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Incidence and pathology of skin tumours in dogs

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Abstract
The present study was undertaken to study the incidence of skin tumours in canine population. A total of 160 dogs were diagnosed with skin tumours in the study period. Mast cell tumours 22 (13.75%), sweat gland adenocarcinoma 22 (13.75%), lipoma 18 (11.25%), histiocytoma 14 (8.75%), trichoblastoma 10 (6.25%) and perianal gland adenoma 10 (6.25%) were the tumours with highest order of occurrence. The tumours were recorded in 16 different breeds with the highest number of cases recorded in non-descript 55 (34.38%) followed by Labrador 46 (28.75%) and Spitz 19 (11.88%). Of the cases recorded, 109 (68.13%) were males and 51 (31.88%) were females. The highest incidence of skin tumours was observed in dogs belonging to the age group of 6-10 years 82 (50.63%) followed by 50 (31.25%) in dogs of 1-5 years age group. In the age group of more than 10 years, 25 (15.63%) cases were recorded. Only 3 (1.88%) cases were observed in young dogs of less than one year old. Location wise, higher incidence was observed in limbs 53 (33.13%), followed by trunk 39 (24.38%), head 32 (20.00%), neck 15 (9.38%), perianal region 15 (9.38%) and tail 6 (3.75%) respectively. Of the skin tumours identified, 73 (45.63%) were benign and87 (54.37%) were malignant.

Keywords: dogs, tumours, skin tumours, cutaneous neoplasms, cytology, histopathology, pathology, mitotic count

Introduction
Cancer is one of the most threatening and tormenting disease throughout the world and is often considered as the emperor of all maladies. Tens of millions of people are diagnosed with cancer around the world every year and more than half of the patients eventually die from it (Ma and Yu, 2006) [1]. A wide array of neoplasms are also recorded in dogs as in human beings, as animals live in the same environment as humans and are exposed to the carcinogens. According to the Cancer facts and figures (2018) [2], skin cancer was found to have the highest order of occurrence of the various cancers recorded in United States in human beings. An increased incidence of skin tumours in dogs is also witnessed in recent times, as skin is an extensive organ, which is exposed directly and indirectly to various carcinogens from the environment. Moreover, skin has a considerable population of cells in growth for continuous renewal and thus have more possibilities to develop mutations in the gene coding during cell cycles of proliferation, eventually ending in cancer initiation and progression (Goldschmidt and Hendrick, 2002) [3]. Hauck (2013) [4] submitted that the percentage of cutaneous tumours in canine biopsy samples was between 25.5 to 43 percent. Roxana *et al*. (2017) [5] also observed a higher percentage of skin tumours in dogs representing 25.86 percent of the total tumours identified in a study on incidence of different tumours over a period of 10 yrs.
Skin tumours are generally classified histologically according to the tissue of origin as epithelial cell and mesenchymal cell tumours and according to individual cells of origin as round cell tumours and spindle cell tumours. Tumours are further classified in terms of the degree of malignancy based on several histologic characteristics, such as the mitotic index and degree of cellular or nuclear atypia (Vail *et al*., 2001) [6]. The common skin tumours popularly encountered in dogs are mast cell tumour, histiocytoma, skin adnexal tumours and lipoma. With this milieu, the present study was carried out to identify thespontaneously occurring skin tumours in dogs based on gross, cytological, histopathologic changes and immunohistochemistry findings with a panel of markers and to study the influence of breed, sex, age and location on the occurrence of various skin tumours.

Materials and Methods
The study was conducted on the skin tumour suspected cases presented to the Small Animal

Surgery-Out Patient ward and Small Animal Operation theatre-Surgery, of Madras Veterinary College Teaching Hospital (MVCTH), Chennai during the period 2016-2019. Particulars of animal like breed, sex and age were recorded. Specific pathological data such as history, clinical manifestation, location, shape, size and weight of growth were also recorded. The samples including fine needle aspirates for cytological examination and excisional biopsies for histopathological examination were collected from animals suspected for cutaneous neoplasms. Blood and serum were collected for haematobiochemical analysis.

Gross pathology
Dogs presented with masses and growths on the skin were ardently observed for various criteria such as the location of the mass in the skin, gross changes in size, shape, colour, margins, orientation, extent, consistency etc. In biopsy samples, cut section of the tumour mass was evaluated for its internal appearance like colour, consistency and the presence of haemorrhage.

Fine needle aspiration biopsy
To obtain fine needle aspiration biopsy (FNAB), the surface of the tumour mass was prepared by clipping off the hair and cleaning with surgical spirit. After stabilizing the mass with one hand, a needle affixed to syringe was introduced gently into the centre of the tumour mass. Strong negative pressure was applied by withdrawing the plunger to about three fourths the volume of the syringe. The needle was moved back and forth repeatedly, passing through about two thirds of the diameter of the mass. For large masses, the needle was redirected to several areas within the mass to increase the amount of tissue sampled. Alternatively several different areas of the mass were sampled with separate collection attempts. Subsequent to sampling several areas in the tumour mass, the negative pressure was released and the needle was removed from the mass. The needle was removed from the syringe and air was drawn into the syringe. The needle was again replaced onto the syringe and the tissue in the barrel and hub of the needle was expelled onto one end of a new, clean, glass microscopic slide by rapidly depressing the plunger. The expelled tissue material was spread gently into a thin layer with the help of another glass slide for further staining and screening procedures (Meinkoth and Cowell, 2002) [7].

Impression smears
Surgically excised skin tumour masses were held firmly and cut with a sharp blade to expose the cut surface. The cut surface was blotted with blotting paper to absorb the blood and tissue fluids. Clean glass microscopic slide was then touched against the blotted surface of the tissue to take multiple imprints on each slide (Cowell and Tyler, 1989) [8].

Fixation of smears
Smears were either air dried immediately or wet fixed (95 percent ethanol or absolute isoproponol). Wet fixation was done immediately by plunging the slides into the fixative for 30 minutes.

Staining of cytology smears
Smears for cytological screening were stained with Leishman-Giemsa (LG) and toluidine blue and Harris haematoxylin and eosin (H&E) stains (Bancroft and Stevens, 1996) [9] in cases of special interest.

Cytological evaluation
Cytology smears were viewed under the microscope for cellularity and critical evaluation of staining characters of the cell, its nucleus and cytoplasm. The criteria for morphologic identification of tumour was based on the cell shape, size, arrangement, the shape of the nuclei, the density of the chromatin, the number, size and distribution of the nucleoli and the volume and basophilia of the cytoplasm, cytoplasmic vacuolation, nucleus to cytoplasm ratio and the presence of mitotic figures.

Histopathology
The tumour samples were fixed in 10 percent neutral buffered formalin for 48 hours, dehydrated in alcohol and cleared in xylene and then embedded in paraffin wax. Paraffin embedded tissues were sectioned to 5µm thickness and stained by haematoxylin and eosin (H&E) for histopathological examination. Wherever necessary, the sections were stained with special stains like Fontana black and toluidine blue (Bancroft and Gamble, 2001) [10].

Identification of the tumours
The tumours were identified and classified based on cytologic and histopathologic findings as per Yager and Wilcock (1994) [11]; Goldschmidt *et al.* (1998) [12]; Hendrick *et al.* (1998) [13] ; Valli, *et al.* (2002) [14] and Gross *et al.* (2005) [15].

Mitotic count
Mitotic count was determined by identifying the most cellular area and mitotically active area of the slide by scanning at 10x magnification and counting the number of mitosis in 10 consecutive high power (400x) fields and the mean was recorded as mitotic index.

Histopathological grading of tumours
Tumours were classified as benign and malignant by morphologic features observed microscopically such as nuclear pleomorphism, mitotic figures and the degree of anaplasia. Histopathological grading of mast cell tumours was done according to 2 tier grading of Kiupel *et al.* (2011) [16] as high and low grade tumours.

Haematology
Haematological parameters such as Haemoglobin (Hb), Packed Cell Volume (PCV), Total Erythrocyte Count (TEC), White Blood Cell count (WBC count), Platelet count (PLT count) and Platelet indices such as Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) and Plateletcrit (PCT) were analyzed using auto haematology analyzer (Mindray - BC-2800 Vet.). Differential Leucocyte Counts (DLC) was done by manual method. Peripheral blood smears were prepared, stained with Leishman-Giemsa stain and examined for Differential Leukocyte Count (DLC), blood parasites and for the changes in blood picture.

Serum biochemistry
Blood samples were centrifuged at 1500 rpm for 20 minutes and serum samples separated were run in a fully automated biochemical analyser (A-15 Biosystem Random Access Analyzer, Biosystems, Barcelona, Spain). Quantitative estimation of Blood Urea Nitrogen (Urease/GLDH method), Creatinine (Modified Jaffe's method), Total protein (Biuret method), Albumin (Bromocresol green method), Alanine Amino Transferase (Lactate dehydrogenase coupled reaction

method), Alkaline Phosphatase (Deutsche Gesellschaft Fur Klinische Chemie-SCE method), Total bilirubin (Modified Dimethyl sulfoxide method), Direct bilirubin (Diazo method), Calcium (O- Cresolphthalein complex method) and phosphorus (Phospho molybdate method) were carried out using specific diagnostic kits supplied by Agappe Diagnostics Private Limited, India, following the manufacturer's recommendations for respective estimations. Sodium, Potassium and Chloride were estimated in an automated electrolyte analyzer (Ion Selective Electrode method/Diestro 103 AP, Argentina). All the selected cases were subjected to routine laboratory investigations as per standard clinical laboratory protocols and interpreted as suggested by Boyd (1984) [17].

Statistical analysis

The data generated from different parameters of the experimental study were subjected to Chi Square test and one-way analysis of variance (ANOVA), followed by the Duncan's multiple range test. Analysis was performed by using IBM SPSS software version 20 for windows. The critical difference and post-hoc analysis for multiple comparisons were done by Duncan's and Dunnett's C Tests. The differences among the groups were considered significant when $p<0.05$.

Results and Discussion

Incidence of canine skin tumours

A total of 160 cases of skin tumours were identified during the study period 2016 to 2018 based on gross, cytological and histopathological findings (Table-1), (Plate1-14). The total number of canine tumours recorded during the period was 2247. This contributed to 7.12 percent of skin tumour incidence in canine population in our study. The increase in skin tumour incidence in our study could be attributed to geographic differences such as the intense ultraviolet exposure in tropical countries and due to increased pollution in urban nature of Chennai city. Earlier, Dobson *et al.* (2002) [18] have reported the incidence of skin and soft tissue tumours as 1.437 percent.

Among the skin tumours diagnosed (n = 160), 80 were epithelial tumours, 35 were mesenchymal tumours, 44 were round cell tumours and one was a case of melanoma. The findings of the study further revealed high incidence of cutaneous adnexal tumours (n = 61) represented by sweat gland tumours (n = 25), perianal gland tumours (n = 18) ceruminous gland tumours (n = 9) and sebaceous gland tumours (n = 9) respectively. The findings showed that mast cell tumour (n = 22) and sweat gland tumour (n = 22) as the cutaneous tumours of high incidence followed by lipoma (n = 18) in Chennai. The high incidence of mast cell tumours recorded in the present study concurred with the findings of Villamil *et al*. (2011) [19] who reported high incidence of mast cell tumours accounting for 11 percent of all skin tumours in their study population.

All the skin tumours identified were found to be solitary except a single case of mast cell tumour which was observed in multiple sites on the skin. All the tumours identified were also found to occur as primary tumours on the skin. This is in agreement with Weiss and Frese (1974) [20] who reported that secondary tumours in the skin are very rare. Metastasis of primary tumour to the skin occurs occasionally in dogs and among the various neoplasms, mammary gland adenocarcinomas, squamous cell carcinomas, transitional cell carcinomas and transmissible venereal tumours had more metastatic potential for secondary cutaneous involvement (Villabos, 2014) [21].

Breedwise incidence of skin tumours

Breed wise incidence of skin tumours in dogs is presented in Fig.1. Totally 16 different breeds were represented. The occurrence of skin tumours in different breeds of dogs were non-descript 55 (34.38%), Labrador 46 (28.75%), Spitz 19 (11.88%), German Shepherd 10 (6.25%) Dachshund 7 (4.38%), Dobermann 6 (3.75%), three (1.88%) each in Great Dane and Pug, two (1.25%) each in Boxer, Rottweiler and Dalmatian and one (0.63%) each in Cocker Spaniel, Lhasa Apso, Golden Retriever, Shih Tsu and Terrier. Similar observations were recorded by Kujur (2005) [22] who reported that non-descript breeds had the highest risk of developing skin tumours. Among the pure breeds, Labrador was mostly affected (n = 46) followed by Spitz (n = 19) and German Shepherd (n = 10). Increase in the occurrence of cutaneous tumours in Labrador among the pure breeds might be due to preference of this breed by pet owners in Chennai city. Hence this breed was more represented in the present study.

Sex-wise incidence of skin tumours

Sex-wise incidence of skin tumours is presented in Fig. 2 and 3. Highly significant difference was recorded between males and females with skin tumours recorded predominantly in males 109 (68.13%) than in females 51 (31.88%). Two of the tumours, papilloma (n = 7) and perianal adenocarcinoma (n = 8) were encountered only in males in the present study. Our findings of high incidence of skin tumours in male are in concordance with the findings of Mukaratirwa *et al*. (2005) [23] who reported a male predominance of 322:218. Tostes *et al.* (2017) [24] also reported a gender bias towards males in the occurrence of canine cutaneous neoplasms. The authors reported skin tumour incidence of males (197/56.77%) compared to females (150/ 43.23%). In contrast, Arya *et al.* (2018) [25] stated sex difference in the metabolism and detoxification of carcinogens, hormonal interaction with receptors in neoplastic tissues, and differential proliferation or growth stimulation effects indicated by gastrin releasing peptide receptors expression markers favouring the development of tumours in female than male dogs. However, Goldschmidt and Shofer (1992) [26] and Pakhrin *et al*. (2007) [27] reported no significant sex linked relationship in the occurrence of skin tumours.

Age wise incidence of skin tumours

The age wise incidence of skin tumours is presented in Fig.4 and 5. The age group of the dogs presented with skin tumours had a wide range of 8 months to 14.5 yrs and the mean age of dogs presented with skin tumours was 7.25. Hasiri *et al.* (2019) [28] have recorded the mean age of 8.22 years in their study on skin tumours in dogs. Highly significant difference was recorded between the tumour groups in our study. The highest incidence of skin tumours was 82 (50.63%) observed in dogs belonging to the age group of 6-10 yrs followed by 50 (31.25%) in dogs of 1-5 yrs age group. In the age group of more than 10 yrs, 25 (15.63%) cases were recorded. Only 3 (1.88%) cases were observed in young dogs of less than one year old. The observations on age wise incidence is in agreement with literature that tumour predisposition occurs with increasing age as seen in 6-10 yrs age group (51.25%). The critical period for the onset of neoplasia in dogs is 6-10

yrs old (Merlo *et al*., 2008 [29] and Butler *et al.,* 2013 [30]). The probable reason for the increase in occurrence of tumours with advancing age is that, long latent period is required for the tumour to get initiated and to progress, the time required for the carcinogens to exert mutagenic effect as well as to the fact that ageing animals are more prone to develop gene mutations than young animals as stated by Arya *et al*. (2018) [25a].

However, tumours were less in very old dogs of more than ten years because the animals would have succumbed to other metabolic and infectious diseases. These findings are in concordance with Villamil *et al*. (2011) [19a] who observed that for all cancers, the incidence increased with age, peaked in the age group of 9-11 yrs and then declined in dogs aged more than 11 yrs. However, histiocytoma has been reported to occur more in young animals. Er and Sutton (1989) [31] recorded 55 of the 80histiocytoma cases in dogsunder 3 years. In our study histiocytoma (n = 14) had a mean age of 5.06 years with the range of 8 months to 9 years.

Location wise incidence of skin tumours

Location of the tumour on the body was categorized into 6 groups: head, neck, trunk, limbs, perianal region and tail. The occurrence of skin tumours in different regions of the skin is presented in Fig. 6 and 7. Highly significant difference was recorded between the tumour groups. Location wise, higher incidence was observed in limbs 53 (33.13%), followed by trunk 39 (24.38%), head 32 (20.00%), neck 15 (9.38%), perianal region 15 (9.38%) and tail 6 (3.75%) respectively. In the head, tumours were located in ears, base of the ear and in the submandibular region. Our findings on location of skin tumours differed with the findings of Mukatirwa *et al*. (2005) [23a] who recorded 22 percent of the neoplasms on the head and neck, 46.1 percent on the trunk and 24.3 percent in the limbs in a study on 540 cases of cutaneous neoplasms. However Simkus *et al*. (2015) [32] have reported high incidence of cutaneous neoplasms in limb as recorded in the present study.

Mitotic count

The mean mitotic count recorded under 10 hpf in different skin tumours in dogs is presented in Fig. 8. Among the different skin tumours studied, the mean mitotic count was highest in squamous cell carcinoma (11.34), transmissible venereal tumour (14.12) and was lowest in lipoma (3.00). The assessment of number of mitotic figures along with the clinical appearance and extent of tumours was of definite clinical significance in assessing the prognosis of canine cutaneous neoplasms. Bostock (1979) [33] reported that tumours with mitotic index less than 3 per 10 hpf had better survival times than those tumours with a mitotic index more than 3 per 10 hpf in their study on post-surgical survival in canine melanoma cases. Similarly, microscopic examination of most benign tumours in the present study revealed only few mitotic figures and less of cell atypia indicating that it was clinically less aggressive compared to the malignant tumours which revealed more number of mitotic figures per 10 hpf.

Histopathological grading of tumours

The tumours were graded histopathologically on the observation of malignancy features of anaplasia, nuclear atypia, presence of mitotic figures and multinucleated cells and invasiveness of tumours. Of the 160 cases of skin tumours diagnosed, 73 (45.63%) tumours were benign and 87 (54.37%) were malignant. This differed with the earlier reports on canine skin tumours which reported higher incidence of benign than malignant skin tumours. Earlier, Graf *et al*. (2018) [34] have diagnosed 57.52 percent tumours as benign and 42.48 percent tumours as malignant in a study on cutaneous tumours in dogs. Machado *et al.* (2018) [35] also reported that skin tumours had more benign biological behaviour (230/468, 49.14%) from their study on 468 cases of canine skin tumours.

Among the epithelial tumours (n = 80), 26 (32.50%) were benign and 54 (67.50%) were malignant. The two cases of squamous cell carcinoma encountered in the present study were graded as poorly differentiated tumours. The tumours showed highly proliferating cells with high anaplasia. Keratin pearls and cell nests characteristic of squamous cell carcinoma could not be seen. More number of mitotic figures were seen. These findings were in accordance with Chandrashekaraiah *et al*. (2011) [36] who have reported poorly differentiated squamous cell carcinoma with high anaplasia and absence of cell nests.

Among the round cell tumours (n = 44), 27 (61.36%) were benign and 17 (38.64%) were malignant. Mast cell tumours were graded histopathologically by the popular grading system of Kiupel *et al*. (2011) [16a]. Of the 22 mast cell tumours recorded in the present study, 15 (68.18%) tumours were identified as low grade and seven (31.82%) tumours as high grade mast cell tumours by Kiupel System (KS). The high grade mast cell tumours had more than seven mitotic figures per hpf, multinucleated cells and atypical nuclei. More than ten mitotic figures per hpf were seen in three out of the seven high grade mast cell tumours. The results of the present study thus showed that most of the mast cell tumours were of low grade.

Among the mesenchymal tumours (n = 35), 19 (54.29%) were benign and 16 (45.71%) were malignant. A lone case of melanoma identified was benign with few mitotic figures.

Haematology

No significant changes in haematological parameters were observed between the apparently healthy and skin tumour bearing dogs in the present study. This was in agreement with the findings of Kujur (2005) [22a] and Nijaguna (2006) [37] in their study on cutaneous neoplasms in dogs in Chennai city.

Serum biochemistry

No significant changes in serum biochemical parameters were observed between the apparently healthy and skin tumour bearing dogs in the present study. The findings differed with the earlier reports of Polton and Brearley (2007) [38] who observed hypercalcaemia and hypophosphataemia in malignant perianal gland carcinomas. Birhan and Chanie (2015) [39] reported hypoproteinaemia, hypoalbuminaemia, hypoglobulinaemia and elevation of BUN and Creatinine in TVT cases. No significant changes of such parameters were recorded in the present study for any type of cutaneous neoplasm.

Conclusion

The present study on skin tumours in dogs identified 160 skin tumours which included 80 epithelial tumours, 35 mesenchymal tumours, 44 round cell tumours and a single case of melanoma. The highest incidence of tumours recorded was mast cell tumour and sweat gland adenocarcinoma. The incidence was more in male dogs and in dogs belonging to 6-

10 years age group. Location wise, the tumours had more predilections to occur in the limb. As skin tumours have overt manifestation on skin, prompt cytopathological screening of the masses may help a large way in diagnosing the tumours in their early stages and guide in effective therapeutic and surgical intervention before the masses become aggressive.

Acknowledgements
The Authors are thankful to the Director of Clinics, Madras Veterinary College, TANUVAS for providing the necessary facilities for the conduct of this study.

Conflicts of interest
There is no conflict of interest.

Table 1: Incidence of different types of canine skin tumours (n = 160)

S. No.	Neoplasms	Total no. of cases	Percentage
A.	**Epithelial tumours (n = 80)**		
1.	Papilloma	7	4.38
2.	Squamous cell carcinoma	2	1.25
3.	Basal cell carcinoma	10	6.25
4.	Ceruminous gland adenoma	2	1.25
5.	Ceruminous gland adenocarcinoma	7	4.38
6.	Perianal gland adenoma	10	6.25
7.	Perianal gland adenocarcinoma	8	5.00
8.	Sweat gland adenoma	3	1.88
9.	Sweat gland adenocarcinoma	22	13.75
10.	Sebaceous gland adenoma	4	2.50
11.	Sebaceous gland adenocarcinoma	5	3.13
B.	**Mesenchymal tumours (n = 35)**		
1	Fibroma	1	0.63
2	Fibrosarcoma	7	4.38
3	Lipoma	18	11.25
4	Liposarcoma	5	3.13
5	Haemangiosarcoma	2	1.25
6	Haemangiopericytoma	2	1.25
C.	**Round cell tumours (n = 44)**		
1	Mast cell tumour	22	13.75
2	Histiocytoma	14	8.75
3	Transmissible venereal tumour	3	1.88
4	Plasma cell tumour	3	1.88
5	Lymphoma	2	1.25
D.	**Melanocytic tumours (n = 1)**		
1.	Melanoma	1	0.63
	Total	160	100.00

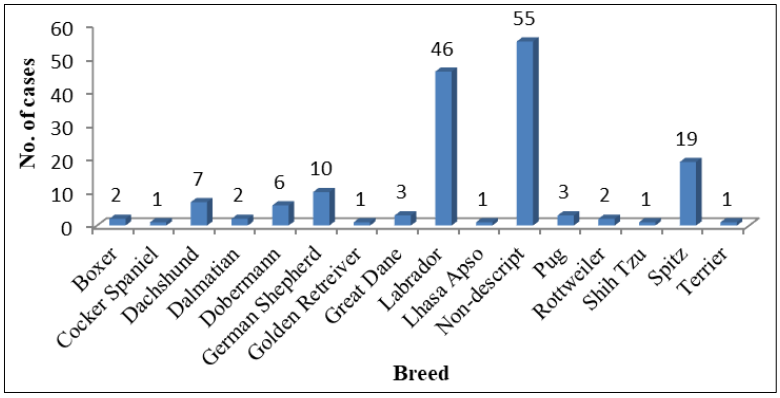

Fig 1: Breed wise incidence of canine skin tumours

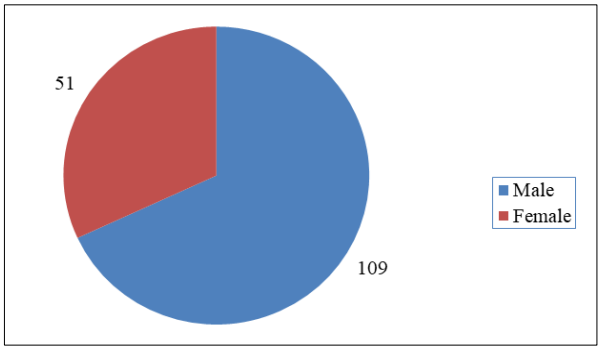

Fig 2: Sex wise incidence of canine skin tumours (n=160)

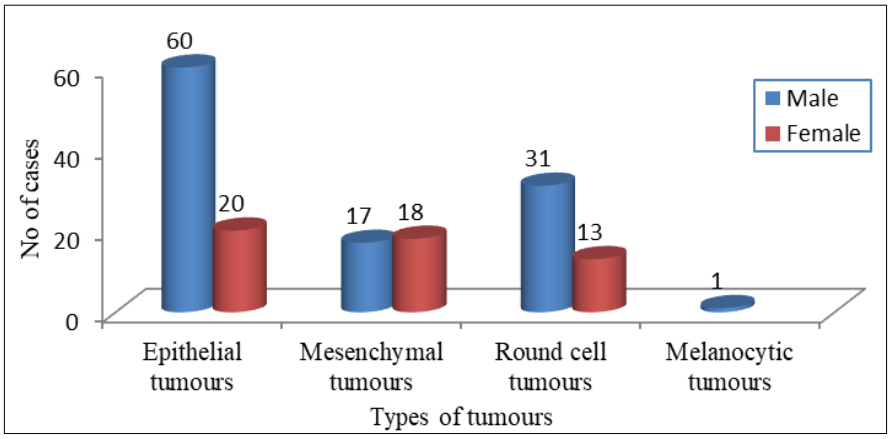

Fig 3: Sex wise incidence of types of canine skin tumours (n=160)

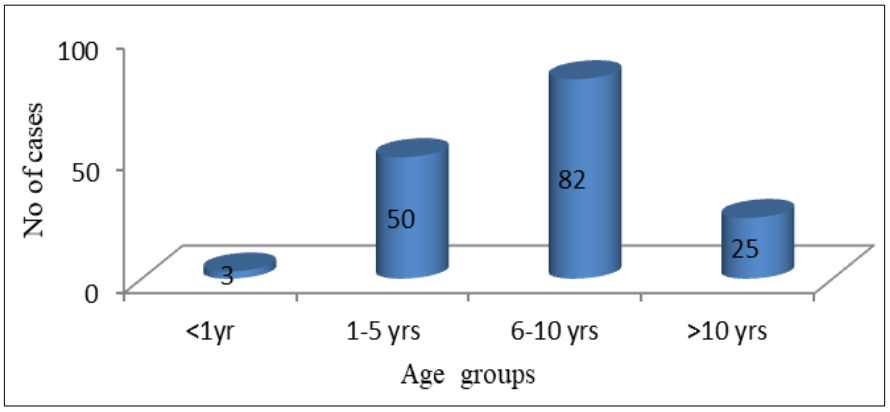

Fig 4: Age wise incidence of canine skin tumours (n=160)

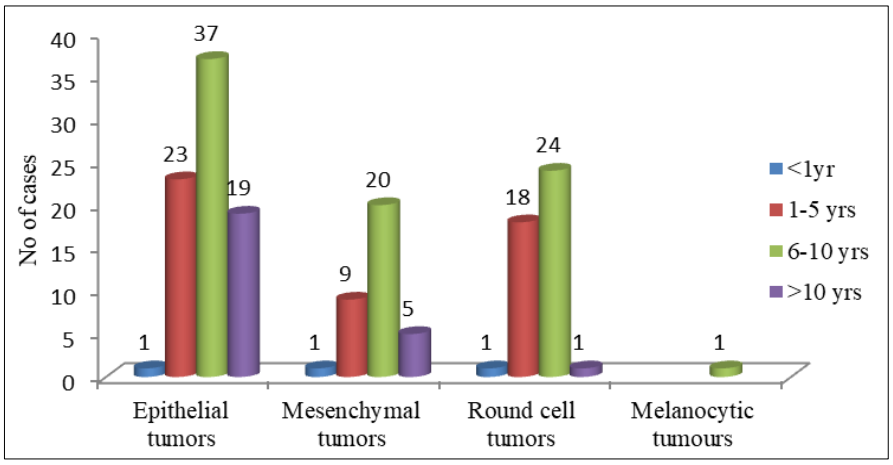

Fig 5: Age wise incidence of types of canine skin tumours (n=160)

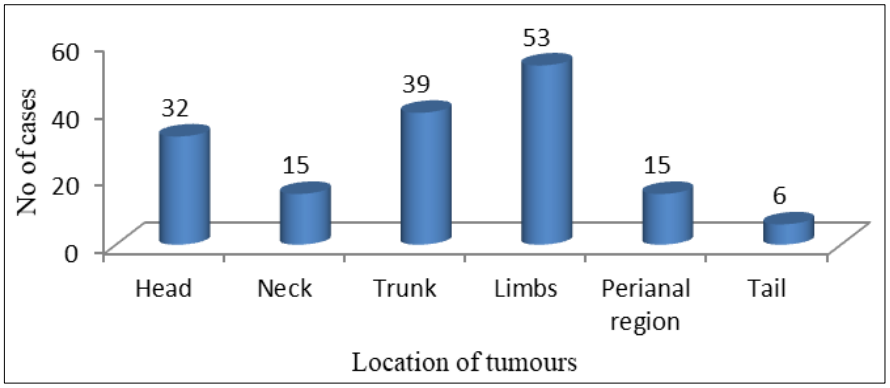

Fig 6: Location wise incidence of canine skin tumours (n=160)

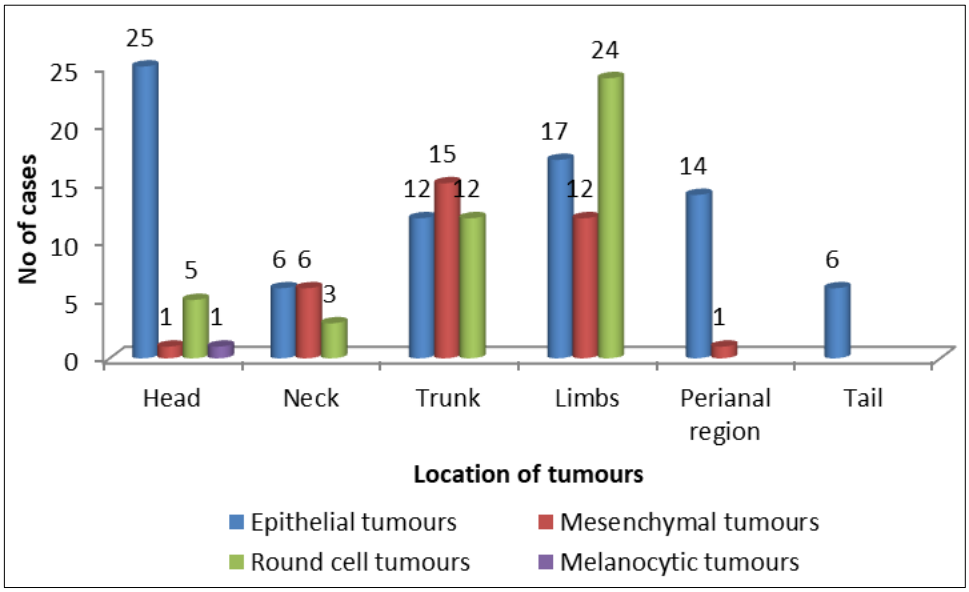

Fig 7: Location wise incidence of types of canine skin tumours (n=160)

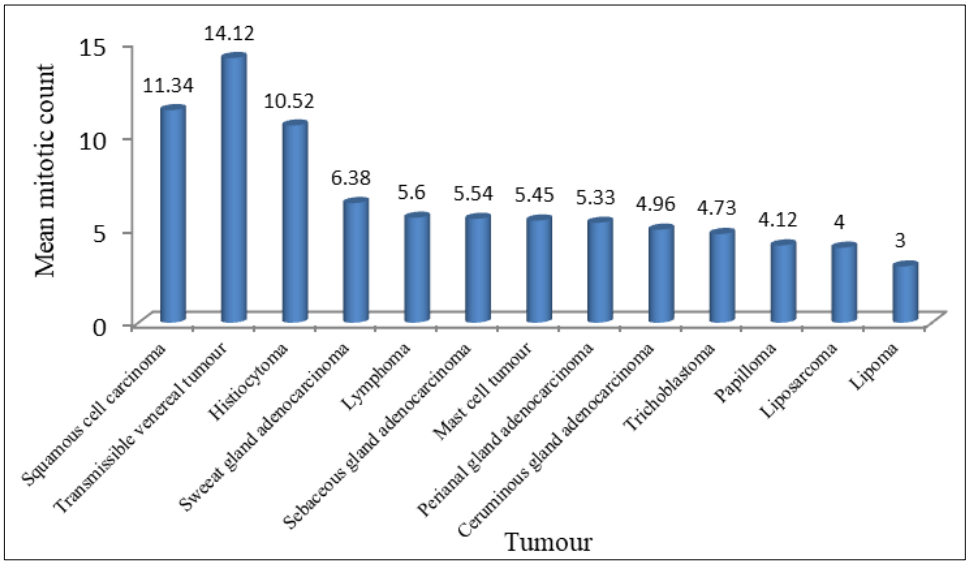

Fig 8: Mean mitotic count in canine skin tumours

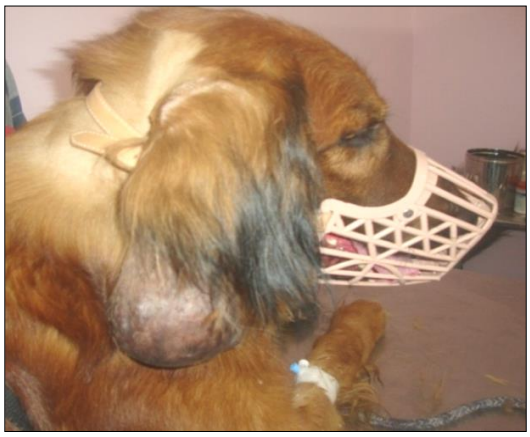

Plate 1: Trichoblastoma-cocker spaniel-ear

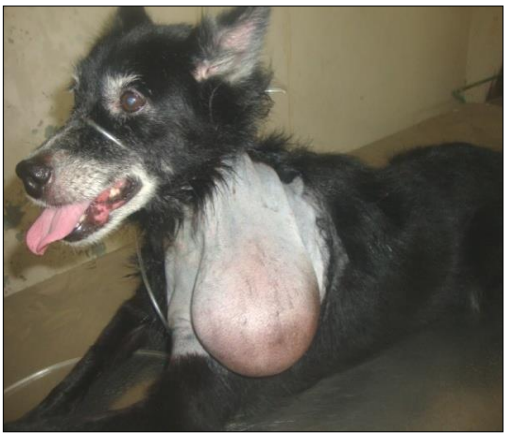

Plate 2: Lipoma-spitz-neck

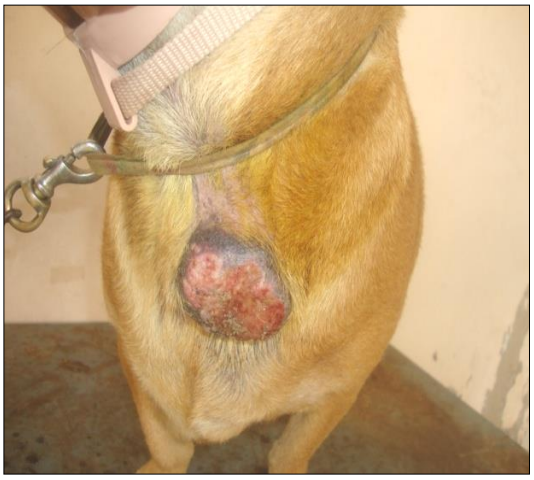

Plate 3: Squamous cell carcinoma-non-descript-thorax

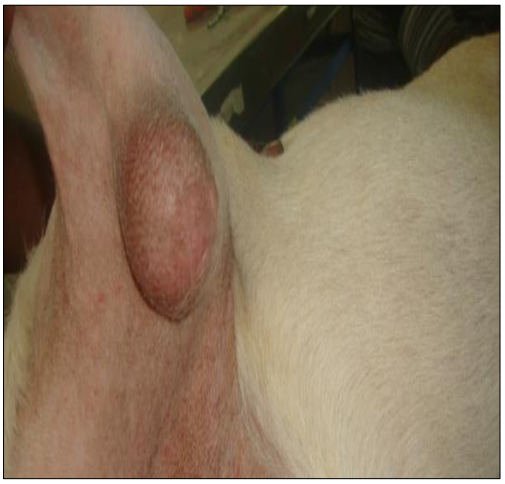

Plate 4: Mast cell tumour-Labrador-thigh

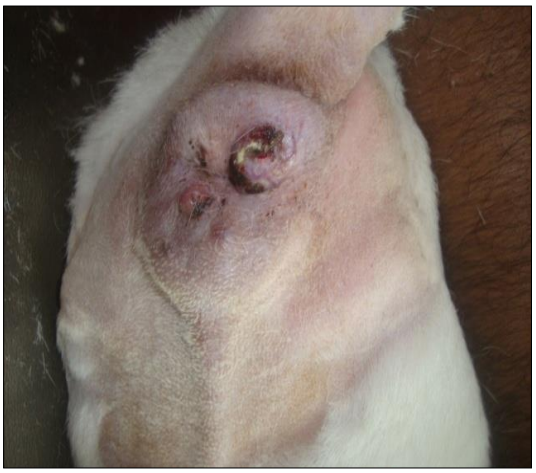

Plate 5: Perianal adenoma-non-descript-perianal region

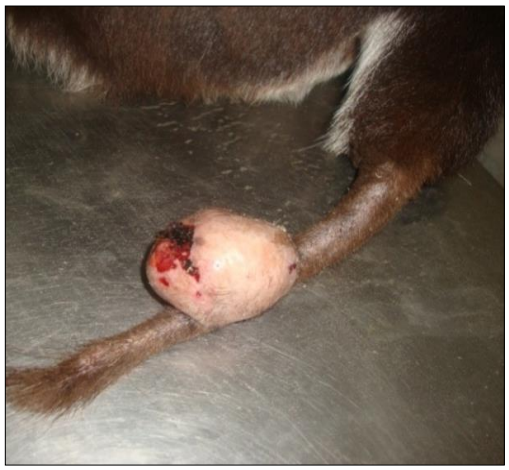

Plate 6: Sweat gland adenocarcinoma-Labrador-tail

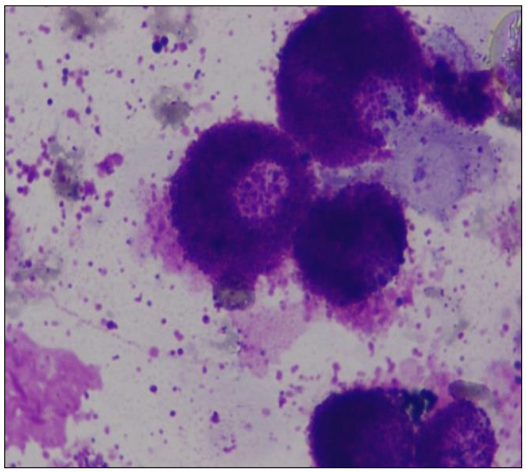

Plate 7: Mast cell tumour-anisocytosis-metachromatic granules- LG x 1000

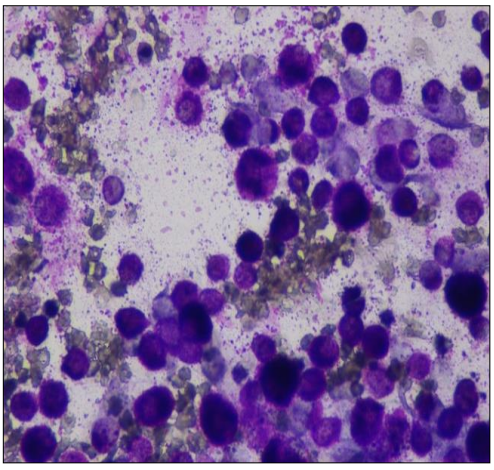

Plate 8: Mast cell tumour-anisocytosis -metachromatic granules LG x 400

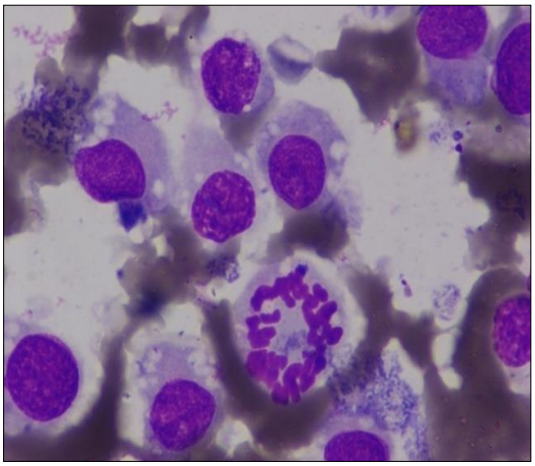

Plate 9: TVT-mitotic figure-LG x 1000

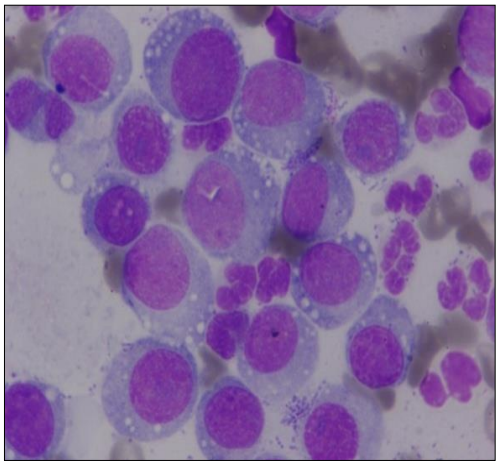

Plate 10: TVT-cytoplasm with coarse chromatin LG x 1000

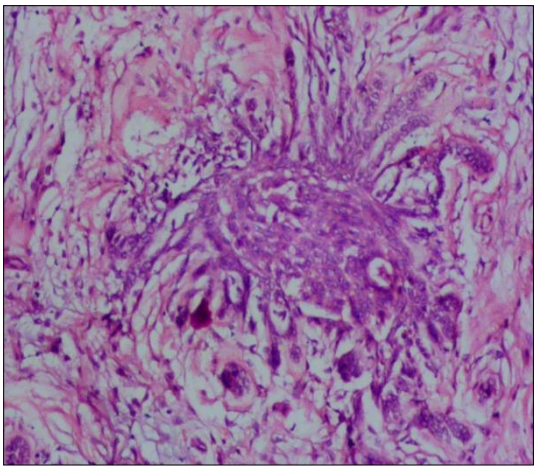

Plate 11: Trichoblastoma-medusa head pattern H&E x 100

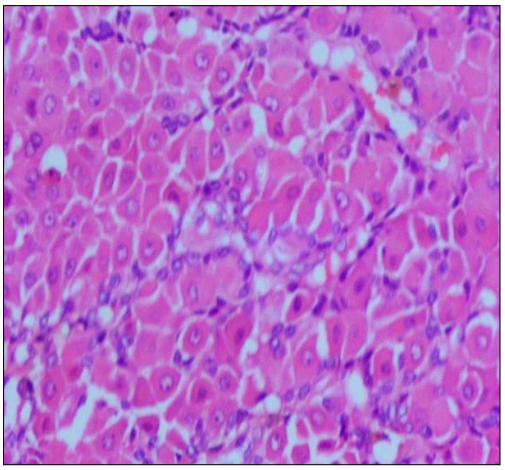

Plate 12: Perianal adenoma-lobules containing hepatoid and reserve cells H&E x 200

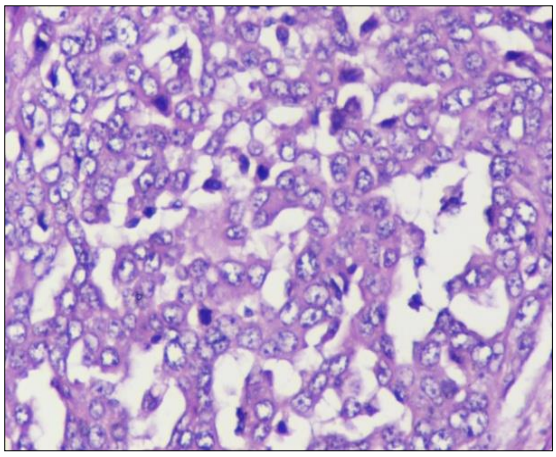

Plate 13: Sweat gland adenocarcinoma-Mitotic figures H&E x 400

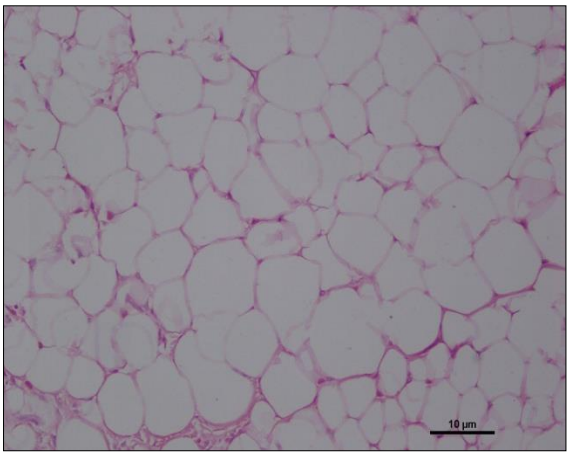

Plate 14: Lipoma-variable sized neoplastic adipocytes with eccentric nuclei H&E x 100

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