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Occurrence and pathology of mammary tumours in dogs of central Kerala

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Abstract
The present study was undertaken to investigate the occurrence, gross, histopathological subtypes and malignancy grades of mammary tumours among dogs of Thrissur district of Kerala. Out of the 25 cases taken for the study, 24 were malignant and only one was a benign tumour. Mean age of occurrence was 8.96 ± 0.56 years. Intact nulliparous females were found to be more susceptible and Dachshunds were seen affected more. Caudal abdominal glands were mostly affected. Mean tumour size was 10.74 ± 2.14 cm. Ductal carcinoma was the most frequent histopathological subtype and majority of the tumours were grade II, six were grade III and three were grade I. High grade tumours were relatively larger than low grade ones. The study also indicated that histopathological subtyping along with malignancy grading could be used as a valuable tool for prediction of prognosis and clinical outcome in canine mammary CMTs.

Keywords: Canine mammary tumours, neoplastic, histological types, grading, prognosis

Introduction
Even though many scientists and physicians have made significant efforts to fight against cancer, it is still one among the major causes of death in the world. (Siegel *et al*., 2012) [18]. This illness is primarily caused by genetic or epigenetic alterations occurring in a cell or group of cells (Hanahan and Weinberg, 2000) [9]. Canine mammary tumours have been suggested as models to study human breast cancer (HBC), due to the close similarities between them, from epidemiological data to the histological patterns of the neoplastic. lesions. They show mutual resemblance even at the molecular levels.

Materials and methods
Twenty-five cases of mammary tumour suspected growths collected from University Veterinary Hospitals at Kokkalai and Mannuthy were considered for the study. Epidemiological factors such as age, breed, sex, and reproductive status of all animals were recorded. Gross pathological features like shape, size, appearance, consistency of the mass, colour of cut surface and other features like presence of cystic cavities, surface ulcerations, abscess formation etc were noted in the excised masses.

Histopathology
After routine paraffin embedding and staining, histopathological characteristics of each sample was evaluated. They were primarily classified into benign and malignant and subsequently to different subtypes as described by Goldschmidt *et al.* (2011) [8].

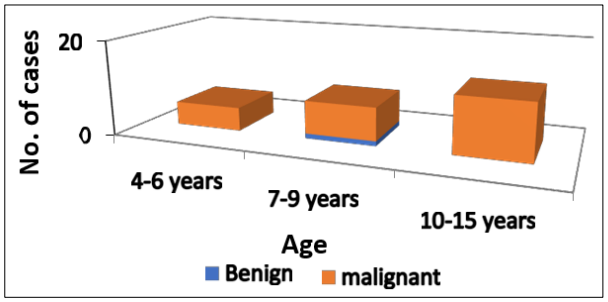
Histological malignancy grading
Grading was done as described by Clemente and co-workers in 2010 [4] which was a modification of Elston and Ellis numeric grading method of HBCs. This system relied on three criteria tubule formation, nuclear pleomorphism and mitotic figures for grading the mammary tumours.

Tubule formation was assessed in the stained sections and a score of one point was given when more than 75 per cent of the area was covered by definite tubules. Two and three points were given when 10-75 per cent and less than 10 per cent of the area was covered by tubules respectively.

Based on the extent of nuclear pleomorphism a score of one to three points was given. When the nuclei were small with minimum variation in size and had uniform chromatin, a score of

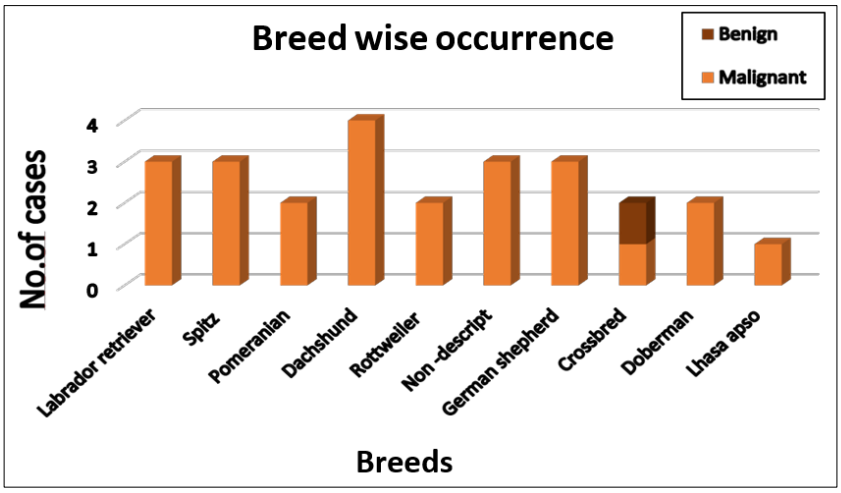
one point was given. When it was larger with moderate anisokaryosis, 2 points was given and when they were vesicular, varying considerably in size and shape with prominent nucleoli, 3 points were given.
If there were upto nine mitotic figures per 10 high power fields (HPF), one point was given. Two points were given for 10 to 19 mitotic figures per 10 HPF and three points for more than 20 mitotic figures per 10 HPF.
These three scores were added finally to get a number between one and nine. If the final score came between three to five points, the tumour was classified as a well differentiated grade I tumour and if it was between six and seven, it was graded as a moderately differentiated grade II tumour. If the score was between eight and nine, it was classified as a poorly differentiated grade III tumour.
Comparison between tumour grades and tumour size was analysed by using one way ANOVA followed by Duncan multiple range test.

Results and Discussions
In the present study, 24 cases (96 percent) were malignant and only one case (4 per cent) was benign.
The occurrence of CMTs was more in dogs of age group between 10 and 14 years (48%) followed by 7 to 9 years age group (32 per cent). The lowest number of cases were identified under the age of six years (20 per cent). The most susceptible age was found to be ten years, and there were no cases recorded under the age of four years. Mathew *et al.* (2019) [13] studied 22 CMT suspected cases and found that majority of the tumours (80 per cent) were in dogs of 7 years and above peaking at 10 years, and that there were no cases recorded in dogs less than 4 years of age. According to Kim *et al.* (2016) [11] CMTs were not common in young females under the age of five. Two spitz dogs of 14 years of age were noticed to have developed mammary tumours and it is the highest age of occurrence recorded in the present study. In larger breeds, the occurrence was mostly found below the age of 10 years. This result is consistent with Sonnenschein *et al.* (1991) [19], who observed that larger breed animals age more rapidly than smaller breed animals and, as a result, develop neoplasms sooner in life due to their shorter lifespans.



Graph 1: Age wise occurrence of CMTs

Dachshunds (16 per cent) were found to be more susceptible followed by Spitz dogs (12 per cent), German Shepherds (12 per cent), non-descript dogs (12 per cent), Labrador retrievers (12 per cent), Rottweilers (8 per cent), Pomeranians (8 per cent), Cross breds (8 per cent) and Dobermanns (8 per cent). Only one case was reported in Lhasa apso (4 per cent). Christy *et al.* (2022) [3] also reported a highest incidence in Dachshunds (24 per cent) followed by Labradors (16 per cent) during their study. Dobson, (2013) [6] also reported that the breed- wise incidence of CMTs varied greatly in different studies from different geographical locations. When sample size and study period vary, breed-specific occurrence also get varied.
Twenty-three cases were in female dogs (92 per cent) and only two were in male dogs (4 per cent). This is consistent with the findings of Brodey *et al.* (1983) [2], that male dogs have a 0 to 2.7 per cent lower incidence of CMTs than female dogs.
Out of the 23 females, fifteen (65.21 per cent) were intact nulliparous dogs and seven (30.43 per cent) had whelped at least once with a whelping rate ranging from one to six. Ovariohysterectomy was done only in one animal (4.34 per cent). Nithya *et al.* (2018) [16] reported that out of the 87 female dogs studied, 90.8 percent were intact, and 37.93 percent had at least whelped once. Animals that had whelped were less in number whereas the number of nulliparous animals was slightly higher.
One of the two male animals presented was a cryptorchid showing feminine characteristics.



Graph 2: Breed wise occurrence of CMTs

Hormonal imbalances might be the reason for CMT in the cryptorchid one. Saba *et al.* (2007) [17] conducted a retrospective study in eight male dogs with histologically proven mammary-gland tumours and observed significant estrogen receptors (ER) expression in fifty per cent of the neoplastic cells from six out of these eight dogs.
In this study, it was found that caudal abdominal and inguinal glands were more affected (56 per cent and 32 per cent

respectively) (32 per cent). In one case (4 per cent) caudal thoracic glands were affected, while in two other cases the cranial abdominal glands were involved (8 per cent). No cases involving cranial thoracic glands have been found. Wey *et al*. (2000) [23] also observed the highest involvement of caudal abdominal glands in their study. An increased tumour incidence in the posterior mammary gland pairs was associated with increased gland volume, estrogen- induced proliferative alterations, and profused secretion during lactation (Meuten, 2016) [14].

In 68 per cent of the cases, the consistency was firm, followed by soft (20 per cent) and hard (12 per cent). Hellmen *et al*. (1993) [10] and Perez *et al*. (1997) [24] studied a correlation between the ulceration of superimposed skin in CMTs with malignancy and found that ulceration was strongly associated with a poor prognosis. Six cases in the current study exhibited ulcerations, and higher-grade tumour masses had more ulceration and pus formation. Abscess formation and cystic cavities were observed in two and three cases respectively. Cut surface was greyish white (64 percent) and were irregular in most of the cases. Cartilage and bone formation was observed in three cases (12 per cent). Kutzler, (2020) [12] reported that cross sections of the masses were usually firm, lobulated, grey tan coloured and mostly contained serosanguineous fluid filled cysts and sometimes the cross sections of mixed mammary neoplasm consisted of recognizable cartilaginous structures or bones in them. Size of the tumour mass varied from 2.5 cm to 20 cm and in three cases, more than one tumour was observed.

In 76 per cent of the cases, the animal was active and alert with normal food and water intake, urination and defaecation. Remaining 24 per cent had shown mild to moderate dullness along with reduced water and food intake, difficulty in urination and defaecation. In 64 per cent of the cases, RBC count and Hb concentration was below normal. Blohmer *et al*. (2005) [1] has found that cancer-related anaemia was a prevalent consequence with an adverse effect on treatment response and prognosis. In 40 per cent animals, high WBC count and in 44 per cent of animals high platelet count was observed. These findings are in line with the observations of Ucmak *et al*. (2021) [22] who has found considerably higher WBC and PLT levels in grade III tumours.

Ductal carcinoma was found to be the most frequently occurring histological subtype (32 per cent) followed by tubulopapillary carcinoma (12 per cent) and solid carcinoma (8 per cent). One case each of cribriform carcinoma, papillary carcinoma, micropapillary carcinoma, intraductal papillary carcinoma, Carcinoma Arising in a Benign Mixed Tumour (CABMT), lipid rich carcinoma, lipoma, complex carcinoma, carcinosarcoma, spindle cell carcinoma, anaplastic carcinoma and mucinous carcinoma were also observed. This agrees with the findings of Devi *et al*. (2022) [5] that the most frequent type of CMT was ductal carcinoma followed by tubulopapillary carcinoma.

Proliferation of neoplastic epithelial cells into cords and tubules surrounding slit like lumen was observed in ductal carcinoma (Fig.7). In tubulopapillary carcinoma, cells were seen proliferating into distinct tubular and papillary patterns, papillae were supported by a fine fibrovascular stalk (Fig.8). The neoplastic cells proliferated in a papillary fashion in papillary carcinoma (Fig.14).

Solid carcinoma was composed of proliferating cells in solid sheets, cords, and masses without a lumina (Fig.15). In cribriform carcinoma, neoplastic cells were arranged in a sieve-like pattern and formed bridges with round lumina (Fig.9). Mucus secreting neoplastic cells with abundant mucin production was observed in mucinous carcinoma (Fig.19). In carcinosarcoma, both epithelial and mesenchymal components were malignant (Fig.10). In micropapillary carcinoma, neoplastic cells formed small papillae and irregular aggregates with no fibrovascular stalk and empty lacunar spaces (Fig.18).

One case of Carcinoma Arising in a Benign Mixed Tumour (CABMT) was observed with areas of both benign and malignant components (Fig.21). Malignant component showed more nuclear and cellular pleomorphism with increased number of mitoses compared to the pre-existing benign epithelial part. One benign case of lipoma was observed characterised by the presence of neoplastic adipocytes with an eccentrically placed nuclei and large cytoplasmic vacuoles separated by a fibrovascular septa (Fig.11).

Round to oval shaped cells with definite cell borders and many small vacuoles at the periphery of nucleus in the cytoplasm was observed in lipid rich carcinoma (Fig.20). One case of complex carcinoma was observed with malignant epithelial component and a benign myoepithelial component (Fig.17). Multi-layered neoplastic cells were arranged in a papillary pattern within the ducts in intraductal papillary carcinoma (Fig.12). Spindle cell carcinoma consisted of spindle shaped cells with a fibrovascular stroma surrounding them (Fig.13). In anaplastic carcinoma, individualized round, oval to polygonal shaped neoplastic cells were observed often grouped in small nests (Fig.16).

All the 24 malignant cases were graded based on three criteria-tubule formation, nuclear pleomorphism and mitotic figures according to Clemente *et al*. (2010) [4]. Out of the 24 malignant tumours, 62.5 per cent were grade II, 25 per cent were grade III and 12.5 per cent were grade I tumours. These observations agree with the findings of Devi *et al*. (2022) [5] who had reported an occurrence of 40 per cent of Grade II tumours, 33 per cent of Grade I tumours and 27 per cent of Grade III tumours. Among the tumour subtypes, ductal carcinoma was categorized under grade I and II, tubulopapillary came under grade II and III. Micropapillary carcinoma, carcinosarcoma and anaplastic carcinoma were graded as III. Solid carcinomas were graded as II and III. Cribriform carcinoma, papillary carcinoma, lipid-rich carcinoma, complex carcinoma, intraductal carcinoma and spindle cell carcinoma were classified as grade III. One case each of CABMT and mucinous carcinoma was classisfied as grade I. Millanta *et al*. (2005) [15] studied a relationship between the histological type and grade of tumours and reported that complex carcinomas were graded under II or III and the most malignant simple carcinomas were graded under III with worse prognosis. Tavasoly *et al*. (2013) [21] observed 37 CMT and found that only 15.6 per cent of the lesions were grade III whereas the complex carcinomas and carcinomas emerging from benign mixed tumours were typically of grade I or II.

Statistical analysis

Comparison of tumour size and tumour grade

Comparison of tumour size and tumour grades was carried out using one way ANOVA followed by Duncan multiple range test and the results are summarised in Table.1.

It was observed that the higher-grade tumours differed significantly in size from low grade tumours. Grade II and III tumours were bigger compared to grade

I. This observation is in concordance with the findings of

Sorenmo *et al*. (2009) [20] that when the tumour size increased, the probability of malignancy also got increased.

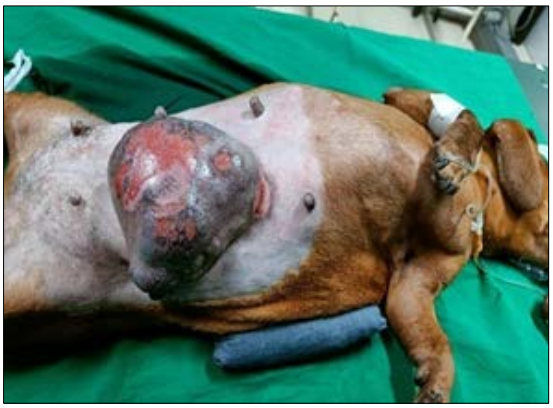


Fig 1: Ulcerated mass involving multiple mammary glands

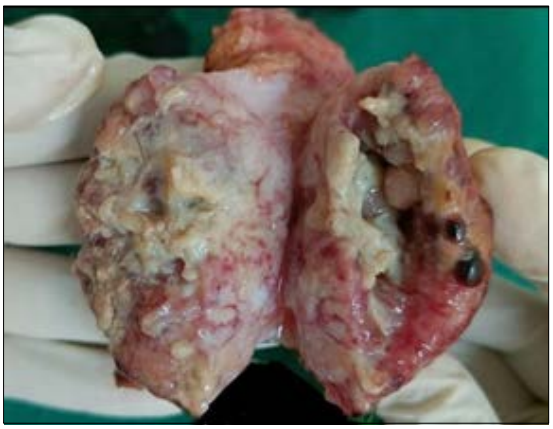


Fig 2: Irregular mass with ulceration and pus formation

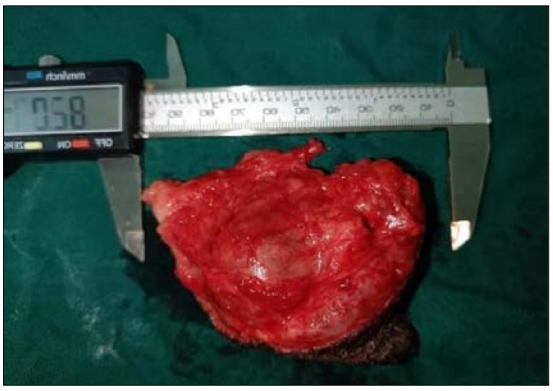


Fig 3: A firm nodular mass

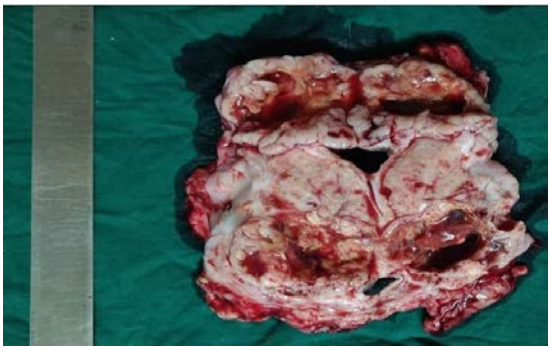


Fig 4: An irregular firm mass with cystic cavities in the cut surface

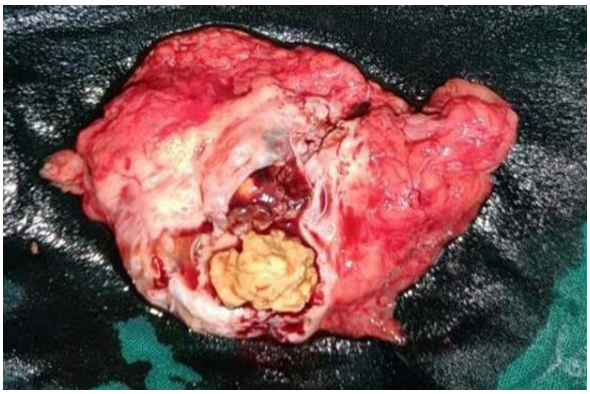


Fig 5: Cut surface of tumour mass with abscess formation

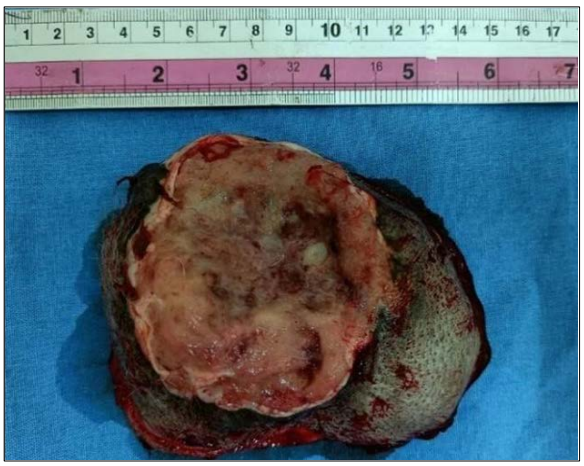


Fig 6: An irregular firm mass with ulceration on the surface

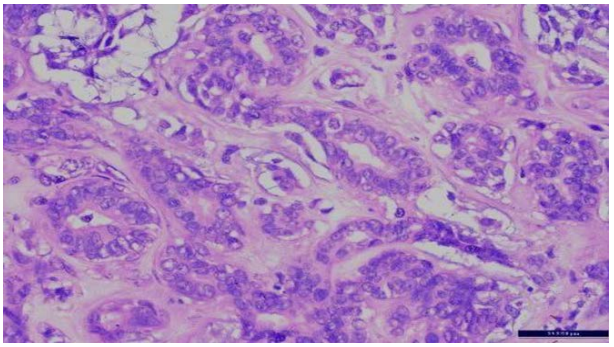


Fig 7: Ductal carcinoma- Neoplastic cells arranged in cords and tubules surrounding slit like lumina (H&E x 200)

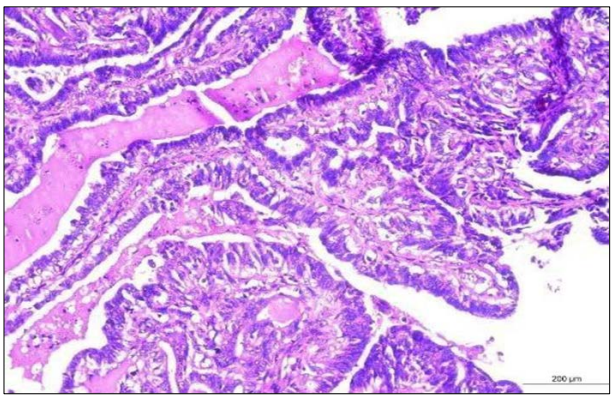


Fig 8: Tubulopapillary carcinoma - Neoplastic cells proliferating in tubular and papillary pattern (H&E x 200)

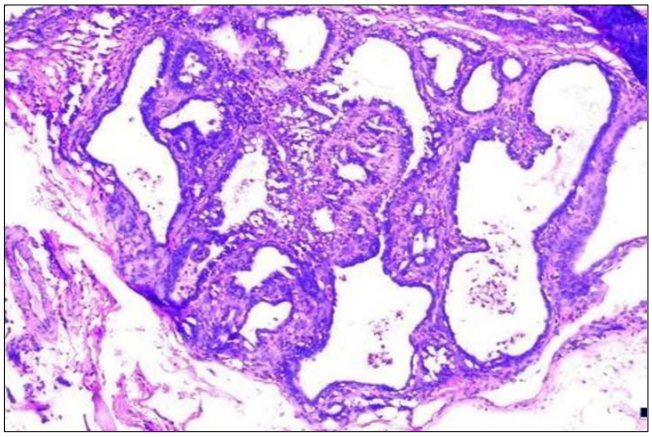


Fig 9: Cribriform carcinoma-Sieve like arrangement of the cells (H&E x 200)

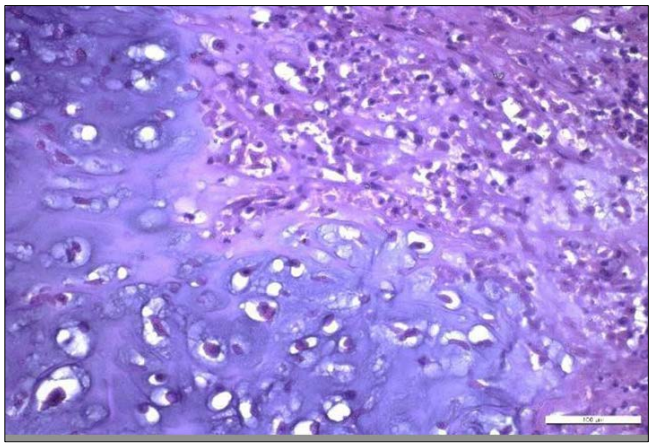


Fig 10: Carcinosarcoma- Both the epithelial and mesenchymal components are malignant (H&E x 400)

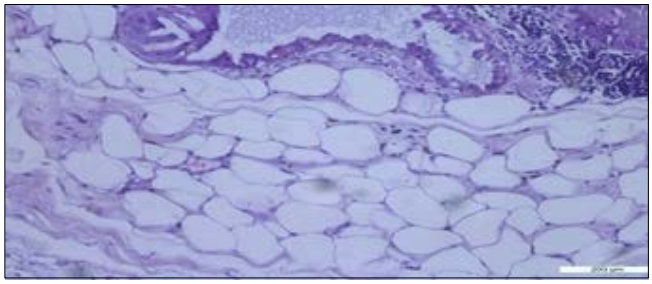


Fig 11: Lipoma- Neoplastic adipocytes with eccentrically placed nuclei and large cytoplasmic vacuoles (H&E x 200)

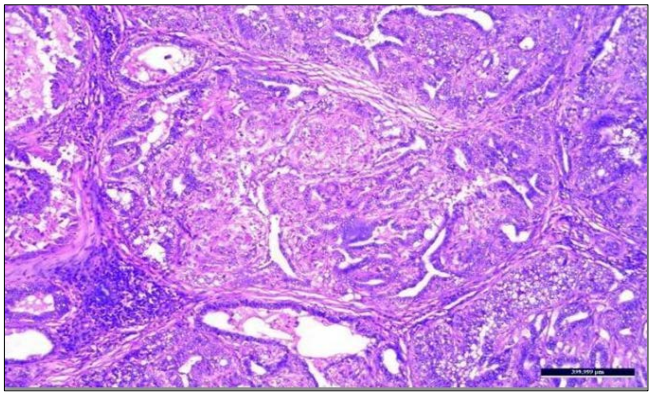


Fig 12: Intraductal Papillary carcinoma – proliferation of neoplastic cells in papillary pattern inside the duct (H&E x 200)

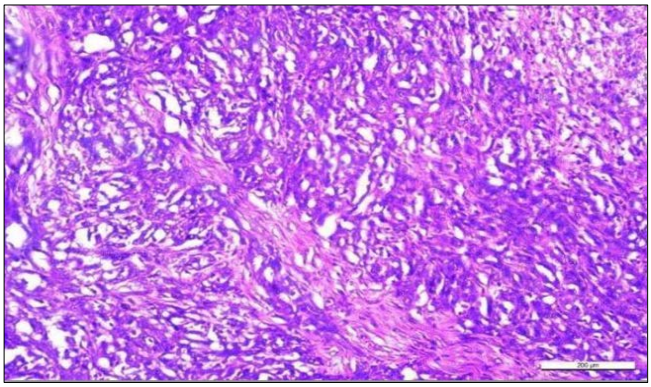


Fig 13: Carcinoma- Spindle cell variant - neoplastic cells were spindle shaped with surrounding fibrovascular stroma (H&E x 200)

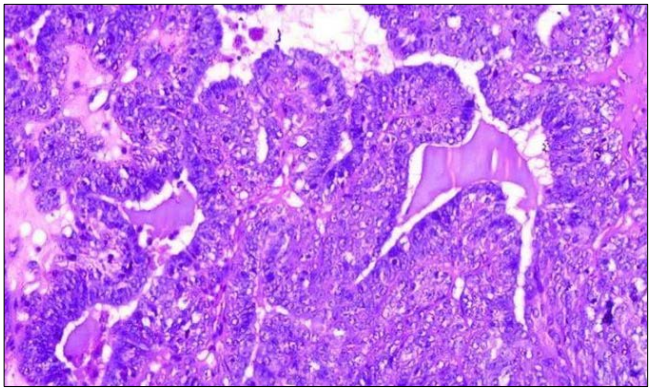


Fig 14: Papillary carcinoma – proliferation of neoplastic cells in papillary pattern (H&E x 200)

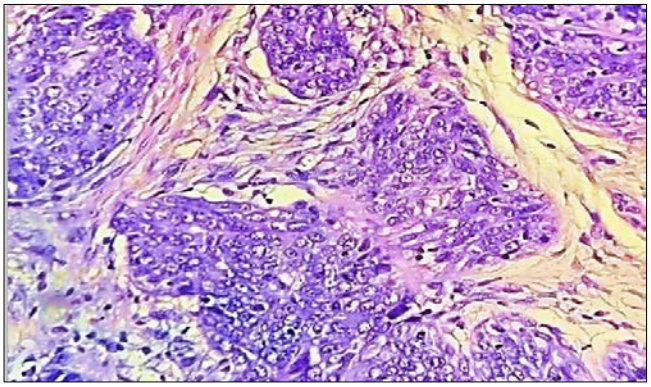


Fig 15: Solid Carcinoma-Closely packed cells with poorly demarcated cell margins and scant cytoplasm (H&E x 200)

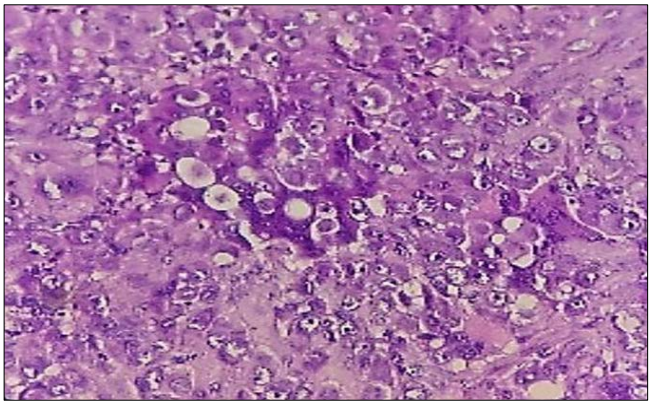


Fig 16: Anaplastic carcinoma- individualized round to oval shaped cells with a prominent nuclei (H&E x 200)

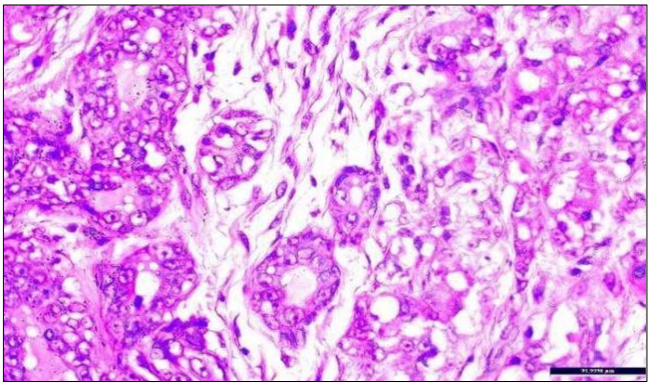


Fig 17: Complex carcinoma – Pleomorphic epithelial cells lining the tubules and non- pleomorphic fusiform myoepithelial cells (H&E x200)

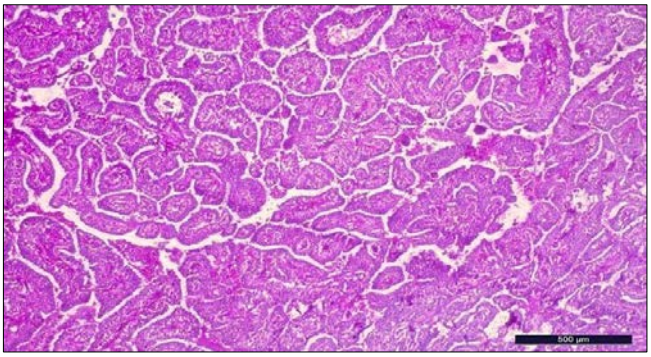


Fig 18: Micropapillary carcinoma- Neoplastic cells formed small papillae and irregular aggregates with no fibrovascular stalk and empty lacunar spaces (H&E x100)

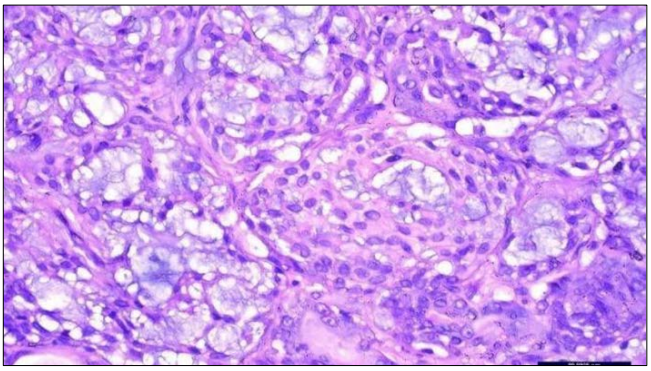


Fig 19: Mucinous carcinoma -abundant mucin production by the neoplastic epithelial mucus secreting cells (H&E x200)

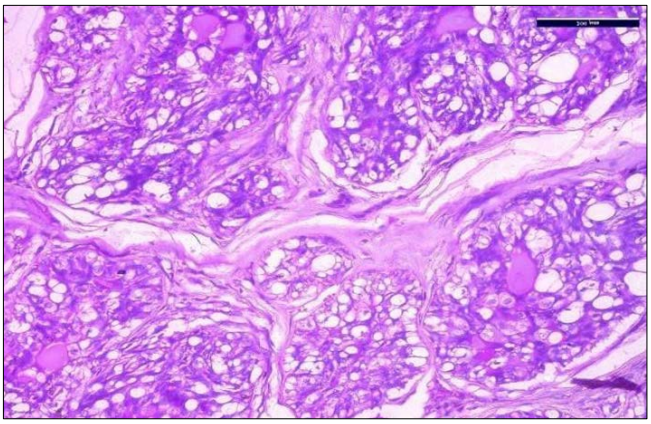


Fig 20: Lipid rich carcinoma-round to oval cells with distinct cell borders and moderate to abundant cytoplasm with numerous small vacuoles that occasionally peripheralized the nucleus (H&Ex200)

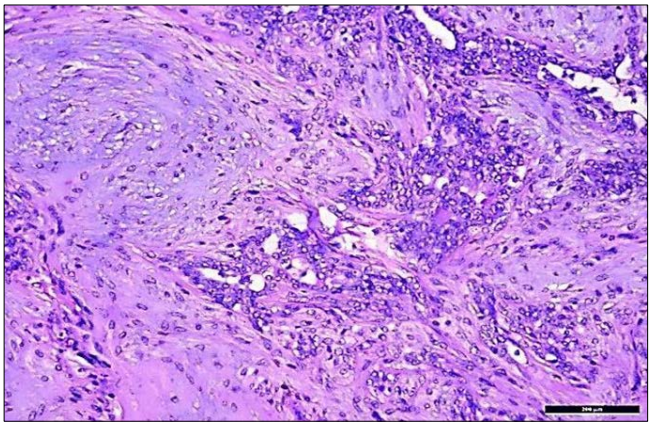


Fig 21: CABMT- Benign cartilaginous areas and areas of malignant epithelial cells with increased cellular and nuclear pleomorphism (H&E x200)

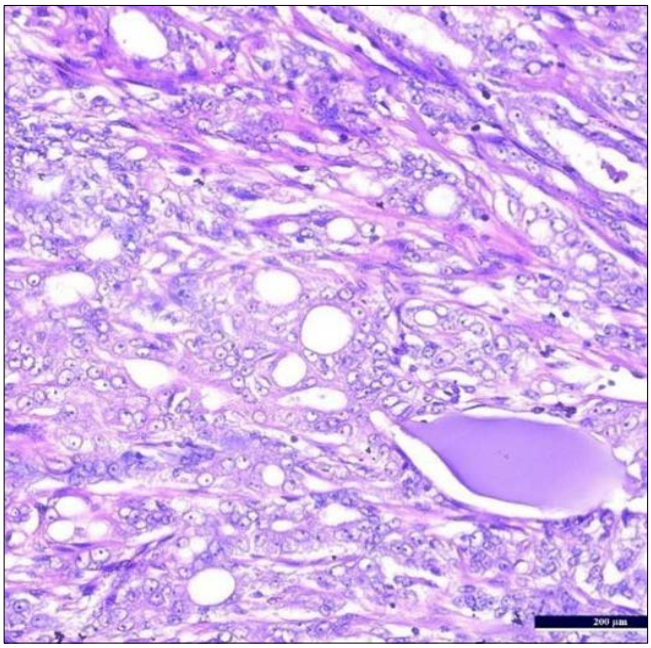


Fig 22: Grade I-more tubule formation, mild anisokaryosis and anisocytosis, well differentiated (H&Ex100)

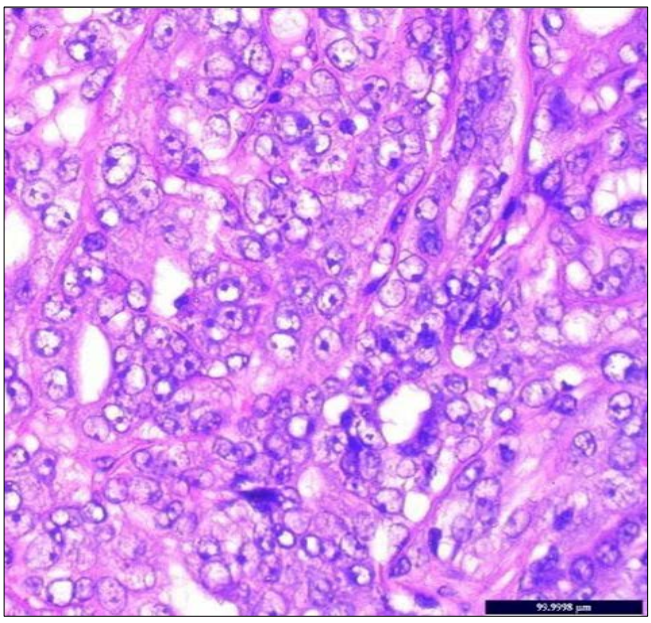


Fig 23: Grade II- moderate anisokaryosis and anisocytosis, moderately differentiated, mitotic figures (H&Ex200)

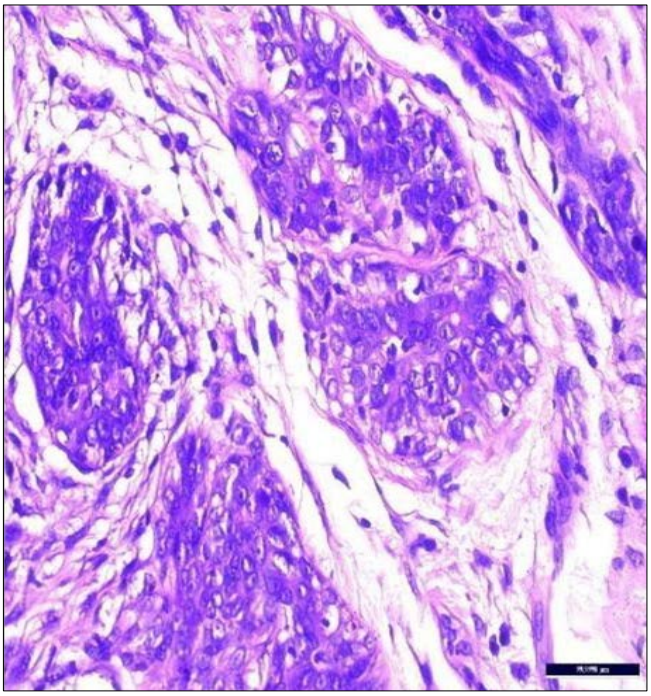


Fig 24: Grade III-severe anisokaryosis and anisocytosis, poorly differentiated, mitotic figures (arrows) (H&Ex200)

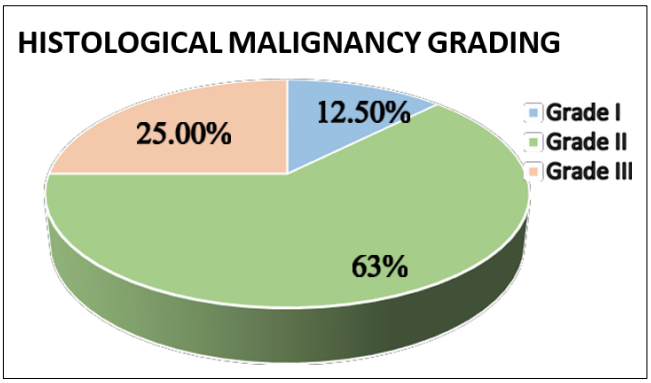


Fig 25: Histological malignancy grading of canine mammary Tumours

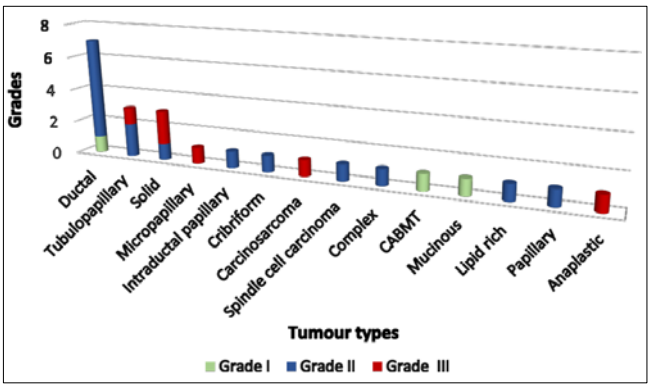


Fig 26: Histological types in relation to grades of tumours

Table 1: Comparison of Tumour size and Tumour grades

Grade	Mean	SE
Grade I	5.17b	1.97
Grade II	10.67a	1.08
Grade III	13.83a	1.68
F-value (P-value)	4.503* (0.024)	

* Significant at 0.05 level

Conclusion

From the present study, it was observed that the intact nulliparous dogs were more susceptible to CMTs. The most common histological type was ductal carcinoma, and the most frequent tumour grade was grade II. The gross and histopathological features of CMTs were very much related to the tumour grades. The grade of CMTs increased as the tumour size increased. A better understanding of these factors would help to predict the prognosis and clinical outcome in CMT cases.

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