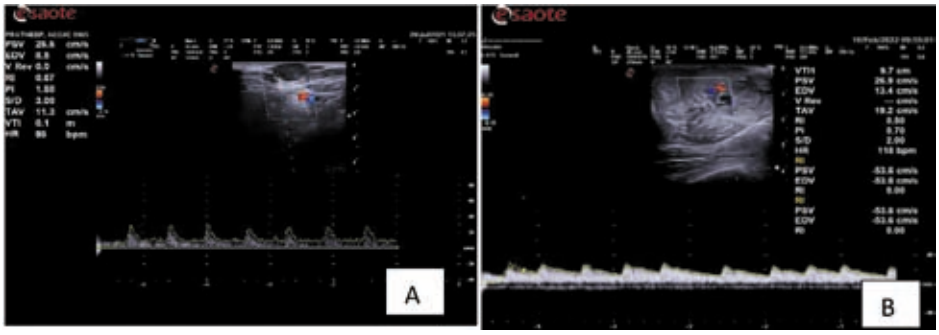

Fig.4. Spectral Doppler ultrasonographic image of mass with tumour neovascularisation and blood flow velocity waveforms in **(A)** A benign mammary neoplasm **(B)** A malignant mammary neoplasm

3). The peripheral pattern was detected in three cases and central pattern in a single case.Among malignant mammary neoplasms (n=12), vascularisation was detected in 11 cases, with most commonly observed flow pattern as central (n=6), followed by peripheral (n=3) and mixed patterns (n=1) (Table 2). The vascular flow pattern was classified into central, peripheral and mixed, based on the distribution of vascular flow.This finding was in accordance with the reports of Felician*oet al*. (2017), who noticed higher proportion of vascularisation in malignant tumours. Similar findings were also given by Nyman *et al*. (2005) who opined that an abnormal vascular supply was induced by malignant tumours and as the result of strong angiogenesis greater number of small vessels were present throughout the tumour.

Peak Systolic Velocity (PSV)

Vascular indices were measured to quantify the blood flow within the tumour vessels and hence the various vascular indices

Table 2. Parameters evaluated by Colour Doppler ultrasonography

Parameters		Tumour classification	
		Benign (n=12)	Malignant (n=6)
Presence of vascular flow	Present	4	11
	Absent	2	1
Flow distribution (Vascular pattern)	Central	1	6
	Peripheral	3	3
	Mixed	nil	2

Table 3. Ultrasonographic parameters evaluated by Spectral Doppler for the different groups of tumours

Parameter	Benign tumour Mean ± SE	Malignant tumour Mean ± SE	p value
Peak Systolic velocity(cm/s)	26.19±1.44	41.16±5.54	0.02*
End Diastolic velocity(cm/s)	7.30±0.55	9.40±1.54	0.21
Resistivity index	0.72±0.01	0.74±0.03	0.60

*Indicates significance at 5 per cent level of significance

were included only for the assessment of clinical cases of mammary neoplasm and not for apparently healthy normal dogs. The mean peak systolic velocity (PSV) obtained for benign and malignant mammary neoplasms are enlisted in Table 3. The mean PSV for benign mammary neoplasm was 26.19±1.44 cm/s, and was found to be lower than that of malignant neoplasm (41.16±5.54 cm/s). There was significant difference in PSV of benign and malignant mammary neoplasms ($p<0.05$). The findings were in accordance with Dock *et al*. (1991) who opined that the absence of non-striated musculature, characteristic tapering seen in normal vessels and presence of similar structural organisation of large capillaries or sinusoids resulted in obstruction, arteriovenous shunts, abnormal course and distribution of tumour vessels which in turn altered the blood flow velocity and manifested as accelerated flow on Doppler sonography.

End Diastolic Velocity (EDV)

The mean end diastolic velocity (EDV) obtained for benign and malignant mammary tumours are enlisted in Table 3. For malignant group of mammary tumours, the mean EDV was 9.40±1.54 cm/s. The mean value for benign lesions was 7.30±0.55 cm/s. Statistically no significant difference was detected for EDV between benign and malignant group of mammary neoplasms. The findings of the present study were in agreement with Soler *et al*. (2016) and Mulazimoglu*et al.* (2016) who found Doppler ultrasonography techniques, insufficient to distinguish malignancy in canine mammary tumours.

Resistivity Index (RI)

The mean RI obtained for benign and malignant canine mammary lesions were 0.72±0.01 and 0.74±0.03 respectively (Table 3). There was no significant difference for the value between benign and malignant mammary lesions. This was in accordance with the findings of Soler *et al*. (2016) and Mulazimoglu*et al.* (2016).

Conclusion

In conclusion, the contours or margin of mammary neoplasm, nature of tumour invasiveness, posterior acoustic enhancement and peak systolic velocity of the tumour vessels were the most consistent ultrasonographic featureswhich showed statistically significant difference between the two group of mammary neoplasm. In order to reach a definitive diagnosis, detailed histological study is required.

Conflict of interest: The authors declare that they have no conflict of interest.

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