INCIDENCE AND HISTOMORPHOLOGICAL CHARACTERIZATION OF CANINE NEOPLASMS

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COLLEGE OF VETERINARY AND ANIMAL SCIENCES

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DECLARATION

I hereby declare that this thesis entitled **"INCIDENCE AND HISTOMORPHOLOGICAL CHARACTERIZATION OF CANINE NEOPLASMS"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Certified that the thesis entitled **"INCIDENCE AND HISTOMORPHOLOGICAL CHARACTERIZATION OF CANINE NEOPLASMS"** is a record of research work done independently by **Dr. Praveena Babu** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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EXTERNAL EXAMINER

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Praveena Babu

DEDICATED TO
MY BELOVED PARENTS
AND
SON

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Introduction

1. INTRODUCTION

Of the late, the incidence of neoplasms appears to be on the increase among pet animals. Neoplasm is an abnormal growth of tissue characterized by persistent, excessive and disorganized cell growth that is unresponsive to normal growth control mechanisms. A neoplasm can be benign or malignant and can originate from virtually any cell in the body.

Canines are more susceptible to cancer than any other domestic animals. The frequency of tumours in dogs is twice and in cats only half when compared to human beings. A high incidence of tumor in canine could be attributed to their population and species involvement. A lower incidence among bovine may be due to slaughter at a younger age and early disposal.

Pet animals with spontaneously developing cancer provide an excellent opportunity to study various aspects of the disease from etiology to treatment (Withrow, 1989). Pets and their owners' share the same environmental factors and so they serve as sentinels for changes in the pattern of development of tumours in humans. Pets have a higher incidence of some cancers like osteosarcoma, non Hodgkin's lymphoma and mammary tumours as compared with humans. Moreover these tumours share many common features with their human counterpart including histological appearance, tumor genetics, biological behavior and response to conventional therapies. Because of their compressed lifespan, tumours in animals progress at a much rapid rate than in humans. They can therefore be studied much faster and the results can be extrapolated to humans. Intense efforts are on among the cancer researchers in western countries to include veterinary oncology and to study tumours in dogs as models for understanding tumor biology as well as for developing drugs and conducting trials for treating human cancer.

The increased concern of animal owners demands accuracy in histopathological and other methods of diagnosis. Enhanced enthusiasm of

veterinary clinicians in diagnosing and treating animal tumours has compelled the veterinary pathologists to conduct in depth studies of these tumours to make an early diagnosis.

Studies on cell proliferation and cell death have emerged as important criteria in the understanding of the pathobiology of cancers. So this study aims to assess the mitotic index and apoptotic index by light microscopy and its reliability in the diagnosis and prognosis of tumours.

Nucleolar organizer regions (NORs) have been recognized as loops of DNA which transcribe to ribosomal RNA. The interphase NORs can be selectively visualized in paraffin- embedded section by the silver staining method (Ploton *et al.*, 1986). AgNOR index appears as a reliable diagnostic marker for tumours and provides additional information about the proliferation rate of tumours (Krishnamurti and Paliwal, 1998).

Neoplasms in pet animals especially in canines appear to be increasing, but in Kerala studies pertaining the incidence are scanty necessitating its detailed documentation.

Hence the present work has been designed with the following objectives.

To study:

1. The incidence of neoplasms in canines in the locality of Thrissur.
2. Classification of canine neoplasms based on histopathology.
3. The use of mitotic index and apoptotic index as proliferation markers in tumours
4. Argyrophilic Nucleolar Organizer Region (AgNOR) count test as an aid in the prognosis of tumours when determining therapeutic options.

Review of Literature

2. REVIEW OF THE LITERATURE

2.1 INCIDENCE

Degloorkar *et al.* (1992) reported that veneral granuloma was the most common tumor in canines.

Rahman and Chakraborthy (1993) reported pulmonary adenocarcinoma, carcinoma of the nasal septum, uterine fibroma, mixed mammary tumour, malignant melanoma and canine veneral granuloma among canines.

Shakir and Sundararaj (1994) observed the occurrence and distribution of skin neoplasms in dogs and described their gross and histological features.

In a study conducted by Viswanath *et al.* (1998) it was evident that squamous papillomas and fibromatous or ossifying epulis were the common benign tumours whereas melanomas and squamous cell carcinomas were the frequent malignant tumours of the oral cavity of dogs.

Sivakumar *et al.* (2004) conducted a study on the occurrence of neoplasms in domestic animals and reported maximum cases in canines.

Out of 48 cases of neoplasms reported among the canines in Bareilly, U.P., 33 (68.75 percent) were diagnosed as benign tumours and 15 as malignant (31.25 percent). They reported a high incidence of mammary tumor followed by skin tumor among canines in that area (Nair *et al*., 2007).

Grieco *et al*. (2008) reported a rise in the incidence and prevalence of canine testicular tumours.

Bhaskara Rao and Malleswara Rao (2009) conducted an epidemiological study of canine neoplasms in Andhra Pradesh and observed that cervix was the frequently affected site for many types of tumours followed by mammary gland.

Dayananda *et al.* (2009a) studied on the incidence of skin and subcutaneous tissue neoplasms in dogs and reported 122 cases which were classified into round cell tumours, epithelial tumours and mesenchymal tumours based on cytology and histopathology.

Kujur *et al.* (2009) conducted a study on the incidence of skin tumours in dog and found that out of 85 cases of skin tumours, epithelial tumours constituted 37 (43.53 percent), mesenchymal tumours 30 (35.35 percent), and round cell tumours 18 (21.17 percent). Out of these, 45.53 percent were benign tumours and 56.47 percent were malignant tumours.

2.2 AGE

The average age of dogs with hepatocellular carcinoma is 10 to 11 years, although they have been reported in dogs as young as four years of age and sixty five percent of the dogs with cholangiocellular carcinoma are greater than 10 years of age (Patnaik *et al*., 1980; Cullen and Popp, 2002).

The highest frequency of occurrence of osteosarcoma was among the dogs aged seven to eight years but approximately ten percent occurred in dogs less than three years of age (Smith and Sulton, 1988; LaRue and Withrow, 1989; Thompson and Pool, 2002).

The mean age of occurrence of hemangiosarcoma in canines is between eight and ten years or younger (MacEven, 1989).

The average age of occurrence of seminomas in dogs is ten years (Postorino, 1989).

The vast majority of vaginal and vulvar tumours are benign and are found in intact female dogs from two to 18 years of age, with an average of 10.8 years (Klein, 1989).

MacEwen and Young (1989) and Jacobs *et al.* (2002) stated that malignant lymphomas were diagnosed at an average age of six - seven years, with a range of six months to more than 15 years.

Most animals with hepatoid gland adenomas are eight years of age or older, while dogs between four and 15 years of age are affected with hepatoid gland carcinoma (Withrow, 1989; Gross *et al*., 1992; Goldschmidt *et al.*, 1998).

The average age of the animals with sebaceous adenoma is 10 years and the peak incidences of melanocytomas are found between the ages of five and 11 years of age (Gross *et al*., 1992; Goldschmidt and Hendrick, 2002).

Lymphoid leukemias among canines tend to be more common in younger individuals (Valli and Parry, 1993; Jacobs *et al*., 2002).

Dogs of all ages can be affected with fibromatous epulis; however, it is rare in dogs less than three years of age. Also dogs as young as three years of age can get affected with pancreatic carcinoma (Head *et al.,* 2002).

The ceruminous gland tumours are relatively common in dogs and cats and occur between four and 13 years of age. Also middle aged or older animals are usually affected with malignant fibrous histiocytoma (Goldschmidt and Hendrick, 2002).

Singh *et al.* (2004) recorded highest incidence of tumours in the age group of 8 to12 years followed by 4 to 8 years and least in 0 to 4 years age group.

Sivakumar *et al.* (2004) reported that the occurrence of canine transmissible venereal tumor was relatively high in younger age group i.e.1 to5 years and mammary tumours were common in bitches aged 6 to15 years of age.

The age wise analysis conducted by Bhaskara Rao and Malleswara Rao (2009) revealed that the majority of tumor bearing animals was in the age group

of 5 to 8 years (46.15 percent) followed by 9 to12 years (30.77 percent), 13 to16 years (17.95 percent) and least in 1to 4 years (5.13 percent).

Dayananda *et al*. (2009a) reported that in canines, skin and subcutaneous neoplasms occurred in all age groups ranging from 5 to 18 years with an average of 7.72 years. The highest incidence was observed in six to eight year age group of dogs.

Sivaseelan *et al.* (2009) noticed that mammary tumours were found only in bitches over five years old.

Srivastava *et al.* (2009) reported that the incidence of mammary tumours among canines was highest between 8-10 years of age followed by 6-8 years and 4-6 years in descending order. Dogs below 2 years and above 12 years were rarely seen with mammary tumor.

Reddy *et al*. (2009b) reported that mammary tumours occurred most frequently in 8-10 years, followed by 6-8 years.

2.3 SEX

Patnaik *et al.* (1980), Postorino (1989) and Cullen and Popp, (2002) reported that most dogs with hepatocellular carcinomas were male, and with bile duct carcinoma were females.

Males are more frequently affected with osteosarcoma than females, except in St.Bernards, where the female is most often affected (LaRue and Withrow, 1989; Thompson and Pool, 2002).

The larger majority of the perianal gland carcinomas occur in older intact males (Gross *et al*., 1992).

There is a slightly higher incidence of sebaceous adenomas in females than in males. (Gross *et al.*, 1992; Goldschmidt and Hendrick, 2002).

Hepatoid gland lesions occur far more frequently in intact males than in females or neutered males (Goldschmidt *et al.*, 1998).

Intact male dogs younger than five years are vulnerable for leukemia (Valli and Parry, 1993; Jacobs *et al.,* 2002).

Neoplastic diseases of skin were observed in twenty dogs by Shakir and Sundararaj (1994*)* and recorded that out of twenty, eighteen occurred in males dogs and two in females. They also reported that perianal gland adenoma was encountered in male dogs only.

Dogs of both sexes and all ages are affected with Transmissible Venereal Tumour (TVT), but are more commonly seen in female dogs that have reached sexual maturity (Degloorkar *et al*., 1992; Shakir and Sundararaj, 1994; Goldschmidt and Hendrick, 2002; Maclachlan and Kennedy, 2002; Thangathurai *et al.*, 2008).

In a study conducted on 46 dogs Singh *et al.* (2004) reported that the occurrence of neoplasms was more in case of male animals (76.47 percent) than in female animals (23.53 percent).In this study skin neoplasms were found more in male animals (80 percent) than in females (20 percent). All cases of perianal adenoma were recorded in male animals of different breeds.

No sex predisposition was noticed for the occurrence of transmissible venereal tumor among canines (Sivakumar *et al.*, 2004).

The sex wise analysis conducted by Bhaskara Rao and Malleswara Rao (2009) revealed that the incidence of tumours was highest in males (56.09 percent) when compared to females (43.91 percent).

Dayananda *et al*. (2009a) reported that male dogs were highly susceptible to skin and subcutaneous tissue tumours than females.

Kujur *et al.* (2009) conducted a study on the incidence of skin tumours in dog and found that 55.30 percent of the affected animals were males and 44.70 percent were females.

Reddy *et al*. (2009a) reported that the occurrence of skin tumours was high in females (55.17 percent) than males (44.83 percent).

2.4 BREED

Dogs of giant breeds generally develop osteosarcomas at a younger age than smaller breeds. Giant breeds like Boxers, Great Danes, Saint Bernards, German Shepherds and Irish Setters are predisposed (Smith and Sulton 1988; LaRue and Withrow, 1989; Thompson and Pool, 2002).

MacEven (1989) reported a higher incidence of hemangiosarcoma among German shepherd dogs.

Certain breeds like Bulldog, Boxer, Bull Mastiff, St.Bernard etc. have a breed predisposition to multicentric lymphoma (MacEwen and Young, 1989; Jacobs *et al.,* 2002).

There is no reported breed predisposition for seminomas (Postorino, 1989).

Cocker Spaniels and Poodles are reported to have higher propensity for sebaceous epithelioma (Gross *et al.*, 1992).

Higher incidence of all melanocytic tumours was reported in breeds with dark pigmentation (Gross *et al*., 1992; Goldschmidt and Hendrick, 2002.).

Hemangiosarcoma can be aggressive, especially so in the German shepherd and Golden retriever breeds, where there is a high incidence of multicentric form, most typically involving the spleen, liver, right auricle, and lungs (Goldschmidt *et al.,*1998).

Sivakumar *et al.* (2004) reported that German Spitz dogs were affected more with mammary tumours.

Krithiga *et al.* (2005b) reported the occurrence of fibromatous epulis in a 10 year old GSD male and in a three year old GSD female.

Grieco *et al*. (2008) reported that seminomas occurred most frequently in German shepherd dogs.

An epidemiological study of canine neoplasms by Bhaskara Rao and Malleswara Rao (2009) found that higher incidence of tumours was in German shepherd dogs (35.90 percent) followed by Pomeranian (30.77 percent), Labrador (23.08 percent), Lhasa apso (5.13 percent), Doberman (2.56 percent) and Dachshund (2.56 percent).

Dayananda *et al*. (2009a) reported that maximum incidence of skin and subcutaneous neoplasms occurred in nondescript dogs (34.42 percent) followed by German shepherd (16.41 percent), Labrador retriever (10.65 percent), Pomeranian (9 percent) and Boxer (5.73 percent).

Out of 85 skin tumours in dogs recorded by Kujur *et al.* (2009), 31.76 percent were found in non-descript animals followed by German Shepherd (14.1 percent), Labrador (14.1 percent) Spitz and Doberman (10.5 percent each) and other breed showed less than 10 percent occurrence.

Sivaseelan *et al.* (2009) noticed that the incidence of mammary tumor was higher in German shepherd dogs.

Srivastava *et al.* (2009) and Reddy *et al.* (2009b) reported that studies on the breed wise distribution of canine mammary tumours revealed highest incidence in GSD, followed by Spitz, and Cross bred/Non-descript breed.

Reddy *et al*. (2009a) studied skin neoplasms among canines at Izatnagar and reported that breed wise distribution of tumours revealed maximum cases in GSD, followed by non descripts.

2.5 HAEMATOLOGY

Patnaik *et al.* (1980) reported that leucocytosis is common in dogs with hepatic neoplasms.

The common hematopoietic changes in neoplasms include leucocytosis, and anemia (Ogilvie, 1989; Cullen *et al*., 2002; Vasudevan *et al*., 2004).

Acute Lymphoblastic Leukemia (ALL) can be distinguished from acute myeloblastic leukemia by the lack of cytoplasmic granulation and in ALL the differential count shows 80 -100 percent of lymphocytes (Valli and Parry, 1993; Jacobs *et al*., 2002).

Aspiration biopsy of the lymph node of a dog with lymphosarcoma revealed the presence of significant number of monomorphic population of \]medium sized lymphocytes with high nucleus: cytoplasm ratio and occasional mitotic figures on Leishman's staining. Leucocytosis and anaemia were the haematological alterations (Lakkawar *et al*., 2002; Pillai *et al.*, 2009)

Pre-operative haematological analysis showed anaemia and leucocytosis with neutrophilia in a German shepherd dog with Sertoli cell tumor (Kujur *et al.*, 2005).

2.6 TUMOURS

2.6.1 Epithelial and Melanocytic Tumours of the Skin

2.6.1.1 Sebaceous Adenoma

Shakir and Sundararaj (1994) reported sebaceous adenoma in the left inguinal region of a non descript dog as a firm grayish lobulated mass.

Histologically the proliferating sebaceous cells were grouped into masses and were surrounded by one or two rows of dark staining palisaded reserve cells.

Sebaceous adenoma is a benign tumor characterized by a preponderance of sebocytes (cells with intracytoplasmic lipid vacuoles) with few basaloid cells and ducts (Goldschmidt *et al*., 1998).

2.6.1.2 Sebaceous Epithelioma

Sebaceous epithelioma is a tumor of low grade malignancy characterized by a preponderance of basaloid cells with few sebocytes and ducts. The tumours may be multiple and surface ulceration is frequent. They occur most often on the head, dorsal neck, and back (Goldschmidt *et al*., 1998).

2.6.1.3 Hepatoid Gland Adenoma (Perianal Gland Adenoma, Circumanal Gland Adenoma)

Gross *et al.* (1992) described perianal adenoma as a well circumscribed nodular mass composed of broad, anastomosing trabeculae of well differentiated hepatoid cells and a peripheral layer of basaloid reserve cells.

Shakir and Sundararaj (1994) reported that perianal gland adenoma was found to be the common tumor in dogs in Madras city amounting to 35 percent of tumours encountered.

Hepatoid gland tumours occur normally in the perianal area, tail, hindlimbs, back and parapreputial area, but occasionally may be found at other sites (Goldschmidt *et al*., 1998).

2.6.1.4 Hepatoid Gland Carcinoma (Perianal Gland Carcinoma, Circumanal Gland Carcinoma)

In hepatoid gland carcinoma, cells showing hepatoid differentiation has a vacuolated cytoplasm that stains less eosinophilic, pleomorphic nuclei and prominent nucleoli (Goldschmidt *et al.*, 1998).

2.6.1.5 Tumours of Sweat Glands

Sweat gland tumours are extremely rare in dogs and cats. Among these tumors eccrine carcinoma involves footpad, dermis and phalangeal bone as swellings and often the overlying epidermis is ulcerated. Irregular tubuloacinar structures lined by one or more layers of cuboidal to polygonal epithelial cells are distributed through an abundant stroma of dense collagen (Gross *et al*., 1992; Goldschmidt and Hendrick, 2002).

Shakir and Sundararaj (1994) reported sweat gland adenoma on the left side of the neck of a Non descript dog as a firm nodular , tan , solitary mass. Histologically, in addition to the cuboidal to columnar lined acini cystic distension with intracystic papillary ingrowths along with connective tissue stroma were present

2.6.1.6 Melanocytoma (Dermal Melanoma, Benign Melanoma)

Cell morphology in melanomas varies from a small spindle cell with melanin granules to large spindle cells, epitheloid cells, polygonal cells, or round cells, which often have a large amount of melanin within the cytoplasm that obscures the nucleus (Gross *et al*., 1992; Goldschmidt and Hendrick, 2002; Krithiga *et al*., 2005b).

Goldschmidt *et al*. (1998) stated that cutaneous and dermal melanocytomas are relatively common in dogs. Dermal melanocytomas occur most often on the trunk and occasionally on the extremities, particularly between the digits.

2.6.2 Mesenchymal Tumours of the Skin and Soft Tissues

2.6.2.1 Fibroma

Fibromas appear as solitary growths ranging from 1 to 5 cm but are occasionally much larger. Ulceration may be present in larger lesions. Limbs and

flanks are frequently reported sites. Histologically the collagen fibres are repetitive and arranged in interwoven fascicles, more rarely in whorls (Gross *et al*., 1992; Goldschmidt and Hendrick, 2002; Krithiga *et al.* 2005b).

2.6.2.2 Benign Peripheral Nerve Sheath Tumor or Neurofibroma

Gross *et al*. (1992) stated that neurofibromas are under diagnosed because of the histopathologic similarity to hemangiopericytoma, fibroma and fibrosarcoma. The confirmation is possible by immunostaining.

2.6.2.3 Liposarcoma

Liposarcomas are malignant neoplasms of adipose tissue that are rare in dogs and cats and the incidence in dogs is less than one percent (Gross *et al*., 1992).

Histologically liposarcoma can be divided into well differentiated, pleomorphic and myxoid subtypes based on cellular morphology (Goldschmidt *et al.*, 1998; Goldschmidt and Hendrick, 2002).

2.6.2.4 Malignant Fibrous Histiocytoma (MFH)

MFH arises in the skin or spleen of the dogs as a single expansile tumor. Microscopically three variants of MFH are reported; Stortiform-pleomorphic, inflammatory and giant cell type. The fibroblasts like cells are arranged in cartwheel patterns along with histiocytes, lymphocytes, plasma cells, neutrophils and eosinophils (Goldschmidt *et al*., 1998; Goldschmidt and Hendrick, 2002).

2.6.2.5 Hemangiosarcoma

MacEven (1989) reported that the site of primary involvement of hemangiosarcoma is usually spleen. Histologically the cells lining the vascular cleft have prominent, bulging nuclei that are pleomorphic and hyperchromatic. Mitotic figures are frequent (Dayananda *et al*., 2009b)

2.6.3 Tumours of Haemolymphatic System.

2.6.3.1 The Lymphoid Leukemias

Acute lymphoblastic leukemia (ALL) accounts for 5 to 10 percent of canine lymphoid neoplasias. In dogs and cats mild hepatomegaly and the moderate and symmetrical enlargement of spleen was present. Microscopically, lymph node and spleen showed follicular atrophy and the pattern of liver involvement was diffuse and sinusoidal with some portal colonization and periacinal ischaemic degeneration. Colonies of tumor cells may be very widespread but are most commonly found in the kidneys, testes, meninges, intestines and pancreas (Valli and Parry, 1993; Jacobs *et al.*, 2002).

2.6.3.2 Canine Multicentric Lymphoma or Malignant Lymphoma

Superficial lymphadenopathy, thoracic involvement, mediastinal and hilar lymphadenopathy and hepatosplenomegaly are some of the common manifestations of canine multicentric lymphoma. Histologically in the spleen subendothelial lymphocyte colonization of large veins within the thick fibromuscular trabeculae and in the liver congregation of neoplastic cells around the portal triads are characteristic (MacEwen and Young, 1989; Jacobs *et al.,* 2002).

2.6.4 Tumours of Bone

2.6.4.1 Osteosarcoma

Smith and Sulton (1988) worked on the osteosarcoma in Brisbane area and reported that proximal humerus and femur as the principal sites of occurrence. They also suggested that confirmation of osteosarcoma is usually dependent on either one or both of radiological examination and biopsy samplings.

Singh and Mouli (1990) reported osteosarcoma in the right foreleg of dogs.

LaRue and Withrow (1989) and Thompson and Pool (2002) reported that the tumor is more likely to occur in the distal radius and proximal humerus. Canine osteosarcoma can vary in the type and quantity of matrix produced and pattern of cell arrangement; it is subclassified as osteoblastic, chondroblastic, fibroblastic and telangiectatic.

Krithiga *et al*. (2005b) reported osteosarcoma in dogs in the lower third of the oesophagus. Histopathology revealed pleomorphic, spindle shaped cells like fibroblasts, which were plumpy and oval to round with basophilic cytoplasm and areas of osteoid formation was also present.

2.6.5 Tumours of Alimentary Tract.

2.6.5.1 Fibromatous Epulis of Periodontal Ligament Origin

Some cases of ossifying epulis showed presence of osteoid or mature lamellated bone (Viswanath *et al.*, 1998).

Odontogenic epithelium is frequently seen in fibromatous epulis of periodontal ligament origin and is considered a secondary feature (Head *et al.*, 2002).

Krithiga *et al.* (2005b) reported the occurrence of fibromatous epulis near the canine teeth of the upper and lower jaws which was 1 cm diameter, oval to spherical, flat, 1.5 -2 g in weight, rubbery to hard and whitish to pinkish cut surface.

Sivaseelan *et al.* (2008) reported the occurrence of mandibular ossifying epulis extending from premolar o molar region, measuring about 5 x 3 cm in size in a seven year old male Doberman dog. In histopathology deeper areas revealed osteoid and mature lamellated bone.

2.6.5.2 Hepatic Tumours

2.6.5.2.1 Hepatocellular Carcinoma

Patnaik *et al.* (1980) and Postorino (1989) described that grossly hepatocellular carcinoma may be massive, nodular or diffuse and the most common metastatic sites are the hepatic lymph nodes, lungs, and peritoneum.

In hepatocellular carcinoma metastatic lesions were reported in the spleen, hepatic and mesenteric lymphnodes and kidney (Phangcho *et al*., 1993).

Histologically the three major diagnostic categories are trabecular, adenoid, and solid. The neoplasm spreads by blood vascular system, lymphatics and direct extension to the omentum and peritoneum (Cullen and Popp, 2002).

2.6.5.2.2 Cholangiocellular Carcinoma

Bile duct carcinomas are highly metastatic tumours; the reported rate of metastases is 87.5 percent. The most common site of metastases is the hepatic lymphnodes, lungs, and peritoneum (Postorino, 1989).

Grossly most tumours are firm because of abundant connective tissue that is typical of these neoplasms. Grossly three forms were described; massive, nodular and diffuse. Histological feature of cholangiocellular carcinoma varies from well differentiated cholangiocarcinomas to less differentiated neoplasms (Cullen and Popp, 2002).

2.6.5.3 Pancreatic Carcinoma

Grossly pancreatic carcinomas produce a mass, often in the mid portion of the pancreas. Adhesion of the affected pancreas to adjacent tissues may occur and metastases to regional lymph nodes are also common. Histologically exocrine pancreatic carcinomas exhibit wide range of differentiation from well differentiated adenocarcinomas with acinar structures to poorly differentiated with solid patterns (Head *et al*., 2002; Cullen, 2007).

2.6.6 Tumours of Eye and Ear.

2.6.6.1 Tumours of the Nictitans Gland

Render and Carlton (2001) reported that neoplasms of the nictitating glands are either adenomas or adenocarcinomas.

Carcinoma of the gland of third eyelid is a rare growth at the base of the nictitans, displacing the globe on the ventro medial conjunctiva (Dubielzig, 2002).

Wilock (2007) reported that adenocarcinoma of the gland of the third eyelid is an uncommon tumor of the dogs and occurs in very old dogs.

2.6.6.2 Ceruminous Gland Carcinoma

Tumours of ceruminous glands resemble those of sweat glands but may be distinguished by the presence of yellowish pigment in the epithelium and secretions. Their cytoplasm usually contains water-insoluble pigment granules which are positive for Periodic Acid Schiff (PAS) staining. (Cankar and Crowley, 1964; Goldschmidt and Hendrick, 2002).

Rani *et al*. (2005) reported ceruminous gland adenocarcinoma in the ear canal of a seven year old non descript dog. Microscopically, though glandular structures were present, the cells lost their polarity and were anaplastic with numerous mitotic figures.

2.6.7 Tumours of the Genital System.

2.6.7.1. Sertoli cell tumor

Ladds (1993) and Foster (2007) described that histologically sertoli cell tumors are of intratubular and diffuse type and the abundant fibrous connective tissue in sertoli cell tumor distinguishes them from other testicular tumors. Also only one-third of the cases produce feminizing effect in dogs.

Sertoli cell tumors are frequently unilateral, cause testicular enlargement, characteristically white and firm and sometimes undergo thrombosis and hemorrhage. (Buergelt, 1997).

Foster (2007) stated that Sertoli cell tumor is the third most common testicular neoplasm of the dog and more than 50 percentage of the cases are located in undesecended testis.

2.6.7.2 Vaginal and Vulvar Tumours

Eighty six percent of vulvar and vaginal tumours are reported to be benign smooth muscles tumours like leiomyoma, fibro leiomyoma, and fibroma (Klein, 1989).

2.6.7.3 Transmissible Venereal Tumor (TVT)

Histologically the TVTs are composed of loose sheets, rows and cords of relatively uniform round to ovoid cells. Nuclei are large, round with a single centrally placed nucleolus surrounded by marginated chromatin. (Shakir and Sundararaj, 1994; Goldschmidt and Hendrick, 2002; Maclachlan and Kennedy, 2002; Thangathurai *et al.*, 2008)

Sivakumar *et al.* (2004) reported that the preponderant tumor among canine was transmissible venereal tumor.

The prominent cytological feature of TVT is the presence of cytoplasmic vacuolation. The cellularity of the cytological smears was high with round individual cells arranged in a sheet like pattern (Krithiga *et al*., 2005a; Thangathurai *et al.*, 2008).

Thangathurai *et al.* (2008) reported that TVT is the most prevalent neoplasia of the external genitalia of the dog in tropical and sub-tropical areas.

2.6.8 Mammary Tumours of Dogs

Moulton (1970) reported that metastatic carcinomatous cells were found in the lymph nodes and lungs of four dogs with malignant mixed tumours.

Roughly 66 percent of canine tumours occur in glands 4 and 5, probably owing to greater volume of breast tissue in these glands (MacEven and Withrow, 1989; Sivakumar *et al*., 2004).

Palmer (1993) stated that extraskeletal osteosarcomas are best known in dogs, especially in the mammary glands.

Canine mammary carcinomas are classified in the order of increasing malignancies as follows: non infiltrating carcinoma, complex carcinoma (two cell types), simple carcinoma (one cell type), simple carcinoma (tubopapillary type), simple carcinoma (solid type) and simple anaplastic carcinoma. Benign mammary tumours are classified as simple adenoma, basaloid adenoma, complex adenoma, benign mixed tumor, fibroadenoma and duct papilloma. Complex adenomas and benign mixed tumours are the most common in dogs (Misdorp, 2002).

Srivastava *et al.* (2009) reported that out of 64 cases of mammary tumours studied, the important the benign type tumours of mammary gland included mixed mammary tumor (22.23 percent), papillary adenoma (17.17 percent), fibroadenoma (12.12 percent), myoepithelioma (10.10 percent) and mucinous adenoma (3.03 percent). The malignant tumours of mammary gland were found in 35 cases which included papillary or mucinous adenocarcinoma (14.14 percent), malignant mixed mammary tumor (10.10 percent), intra acinar mammary carcinoma solid type (9.09 percent) and intra ductal carcinoma (2.02 percent).

2.7 APOPTOTIC INDEX (AI)

Wyllie (1987) stated that the four cardinal elements involved in apoptosis are rapid volume reduction, chromatin condensation, recognition by phagocytic cells and dependence on active protein synthesis.

DNA condensation and fragmentation are hallmarks of apoptosis. By measuring the area and the intensities of the brightest portions of the nuclear image, the bright, punctate nuclear imagery of apoptotic cells can be distinguished from the evenly stained nuclear imagery of a normal, healthy nucleus (Singh and Anand, 1995).

Apoptosis is characterized by a series of well-documented morphological changes, which can be observed by light and electron microscopy. In histological tumor material, apoptotic index is defined as the number of apoptoic cells per 1000 tumor cells or number of apoptotic cells per 10 high power fields (HPF) (Langlois *et al*., 2000).

There is a wide variation in the extent of apoptosis not only between different tumours but also within a tumor type and there exists a positive correlation between and apoptosis and proliferation (Soini *et al*., 1998).

Hassan and Walker (1998) conducted a study on human breast cancers and their overall results showed that the apoptotic index was higher in the normal/benign tissue than the cancer containing breast.

Sinicrope *et al.* (1999) reported that they failed to detect a significant correlation between AI and MI ($P = 0.27$) in all tumours or when stratified by tumor site.

Jain *et al*. (2009) concluded that tumours that exhibit more apoptosis may be slower growing and therefore may be biologically less aggressive and that a low apoptosis suggests a poor prognosis.

2.8 MITOTIC INDEX (MI)

In routine histopathology, the total number of mitosis was obtained by examining at least ten fields, mostly at the tumor periphery, using a standard light microscope equipped with an X 10 ocular and an X 40 objective (Pich *et al.*, 1994).

Mitotic index of canine TVT is very high (Shakir and Sundararaj, 1994; Goldschmidt and Hendrick, 2002; Maclachlan and Kennedy, 2002; Thangathurai *et al.*, 2008).

According to Biesterfeld (1997) mitotic activity should be calculated in areas of tumor sections where there are numerous mitotic tumor cells. Additionally, only cells with nuclei showing lysed nuclear membranes and identifiable single chromosomes at higher magnification ought to be considered in the calculation.

In the dogs with melanocytoma, finding more than three mitoses per 10 high power fields is indicative of malignancy (Goldschmidt *et al.*, 1998).

An abundance of mitotic figures is a distinctive feature of cholangiocarcinomas and assists in distinguishing these neoplasms from hepatocellular carcinomas (Cullen and Popp, 2002).

Mikaelian and Gross (2002) reported that mitoses were rare in fibroma and was low in fibrosarcomas with one or two mitoses per 10 high power fields (HPF).

In sertoli cell tumors mitotic figures are rare, ranging from zero to one per HPF (Grieco *et al.,* 2008).

Bukhari *et al.* (2007) suggested that there exists a relationship between cell proliferation and NORs.

Romansik *et al.* (2007) stated that there was a significant association between mitotic index and rate of metastatic disease, where higher the MI, the greater was the risk of metastatic disease.

2.9 ARGYROPHILIC NUCLEOLAR ORGANISER REGION COUNT TEST (AgNORs.)

Crocker *et al.* (1989) suggested that total AgNOR dots, both intra and extra- nucleolar, be enumerated. According to these recommendations, a dot aggregation cannot be resolved in individual NORs by focusing, but each cluster must be considered as one AgNOR.

The interphase NORs of cancer cells are more intensely stained in sections fixed with ethanol or methcarn solution than in sections fixed with formalin or Bouin's fluid (Derenzini and Trere, 1991).

Orrell *et al.* (1991) stated that counting the mean number of total AgNORs (both intra and extranucleolar) per nucleus is the best means of discriminating benign melanonaevi from malignant melanoma.

Evans *et al.* (1992) suggested that among the cutaneous melanomas of humans, there exists no correlation between the mitotic index and total AgNORs.

Johnson *et al*. (1995) stated that the mean number of AgNORs per nucleus accurately correlates with mitotic rate in tumor cell line sand AgNOR counts may thus provide an indirect measurement of mitotic rate and also AgNOR counts and mitotic indexes are capable of differentiating benign from malignant smooth muscle tumours.

Preziosi *et al*. (1995) shown that AgNOR proteins are useful in the evaluation of the proliferative activity of perianal gland tumours.

Radhakrishnan *et al.* (1995) stated that tumours with higher AgNOR number manifested recurrences much earlier than tumours with lower AgNOR number.

Krishnamurty and Paliwal (1998) reported that AgNORs in malignant cells were irregular in size and shape and dispersed throughout the nucleus. In contrast, AgNORs in control and benign lesions were larger, rounded and sharply defined usually confining to the nucleolus.

Derenzini, (2000) reported that the interphase AgNOR quantitative evaluation together with those parameters indicating the number of proliferating cells is the only method which permits information to be obtained on the rapidity of cell proliferation in routinely processed tissue samples.

Pawaiya *et al*. (2006) stated that the small size and high numbers of AgNORs are correlated with aggressive proliferative activity of the cell, while large sized AgNORs in low numbers indicate less or minimum proliferative activity of the cell.

Dayananda *et al.* (2009b) reported that in hemangioma the AgNOR distribution was single and undispersed whereas in hemangiosarcoma, there was multiple dispersed NOR indicating increased activity of the cell.

Materials and Methods

3. MATERIALS AND METHODS

3.1 COLLECTION OF SAMPLES AND PROCESSING

The study was conducted in sixty one selected number of cases presented for treatment to the Veterinary hospitals of Mannuthy and Kokkalai and carcasses brought for the post mortem examination in the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy during a period of one year i.e., from December 2008 to December 2009. Any abnormal growths detected were examined in detail. The tissues collected were preserved in 10% neutral buffered formalin, dehydrated through ascending grades of alcohol, cleared in xylene and embedded in paraffin. Sections were cut at 4 -5 micron thickness and stained with routine Haematoxylin and Eosin stain (Bancroft and Gamble, 2002).

3.2 INCIDENCE

The tissue samples confirmed as neoplasms were included for study. The the age, sex and breed of the tumour bearing animals were recorded and analysed. For age wise analysis the tumour bearing animals were grouped into four groups. *i.e.*, 0 to 4 years, 5 to 8 years, 9 to 12 years and greater than 12 year group.

3.3 CLASSIFICATION OF TUMORS

The tumors were classified based on their nature of malignancy (i.e., either benign or malignant) and anatomoical location. The recommendations of World Health Organization and Armed Forces Institute of Pathology for the tumors in domestic animals were adopted for the location or system wise classification of tumors.

3.4 HAEMATOLOGY

Blood samples were collected from live tumor bearing animals using EDTA (2 mg/ml) as anticoagulant. Total erythrocyte count (TEC) and

concentration of haemoglobin (Hb) was estimated by acid haematin method as described by Feldman *et al*. (2000). Total Leukocyte count (TLC), Packed Cell Volume (PCV), Erythrocyte sedimentation rate (ESR) and Differential Leucocyte count (DLC) were estimated by the method suggested by Thrall *et al*. (2004).

3.5 SPECIAL STAINING

The gross and histological features of the tumours were observed in detail and special stains were used whereever necessary.

3.5.1 Masson's Trichrome for demonstrating collagen

The paraffin sections cut at 4-5 micron thickness were cleared in xylene and hydrated to water. Mordant sections of formalin-fixed tissues in Bouin's fluid for one hour at 56^0C. Cool and washed thoroughly until the yellow colour disappeared. Rinsed in distilled water after each step. Stained in Weigert's iron haematoxylin solution for 10 minutes followed by Biebrich scarlet – acid fuchsin solution for 15 minutes. Again mordant in phosphomolybdic acid – phosphotungstic acid solution for 15 minutes. Then counterstained with aniline blue solution for 10 minutes followed by treatment with one percent acetic acid solution for five minutes. Then dehydrated in two changes of absolute alcohol, cleared in xylene and mounted in DPX (Luna, 1968). After staining nuclei took black colour, cytoplasm, appeared red and collagen stained blue.

3.6 APOPTOTIC INDEX (AI)

The haematoxylin and eosin stained sections were examined under oil immersion (x 1000). From each section, fields devoid of any preservation or fixation artifact, inflammation and necrosis were selected. Average AI was calculated as the number of apoptotic cells per 10 high power fields (Soini *et al*., 1998; Jain *et al*., 2009).

3.7 MITOTIC INDEX (MI)

The haematoxylin and eosin stained sections were examined under high power fields (x 400). The regions of tumor cells having highest overall mitotic activity were chosen for evaluation. Mitotic cells in 10 high power fields were counted. Average MI is the number of mitosis divided by 10 high power fields (Romansik *et al.,* 2007).

3.8 MODIFIED SILVER COLLOID STAINING FOR AgNORs

AgNOR stain

a) Silver nitrate solution: 50 percent solution of AR grade silver nitrate was prepared in de- ionized distilled water and stored in a polypropylene container away from the light.

b) Gelatin solution: 2 percent Gelatin solution was prepared in de-ionized distilled water and pure formic acid was added to this to a final concentration of 1 percent.

c) Working solution: (a) and (b) was mixed in a ratio of 2:1

Paraffin embedded tissue sections were cut at 4 micron thickness and sections were taken on to glass slide. Sections were deparaffinized and hydrated through graded alcohol and then washed in deionized distilled water. The staining solution was poured over the tissue sections and left for 60 minutes at room temperature. The silver colloid was then washed off with deionized water. The sections were dehydrated to xylene and mounted in DPX. The sections were then evaluated for AgNOR counts under oil immersion objectives. Fields were selected at random and 100 neoplastic cells were examined. All visible AgNORs (both intranucleolar and extranucleolar dots) were counted by careful focusing. The mean number of AgNOR per cell was then calculated (Crocker and Nar, 1987)

3.9 STATISTICAL ANALYSIS

Karl Pearson coefficient of correlation between mitotic indices, apoptotic indices and AgNOR counts of the tumors were worked out. Equality of means between mitotic indices, apoptotic indices, AgNOR counts and haematological parameters were analysed using Independent Sample‘t’ test (Snedecor and Cochran, 1994).

Results

4. RESULTS

4.1 INCIDENCE

During the period of study a total of sixty one tumour cases were reported in sixty dogs. This was due to the presence of multiple tumours in a dog.

4.2 CLASSIFICATION OF TUMORS

Out of 61 tumor cases, 40 (66 percent) were benign and 21 (34 percent) were malignant (Fig.1).

Location or system wise classification of the tumors showed that the skin and soft tissue tumors were in maximum number - 20 cases (32.8 percent). Mammary tumors of the dogs ranked second - 18 cases (29.51 percent). Thirteen cases (21.31 percent) of tumors pertained to the genital system. Four cases (6.56 percent) from the alimentary tract included pancreatic and hepatic tumors. Three cases (4.92 percent) from the haemolymphatic system, two cases (3.3 percent) of eye and ear origin and one case (1.64 percent) from bone (Table.1). Details pertaining to individual cases were tabulated in the tables 2 to 8.

4.3 AGE OF THE ANIMALS

The tumor bearing animals were grouped into four age groups namely 1 to 4 years, 5 to 8 years, 9 to 12 years and greater than 12 year groups. Maximum cases were observed in 5 to 8 years group. i.e., Thirty three out of sixty (55 percent) tumor bearing animals were in this group. Thirteen cases (21.67 percent) were in 9 to 12 year group. The third highest was in 1 to 4 year group. i.e., ten (16.67 percent) out of sixty cases. Four cases (6.67 percent) were reported in greater than 12 year group (Fig.2).

4.4 SEX OF THE ANIMALS

Out of 60 tumor bearing animals 35 (58 percent) were females and 25 (42 percent) were males (Fig.3).

4.5 BREED OF THE ANIMALS

Sixty one cases were recorded in 60 animals. This is because of the presence of concomittent tumor in a Mongrel dog. i.e., malignant fibrous histiocytoma and sebaceous adenoma occurred together in the same dog. Out of these 60 affected animals , 17 (28.33 percent) were German shepherds, 13 (22 percent) were Mongrels, six cases (10 percent) each were Labrador and Rottweiler, three (5 percent) were Dobermann, two cases (3.33 percent) each of Cocker spaniel and Boxer and one case (1.76 percent) each of Dalmatian, Spitz, Lhasa apso and Bull mastiff (Fig.4).

4.6 HAEMATOLOGY

The haematological parameters of the tumor bearing animals were listed in the table 9 and 10. While the mean values of haemoglobin concentration and packed cell volume of all animals and total erythrocyte count of the malignant tumor bearing animals showed a slight reduction from the normal ranges, the total erythrocyte counts of the benign tumor bearing animals were within the lower limit of normal range. The erythrocyte sedimentation rate of all animals was within the normal range. The total leucocyte counts were elevated in all animals. The mean neutrophil and lymphocyte percentage also fell within the normal range. There was no significant difference between the mean hematological values of animals bearing benign and malignant tumors.

Table 1. Location wise distribution of tumors

No.	TUMORS	INCIDENCE
1	Tumors of the skin and soft tissues	20
2	Mammary tumors of dog	18
3	Tumors of the genital system	13
4	Tumors of alimentary tract	4
5	Tumors of hemolymphatic system	3
6	Tumors of eye and ear	2
7	Tumors of bone	1
TOTAL		61*

*Incidence of tumors was observed in 60 animals. But a total of 61 cases, because of the occurrence of concomittant tumor in a dog.

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Table 2. Tumors of the skin and soft tissues

Epithelial and melanocytic tumors of the skin					
No.	TUMOR	AGE (years)	SEX	BREED	LOCATION
BENIGN					
1	Sebaceous adenoma	10	F	Mongrel	Interdigital space
2	Sebaceous adenoma	7	M	Lhasa apso	Interdigital space
3	Sebaceous adenoma	10	M	Mongrel	Ear pinna and anal region
4	Sebaceous epithelioma	11	F	Cocker Spaniel	Ear pinna
5	Melanocytoma	5	M	Rottweiler	Interdigital space
6	Hepatoid gland adenoma	15	M	Dachshund	Perianal region
7	Hepatoid gland adenoma	8	M	Mongrel	Perianal region
MALIGNANT					
8	Hepatoid gland carcinoma	10	M	Mongrel	Perianal region
9	Hepatoid gland carcinoma	9	M	Rottweiler	Perianal region
10	Eccrine carcinoma	7	M	GSD	Foot pad and hindlimb
Mesenchymal tumors of the skin and soft tissues					
BENIGN					
11	Fibroma	11	F	Mongrel	Uterus
12	Fibroma	8	F	Labrador	Vagina
13	Fibroma	7	F	Dachshund	Vagina
14	Fibroma	5	F	Mongrel	Vagina
15	Fibroma	7	F	Dachshund	Vagina
16	Fibroma	9	F	Spitz	Vagina
17	Neurofibroma	8	M	GSD	Hock joint
MALIGNANT					
18	Malignant fibrous histiocytoma (MFH)	10	M	Mongrel	Elbow
19	Liposarcoma	8	M	Labrador	Perineal region
20	Hemangiosarcoma	8	M	GSD	Spleen

n=20

Table 3. Tumors of bone

No.	TUMOR	AGE (years)	SEX	BREED	LOCATION
MALIGNANT					
1	Osteosarcoma	2	F	Boxer	Distal radius

n=1

Table 4. Tumors of haemolymphatic system

No.	TUMOR	AGE (years)	SEX	BREED	LOCATION
MALIGNANT					
1	Lymphosarcoma	7.5	M	Mongrel	Lymph nodes
2	Acute Lymphoblastic Leukemia	2.5	M	Boxer	Bone marrow
3	Canine multicentric lymphoma		M	Bull mastiff	Lymph nodes

n=3

Table 5.**Tumors of alimentary tract**

No.	TUMOR	AGE (years)	SEX	BREED	LOCATION
BENIGN					
1	Ossifying epulis	8	M	Labrador	Lower gum
MALIGNANT					
2	Hepatocellular carcinoma	4	M	GSD	Liver
3	Cholangiocellular carcinoma	4.5	M	Dalmatian	Liver
4	Pancreatic carcinoma	3	F	Doberman	Pancreas

n=4

Table 6. Tumors of eye and ear

No.	TUMOR	AGE	SEX	BREED	LOCATION
1	Adenoma of nictitans gland	8	M	Mongrel	Inner canthus of right eye
2	Ceruminous carcinoma	8	M	Mongrel	Base of the ear

n=2

Table 7. Tumors of the genital system

No.	TUMOR	AGE (years)	SEX	BREED	LOCATION
1	Sertoli cell tumor	4	M	GSD	Inguinal region
2	Sertoli cell tumor	13	M	Dachshund	Testis
3	TVT	3	F	GSD	Vulva
4	TVT	4	F	Rottweiler	Vulva
5	TVT	5	F	GSD	Vulva
6	TVT	5	F	GSD	Vulva
7	TVT	5	F	Mongrel	Vulva
8	TVT	5	F	GSD	Vulva
9	TVT	6.5	F	Labrador	Vulva
10	TVT	6	F	Rottweiler	Vulva
11	TVT	5	M	Dachshund	Penis
12	TVT	3.5	M	Doberman	Penis
13	TVT	4	M	Dachshund	Penis

n=13

Table 8. Mammary tumors of dogs

No.	TUMOR	AGE (years)	SEX	BREED	LOCATION
Benign mammary tumors					
1	Benign mixed tumor	10	F	Dobermann	Caudal abdominal
2	Benign mixed tumor	10	F	Mongrel	Cranial thoracic
3	Benign mixed tumor	6	F	Rottweiler	Cranial abdominal
4	Benign mixed tumor	7	F	Labrador	Caudal abdominal
5	Benign mixed tumor	8	F	Rottweiler	Caudal abdominal
6	Benign mixed tumor	7	F	Labrador	Cranial thoracic
7	Simple adenoma	9	F	GSD	Inguinal
8	Simple adenoma	9	F	GSD	Inguinal
9	Myoepithelioma	7	F	GSD	Caudal abdominal
10	Complex adenoma	6	F	GSD	Cranial abdominal and caudal thoracic
11	Fibroadenoma	12	F	GSD	Caudal abdominal
Malignant mammary tumors					
12	Solid carcinoma	8	F	Dachshund	Caudal abdominal and inguinal
13	Simple carcinoma	13	F	Cocker Spaniel	Inguinal
Mammary sarcomas					
14	Carcinosarcoma	5	F	GSD	Caudal abdominal
15	Carcinosarcoma	8	F	GSD	Caudal abdominal
16	Carcinosarcoma	13	F	Mongrel	Inguinal and cranial thoracic
17	Osteosarcoma	9	F	GSD	Caudal abdominal
18	Fibrosarcoma	7	F	GSD	Caudal thoracic and cranial abdominal

n=18

Table 9. Haematological parameters of animals with benign tumors

No.	TUMOR	Hb (gm/dl)	PCV (percent)	ESR (mm/hr)	TEC (10^6/µl)	TLC (thousands/mm^3)
1	Benign mixed mammary tumor	16.0±1.06	49.0±2.88	5.0±1.82	8.9±0.45	12 400±2434
2	Complex adenoma of mammary gland	14.0±1.06	41.0±2.88	2.0±1.82	7.0±0.45	13 500±2434
3	Simple adenoma	12.0±1.06	36.0±2.88	5.0±1.82	6.6±0.45	9 800±2434
4	Epulis	8.6±1.06	26.0±2.88	7.0±1.82	4.8±0.45	9 800±2434
5	Sertoli cell tumor	8.8±1.06	34.0±2.88	15.0±1.82	5.8±0.45	28 050±2434
6	Melanoma	14.2±1.06	32.0±2.88	14.0±1.82	5.7±0.45	19 100±2434
7	Sebaceous epithelioma	7.2±1.06	24.0±2.88	22.0±1.82	5.2±0.45	8 300±2434
8	Sebaceous adenoma	16.2±1.06	45.2±2.88	2.0±1.82	6.6±0.45	7 740±2434
9	Neurofibroma	10.8±1.06	40.0±2.88	3.2±1.82	6.3±0.45	10 500±2434
10	Fibroma	4.8±1.06	13.3±2.88	2.0±1.82	2.5±0.45	34 600±2434
11	Hepatoid gland adenoma	15.0±1.06	42±2.88	5.0±1.82	7.2±0.45	14 500±2434
12	TVT	11.0±1.06	36.0±2.88	7.0±1.82	7.0±0.45	21 100±2434
	Mean values	**11.55±1.06**	**34.9±2.88**	**7.4±1.82**	**6.13±0.45**	**15782±2434**

Differential Leucocyte Count (DLC)

		Neutrophils (percent)	Lymphocytes (percent)	Eosinophils (percent)	Monocytes (percent)
1	Benign mixed mammary tumor	65±2.68	32±2.31	3	-
2	Complex adenoma	67±2.68	29±2.31	2	2
3	Simple adenoma	70±2.68	30±2.31	-	-
4	Epulis	56±2.68	43±2.31	1	-
5	Sertoli cell tumor	72±2.68	24±2.31	4	-
6	Melanoma	65±2.68	35±2.31	-	-
7	Sebaceous epithelioma	70±2.68	24±2.31	6	-
8	Sebaceous adenoma	44±2.68	45±2.31	11	-
9	Neurofibroma	62±2.68	38±2.31	2	-
10	Fibroma	78±2.68	20±2.31	-	2
11	Hepatoid gland adenoma	72±2.68	26±2.31	2	-
12	TVT	76±2.68	24±2.31	-	-
	Mean values	**66.42±2.68**	**30.83±2.31**		

Table 10. Haematological parameters of animals with malignant tumors

No	TUMOR	Hb (gm/dl)	PCV (percent)	ESR (mm/hr)	TEC (10^6/µl)	(TLC) (thousands/mm^3)
1	Simple carcinoma of mammary gland	14.0±1.2	32.0±4.01	7.0±6.98	4.64±0.33	7 150±3070
2	Carcinosarcoma of mammary gland	16.2±1.2	51.0±4.01	1.0±6.98	5.26±0.33	18 650±3070
3	Liposarcoma	7.6±1.2	23.0±4.01	2.2±6.98	3.19±0.33	7 000±3070
4	MFH	5.0±1.2	14.9±4.01	10.0±6.98	3.67±0.33	16 000±3070
5	ALL	7.4±1.2	20.5±4.01	51.0±6.98	3.22±0.33	23 900±3070
6	Lymphosarcoma	8.1±1.2	23.8±4.01	46.0±6.98	4.24±0.33	29 016±3070
7	Eccrine carcinoma	12.2±1.2	36.0±4.01	10.0±6.98	6.31±0.33	13 100±3070
8	Hepatoid gland carcinoma	11.4±1.2	46.0±4.01	1.2±6.98	3.94±0.33	20 250±3070
9	Hepatocellular carcinoma	9.2±1.2	26.0±4.01	42.0±6.98	3.86±0.33	34 000±3070
	Mean values	**10.1±1.2**	**30.4±4.01**	**18.9±6.98**	**4.25±0.33**	**9211.6±3070**

Differential Leucocyte Count (DLC)

		Neutrophils (percent)	Lymphocytes (percent)	Eosinophils (percent)	Monocytes (percent)
1	Simple carcinoma of mammary gland	75±5.96	24±6.43	1	-
2	Carcinosarcoma of mammary gland	69±5.96	31±6.43	-	-
3	Liposarcoma	76±5.96	21±6.43	2	1
4	MFH	82±5.96	10±6.43	8	-
5	ALL	24±5.96	76±6.43	-	-
6	Lymphosarcoma	58±5.96	39±6.43	5	-
7	Eccrine carcinoma	76±5.96	24±6.43	-	-
8	Hepatoid gland carcinoma	70±5.96	28±6.43	2	-
9	Hepatocellular carcinoma	80±5.96	15±6.43	5	4
	Mean values	**67.78±5.96**	**29.78±6.43**		

4.7 GROSS AND HISTOPATHOLOGICAL FEATURES OF THE TUMORS

4.7.1 Epithelial and Melanocytic Tumors of the Skin

4.7.1.1 Sebaceous Adenoma

Grossly solitary dome shaped growth in the interdigital space of hindlimb with alopecia and ulceration of the overlying skin was detected in two dogs (Fig.5). A third case was of multiple growths in the ear pinna and in the anal region of a ten year old Mongrel dog.

Histologically there was a preponderance of mature sebocytes in multiple groups and several layers of basaloid reserve cells at the periphery of the lobules. The groups of the sebocytes were not oriented around the ducts and were separated by thick hyalinised bands of fibrocollagenous tissue. Focal areas of squamous metaplasia with a few keratin nests were present (Fig.6). Stroma showed dense infiltration of lymphocytes, plasma cells and polymorphs. Areas of cystic degeneration were also present.

4.7.1.2 Sebaceous Epithelioma

Multiple firm small nodular growths were present in the face, ear and thoracic region of a 13 year old Cocker Spaniel bitch. The mass in the ear pinna bearing surface ulceration was about 2 cm in diameter (Fig.7).

Histologically there was a preponderance of basaloid cells with a few sebocytes arranged individually or in small aggregates (Fig.8). Basaloid cells exhibited marked mitotic activity and possessed scant cytoplasm with hyperchromatic nuclei. Foci of ductal differentiation were found as small horn cysts. The connective tissue stroma separating the tumor growth appeared blue in Masson's trichrome (Fig.9).

4.7.1.3 Hepatoid Gland Carcinoma

Multiple small and firm nodular growths were observed in the perianal region of two dogs (Fig.10). In one case bleeding from the rectum was observed

and on per rectal examination numerous small nodular growths could be appreciated on the wall and floor of the rectum.

Histologically there was irregular arrangement of the hepatoid cells which showed varying degrees of maturation. Nuclear pleomorphism was present in the reserve cells and mitotic figures were moderate in number. Maturation patterns from reserve cells to hepatoid cells were somewhat haphazard. Hepatoid cells exhibited vacuolated cytoplasm with large nuclei and several prominent nucleoli (Fig.11). Infiltration of inflammatory leucocytes was noticed in the stroma.

4.7.1.4 Hepatoid Gland Adenoma

Hepatoid gland adenomas appeared as well circumscribed, firm and nodular growths in the perianal region. Cut section revealed multilobulated appearance with focal hemorrhagic areas.

Histologically the cells resembling hepatocytes were arranged in cords with anastomosing trabeculae (Fig.12). The cells were polyhedral with centrally located vesicular nucleus and eosinophilic cytoplasm. Basaloid reserve cells with hyperchromatic nuclei and little cytoplasm were present at the periphery of the lobules. A rich vascular interlobular stroma was observed. Sinusoidal blood vessels were dilated separating the epithelial trabeculae. Few mitotic figures present were confined to the reserve cells.

4.7.1.5 Eccrine Carcinoma

Swellings of about 3.2 cm in diameter with ulcerations of the overlying epidermis were noticed in the foot pad and hind limb of a seven year old GSD dog for the last three months (Fig.13 and 14).. The mass was firm in consistency with a central depressed area and white cut surface.

Histologically cuboidal neoplastic cells possessed eosinophilic cytoplasm and hyperchromatic nuclei with prominent nucleoli. Small foci of keratinization and intraluminal eosinophilic secretions were observed. Epidermal ulceration was

also evident (Fig.15). Irregular tubuloacinar structures were lined by one or more layers of cuboidal epithelial cells lying distributed in an abundant stroma of dense collagen that stained blue with Masson's trichrome (Fig.16).

4.7.1.6 Melanocytoma

Grossly melanoma was observed as a solitary, black colored alopecic nodule of about 12 cm diameters in the interdigital space of a Rottweiler (Fig.17). The growth was fleshy in consistency with a blackish cut surface.

Microscopically spindle shaped neoplastic cells with intracytoplasmic melanin granules (Fig.18) and variable amount of collagenous stroma separating the neoplastic cells could be appreciated in the dermis (Fig.19). Marked cellular pleomorphism was also noticed. The epidermis was intact.

4.7.2 Mesenchymal Tumors of the Skin and Soft Tissues

4.7.2.1. Fibroma

Grossly fibromas were very firm, well circumscribed, subcutaneous masses (Fig.20) with an average of 17 cm diameter and 79 gm in weight. The cut surface was whitish with a glistening appearance (Fig.21). Ulcerated surface was noticed in some larger masses. All cases of fibromas were obtained from the female genital system.

Histologically interlacing bundles of spindle shaped fibroblasts and collagen fibres oriented in various directions could be appreciated (Fig.22). The neoplastic fibrocytes were uniform with oval normochromatic nuclei and indistinct cytoplasm. Mitotic figures were rarely observed. Sections stained with Masson's trichrome predominantly took blue colour indicating the abundance of collagen (Fig.23).

4.7.2.2 Benign Peripheral Nerve Sheath Tumor / Neurofibroma

A well circumscribed unencapsulated lobulated growth of about 20 cm diameter and weighing 200 gm was seen on the lateral aspect of the right hind limb, just above the hock joint and parallel to the saphenous vein (Fig.24).

Histologically the sections revealed small spindle cells arranged in wavy bundles, palisades and partial whorls (Fig.25). Cellularity was moderately low. There was moderate amount of collagenous stroma and the collagen fibres were more delicate than in fibromatous neoplasms (Fig.26). Mitotic figures were rare. In some areas palisades of spindle cells were oriented around small zones of sclerotic collagen, while in other areas cells were loosely distributed in a fibrillar matrix.

4.7.2.3 Liposarcoma

Grossly liposarcoma observed as a firm subcutaneous growth of about 14 cm diameter and weighing 93 gm in the perineal region (Fig.27). The mass was soft in consistency with grayish white colour and oily cut surface. Focal areas of necrosis were also noticed in the cut surface.

Histologically features were suggestive of well differentiated subtype. Most of the cells resembled normal adipocytes with single clear fat vacuoles pushing the nuclei to the periphery. Other cells contain abundant cytoplasm with variably sized lipid droplets (Fig.28). Collagenous stroma was scanty and appeared blue with Masson's trichrome stain (Fig.29). Nuclear pleomorphism, atypical mitosis and a few giant cells were observed.

4.7.2.4 Malignant Fibrous Histiocytoma (MFH)

A firm unencapsulated whitish colored growth with a distinct margin was seen on the elbow joint of a ten year old Mongrel dog (Fig.30).

Histologically the fibroblasts like cells were arranged in storiform patterns (Fig.31). There was an abundance of histiocytoid cells, plasma cells, neutrophils,

eosinophils and lymphocytes (Fig.32). Histiocytoid cells were frequently karyomegalic or multinucleate, with nuclear atypia. Patchy zones of collagenous stroma were also present.

4.7.2.5 Hemangiosarcoma

An eight year old German shepherd dog was presented to the University Veterinary Hospital with complaints of anorexia, emesis and weakness. Besides this abdominal distension was noticed for the last one week. On abdominal palpation, a hard mass on the ventral aspect more towards the right side of the abdomen was appreciated. Ultrasonographically a mixed echogenic mass was confirmed within the spleen.

On exploratory laparotomy a tumor mass was found in the hilus of the spleen. Single large well defined growth of about 3.67 kg was obtained from the spleen after perfoming splenectomy. The mass was firm in consistency, brown in color and was attached to the hilus portion of the spleen (Fig.33). Cut section revealed blood pockets from which blood was oozing out. Adjacent areas of liver which showed whitish raised degenerative changes were also resected during surgery.

Histologically neoplastic cells were pleomorphic, ranging from spindle shaped to ovoid and formed vascular clefts in the tumor (Fig.34). The cells lining the clefts had pleomorphic nuclei which were prominent, bulging and hyperchromatic. Mitotic figures were common. In some areas stroma between the clefts was acellular and brightly eosinophilic. In the liver, the hepatocytes showed areas of acute cell swelling and severe degrees of fatty change without a zonal pattern of distribution (Fig.35). Areas of marked sinusoidal dilatation were also appreciated.

4.7.3 Tumors of Bone

4.7.3.1 Osteosarcoma

A warm and painful swelling extending from the middle of the forearm down to the distal end of metacarpals with exudation of serosanguinous fluid was noticed in a two year old female Boxer for the last two months (Fig.36). The mass was soft in consistency and the cut section was whitish with focal areas of haemorrhage and necrosis. Small bony spicules were seen embedded in the mass.

Histologically the tumor was predominantly cellular and highly vascular with sparse osteoid production suggestive of productive osteoblastic osteosarcoma. Malignant osteoblasts varied from pleomorphic spindle shaped cells to plump oval to rounded cells with basophilic cytoplasm and eccentric hyperchromatic nuclei. Mitotic figures were common and multinucleated giant cells with stacked up nuclei were seen (Fig.37). Islands of osteoid were surrounded by malignant osteoblasts, some of which were incorporated in the tumor osteoid. Osteoid was present as delicate network which stained blue in Masson's Trichrome (Fig.38). Large areas of haemorrhage lined by tumor cells were noticed in one section.

4.7.4 Tumors of Hemolymphatic System

4.7.4.1 Lymphosarcoma

A Mongrel male dog of 7.5 years was presented at the University Veterinary Hospital with a history of listlessness, excessive panting and severe cough. Generalized lymphadenopathy was noticed (Fig.39). The superficial lymph nodes were highly enlarged, nodular and hard in consistency. Ultra sound scanning revealed the presence of hyperechoic mass in the spleenic area, enlargement of the mediastinal lymph nodes and changes in the kidney.

Cytological evaluation of the lymph node aspirate revealed a monotonus population of pleomorphic lymphocytes (Fig.40). On the basis of lymph node aspiration biopsy, ultrasonography and haemogram, the disease was diagnosed to

be multicentric lymphosarcoma. Though the animal was subjected to chemotherapeutic treatment, it was reported that it succumbed after one month.

4.7.4.2 Acute Lymphoblastic Leukemia (ALL)

A male Boxer 2.5 year old was presented at the University Veterinary Hospital, Mannuthy, Thrissur with complaints of recurring fever and severe weight loss since 80 days and was not responding to antibiotics and other supporting medicines (Fig.41).

Haematological observations revealed that the animal was anaemic with leucocytosis. Differential leucocyte count revealed seventy four percentage large atypical cells having features of blast cells (Fig.42). On ultrasonography splenomegaly could be appreciated. Bone marrow examination revealed large number of atypical blast cells suggestive of lymphoblastic leukemia. On the next day the animal succumbed and a detailed post mortem examination was conducted.

On post mortem examination, severe hepatomegaly and congestion of the liver was noticed (Fig.43). Splenomegaly of about two to three times could be well appreciated. Also pea sized nodular growths were present along the margins of the spleen (Fig.44). Kidneys showed focal areas of degeneration. Areas of congestion and emphysema were noticed in lungs. Intestinal mucosa showed congestion with excess mucus secretion. Urinary bladder was filled with thick viscous yellowish mucous like secretion and the mucosa revealed petechiae along with black spots. The prostate was enlarged and was firm in consistency.

Histology of the liver showed diffuse involvement with dilatation of sinusoids along with cells of lymphoblast series. Hepatocytes showed varying degrees of vacuolar degeneration. Sinusoidal congestion was noticed in focal areas (Fig.45).

Spleen showed massive infiltration with cells of lymphoblast series. The red pulp was completely depleted and foci of necrosis were also noticed (Fig.46).

In sections of the kidneys collection of neoplastic cells was observed in the interstitium and the tubular epithelium was degenerated. Focal areas of necrosis and desquamation of tubular epithelial cells were also seen. Areas of congestion and emphysema were present in the lungs. Myocardial congestion and fatty change involving the myocardial fibres were present. Intestinal features were typical of catarrhal inflammation characterized by infiltration of the lamina propria with inflammatory cells and goblet cell hyperplasia. The urinary bladder showed infiltration of the inflammatory cells into the mucosa with excessive mucous production. The prostate was marked by glandular hyperplasia. All the glands were cystic with an increase in the amount of fibromuscular stroma. Presence of chronic inflammatory cells and focal areas of papillary infoldings with single layer lining of epithelial cells were evident.

All the above findings led to the conclusion that the case was of Acute Lymphoblastic Leukemia (ALL).

4.7.4.3 Canine Multicentric Lymphoma

A three year old male Bull mastiff was brought for necropsy to the Centre of Excellence in Pathology with a vague history of illness for the last five months not responding to treatment.

On examination, the carcass was found to be emaciated with bilateral and symmetrical enlargement of all the peripheral lymph nodes (Fig.47). The skin was dehydrated with pale and sunken mucous membrane. Visceral examination revealed the enlargement of all the lymph nodes in the body. All the peripheral lymph nodes namely submandibular, prescapular and popliteal and visceral lymph nodes namely bronchial, mediastinal, mesenteric, renal and parotid lymph nodes were enlarged and smooth in consistency. Central necrotic areas were noticed in the lymph nodes. Cut surface of the enlarged lymph nodes bulged out and had a homogenous appearance with no corticomedullary differentiation. Marked hepatomegaly (Fig.48) and splenomegaly (Fig.49) was noticed. The liver and spleen was dark brown in colour with soft consistency. The mucosa of the

stomach and intestine were congested. The organs were collected in neutral buffered formalin and processed.

Histologically the lymph nodes were infiltrated with sheets of monomorphic cells. Effacement of the normal architecture without differentiation of the cortex and medulla was noticed (Fig.50). Diffuse splenic involvement was noticed. The most characteristic histologic lesion in the spleen was the atrophy of the periarteriolar lymphoid sheaths and subendothelial lymphocyte colonization of large veins within the thick fibromuscular trabeculae. (Fig.51). The cells were uniformly small but cleaved. i.e., diffuse small cleaved cell (DSC) (Fig.52). The nuclei were small and irregular in outline and there was little internal nuclear detail, and the nucleoli and mitoses were rarely found. There was multifocal hepatic involvement characterized by focal congregation of neoplastic cells around portal triads (Fig.53). Extreme dilatation of sinusoids and atrophy of hepatic cords in the centrilobular areas were noticed. Cytoplasm of the hepatocytes revealed vacuolar changes.

In the kidney, focal collections of neoplastic cells were noticed in the intertubular regions of the cortex. Medulla was scarcely affected when compared to cortex. Scattered areas of intertubular haemorrhages were noticed. Tubules showed mild vacuolar changes. In the lungs aggregations of the neoplastic cells were found perivascularly and also around the lymphatics (Fig.54). The sections of the intestines revealed infiltration of the inflammatory cells and hyperemia of the mucosa.

4.7.5 Tumors of Alimentary Tract

4.7.5.1 Ossifying Epulis

Grossly a whitish hard mass of 4 cm diameter was attached to the lower gum of a Labrador (Fig.55). The cut surface showed lobulations and granularity indicating calcification.

Histology revealed rete pegs of the overlying epithelium extending deep into the mass (Fig.56). The peripheral cells of these cords were columnar and in palisade arrangement. Deeper areas revealed osteoid and mature lamellated bone (Fig.57). Scattered proliferation of stellate and spindle cells with moderate collagen were observed throughout the tissue. These cells showed elongated to oval nuclei and eosinophilic cytoplasm. Since the bone tissues occur in the ossifying epulis, the tumor might arise from odontogenic epithelial cells of periodontal membrane. Clusters of apoptotic bodies were observed in the section (Fig. 58).

4.7.5.2 Hepatocellular Carcinoma

A four years old male GSD was brought to the University Veterinary Hospital with the history of inappetance, severe anaemia and recurring fever. Ultrasonography revealed the presence of hepatomegaly with some abnormal growths. Punch biopsy was taken and the results confirmed hepatocellular carcinoma. It was decided to conduct an explorative laprotomy to remove the affected portions of the liver. While opening it was seen that the entire peritoneum was studded with nodular growths. The liver showed multiple nodular growths with a little area of normal appearance. The animal was moribund and so resorted to euthanasia. The carcass was submitted for post mortem examination at the Department of Pathology.

On opening the carcass, hepatomegaly and diffuse, large nodular growths which appeared as white lumps was present in the liver with a few normal brownish areas. Multiple indistinct masses were present throughout the liver suggestive of nodular type of hepatocellular carcinoma (Fig.59). The liver had a mottled appearance with a friable consistency. The centre of the neoplasm showed necrotic areas. The entire peritoneal folds and diaphragm were studded with metastatic nodular growths indicating transcoelomic spread (Fig.60).

Whitish nodular growths were present in the lungs with areas of collapse and emphysema. Metastatic growths were seen in the pleura. Right ventricular

dilatation was present. Whitish nodular growths were seen attached to the capsule of the kidneys. The mesenteric folds were studded with nodular growths. A mesenteric lymph node was seen severely enlarged with irregular borders, very hard to cut and gritty consistency (Fig.61). Stomach was empty and the mucosa showed areas of congestion. Catarrhal enteritis was present.

Histology of the liver showed islands of neoplastic cells embedded in the hepatic parenchyma. The neoplastic cells were arranged in trabecular (Fig.62) and adenoid patterns (Fig.63) (mixed type). In some areas neoplastic cells were arranged in sheets with trabeculae, while in some areas neoplastic cells revealed acinar pattern with lumens varying in size. The acini were lined by well differentiated hepatocytes with scant connective tissue stroma present between the acini. Severe sinusoidal dilatation, fatty change and haemorrhages were seen in focal areas. Areas of infiltration of inflammatory cells (PMNs) and necrosis were present among the hepatocytes. The neoplastic cells showed cellular and nuclear pleomorphism, vacuolated, scant and basophilic cytoplasm. Mitotic figures were present frequently.

. The entire architecture of the mesenteric lymph nodes was replaced by infiltrating neoplastic cells and collagenous connective tissue stroma. Neoplastic cells showed a tendency to form glandular pattern. Occasional areas of calcification were seen (Fig.64). There was thickening of the capsule along with massive proliferation of the granulation tissue. In the lungs extreme alveolar and interstitial emphysema were present. Focal collections of neoplastic hepatocytes tending to form acini were deposited in the lung parenchyma (Fig.65).

In the kidneys tubular degeneration was evident with a few tubules showing desquamation and denudation of the tubular epithelial cells. Areas of intertubular haemorhages were also present. Neoplastic cells arranged in trabecular and adenoid pattern were seen attached to the renal capsule by a connective tissue strand. Splenic tissue showed nodular hyperplasia of the white pulp, congestion and haemorrhage in red pulp.

4.7.5.3 Cholangiocellular Carcinoma

The carcass of a male Dalmatian dog of four years and five months old was brought for postmortem examination to the Centre of Excellence in Pathology. The history revealed that the animal was off fed for 45 days and was under the treatment for ascitis. On post mortem about 4 litres of serosanguineous fluid was seen in the abdominal cavity. Multiple lobulated growths were seen in the ventral aspect of left lateral lobe of the liver (Fig.66). The liver was firm in texture with yellowish brown cut surface. Cystic areas containing yellow brown viscous fluid was found focally. Nodular growths were present in the diaphragm (Fig.67), lungs (Fig.68), pleura, and in the peritoneal attachment of the spleen indicating a transcoelomic spread of the neoplasm. There were focal areas of pulmonary congestion and right ventricular dilatation of the heart.

The capsule of the kidney was firmly adherent to the cortical surface bearing diffuse subcapsular pinpoint depressed areas. The stomach was empty and the mucosa revealed areas of congestion. The pancreas was prominent due to the firm texture and thickening. The serosa of intestine and mesentery was covered with soft greasy fat. Intestinal loops showed non-separable adherence to each other and with the mesentery. Urinary bladder was full with yellowish coloured urine.

Sections of the liver revealed tubular or acinar arrangement of cells. The tubules were lined by multiple layers of cuboidal to columnar epithelial cells. Islands of tubules surrounded by abundant connective tissue were noticed (Fig.69 and 70). Papillary infoldings of the tubular epithelium was observed in focal areas (Fig.71). Neoplastic cells were cuboidal to columnar and possessed a moderate amount of pale eosinophilic and slightly granular cytoplasm. Nuclear pleomorphism was present. Presence of mucin within the lumen of neoplastic tubules could be noticed in certain areas. Based on the histological features it was confirmed as a well differentiated type of cholangiocellular carcinoma.

In the diaphragm clusters of neoplastic cells were arranged as tubules or acini with deeply basophilic nuclei. Nuclear pleomorphism was prominent. Goblet cell hyperplasia and infiltration of lamina propria and sub mucosa with inflammatory cells was seen indicating catarrhal enteritis (Fig.72).

In the lungs tumor emboli were seen in the blood vessels, lymphatics and bronchioles (Fig.73). Focal areas of massive congestion and emphysema were present. Sub pleural and alveolar haemorrhages were present. In the spleen, trabeculae were prominent and the pulp, severely atrophied. A firm nodular growth was attached to the splenic capsule and its microscopic appearance revealed the predominance of fibrous connective tissue within which well differentiated tubules were embedded and was attached to the splenic tissue by a strand of connective tissue. Exocrine glandular hyperplasia was present in the pancreas.

4.7.5.4 Pancreatic Carcinoma

A three year old female Doberman was presented in the University Veterinary Hospital, Mannuthy from Namakkal area with the complaint that animal was not taking any solid food and was having melena since four months and was not responding to any treatment.

On abdominal palpation crepitation could be felt in the intestinal loops and also a foreign body like hard mass was felt in the anterior abdominal region. On X- ray and endoscopy, no obstruction was seen till the duodenal region. The animal was subjected to explorative laparotomy and a hard mass was seen starting from the distal portion of the pancreas and besides this the posterior duodenum jejunum and ileum along with the mesenteric folds were seen adhered to the mass. The pancreatoduodenal lymph node was highly enlarged with areas of necrosis at the centre (Fig.74). The affected parts were exteriorized and excised and the proximal duodenum was anastomosed with the colon. Post operative antibiotic and supplementary treatments were suggested. But the animal succumbed after a day.

The removed tissues were subjected to histopathological studies. Histologically highly anaplastic or undifferentiated neoplastic cells with no resemblance to pancreatic acini were observed. A dense amount of collagenous supporting stroma was present. The nuclei were crowded together exhibiting a high degree of pleomorphism (Fig.75 and 76). Mitotic index was very high, four or five mitotic figures were observed in each field.

Sections of the pancreatoduodenal lymph node revealed infiltration of the neoplastic cells along with aggregations of plasma cells, lymphocytes and a few macrophages indicating a reactive lymph node (Fig.77). Giant cells and mitotic figures were frequently present. The serosa of the duodenum and the neoplastic pancreas tightly adhered to each other. The muscular coat of the duodenum was intact and infiltration of the neoplastic cells was observed in the submucosa (Fig.78). So the case was a highly undifferentiated pancreatic carcinoma with metastases to adjoining lymph nodes and duodenum.

4.7.6 Tumors of Eye and Ear

4.7.6.1 Adenoma of Nictitans Gland

An eight year old Mongrel dog was presented to the University Veterinary Hospital, Kokkalai with a growth on the inner canthus of the right eye, blocking the normal vision (Fig.79). The growth was observed for about three months. The enlarged nictitans gland was resected and subjected to histopathological studies.

Histologically most of the tubules were completely obliterated by proliferating neoplastic cells (Fig.80). Some part of the tissue showed multiple layering of the acinar lining cells and also stromal invasion of the neoplastic cells (Fig.81). Cells revealed nuclear hyperchromasia and pleomorphism. Cytoplasm was moderately basophilic, whereas in other parts of the tissue, lesions were characteristic of adenoma where the tubules were lined by a single layer of low cuboidal cells. The acini contained secretions. Infiltration of lymphocytes was also noticed. An epithelial lining was noticed at the periphery of the growth and the nuclei were retained in the stratum corneum layer. Areas of haemorrhage

were noticed between the layers of the epithelium. All these suggested that the growth was in its transition stage from benign to malignant, thus leading to the conclusion of this as a case of adenoma of nictitans gland with border line malignancy.

4.7.6.2 Ceruminous Gland Carcinoma

Grossly a pedunculated ulcerating mass was present at the base of the ear of a Mongrel dog. The growth was firm in consistency and the cut surface was creamy white in color with dark brown areas (Fig.82).

Histologically the tumor was subdivided into lobules by fibrous trabeculae. In the glandular lumen multiple layers of lining epithelial cells was present.Nuclear pleomorphism with large prominent nucleoli was present and the cell borders were distinct. The epidermis was intact. A brown material was present in the glandular lumen occasionally (Fig.83).

4.7.7 Tumors of the Genital System

4.7.7.1 Sertoli cell tumor

Out of two cases, one was from a 13 years old male Dachshund and was in the right testicle (Fig.84). Cystic dilatation of the left scrotum with atrophy of the left testicle was noticed. The growth weighed 460 gm and was 26cm in diameter. The second case was from the left inguinal region of a four years old cryptorchid GSD which appeared since four months (Fig.85). The growth was in the testis retained in the inguinal region. The mass weighed 370 gm and 21 cm diameter. The growths were firm, white and lobulated (Fig.86). Upon squeezing a milky secretion exuded from the cut surface. Areas of haemorrhage and necrosis were reflected in the cut section of second case.

Histology of both showed intratubular type of Sertoli cell tumor. Neoplastic Sertoli's cells had a clear elongated cytoplasm and an elongated oval, hyperchromatic, vesicular nucleus. Cells are lined up like palisades, perpendicular to the tubular basement membrane (Fig.87). The abundant stromal

tissues provoked a pseudotubular pattern in which the neoplastic cells palisaded. The adjacent neoplastic tubules like structures were separated by prominent coarse bands of collagenous stroma. (Fig.88). Massive areas of interstitial as well as intraluminal haemorrhages and necrotic areas were present. Haemorrhagic and necrotic areas was absent in the first case.

4.7.7.2 Transmissible Venereal Tumor

Grossly nodular masses were seen protruding from the surface of the vulva (Fig.89) or penis (Fig.90). The surface was ulcerated and friable with a smooth appearance.

Cytologically when stained with Wright - Giemsa stain the tumor was highly cellular with a homogenous population of round to slightly polyhedral cells arranged in a sheet like pattern. Anisocytosis and anisokaryosis were observed. The nucleus was round to oval and centrally placed with reticulated chromatin. The cytoplasm showed distinct vacuoles (Fig. 91). The nuclear to cytoplasmic ratio was high and mitotic figures were prominent.

4.7.8 Mammary Tumors of dogs

A total of 18 cases of mammary tumors were obtained. Among these 11 cases (61.1 percent) were benign tumors, two cases were malignant tumors (11.1 percent) and five cases (27.8 percent) were mammary sarcomas.

Among mammary glands, the abdominal pairs, i.e., third and fourth pairs, especially the fourth pair was the most affected. Second largest involvement was seen in the inguinal glands, i.e., the fifth pair and thoracic pairs (first and second pairs) held the third position of involvement.

4.7.8.1 Benign Mammary Tumors.

4.7.8.1.1 Benign Mixed Mammary Tumors

Seven cases of benign mixed mammary tumors were obtained. Grossly five tumors had a firm consistency, creamy or grayish cut surface with focal areas

of haemorrhage in some. Two cases were encapsulated hard bony masses with a gritty feel to cut.

The histology of the bony masses revealed extensive formation of well formed cartilage and bone with low cellularity (Fig.92). Proliferation of myoepithelial cells was seen. No glandular structures were found. Cross sections of small capillaries were present indicating the high vascularity of the tumor. Well formed osteoid tissues were lined by deeply basophilic and plumb osteoblasts. The transition stages of myoepithelial cells to cartilage and that of cartilaginous components to bone were evident.

Histologically in other sections, proliferation of luminal epithelial cells and myoepithelial cells mixed with cartilage producing mesenchymal cells appearing as bluish areas were evident in the H & E sections (Fig.93). In some sections, glandular proliferations were evident while in others, the lining intraductal epithelium formed papillary infoldings along with cartilage formation.

4.7.8.1.2 Simple Adenoma

Two cases of simple adenoma revealed encapsulated firm masses with white cut surface. Microscopically one was a case of myoepithelioma which showed a predominant proliferation of spindle shaped myoepithelial cells disorderly arranged in the form of interlacing bundles (Fig.94). Histology of the second mass showed proliferation of the glandular elements without any stromal invasion and the glands were lined by single layer of low cuboidal epithelium (Fig.95).

4.7.8.1.3 Complex Adenoma

Grossly an elongated nodular firm mass with 12 cm diameter and weighing 67 gm with a cystic cavity was present in the mammary gland of a GSD involving the caudal thoracic and cranial abdominal glands.

Histologically proliferation of both glandular and myoepithelial cells were present (Fig.96). The spindle shaped cells of myoepithelium were arranged in a reticulated pattern.

4.7.8.1.4 Fibroadenoma

Grossly a large ulcerating growth was present in the caudal abdominal mammary gland of a GSD. The growth was firm in consistency with blood oozing from the whitish cut surface (Fig.97).

Histology showed acinar cells of varying size and shape in the stroma of proliferating neoplastic fibroblasts (Fig.98). Ulceration of the overlying epidermis and leucocytic infiltration in the neoplastic tissue was also present.

4.7.8.2 Malignant Mammary Tumors

4.7.8.2.1 Simple Carcinoma

Two cases of simple carcinomas showed encapsulated firm masses with surface ulcerations in one case (Fig.99). Both weighed an average of 110 gm and 21 cm diameter.

Histology of one revealed simple solid carcinoma wherein the neoplastic cells were arranged in solid sheets or cords with small amount of stroma (Fig.100). There was a substantial reduction in the amount of glandular tissue. A thick collagenous covering was also present. Abundant mitotic figures of about 5 - 6 per HPF were present. The second case was of simple tubulopapillary type which was characterized by the formation of tubules with papillary projections into the lumen (Fig.101). Proliferation of glandular components was also present. The stroma was scanty and the entire parenchyma was invaded by neoplastic cells.

4.7.8.3 Mammary Sarcomas

Five cases of carcinosarcomas were obtained

4.7.8.3.1 Fibrosarcoma

Grossly this was in the form of a firm mass in the abdominal pairs of mammary glands with whitish cut surface and focal areas of congestion (Fig.102). Histologically proliferation of both epithelial and myoepithelial cells and absence of glandular cells were observed. High vascularity was seen. The spindle cell type of myoepithelium formed collagenous fibres and some of these fibres were concentrically arranged around proliferating blood vessels imparting a hemangiopericytoma like pattern (Fig.103). The fibroblast and collagen were arranged haphazardly (Fig.104).

4.7.8.3.2 Osteosarcoma

Grossly a lobulated soft mass having white cut surface with focal areas of haemorrhage was noticed. It weighed 120 gm and had a diameter of 17 cm. Histology showed spicules of osteoid produced by tumor cells. A combination of bone, fibrous and cartilagenous components were found. High vascularity was also a feature (Fig.105). Mitotic figures were moderate in number.

4.7.8.3.3 Carcinosarcoma

Three cases of carcinosarcomas were found. The growths were firm or hard and in one case necrotic areas were present at the centre. In one case the growth was obtained from the inguinal mammary gland during necropsy (Fig.106), the lungs of which showed multiple nodular growths (Fig.107).

Histologically the growth obtained during necropsy revealed proliferation of myoepithelial cells with a border line malignancy and metaplasia to chondroblasts and bone were noticed (Fig.108). In the lungs, metastatic undifferentiated carcinoma with areas of cartilaginous metaplasia was seen (Fig.109). The lining cells were of different cell types, namely columnar,

cuboidal and squamous cell types. Mitotic figures and tumor giant cells were also seen.

In the other two cases intraductal carcinoma with multiple layers of ductular epithelium thrown into papillary infoldings of several cell thick was noticed. Extensive bone formations were also present (Fig.110). Cellular and nuclear pleomorphism was evident.

4.8 APOPTOTIC INDEX (AI)

The mean values of apoptotic index for benign tumors were 15.79 ± 2.34 and that of malignant tumors was 11.59 ± 0.86. Though the value of benign tumors were higher than that of malignant tumors, no significant difference existed between this values since $p<0.05$

4.9 MITOTIC INDEX (MI)

The mean values of mitotic index for benign tumours were 10.93 ± 1.51, while that of malignant tumors was 32.82 ± 4.39. A significant difference existed between this values since $p>0.05$.

4.10 ARGYROPHILIC NUCLEOLAR ORGANIZER REGION COUNTS (AgNOR counts)

AgNOR staining showed the presence of multiple AgNOR dots in the nuclei of various neoplastic cells, which appeared as dark brown or black dots in yellow background (Fig.111 to Fig.114).

The mean AgNOR count of the benign tumours was 234.43±11.81 and that of malignant tumours were 359 ± 22.46. A significant difference existed between this values at the level of $p>0.05$.

All the values were tabulated in the tables 11 and 12

Karl Pearson coefficient of correlation between mitotic indices, apoptotic indices and AgNOR counts of the tumors were worked out. MI and AgNOR

counts of the tumors are highly correlated (r =0.949(**))*. But no correlation was found to exist between MI and AI and also between AI and AgNOR counts.

(**) - Highly significant

* Karl Pearson's Coefficient of Correlation (r)

Table 11. Mitotic indices (MI), apoptotic indices (AI) and AgNOR counts of benign tumors

No.	TUMOR	MI (per 10 HPF)	AI (per 10 HPF)	AgNOR COUNTS (per cell)
1	Sebaceous adenoma	10 ± 1.51	21 ± 2.34	238 ± 11.81
2	Sebaceous epithelioma	21 ± 1.51	28 ± 2.34	322 ± 11.81
3	Melanoma	10 ± 1.51	8 ± 2.34	201 ± 11.81
4	Hepatoid gland adenoma	11 ± 1.51	24 ± 2.34	216 ± 11.81
5	Fibroma	4 ± 1.51	1 ± 2.34	190 ± 11.81
6	Neurofibroma	12 ± 1.51	14 ± 2.34	220 ± 11.81
7	Epulis	5 ± 1.51	27 ± 2.34	188 ± 11.81
8	Sertoli cell tumor	12 ± 1.51	16 ± 2.34	239 ± 11.81
9	Adenoma of nictitans gland	15 ± 1.51	24 ± 2.34	269 ± 11.81
10	Benign mixed mammary tumor	9 ± 1.51	14 ± 2.34	231 ± 11.81
11	Complex adenoma of mammary gland	7 ± 1.51	11 ± 2.34	214 ± 11.81
12	Simple adenoma	14 ± 1.51	22 ± 2.34	262 ± 11.81
13	Fibroadenoma	2 ± 1.51	4 ± 2.34	178 ± 11.81
	Mean values	**10.93 ± 1.51**	**15.79 ± 2.34**	**234.43 ± 11.81**

Table 12. Mitotic indices (MI), apoptotic indices (AI) and AgNOR counts of malignant tumors

No.	TUMOR	MI (per 10 HPF)	AI (per 10 HPF)	AgNOR COUNTS (per cell)
1	Hepatoid gland carcinoma	22 ± 4.39	11 ± 0.86	301 ± 22.46
2	Ceruminous gland carcinoma	16 ± 4.39	8 ± 0.86	280 ± 22.46
3	Eccrine carcinoma	10 ± 4.39	11 ± 0.86	188 ± 22.46
4	Liposarcoma	35 ± 4.39	11 ± 0.86	400 ± 22.46
5	MFH	24 ± 4.39	10 ± 0.86	332 ± 22.46
6	Haemangiosarcoma	21 ± 4.39	7 ± 0.86	330 ± 22.46
7	ALL - Liver	52 ± 4.39	12 ± 0.86	490 ± 22.46
	ALL - Spleen	68 ± 4.39	18 ± 0.86	568 ± 22.46
8	Multicentric lymphoma	21 ± 4.39	18 ± 0.86	341 ± 22.46
9	Hepatocellular carcinoma	54 ± 4.39	12 ± 0.86	425 ± 22.46
10	Cholangiocellular carcinoma	49 ± 4.39	12 ± 0.86	398 ± 22.46
11	Pancreatic carcinoma	52 ± 4.39	11 ± 0.86	426 ± 22.46
12	Osteosarcoma	25 ± 4.39	14 ± 0.86	380 ± 22.46
13	Simple solid carcinoma of mammary gland	55 ± 4.39	17 ± 0.86	412 ± 22.46
14	Simple carcinoma of mammary gland	24 ± 4.39	11 ± 0.86	281 ± 22.46
15	Fibrosarcoma of mammary gland	14 ± 4.39	6 ± 0.86	263 ± 22.46
16	Osteosarcoma of mammary gland	16 ± 4.39	8 ± 0.86	288 ± 22.46
17	Carcinosarcoma	21 ± 4.39	7 ± 0.86	314 ± 22.46
	Mean values	**32.82 ± 4.39**	**11.59 ± 0.86**	**359 ± 22.46**

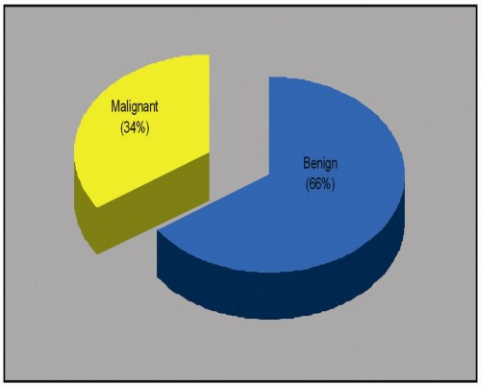

Fig.1
Benign and malignant proportion of tumors

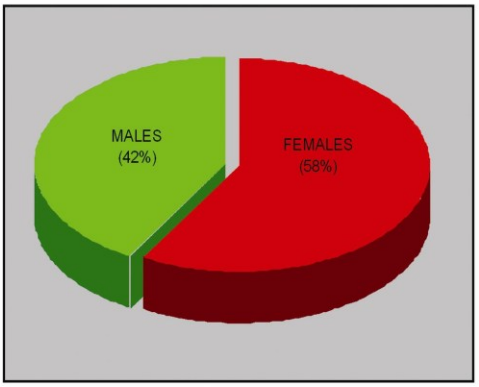

Fig. 2
Sex wise distribution of tumors

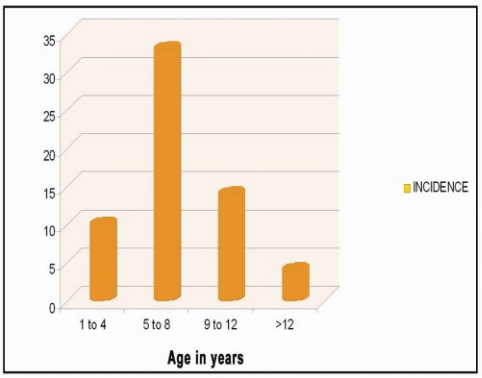

Fig. 3
Age wise distribution of tumors

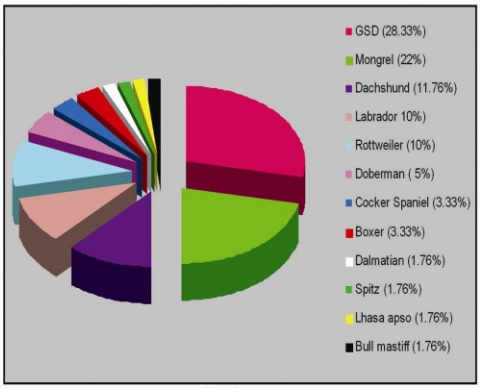

Fig. 4
Breed wise distribution of tumors

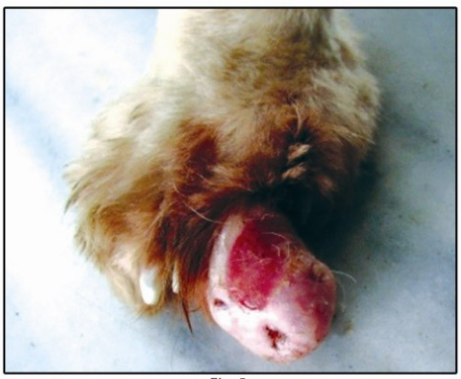

Fig. 5

Sebaceous adenoma. Solitary hard mass in the interdigital space

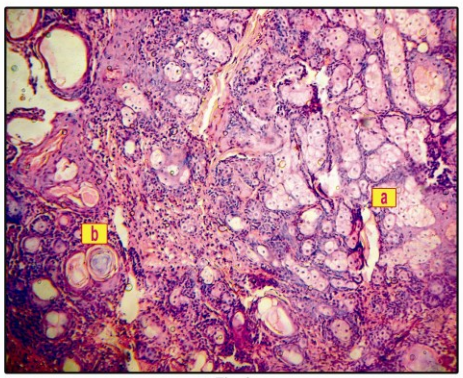

Fig. 6

Sebaceous adenoma. Multiple groups of mature sebocytes with fewer basaloid cells(a) and a few keratin nests(b) (H&E x 100)

Fig. 7

Sebaceous epithelioma. Nodular ulcerative growth in the ear pinna of a Cocker Spaniel.

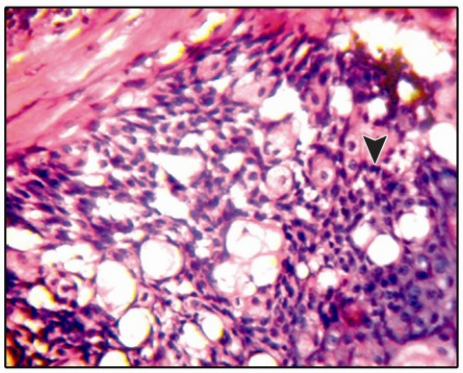

Fig. 8

Sebocytes arranged individually and in small clusters with preponderance of basaloid reserve cells.Mitotic figures(arrow head) (H&E x400)

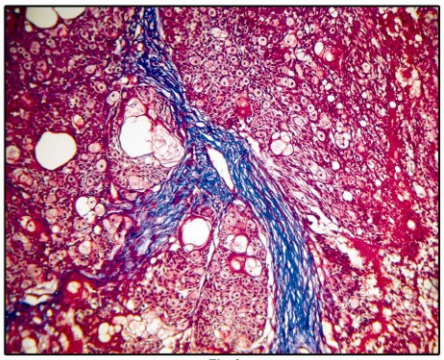

Fig. 9

The stromal collagen in blue colour in sebaceous epithelioma. (Masson's trichrome x100)

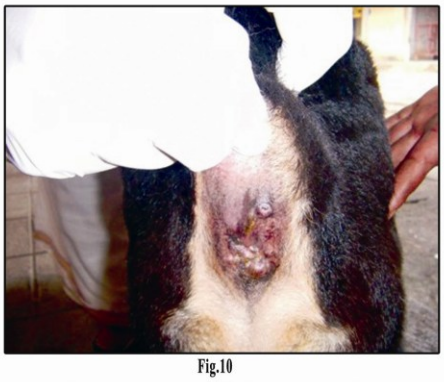

Fig.10

Hepatoid gland carcinoma. Multiple nodular growths in the perianal region of a Rottweiler.

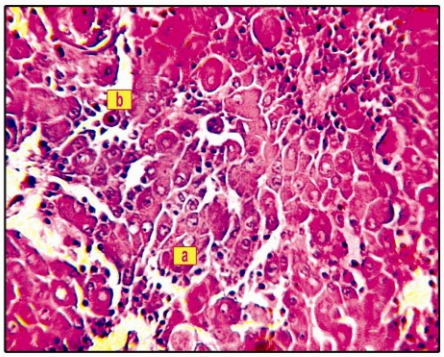

Fig. 11

Hepatoid gland carcinoma. Irregular arrangement of hepatoid cells(a) with vacuolated cytoplasm and prominent nuclei(b)(H&E x 400)

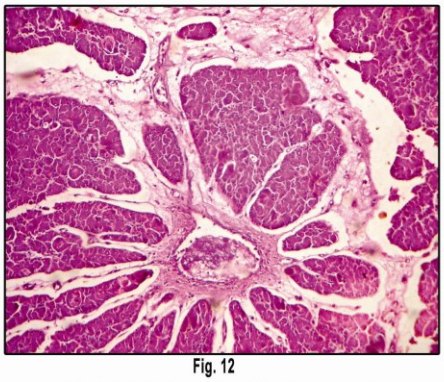

Fig. 12

Hepatoid gland adenoma. Hepatoid cells are arranged in clusters and an abundance of interlobular stroma. (H&E x 100)

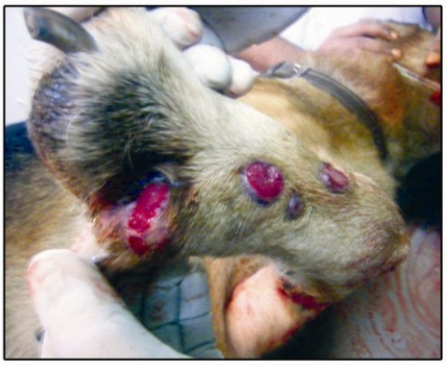

Fig. 13

Fig. 14

Eccrine carcinoma. Swelling with ulceration of the overlying epidermis in the footpad and hindlimb

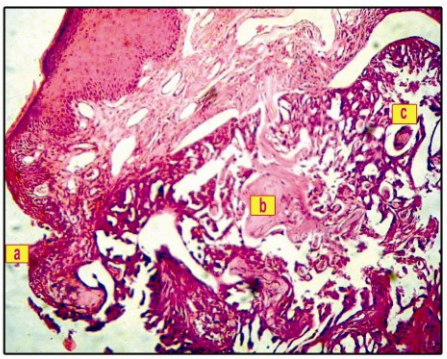

Fig. 15

Eccrine carcinoma. Epidermal ulceration(a), foci of keratinization(b), and eosinophilic secretions in the lumen(c) (H&E x 100).

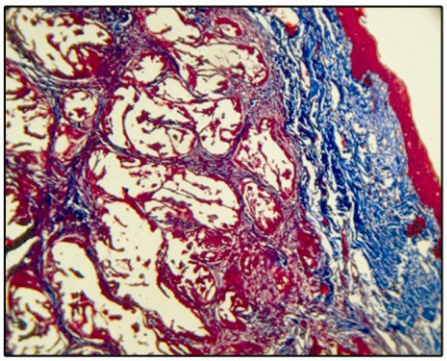

Fig. 16

Eccrine carcinoma. Collagen strands dividing the glandular lumen. (Masson's trichrome x 100)

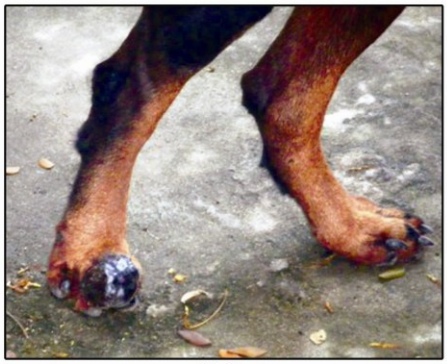

Fig. 17

Melanocytoma.Solitary black coloured growth in the interdigital space of a Rottweiler.

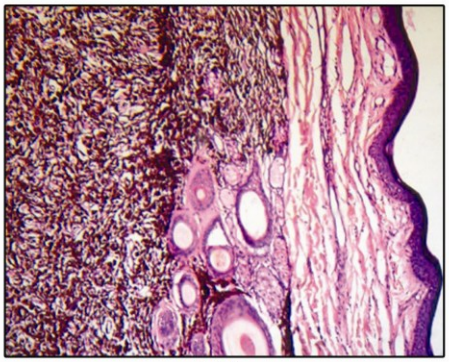

Fig. 18

Melanocytoma. Spindle shaped neoplastic cells with intracytoplasmic melanin granules. Epidermis is intact. (H&E x 100)

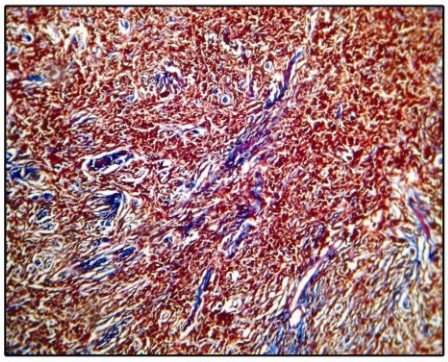

Fig. 19

Melanocytoma. Collagenous stroma separating the neoplastic cells appears blue. (Masson's trichrome x 100)

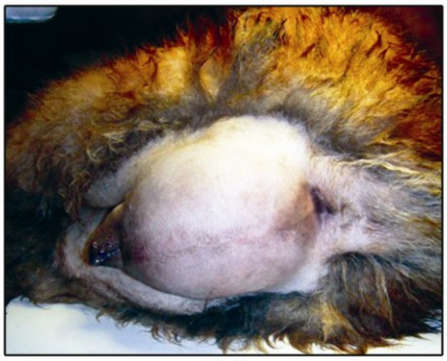

Fig. 20

Fibroma. Firm subcutaneous growth in the vaginal region of a bitch.

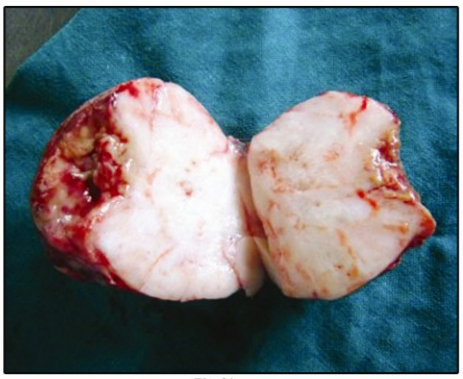

Fig. 21

Cut surface of the fibroma showing whitish glistening appearance.

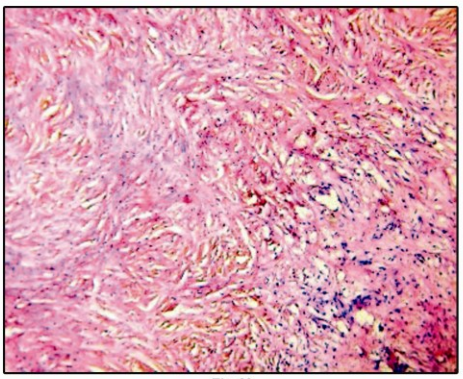

Fig. 22

Interlacing bundles of collagen and fibroblasts running in different directions and presence of inflammatory cells (H & E x400)

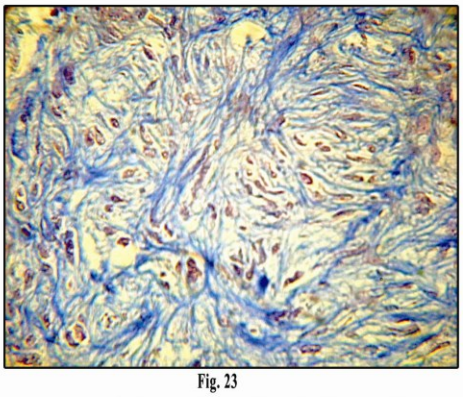

Fig. 23

Abundance of collagen in fibroma.(Masson's trichrome x 400)

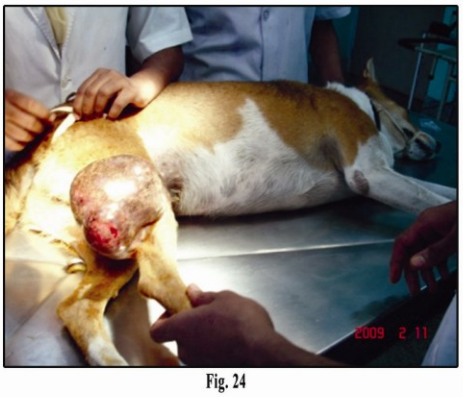

Fig. 24

Neurofibroma. A well circumscribed lobulated growth on the lateral aspect of the right hind limb, just above the hock joint

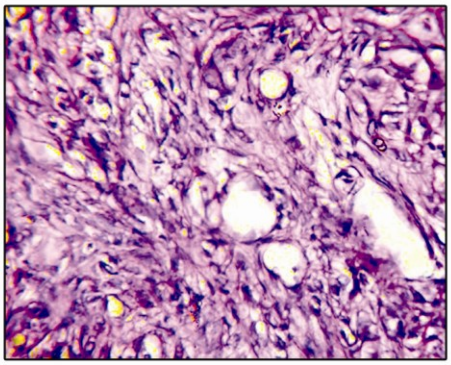

Fig. 25
Neurofibroma. Spindle cells arranged in wavy bundles and partial whorls with moderately low cellularity. (H & E x 400)

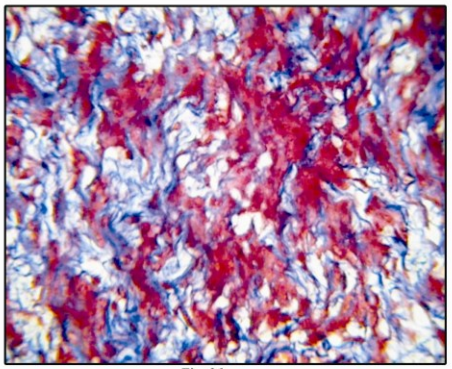

Fig. 26
Neurofibroma. Moderate amount of delicate blue collagen fibres and low cellularity. (Masson's trichrome x 400)

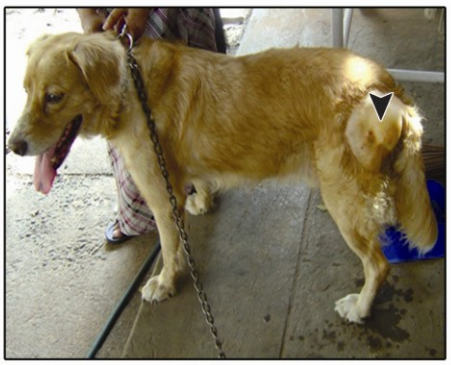

Fig. 27
Liposarcoma. Soft raised subcutaneous growth in the perineal region.

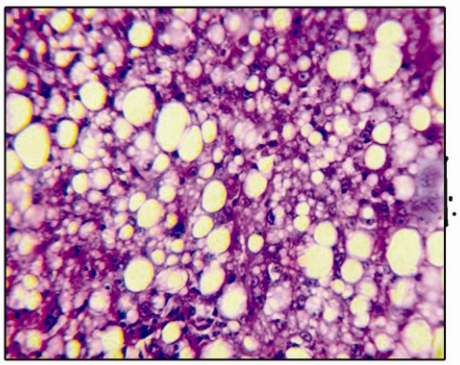

Fig. 28
Liposarcoma. Well differentiated liposarcoma with adipocytes containing lipid vacuoles of varying size. (H & E x400)

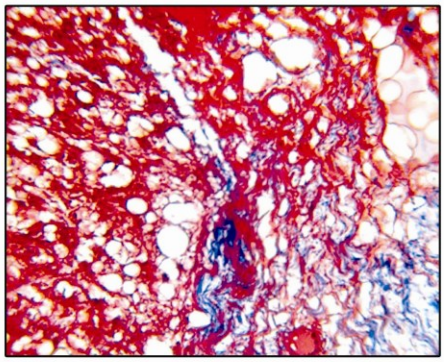

Fig. 29

The blue collagenous stroma was scanty in liposarcoma.
(Masson's trichrome x 100)

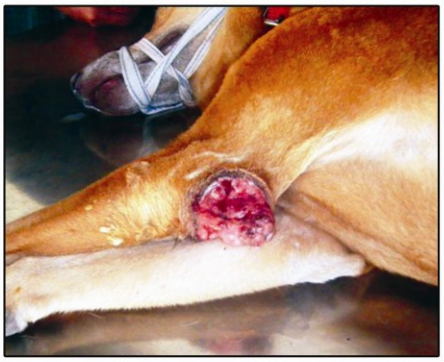

Fig. 30

Malignant fibrous histiocytoma. An unencapsulated growth with distinct margin on the elbow joint.

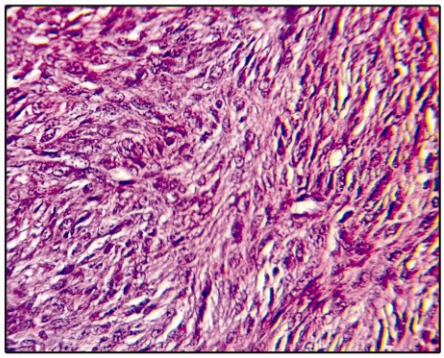

Fig.31

Malignant fibrous histiocytoma. Storiform pattern of arrangement of cells. (H&E x 400)

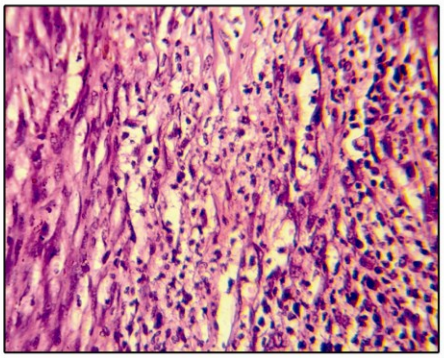

Fig. 32

Presence of inflammatory cells in malignant fibrous histiocytoma (H&E x 400)

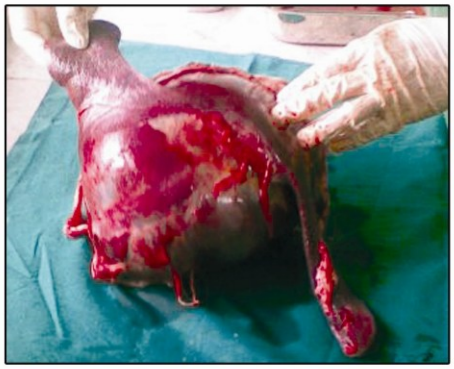

Fig. 33

Hemangiosarcoma. Large firm brown colored growth attached to the hilus portion of the spleen.

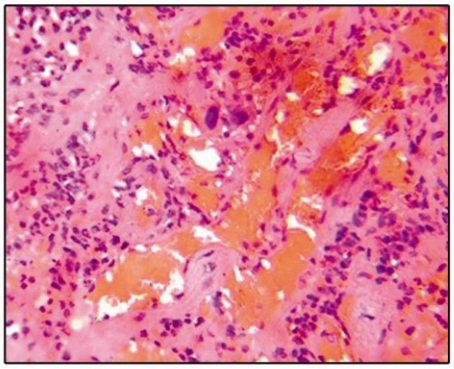

Fig.34

Hemangiosarcoma. Pleomorphic neoplastc cells lining the vascular clefts in the spleen. (H & E x400)

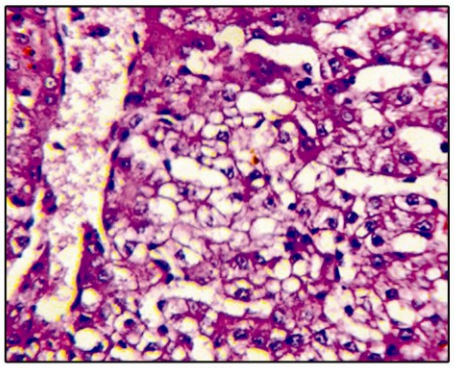

Fig. 35

Hemangiosarcoma. Acute cell swelling and severe fatty change in the liver. (H & E x400)

Fig. 36

Osteosarcoma. Growth extending from the middle of the forearm down to the distal end of metacarpals in a Boxer.

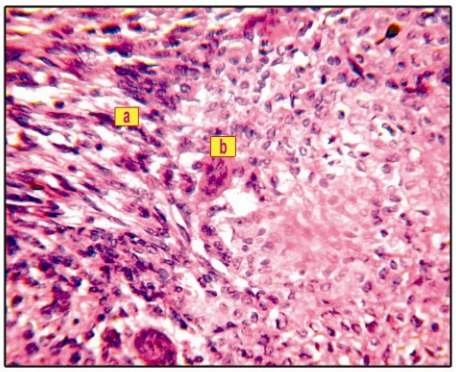

Fig. 37

Osteosarcoma. Highly cellular with pleomorphic osteoblasts(a) and multinucleated giant cells(b). (H&E x 400)

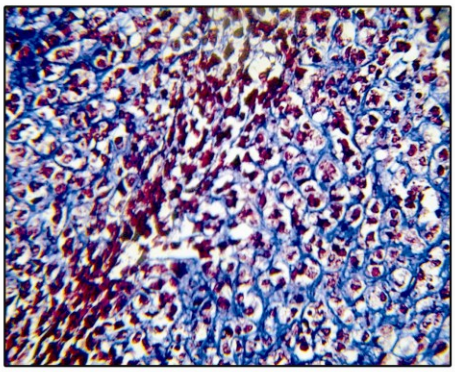

Fig. 38

Delicate starnds of osteoid produced in osteosarcoma (Masson's trichrome x 400)

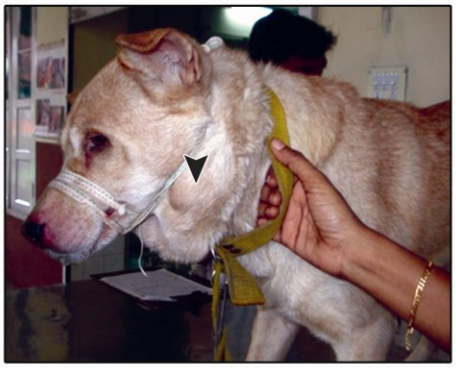

Fig. 39

Mandibular lymphadenopathy in a lymphosarcoma case.

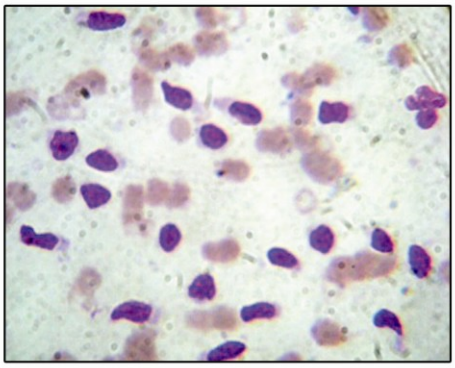

Fig. 40

Lymphosarcoma. Pleomorphic lymphocytes in the lymph node aspirate smear. (Leishman's stain x 1000)

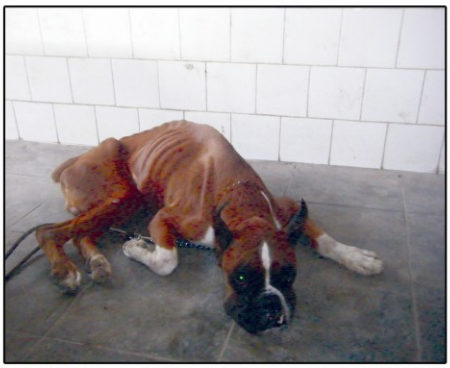

Fig. 41

Acute Lymphoblastic Leukemia(ALL). Weak and emaciated appearance.

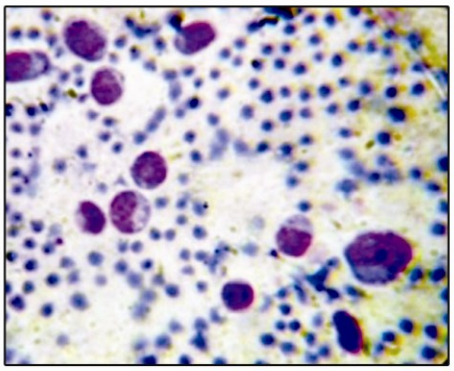

Fig. 42

ALL. Blood smear showing atypical blast series of lymphocytes. (Leishman's stain x 1000)

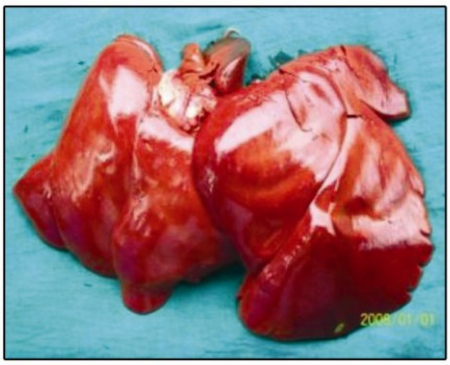

Fig. 43

Hepatomegaly and congestion of the liver in ALL

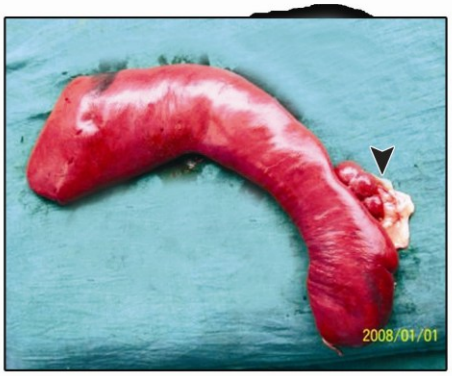

Fig. 44

Splenomegaly and pea sized nodules along the margin in ALL.

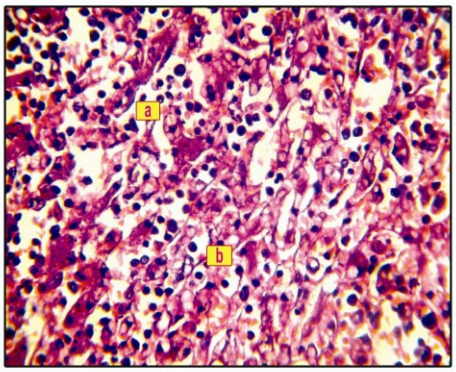

Fig. 45

Dialatation of sinusoids with infiltrating lymphoblast series of cells(a) in ALL.Hepatocytes showed varying degrees of vacuolar degeneration(b). (H&E x 400)

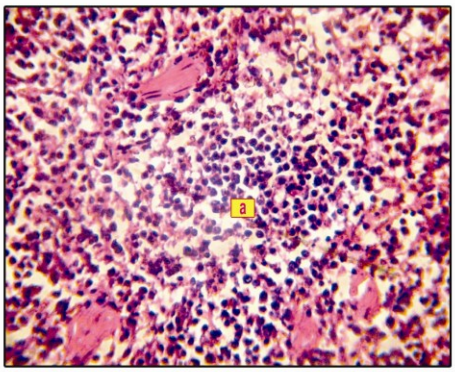

Fig. 46

ALL . Massive infiltration of spleen with lymphoblast series of cells(a) and the red pulp was obliterated. (H&E x 400)

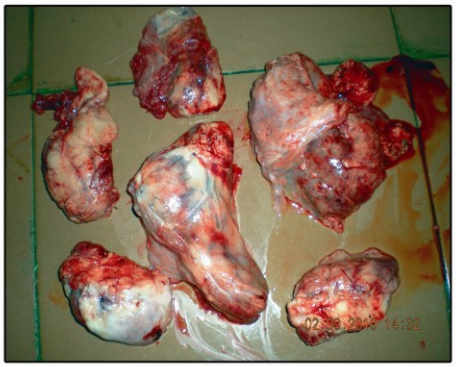

Fig. 47

Multicentric lymphoma. Enlargement of peripheral lymphodes.

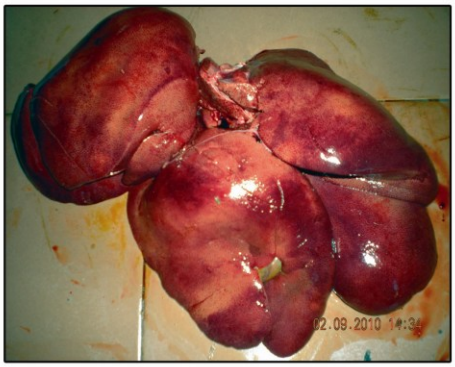

Fig. 48

Hepatomegaly in multicentric lymphoma.

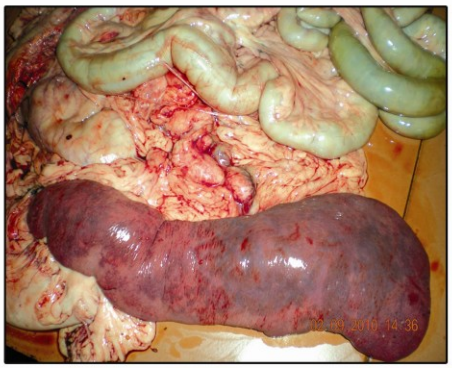

Fig. 49

Splenomegaly in multicentric lymphoma.

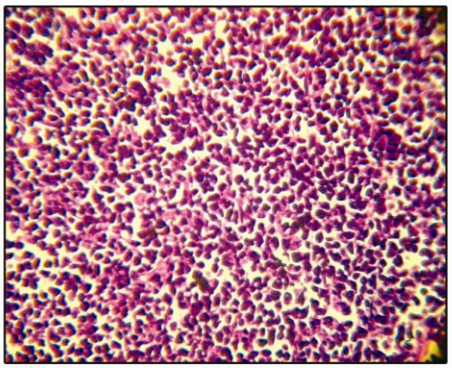

Fig. 50

Invasion of the lymphnodes with monomorphic cells and effacement of the normal architecture in multicentric lymphoma.(H&E x 400)

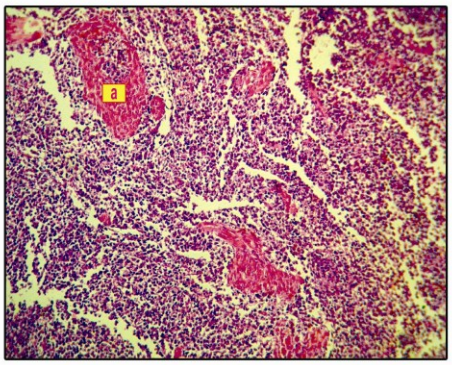

Fig. 51

Multicentric lymphoma. Diffuse spleenic involvement and subendothelial lymphocytic colonization within the fibromuscular trabeculae. (H&E x 400)

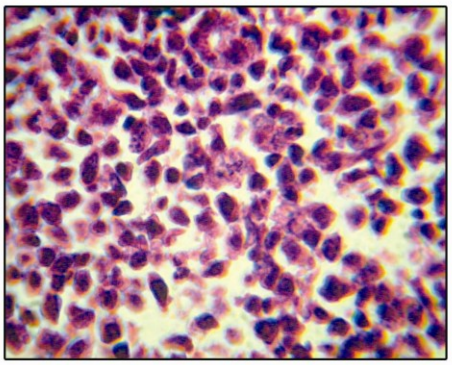

Fig. 52

Multicentric lymphoma. Cells are uniformly small and cleaved (H&E x 1000)

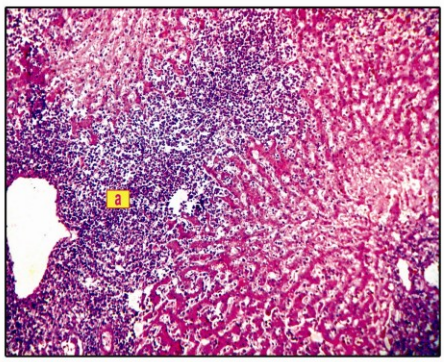

Fig. 53

Multicentric lymphoma. Congregation of neoplastic cells around the portal triads in liver (a). (H&E x 100)

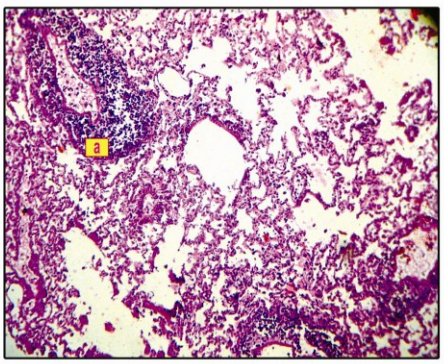

Fig. 54

Multicentric lymphoma. Perivascular aggregation of neoplastic cells in the lungs (a). (H&E x 100)

Fig. 55

Ossifying epulis. Growth in the lower jaw.

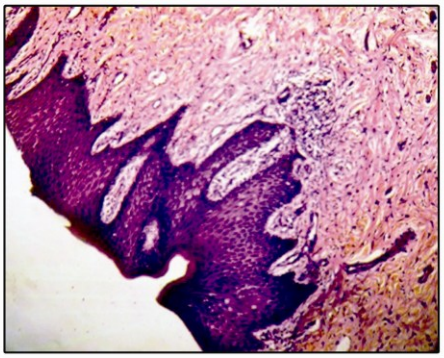

Fig. 56

Rete pegs of the overlying epithelium in ossifying epulis. (H&E x 100)

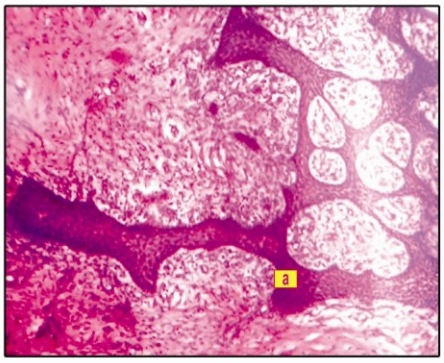

Fig. 57

Mature lamellated bone(a) and fibroblasts in the deeper areas of ossifying epulis. (H&E x 100)

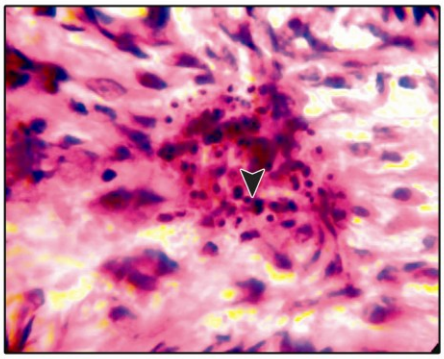

Fig.58

Cluster of apoptotic bodies(arrow head) in the section of ossifying epulis. (H&E x 1000)

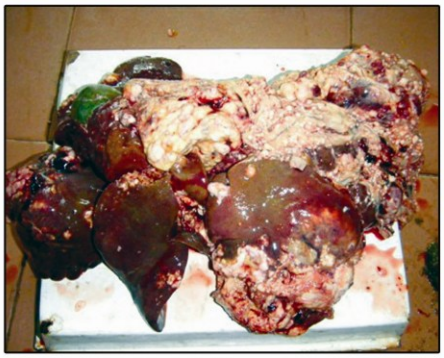

Fig. 59

Hepatocellular carcinoma. Hepatomegaly and diffuse large necrotic areas in the liver.

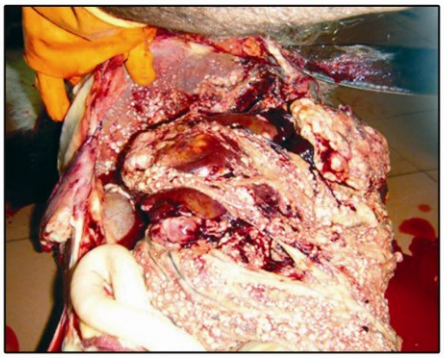

Fig. 60

Metastatic nodular growths in the diaphragm and peritoneal folds in hepatocellular carcinoma

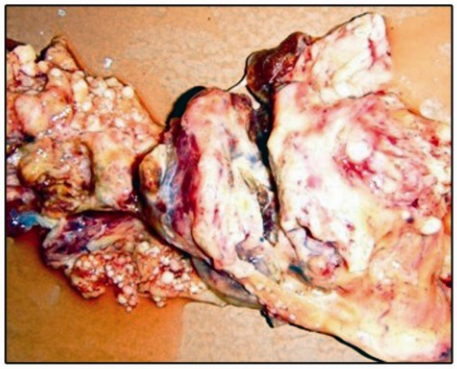

Fig.61
Enlarged mesentric lymph node with irregular borders and grittiness in hepatocellular carcinoma.

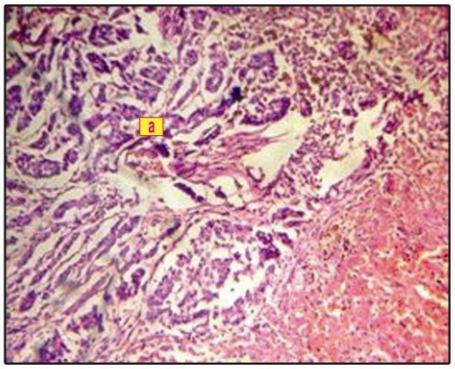

Fig. 62
Trabecular pattern of arrangement of neoplastic cells(a) among the hepatocytes in hepatocellular carcinoma. (H&E x 100)

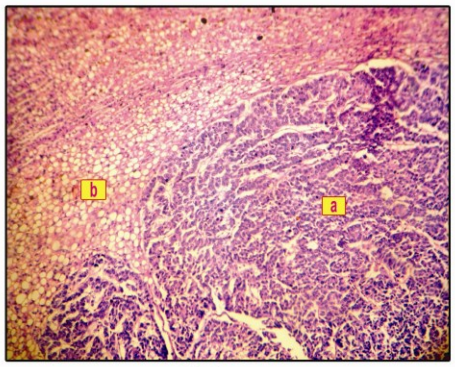

Fig. 63
Hepatocellular carcinoma. Neoplastic cells arranged in adenoid pattern(a)and fatty change in the adjoining hepatocytes(b) (H&E x 100)

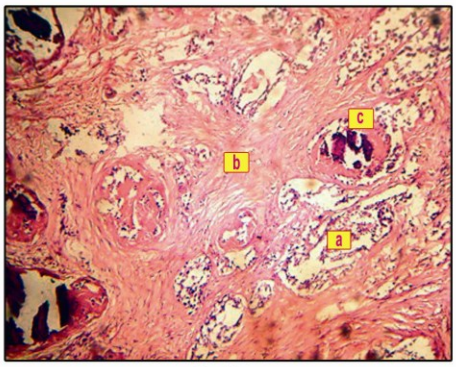

Fig. 64
Hepatocellular carcinoma. Infiltration of neoplastic cells(a), collagenous stroma(b) and areas of calcification(c) in mesentric lymph node. (H&E x 100)

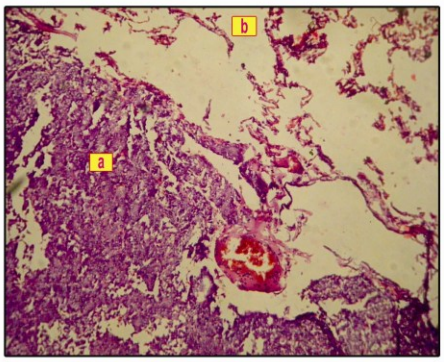

Fig. 65
Hepatocellular carcinoma. Collection of neoplastic cells and extreme emphysema in the lungs. (H&E x 100)

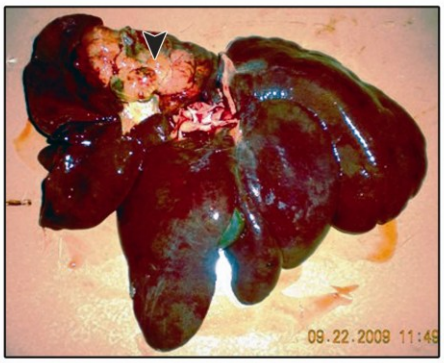

Fig. 66
Cholangiocellular carcinoma. Multiple lobulated growths in the ventral aspect of left lateral lobe.

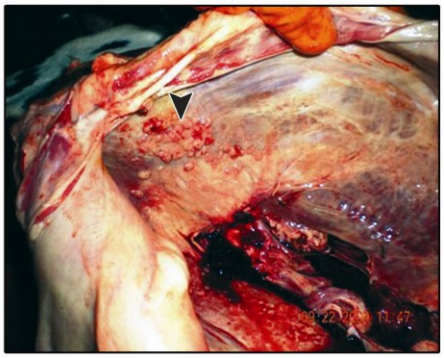

Fig. 67
Nodular growths in the diaphragm in cholangiocellular carcinoma.

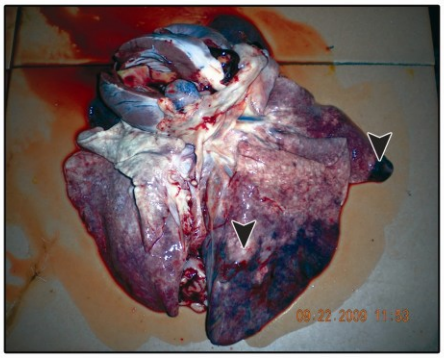

Fig. 68
Nodules in the lungs in cholangiocellular carcinoma.

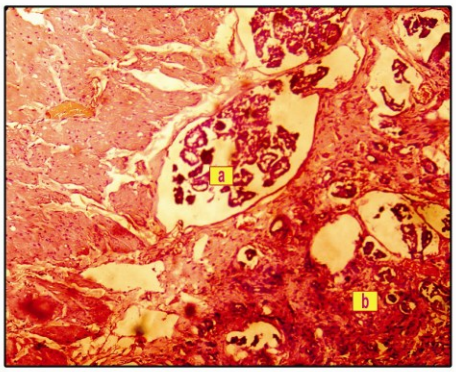

Fig.69
Cholangiocellular carcinoma. Islands of neoplastic cells(a) andfibrous tissue proliferation in the liver(b) (H&E x 100)

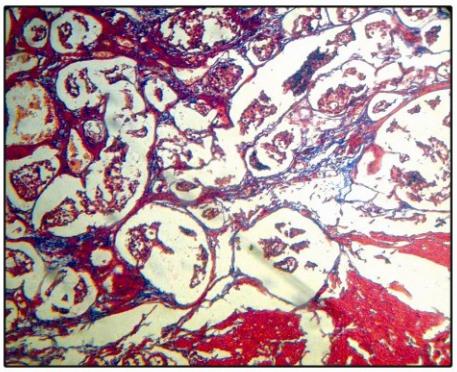

Fig.70
Collagen in the liver in cholangiocellular carcinoma took blue color (Masson's trichrome x 100)

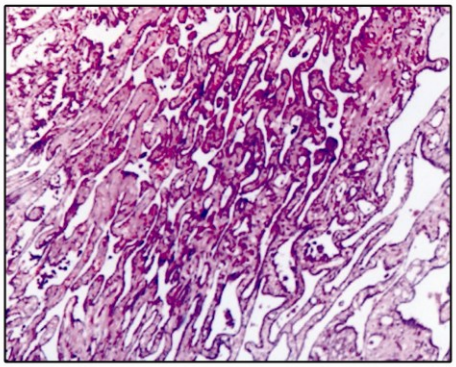

Fig. 71

Papillary infoldings of the biliary tubular epithelium in cholangiocelluar carcinoma.(H&E x 400)

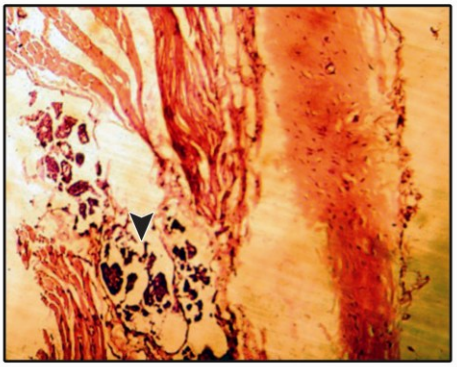

Fig. 72

Clusters of neoplastic cells in the section of diaphragm(arrow head) in cholangiocellular carcinoma. (H&E x 400)

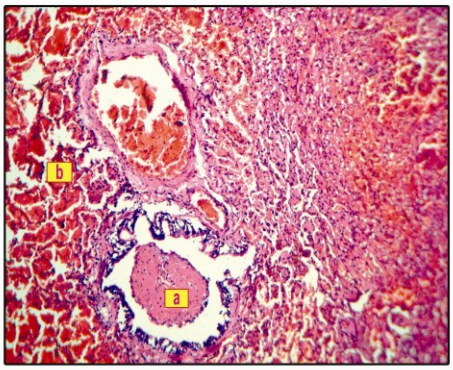

Fig. 73
Cholangiocellular carcinoma. Tumor emboli(a) and areas of congestion in the lungs (b). (H&E x 100)

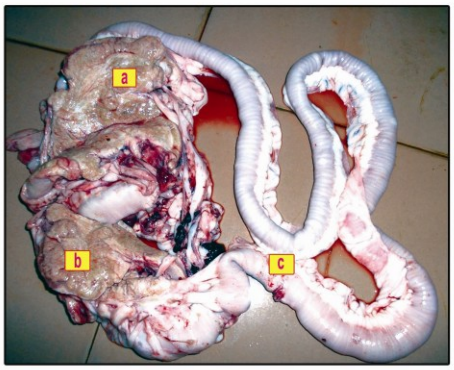

Fig. 74
Pancreatic carcinoma. Cancerous part of the pancreas(a), enlarged lymphnode(b) and adhesion of different parts(c)

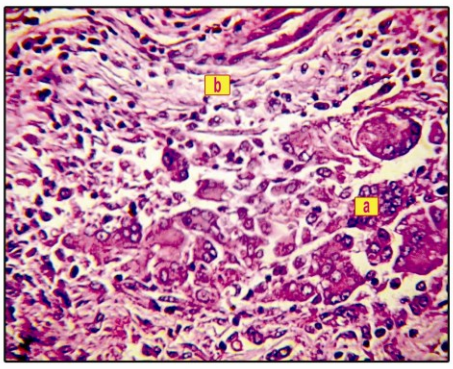

Fig. 75
Undifferentiated pancreatic carcinoma. Nuclear pleomorphism, crowding of the nuclei(a) and dense collagenous stroma(b). (H&E x 400)

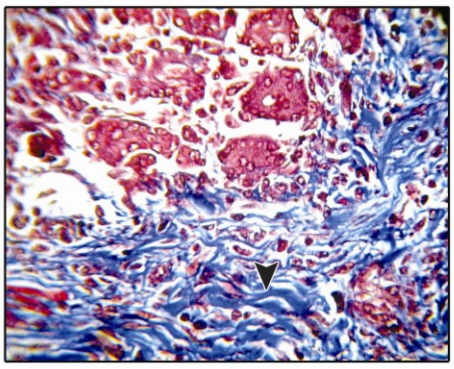

Fig. 76
Dense collagenous stroma(arrow head) in undifferentiated pancreatic carcinoma.(Masson's trichrome x 400)

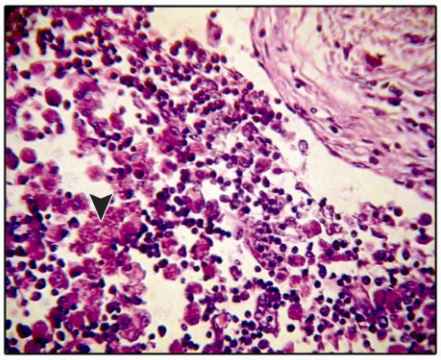

Fig. 77

Reactive pancreatoduodenal lymphnode with infiltration of neoplastic cells(arrow head) in undifferentiated pancreatic carcinoma. (H&E x 400)

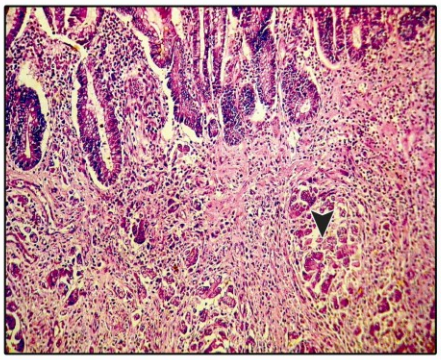

Fig. 78

Infiltration of neoplastic cells in the submucosa of the duodenum (arrow head) in undifferentiated pancreatic carcinoma. (H&E x 100)

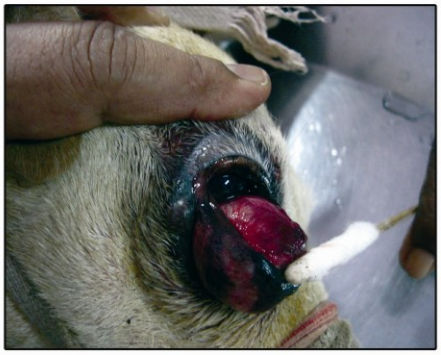

Fig. 79

Adenoma of the nictitans gland. Growth in the inner canthus of the right eye.

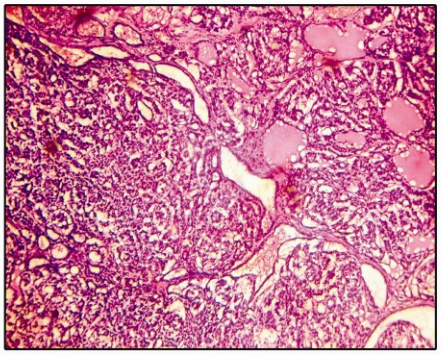

Fig. 80

Adenoma of the nictitans gland. Obliteration of the tubules by the proliferating neoplastic cells and acini with secretions. (H&E x 100)

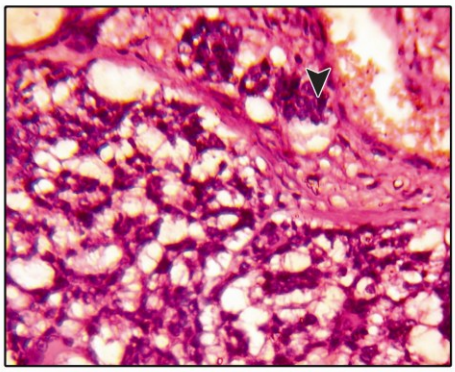

Fig. 81

Stromal invasion of the neoplastic cells (arrow head) in adenoma of the nictitans gland. (H&E x 400)

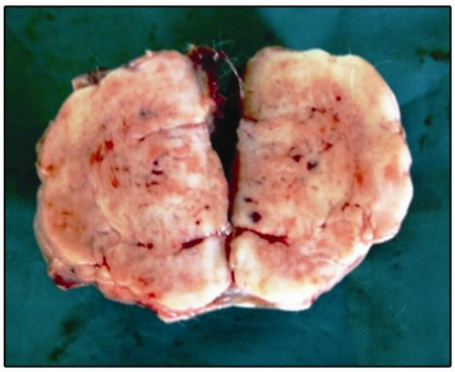

Fig. 82

Cut section of ceruminous gland carcinoma with dark brown areas.

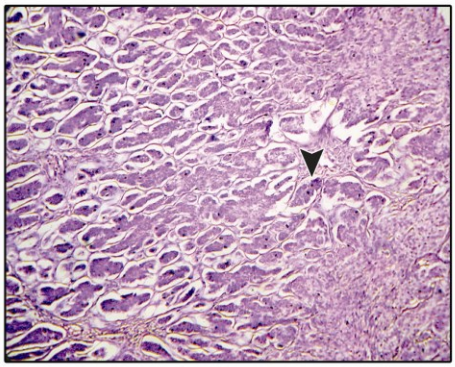

Fig. 83

Glandular lumen lined with multiple layers of epithelial cells and presence of a brown material (arrow head) in ceruminous gland carcinoma (H&E x 100)

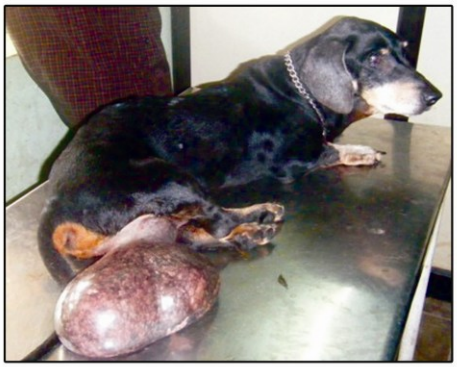

Fig. 84

Sertoli cell tumor in the right testicle of Dachshund.

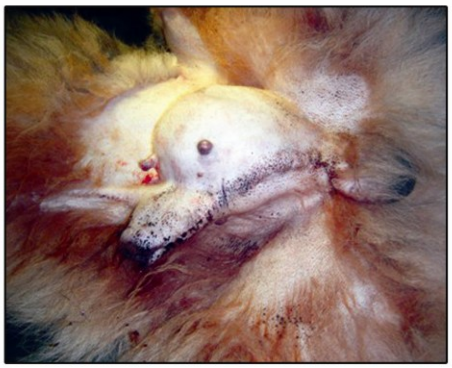

Fig.85

Sertoli cell tumor in the inguinal region of a cryptorchid dog.

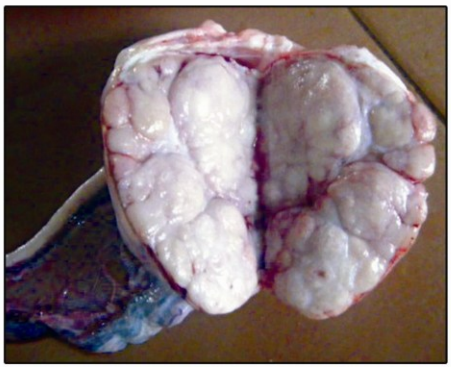

Fig.86

Sertoli cell tumor. Cut surface of the testis; whitish and lobulated.

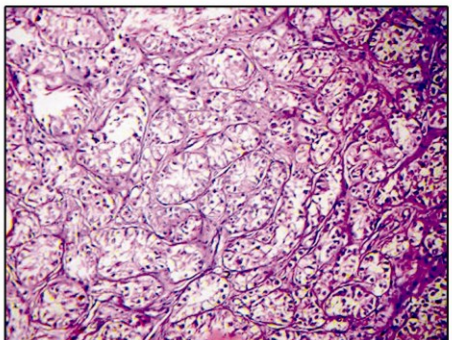

Fig.87

Intratubular presence of neoplastic cells in sertoli cell tumor (H&E x 100).

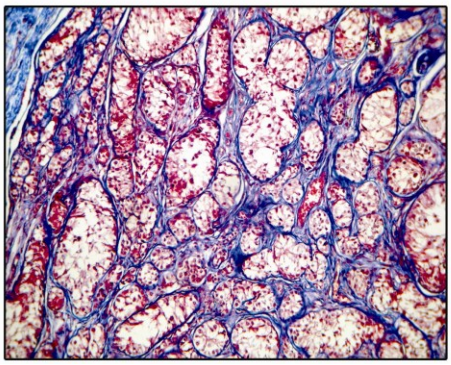

Fig.88

Proliferation of collagenous stroma (blue) in sertoli cell tumor (Masson's trichrome x 100).

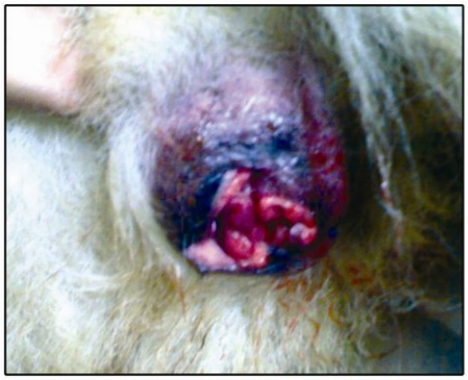

Fig. 89

TVT - Proliferative growths in the vulva

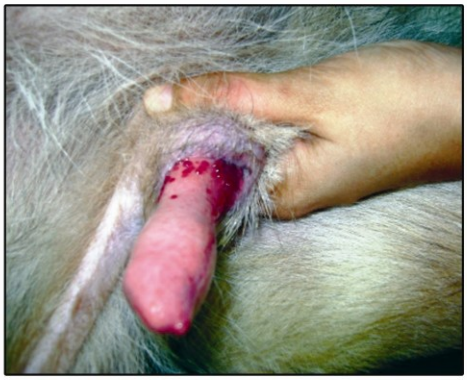

Fig. 90

TVT. Initial stage of growth in the penis.

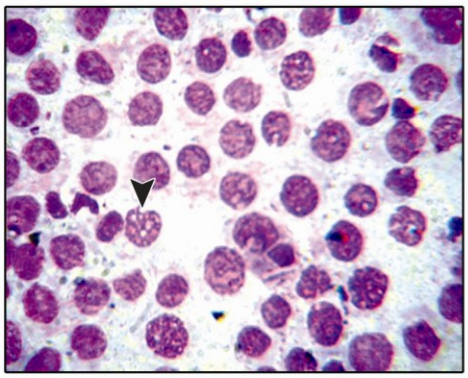

Fig. 91

Impression smear of TVT showing cells with cytoplasmic vacuolation (arrow head) arranged in sheets. (Wright- Giemsa x 1000)

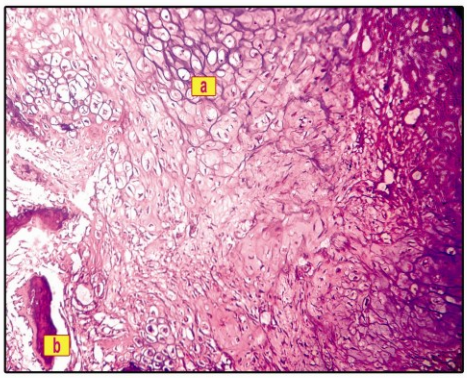

Fig. 92

Benign mixed mammary tumor. Proliferation of myoepithelial cells along with well formed cartilage(b) and bone(a). (H&E x 100)

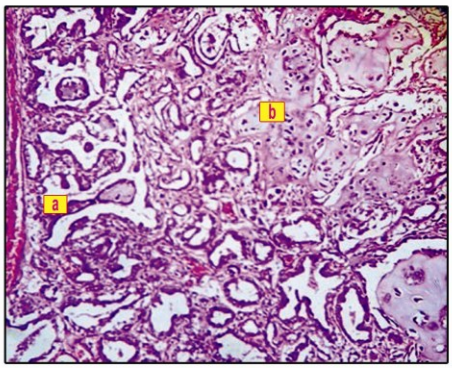

Fig. 93
Benign mixed mammary tumor. Proliferation of luminal epithelial cells(a) and myoepithelial cells and cartilage formation (b). (H&E x 400)

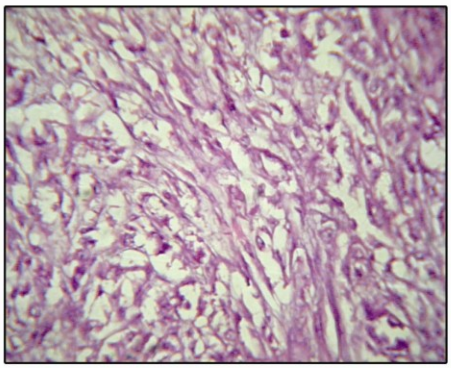

Fig. 94
Simple adenoma - myoepithelioma in the mammary gland with predominent proliferation of spindle shaped myoepithelial cells. (H&E x 400)

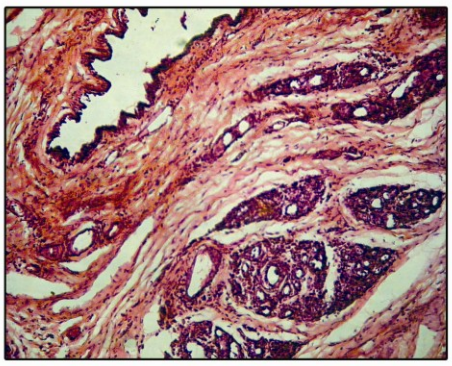

Fig. 95
Simple adenoma of the mammary gland. Glandular proliferation without stromal invasion. (H&E x 400)

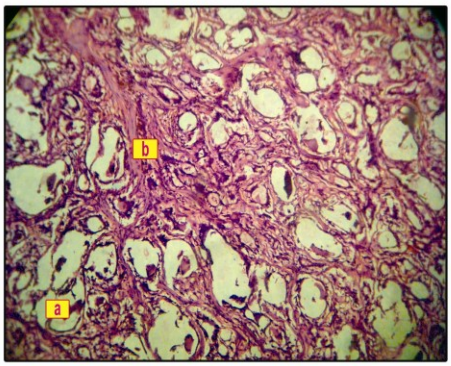

Fig. 96
Complex adenoma of the mammary gland. Proliferation of both glandular (a) and myoepihelial cells (b). (H&E x 100)

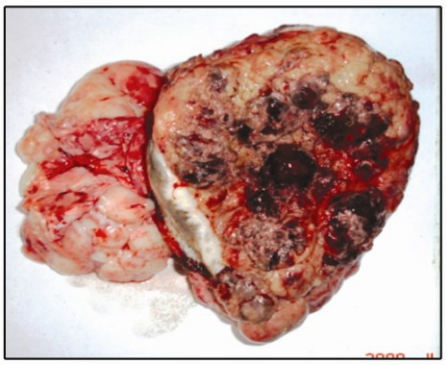

Fig. 97
Fibroadenomatous growth removed from the mammary gland of a bitch.

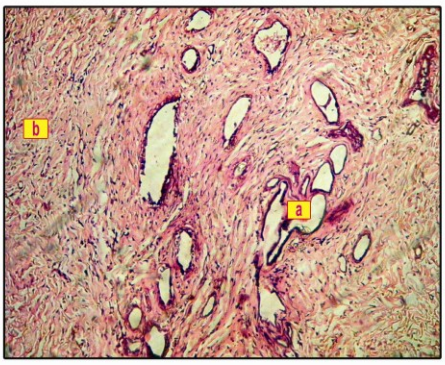

Fig. 98
Acinar cells (a) in the stroma of proliferating fibroblasts (b) in fibroadenoma. (H&E x 100)

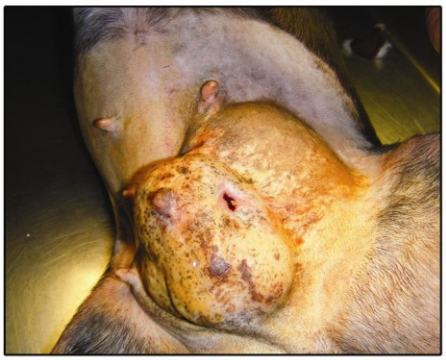

Fig. 99
Solid carcinoma in the inguinal and caudal abdominal mammary glands of a Dachshund.

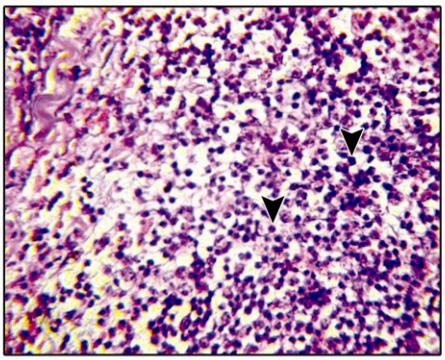

Fig. 100
Replacement of the glandular tissue with solid sheets of neoplastic cells in solid carcinoma. Numerous mitotic figures (arrow head) also present. (H&E x 400)

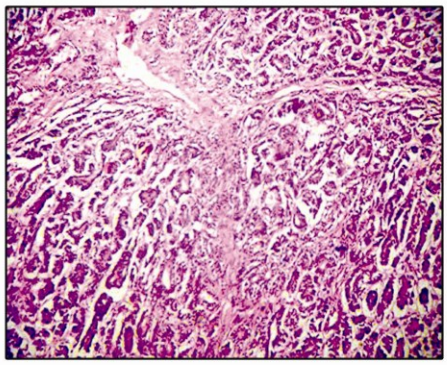

Fig. 101

Simple carcinoma of the mammary gland - tubulopapillary type. (H&E x 100)

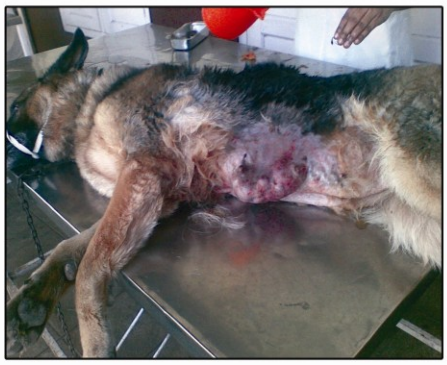

Fig. 102

Fibrosarcoma in the abdominal pairs of mammary gland.

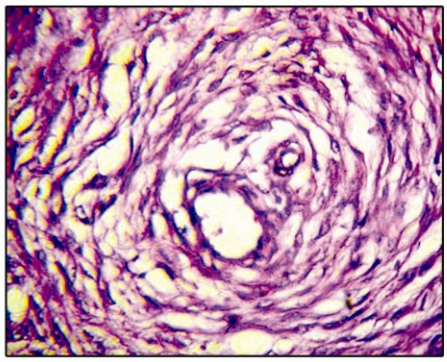

Fig. 103

Fibrosarcoma of mammary gland. Concentric arrangement of collagen fibres around a proliferating blood vessel-hemangiopericytoma like pattern. (H&E x 400)

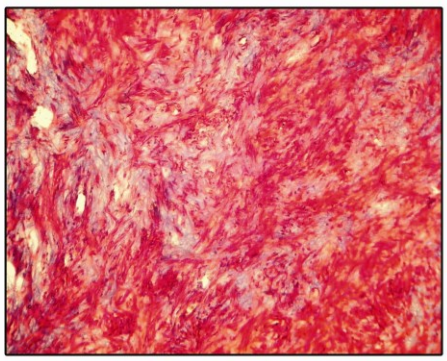

Fig. 104

Fibrosarcoma of mammary gland showing haphazard arrangement of fibroblasts and collagen. (Masson's trichrome x 100)

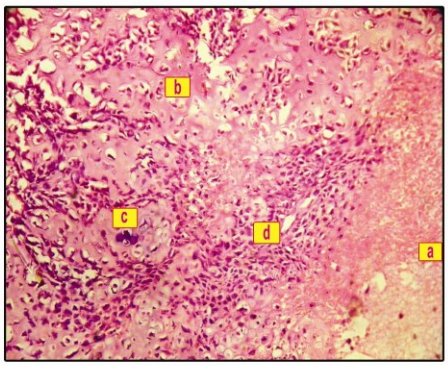

Fig. 105

Osteosarcoma in the mammary gland. High vascularity (a) and combination of osteoid (b), cartilage (c) and fibroblasts (d). (H&E x 100)

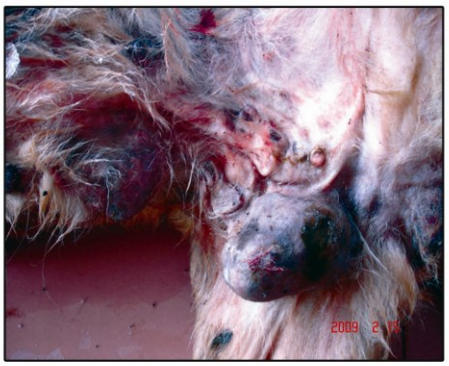

Fig. 106

Carcinosarcoma in the inguinal mammary gland of a bitch.

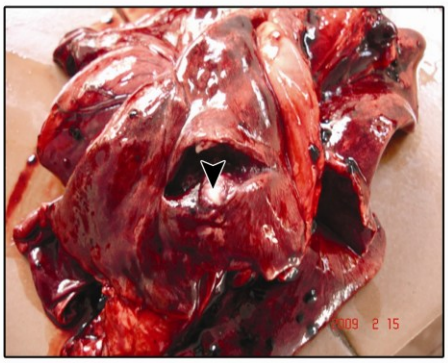

Fig. 107

Nodules in the lungs of the same carcinosarcoma case above.

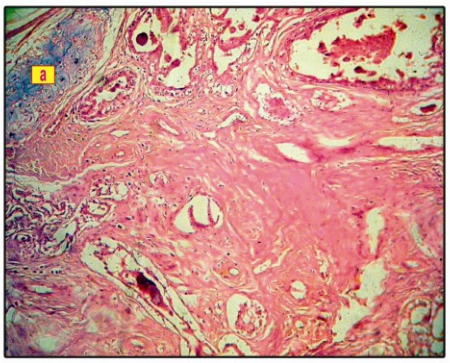

Fig. 108

Proliferation of myoepithelial cells and its metaplasia to chondroblasts (a) in carcinosarcoma. (H&E x 100)

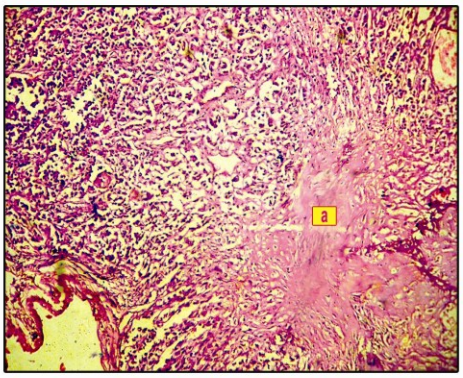

Fig. 109
Metastatic undifferentiated carcinoma with cartilaginous metaplasia (a) in the lungs of dog with carcinosarcoma. (H&E x 100)

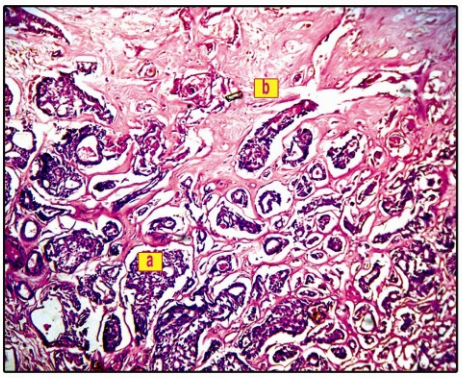

Fig. 110
Intraductal carcinoma (a) along with bone formation (b) in carcinosarcoma.(H&E x 100)

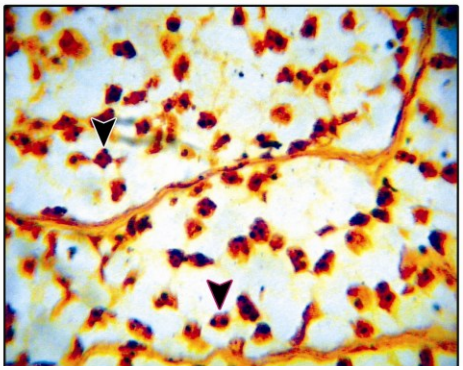

Fig.111

Sertoli cell tumor.Occasional multiple black AgNOR dots in the nucleus (x1000).

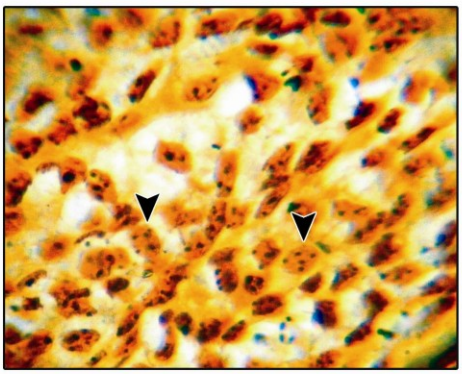

Fig. 112
Sebaceous epithelioma. Multiple AgNOR dots in the nucleus. (x1000)

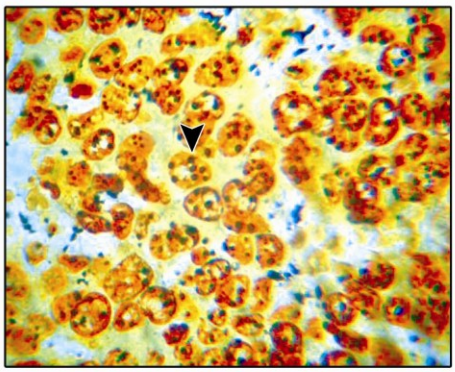

Fig. 113

Multiple AgNOR dots in hepatocellular carcinoma. (x1000)

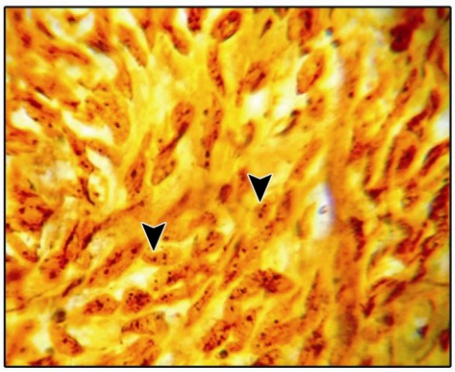

Fig. 114

Multiple small AgNOR dots in the nucleus of fibroblasts in the fibrosarcoma of the mammary gland. (x1000)

Discussion

5. DISCUSSION

Advanced diagnostic techniques and better health care has led to an increase in the life span of the pet animals which in turn has contributed to the increased occurrence of old age diseases especially cancer.

The present study was undertaken to investigate the incidence of tumours among canines in Thrissur district of Kerala State. This study attempted to evaluate the gross and histopathological features of the tumor samples obtained. Furthermore the mitotic index, apoptotic index and AgNOR counts of the tumor samples were calculated and their correlation was investigated.

The common observations are included under the sections 5.1, 5.2, 5.3 5.4 and 5.5. The details pertaining to individual tumor types or groups are discussed along with respective tumor headings.

5.1 INCIDENCE

During the period of study from December 2008 to December 2009, a total of 61 cases of tumours were recorded. This included 24 types of tumours belonging to different systems all of which are discussed below.

5.2 CLASSIFICATION OF TUMOURS

Tumours were classified based on the nature of their malignancy and anatomical location as per the recommendations of World Health Organization and Armed Forces Institute of Pathology for the tumours in domestic animals.

Tumours are broadly classified as benign and malignant. In the present study benign and malignant tumours were 66 percent and 34 percent respectively.

Location or system wise classification revealed that tumours of the skin and soft tissues constituted 32.8 percent, mammary gland tumours of bitches 29.51 percent, and tumours of the genital system 21.31 percent. Tumours of the alimentary tract contributed 6.56 percent, tumours of the haemolymphatic system

4.92 percent, tumours of eye and ear origin 3.3 percent and one tumor case from bone (1.64 percent).

The present observations were is in accordance with the observations of Nair *et al*. 2007 who reported that 68.75 percent of canine tumours as benign and 31.25 percent as malignant. They also reported a high incidence of mammary tumours (41.6 percent) followed by skin tumors (31.25 percent) in canines which disagreed with the present observation where skin and soft tissue tumours were of high incidence followed by mammary tumours.

5.3 AGE OF THE ANIMALS

Fifty five percent of the tumours bearing animals were in 5 to 8 year group, 21.67 percent in 9 to 12 year group, 16.67 percent in 1 to 4 year group and 6.67 percent were reported in greater than 12 year group.

Singh *et al.* (2004) recorded highest incidence of tumours in the age group of 8 to12 years followed by 4 to 8 years and least in 0 to 4 years age group. But the results of the present study is in accordance with the observations of Bhaskara Rao and Malleswara Rao (2009) who stated that the majority of tumor bearing animals were in the age group of 5 to 8 years (46.15 percent) followed by 9 to 12 years (30.77 percent), 13 to16 years (17.95 percent) and least in 1 to 4 years (5.13 percent).

5.4 SEX OF THE ANIMALS

Out of 60 tumor bearing animals 35 (58 percent) were females and 25 (42 percent) were males. But Singh *et al.* (2004) reported that the occurrence of neoplasms was more in case of male animals (76.47 percent) than in female animals (23.53 percent). Moreover Bhaskara Rao and Malleswara Rao (2009) also reported that the incidence of tumours was highest in males (56.09 percent) when compared to females (43.91 percent).

In the present study 29.5 percent of cases (18 out of 61) reported was mammary tumours in bitches and also 73 percent of the TVT cases (eight out of 11) were in females. This contributed to an increase in the percentage of females.

5.5 BREED OF THE ANIMALS

German shepherds were the most affected, i.e., 17 cases (28.33 percent). Thirteen cases (22 percent) were Mongrels, six cases (10 percent) each from Labrador and Rottweiler, three cases (5 percent) from Doberman, two cases (3.33 percent) each from Cocker spaniel and Boxer and one case (1.76 percent) each in Dalmatian, Spitz, Lhasa apso and Bull mastiff.

The present result was in accordance with Bhaskara Rao and Malleswara Rao (2009) who reported that higher incidence of tumours was in German shepherd (35.90 percent) followed by Pomeranian (30.77 percent), Labrador (23.08 percent), Lhasa apso (5.13 percent), Doberman (2.56 percent) and Dachshund (2.56 percent).

The report on the occurrence of tumours depends on different patterns of breed distribution in the areas of study. In the present area of study GSD dogs are found to be the most popular ones, which contributed to their high percentage.

5.6 HAEMATOLOGY

Anaemia and leucocytosis were the haematological changes noticed in tumour bearing animals. The present result was in consonance with the observation that the common hematopoietic changes in dogs having various neoplasms includes leucocytosis and anaemia (Patnaik *et al.*, 1980; Ogilvie, 1989; Cullen *et al*., 2002; Jacobs *et al*., 2002; Lakkawar *et al*., 2002; Vasudevan *et al*., 2004; Kujur *et al.,* 2005; Pillai *et al.,* 2009).

Differential leucocyte count did not show much variation except in ALL wherein lymphocytes belonging to blast series of cells constituted 76 percent. This was in consonance with the reports of Valli and Parry (1993).

5.7 TUMOURS OF THE SKIN AND SOFT TISSUES

Skin and soft tissue tumours included epithelial, melanocytic and mesenchymal tumours. A total of 20 cases (32.8 percent) belonged to this category. Of these 70 percent were benign tumours and 30 percent were malignant tumours. Sex wise analysis revealed that 60 percent of the affected animals were males and 40 percent were females. Age wise data showed that 55 percent of the animals were in 5 to 8 year group, 40 percent in 9 to 12 year group and five percent in >12 year group. It was found that Mongrels accounted for 35 percent of the affected dogs, GSD and Dachshund contributed 15 percent each, Rottweiler and Labrador constituted 10 percent each and Spitz, Lhasa apso and Cocker spaniel contributed five percent each.

This was in accordance with the observations of Shakir and Sundararaj (1994*)*, Singh *et al.* (2004), Dayananda *et al*. (2009a) and Kujur *et al.* (2009) who reported that skin neoplasms were found more in male animals than in females and also benign skin tumours are more common than malignant.

The present observation was also in agreement with Dayananda *et al*. (2009a) and Kujur *et al.* (2009) who reported that maximum incidence of skin and subcutaneous neoplasms occurred among non descript dogs followed by German shepherds and the maximum incidence were in the 5 to 8 year group. But this observation disagreed with Reddy *et al*. (2009a) who reported that the incidence is more in females than in males and also maximum cases were reported in GSD followed by non descript.

5.7.1 Epithelial and Melanocytic Tumours of the Skin

5.7.1.1 Sebaceous Gland Tumours

5.7.1.1.1 Sebaceous Adenoma

Three cases of sebaceous adenomas were observed in two males and one female with an average 9 years of age. But the findings of the present study were at variance with the observation that the average age of the animals with

sebaceous adenoma was 10 years and there was a slightly higher incidence of sebaceous adenomas in females than in males (Gross *et al*., 1992; Goldschmidt and Hendrick, 2002).

Microscopically solitary masses and grayish lobulated growths revealed a preponderance of mature sebocytes in multiple groups with little orientation around the ducts and several layers of basaloid reserve cells at the periphery of the lobules. Focal areas of squamous metaplasia and a few keratin nests were present. These features were in accordance with the description of Gross *et al.* (1992) and Goldschmidt and Hendrick (2002).

5.7.1.1.2 Sebaceous Epithelioma

Sebaceous epithelioma appeared as multiple firm small nodular growths in the face, ear and thoracic region of a 13 year old Cocker Spaniel bitch. Gross *et al.* 1992 reported that Cocker Spaniels and Poodles are highly prone to sebaceous epitheliomas and there is no known age or sex prediliction for sebaceous epithelioma.

Histologically, there was preponderance of basaloid cells with a few sebocytes arranged individually or in small aggregates as stated by Goldschmidt *et al*. (1998) and Goldschmidt and Hendrick, (2002).

5.7.1.1.3 Hepatoid Gland Tumours.

Four cases, two adenomas and two carcinomas were obtained. All cases were reported in the perianal region of intact male dogs with an average age of 10.5 years and this is in accordance with the reports of Withrow (1989), Gross *et al*. (1992), Goldschmidt *et al*. (1998) and Singh *et al.* (2004).

Grossly, they appeared as well circumscribed and multilobulated growths with focal hemorrhagic areas. Histologically in adenoma, the well differentiated hepatoid cells were arranged in anastomosing trabeculae. The interlobular septa separating the epithelial trabeculae were rich in blood vessels and the dilated

sinusoidal blood vessels and this account for the presence of haemorrhagic areas in the cut sections. The picture of carcinomas were similar to adenoma but with a tendency towards infiltration. Besides, in carcinomas nuclear pleomorphism and mitotic activity were evident and the hepatoid cells exhibited vacuolated cytoplasm with prominent nuclei and nucleoli. These findings were in accordance with Gross *et al*. (1992), Shakir and Sundararaj (1998), Goldschmidt *et al*. (1998) and Singh *et al.* (2004).

5.7.1.2 Eccrine Carcinoma

Swellings with ulcerations of the overlying epidermis were noticed in the foot pad and hind limb of a seven year old GSD dog for three months.

Histologically irregular tubuloacinar structures lined by one or more layers of cuboidal epithelial cells distributed in an abundant stroma of dense collagen were noticed. Small foci of keratinization and intraluminal eosinophilic secretions were found.

Gross *et al*. (1992) and Goldschmidt and Hendrick, (2002) described eccrine carcinomas as extremely rare sweat gland neoplasms of dogs that are limited to the foot pads. The results of the present study were in accordance with their findings.

5.7.1.3 Melanocytoma

Grossly melanoma was observed as a solitary, black colored alopecic nodule in the interdigital space of a five year old Rottweiler male. Microscopically spindle shaped neoplastic cells with intracytoplasmic melanin granules and variable amount of collagenous stroma separating the neoplastic cells could be appreciated in the dermis. Marked cellular pleomorphism was also noticed. The epidermis was intact.

The present finding were in agreement with the statement that higher incidence of all melanocytic tumours was reported in breeds with dark

pigmentation and the peak incidence of melanocytomas was found between five and 11 years of age. More over the dermal melanocytomas occur occasionally on the extremities, particularly between the digits (Gross *et al*., 1992; Goldschmidt and Hendrick, 2002)

5.7.2 Mesenchymal Tumours of the Skin and Soft Tissues

5.7.2.1 Fibroma

Grossly fibromas were very firm, well circumscribed, subcutaneous masses with white, glistening cut surface. Ulcerated surfaces were noticed in some larger masses. All cases of fibromas were obtained from the genital system of intact female bitches with an average age of 7.8 years. This was in agreement with Klein (1989) who reported that the vast majority of vaginal and vulvar tumours were benign smooth muscle tumours like fibroma, leiomyoma and fibro leiomyoma and are found in intact female dogs.

Histologically interlacing bundles of spindle shaped fibroblasts and collagen fibres running in various directions were observed and mitotic figures were rarely observed as stated by Krithiga *et al.* (2005b).

5.7.2.2 Benign Peripheral Nerve Sheath Tumor / Neurofibroma

A well circumscribed unencapsulated lobulated growth was noticed on the lateral aspect of the right hind limb, just above the hock joint and parallel to the saphenous vein.

Features like small spindle cells arranged in wavy bundles, palisades and partial whorls with moderately low cellularity and delicate collagen fibres suggested neurofibroma. Gross *et al*. (1992) and Goldschmidt and Hendrick (2002) stated that neurofibromas are rare in dogs and the features described were similar to the observations of the present study.

5.7.2.3 Liposarcoma

A firm subcutaneous growth, soft in consistency with a gray white and oily cut surface was observed in the perineal region of a Labrador. Focal areas of necrosis were also noticed on the cut surface. Histologically, features were suggestive of a well differentiated liposarcoma. These observations were in agreement with the findings of Gross *et al*. (1992), Goldschmidt *et al.* (1998) and Goldschmidt and Hendrick, (2002).

5.7.2.4 Malignant Fibrous Histiocytoma (MFH)

A firm unencapsulated white colored growth with a distinct margin was seen on the elbow joint of a ten year old Mongrel dog. Histologically the fibroblasts like cells were arranged in storiform patterns. There was an abundance of histiocytoid cells, plasma cells, neutrophils, eosinophils and lymphocytes and patchy zones of collagenous stroma. All the above findings were in agreement with that of Goldschmidt *et al.* (1998) and Goldschmidt and Hendrick, (2002).

5.7.2.5 Hemangiosarcoma

Hemangiosarcoma occurred as a single large well defined growth in the spleen of an eight year old male GSD. The liver showed raised areas of fatty change. Histologically neoplastic cells were pleomorphic, ranging from spindle shaped to ovoid and forming vascular clefts in the tumour. In the liver, the hepatocytes showed areas of acute cell swelling and severe degrees of fatty change with no zonal pattern of distribution which could be due to the hypoxia caused by the pressure of spleenic mass.

These observations were in consonance with the finding that there exists a higher incidence of hemangiosarcoma among males between 8 and 10 years or younger and in German shepherd breeds (MacEven, 1989). Goldschmidt *et al.*, (1998) also reported that hemangiosarcoma can be aggressive, especially in the German shepherd and Golden retriever breeds, where there is a high incidence of

multicentric form, most typically involving the spleen, liver, right auricle, and lungs, which proved true in the present study also.

5.8 TUMOURS OF BONE

5.8.1 Osteosarcoma

Osteosarcoma appeared grossly as a warm, painful and soft swelling extending from the middle of the forearm down to the distal end of the metacarpals of a two year old female Boxer. This was seen for 2 months. The cut section was whitish with focal areas of haemorrhage and necrosis.

The present observation was in agreement with Smith and Sulton (1988) who reported that the tumor is more likely to occur in the forelimbs than the rear, and the distal radius and proximal humerus are the most common locations and besides this approximately 10 percent occurred in dogs less than three years of age.

Histologically the features were similar to that of simple productive osteoblastic osteosarcomas with large areas of haemorrhage lined by neoplastic cells in some areas indicating their rapid growth. This was in accordance with the findings of LaRue and Withrow (1989) and Thompson and Pool (2002). They also reported that dogs of giant breeds generally develop osteosarcomas at a younger age than dogs of smaller breeds. Giant breeds like Boxers, Great Danes, Saint Bernard, German Shepherds and Irish Setters are predisposed, which were similar to the present observation.

5.9 TUMOURS OF HAEMOLYMPHATIC SYSTEM

5.9.1 Lymphosarcoma

A Mongrel male dog was presented to the University Veterinary Hospital with generalized lymphadenopathy and enlarged superficial lymph nodes with hard consistency. Ultrasound scanning revealed the presence of hyperechoic mass

in splenic area, enlargement of mediastinal lymph nodes and changes in the kidney.

Cytological evaluation of lymph node aspirate revealed a monotonous population of pleomorphic lymphocytes. On the basis of lymph node aspiration biopsy, ultrasonography and haemogram, the disease was diagnosed to be multicentric lymphosarcoma. Lakkawar *et al*. (2002) and Pillai *et al.* (2009) made similar observation in dogs affected with lymphosarcoma

5.9.2 Acute Lymphoblastic Leukemia (ALL)

Differential leucocyte count of a male Boxer aged two and a half years revealed seventy four percentage large atypical cells having features of blast cells. On ultrasonography, splenomegaly could be appreciated. Bone marrow examination revealed large number of atypical blast cells suggestive of lymphoblastic leukemia.

On post mortem examination, hepatomegaly, splenomegaly with pea sized nodular growths along the margin, pulmonary congestion and emphysema, degenerative changes in the kidney, catarrhal enteritis, cystitis and prostatic enlargement were evident.

Histology of the liver showed diffuse involvement with dilatation of sinusoids with infiltration by cells of the lymphoblast series. Spleen showed massive infiltration with these cells resulting in complete obliteration of the red pulp. There was collection of neoplastic cells in the interstitium of the kidneys. Microscopically prostate gland was characterized by glandular hyperplasia which may be attributed to a reactive alteration to the presence of neoplasm.

Valli and Parry (1993) and Jacobs *et al.* (2002) reported that intact male dogs younger than 5 years are vulnerable for leukemia and ALL can be distinguished from acute myeloblastic leukemia by the lack of cytoplasmic granulation. Besides, the gross and histological features of the liver and spleen were in accordance with their findings.

5.9.3 Canine Multicentric Lymphoma

A three year old male Bull mastiff was brought for necropsy to the Centre of Excellence in Pathology. On examination the carcass was emaciated with bilateral and symmetrical enlargement of all the peripheral lymph nodes.

The signalment of the animal were in accordance with the observations of McEwen and Young (1989) and Jacobs *et al.* (2002) who stated that malignant lymphomas were diagnosed at an average age of 6-7 years, with a range of six months to more than 15 years. They also reported a breed predisposition of certain breeds of dogs like Bulldog, Boxer, Bull Mastiff, St.Bernard etc. to multicentric lymphoma.

On opening the carcass, the most prominent feature noticed was the enlargement of all lymph nodes in the body including bronchial, mediastinal, hilar, mesenteric, renal, mandibular and parotid lymph nodes. The lymph nodes were smooth in consistency with necrosis at the centre of the much enlarged ones. Cut surface showed little corticomedullary differentiation. Marked hepatomegaly and splenomegaly were noticed.

Histologically, the lymph nodes showed effacement of the normal architecture and invasion by diffuse sheets of monomorphic cells. The cells were uniformly small but cleaved. i.e., diffuse small cleaved cell (DSC). Multifocal hepatic involvement with the masses of neoplastic cells congregating around portal triads was noticed. In the kidney, focal collections of neoplastic cells were noticed in the intertubular regions of the cortex. The most characteristic histologic lesion in the spleen was the atrophy of the periarteriolar lymphoid sheaths and subendothelial lymphocyte colonization of large veins within the thick fibromuscular trabeculae. These features were strictly compatible with the observations made by Jacobs *et al.* (2002). In the lungs aggregations of the neoplastic cells were found perivascularly and also around the lymphatics indicating the hematogenous and lymphogenous spread of the tumor.

5.10 TUMOURS OF ALIMENTARY TRACT

5.10.1 Ossifying Epulis

A whitish hard mass was seen in the lower jaw of an eight year old male Labrador. The cut surface showed lobulations and granularity indicating calcification. Microscopically rete pegs of the overlying epithelium were seen extending deep into the mass. The deeper areas revealed osteoid and mature lamellated bone with rich bone forming matrix. Scattered proliferation of stellate and spindle cells with moderate collagen were observed throughout the tissue section.

These bony spicules in the tumor appeared to arise by metaplasia of fibrous tissue as suggested by Viswanath *et al.* (1998). Head *et al.* (2002) suggested since the bone tissues occur in the ossifying epulis, the tumor might arise from odontogenic epithelial cells of periodontal membrane. Krithiga *et al.* (2005b) and Sivaseelan *et al.* (2008) also reported mandibular ossifying epulis in canines.

5.10.2 Hepatic Tumours

One case each of hepatocellular carcinoma and cholangiocellular carcinoma were observed in the study.

Cholangiocellular carcinoma was seen in a male Dalmatian of four years and five months old and hepatocellular carcinoma was reported in a four years old male GSD. This was at variance with the observations that bile duct carcinomas tend to occur more frequently in female dogs and at the same time it was in agreement with the report that males appear to be at increased risk of developing hepatocellular carcinomas (Patnaik *et al.,* 1980; Postorino, 1989; Cullen and Popp, 2002). They also reported that dogs as young as four years of age can get affected with hepatocellular carcinomas.

The gross features of hepatocellular carcinoma were hepatomegaly with multiple nodular growths and with a few normal brownish areas. Gross features

suggested nodular hepatocellular carcinoma. The centre of the neoplasm showed necrotic areas. Metastatic growths indicating a transcoelomic spread were seen in the pleura, lungs, diaphragm, serosa of the kidney, peritoneum, mesenteric folds and mesentric lymph nodes

In cholangiocellular carcinoma, liver showed multiple lobulated growths in the ventral aspect of left lateral lobe and was firm in texture. Metastatic nodular growths were present in the lungs, pleura, diaphragm and in the peritoneal attachment of the spleen indicating a transcoelomic spread of the neoplasm. The pancreas was prominent due to the firm texture and thickening.

Histologically in hepatocellular carcinoma the liver showed islands of neoplastic cells embedded in the hepatic parenchyma and arranged predominantly in an adenoid pattern, wherein the neoplastic cells formed acini of various shapes. But in some areas neoplastic cells were arranged in sheets with trabeculae. So this was diagnosed as a case of mixed type hepatocellular carcinoma. Focal collections of neoplastic hepatocytes tending to form acini were deposited in the lung parenchyma. Splenic tissue showed nodular hyperplasia of the white pulp, congestion and haemorrhage in the red pulp. The entire architecture of the mesenteric lymph nodes was replaced by infiltrating neoplastic cells, collagenous connective tissue stroma and foci of calcification.

Histology of the liver in cholangiocellular carcinoma showed arrangement of neoplastic cells in tubular or acinar pattern and the tubules were lined by multiple layers of cuboidal to columnar epithelial cells suggestive of a well differentiated subtype. Islands of tubules surrounded by an abundance of proliferating connective tissue gave a firm texture to the liver. Metastatic tumor emboli were seen embedded in the blood vessels and lymphatics in the lungs, diaphragm and were also seen attached to the splenic capsule. So transcoelomic, haematogenous and lymphogenous spread were evident. Exocrine glandular hyperplasia was present in the pancreas.

The features described above were in agreement with the observations of Patnaik *et al.* (1980) and Cullen and Popp, (2002) who histologically classified hepatocellular carcinomas as trabecular, adenoid, and solid patterns and cholangiocelluar carcinomas as well-differentiated and poorly differentiated or anaplastic subtypes.

Phangcho *et al*. (1993) reported metastatic lesions of hepatocellular carcinoma in the spleen, hepatic and mesenteric lymph nodes and kidneys.

5.10.3 Pancreatic Carcinoma

Pancreatic carcinoma was reported in a three years old female Doberman. A hard mass was seen starting from the distal portion of the pancreas and the posterior duodenum, jejunum and ileum with the mesenteric folds adherent to the mass. The pancreatoduodenal lymph node was highly enlarged with areas of necrosis at the centre.

Histology revealed that it was a case of highly anaplastic or undifferentiated pancreatic carcinoma with metastases to the adjoining lymph nodes and duodenum.

The observations are in agreement with the reports of Head *et al.* (2002) that pancreatic tumours produce a mass, often in the mid portion of the pancreas. Cullen (2007) reported that pancreatic carcinoma can occur in dogs as young as three years. They also reported adhesion of the affected pancreas to the adjacent tissues and metastasis to adjoining areas.

5.11 TUMOURS OF EYE AND EAR

5.11.1 Adenoma of Nictitans Gland

A proliferative growth was noticed in the inner canthus of the right eye of an eight year old Mongrel dog, blocking the normal vision. Histologically part of the tissue showed multiple layering of the acinar lining cells and also stromal invasion of the neoplastic cells, while in other parts of the tissue, lesions were

characteristic of adenoma wherein the tubules are lined by a single layer of low cuboidal cells and secretion containing acini. Infiltrations of lymphocytes were also noticed. All these suggested that the growth was in its transition stage from benign to malignant. So the case was diagnosed as adenoma of nictitans gland with border line malignancy.

Limited reports are available on the tumours of the nictitans gland. Render and Carlton (2001) described benign and malignant neoplasms of the nictitans gland and opined that they are uncommon in dogs. Wilcock (2007) reported adenocarcinoma in the gland of the third eyelid of very old dogs and described it as tubular carcinomas with abundant squamous metaplasia.

5.11.2 Ceruminous Gland Carcinoma

A pedunculated ulcerating mass was noticed at the base of the ear. Histologically the tumor was subdivided into lobules by fibrous trabeculae. In the glandular lumen multiple layers of lining epithelial cells were present. A brown material was present in the glandular lumen occasionally.

Cankar and Crowley (1964), Goldschmidt and Hendrick (2002) and Rani *et al*. (2005) reported ceruminous gland adenocarcinoma in the ear canal of dogs.

5.12 TUMOURS OF THE GENITAL SYSTEM

5.12.1 Sertoli cell tumor

Sertoli cell tumor was observed in the left inguinal region of a four years old male GSD which was a unilateral cryptorchid and in the right testicle of a 13 years old male Dachshund. In both cases no sign of feminization was observed. The cut surfaces were whitish and lobulated in both with haemorrhagic areas in the cryptorchid case.This was in accordance with findings of Buergelt (1997).

Histology of both showed intratubular type of Sertoli cell tumor. The adjacent neoplastic tubules like structures were separated by prominent coarse bands of collagenous stroma.

The observations made in the present study were in consonance with the reports of Ladds (1993) and Foster (2007) who described that histologically sertoli cell tumors are of intratubular and diffuse type and the abundant fibrous connective tissue in sertoli cell tumor distinguishes them from other testicular tumors. Also only one-third of the cases produce feminizing effect in dogs.

5.12.2 Transmissible Venereal Tumor

A total of eleven cases were reported, of which eight were from the vulva of females and three from the penis of males.

The present result was in consonance with the observations of Degloorkar *et al.*(1992), Shakir and Sundararaj (1994), Goldschmidt and Hendrick (2002), Maclachlan and Kennedy (2002) and Thangathurai *et al. (*2008) who reported that dogs of both sexes and all ages were affected with TVT, but are more commonly seen in female dogs that have reached sexual maturity.

Furthermore, among these 11 animals, seven were in the 5 to 8 year group and four were in the 1 to 4 year group. But Sivakumar *et al.* (2004) reported that the occurrence of transmissible venereal was relatively high in younger age group i.e.1-5 years which was not in accordance with the present observation.

As regards the breed wise occurrence, four cases were from GSD, two each from Rottweiler and Dachshund and one each from Mongrel, Labrador and Doberman. This may be due to the increased population of GSD in the area of study.

Grossly nodular masses were seen protruding from the surface of the penis or vulva. Cytologically when stained with Wright - Giemsa stain the tumor was highly cellular with a homogenous population of round to slightly polyhedral individual cells arranged in a sheet like pattern, the cytoplasm of which showed distinct vacuoles. The nuclear to cytoplasmic ratio was high and mitotic figures were prominent. Krithiga *et al*. (2005a) and Thangathurai *et al.* (2008) stated that

the prominent cytological feature of TVT is the presence of cytoplasmic vacuolation, which was noticed in the present study also.

5.13 MAMMARY TUMOURS OF DOGS

A total of 18 cases of mammary tumours were obtained. Among the mammary glands caudal thoracic (4^{th} pair) was the most affected gland, i.e., eight cases. Three cases involved inguinal (5^{th} pair), two cranial thoracic (1^{st} pair) and the least affected were cranial abdominal (3^{rd} pair). Multiple glandular involvement was noticed in four cases, two cases involved caudal thoracic and cranial abdominal, one case of caudal abdominal and inguinal and one report involving both cranial thoracic and inguinal glands.

The above observations were in agreement with the findings of MacEven and Withrow (1989) and Sivakumar *et al*. (2004) who reported that roughly 66 percent of canine tumours occur in glands 4 and 5, probably owing to greater volume of breast tissue in these glands.

Out of eighteen cases, ten cases (55.6 percent) belonged to 5 to 8 year group, six cases (33.3 percent) in 9 to 12 year group and two cases (11.1 percent) were in greater than 12 year group. Breed wise analysis revealed maximum cases among GSD (50 percent), two cases (11.11 percent) each from Mongrel, Rottweiler and Labrador and one case (5.55 percent) each from Doberman, Dachshund and Cocker spaniel. This may be due to the increased population of GSD breed in the area of the study.

The results of age wise analysis were in accordance with the observations of Sivakumar *et al.* (2004) who reported that mammary tumours were common in bitches aged 6 to15 years of age. Sivaseelan *et al.* (2009) also noticed that mammary tumours were found only in bitches over five years old. But Reddy *et al.* (2009b) and Srivastava *et al.* (2009) reported that the incidence of mammary tumours among canines was highest between 8 to10 years of age followed by 6 to 8 years and 4 to6 years.

Sivakumar *et al.* (2004), Sivaseelan *et al.* (2009) and Srivastava *et al.* (2009) noticed that the incidence of mammary tumor was higher in German shepherd dogs, which was similar to the present results.

Among these 18 cases, 11 cases (61.1 percent) were benign tumours, 2 cases were malignant tumours (11.1 percent) and 5 cases (27.8 percent) were mammary sarcomas. Srivastava *et al.* (2009) reported that 64.65 percent of the mammary tumours observed were of benign type, while malignant tumours constituted 35.35 percent. But Reddy *et al.* (2009b) reported that out of the mammary tumours observed, malignant tumours comprised 81.25 percent and benign tumours, 18.75 percent which was incompatible with the present observation

Benign tumours included six benign mixed mammary tumours, two simple adenomas, one myoepithelioma, one complex adenoma and one fibroadenoma. Two cases of malignant mammary tumours were of simple carcinomas, one of which was a tubulopapillary type while the other was solid carcinoma.

Mammary sarcomas included fibrosarcoma, osteosarcoma and carcinosarcoma or malignant mixed mammary tumor. Metastatic undifferentiated carcinoma with areas of cartilaginous metaplasia was seen in the lungs of one carcinosarcoma case. Moulton (1970) reported carcinomatous metastasis in the lungs in malignant mixed tumor.

In the carcinosarcomas, mixture of all types of carcinomatous components was recognized and merger between carcinomatous and chondrosarcomatous parts are suggestive of transformation as described by Misdorp (2002).

5.14 APOPTOTIC INDEX (AI), MITOTIC INDEX (MI) AND ARGYROPHILIC NUCLEOLAR ORGANIZER REGION COUNTS (AgNOR counts)

The mean AI of benign tumours were higher than that of malignant tumours but there existed no significant difference since $p<0.05$. This was in accordance with Hassan and Walker (1998) who stated that the apoptotic index was higher in the normal/benign tissue than the cancer containing breast.

The mean values of mitotic index and AgNOR count for malignant tumours were significantly higher than that of benign tumours ($p>0.05$). Also MI and AgNOR counts of the tumours are highly correlated ($r = 0.949(**)$). But no correlation was found to exist between MI and AI and also between AI and AgNOR counts.

The present observation agreed with Johnson *et. al*. (1995) and Bukhari *et al.* (2007 who reported that the mean number of AgNORs per nucleus accurately correlated with the mitotic rate in tumor cell lines and AgNOR counts could thus provide an indirect measurement of mitotic rate. They suggested that AgNOR counts and mitotic indices could be of use in differentiating benign from malignant tumours. But Evans *et al.* (1992) suggested that among the cutaneous melanomas of humans, there existed no correlation between the mitotic index and total AgNORs, which was not in accordance with the present study also.

Soini *et al*. (1998) stated that and there exists a positive correlation between apoptosis and proliferation and also there was a wide variation in the extent of apoptosis not only between different tumours but also within a tumor type. But Sinicrope *et al.* (1999) failed to detect a significant correlation between AI and MI ($P = 0.27$) in all tumours or when stratified by tumor site which was in consonance with the present result.

So we can conclude from the present study that there exists a positive relationship between mitotic index and AgNOR counts, which can be suggested

as reliable proliferation markers and can also be utilized for differentiating benign from malignant tumours. Apoptotic index failed to establish any correlation with mitotic index and AgNOR counts. Even though the mean apoptotic indices of the benign tumours were higher than malignant tumours, no significant difference was noticed. That could be due to the wide varieties of the tumor types included in the study.

From the present study it was evident that the incidence of tumours among animals is high and is on the increase. Skin and soft tissue tumours, mammary tumours, and Transmissible Venereal tumor are the predominant tumours among canines in the area of the present study. Even though several theories and concepts were proposed to explain the occurrence of cancer, the picture is still not clear. In this study, undertaking histopathological studies familiarized with the features of the different tumours, which enables easier and quicker diagnosis in the future. The mitotic index and AgNOR counts of the tumours as reliable proliferation markers facilitate classification of tumours into benign and malignant. All these equips us to adopt appropriate treatment regimes for the tumor bearing animals in pursuit of saving or prolonging their lives

Summary

6. SUMMARY

The present study was undertaken to assess the incidence and pathology of tumours among canines in Thrissur locality and also to assess the significance of apoptotic index, mitotic index and AgNOR counts in the identification of different types of tumours.

Canine cases presented to the Veterinary hospitals of Mannuthy and Kokkalai and carcass brought for the post mortem examination to the Centre of Excellence in Pathology for a period of 12 months *i.e*., from December 2008 to December 2009 was utilized for the study. Any abnormal mass detected was examined in detail. The tissues collected were preserved in 10 percent neutral buffered formalin. Histopathological studies were performed on the tissues after processing, paraffin embedding and haematoxylin and eosin staining. Besides mitotic and apoptotic indices of the tumor was assessed. Special stains were used wherever needed. Duplicate sections were stained for AgNOR count test and assessed. Routine haematological parameters like haemoglobin, packed cell volume, erythrocyte sedimentation rate, total WBC count, total RBC count and differential count were estimated in case of live animals. Detailed epidemiological data was collected in each case and the age, sex, breed and location wise occurrence of various tumours in dogs were recorded.

The present study encountered 61 tumours in 60 animals which included multiple tumours in one dog. Age wise analysis revealed that 33 out of 60 (55 percent) tumor bearing animals were in 5 to 8 year group. 21.67 percent in 9 to 12 year group, 16.67 percent in 1 to 4 year group and four cases (6.67 percent) were reported in greater than 12 year group. Sex wise, 58 percent of the affected animals were females and 42 percent were males. Breed wise analysis of the animals revealed that German shepherds, were the most affected - (28.33 percent), Mongrels (22 percent), Labrador and Rottweiler (10 percent) each, Doberman (5 percent), two cases (3.33 percent) each from Cocker spaniel and

Boxer and one case (1.76 percent) each in Dalmatian, Spitz, Lhasa apso and Bull mastiff.

In the present study benign tumours comprised 66 percent and malignant tumours 34 percent. Tumours were also classified based on their anatomical location and following the recommendations of World Health Organization and Armed Forces Institute of Pathology for the tumours in domestic animals.

In this study, tumours of the skin and soft tissues constituted 32.8 percent, 29.51 percent tumours had their origin from mammary glands of bitches and 21.31 percent belonged to the genital system especially TVT. Tumours of the alimentary tract contributed 6.56 percent, tumours of the haemolymphatic system 4.92 percent, tumours of eye and ear origin 3.3 percent and one from bone (1.64 percent).

Anaemia and leucocytosis were the haematological changes noticed in tumor bearing animals. Differential leucocyte count did not show much variation except in ALL wherein lymphocytes belonging to blast series constituted 76 percent.

Tumours of the skin and soft tissues included epithelial and melanocytic tumours of the skin and mesenchymal tumours of the skin and soft tissue. Of the 20 cases in this category, 70 percent were benign tumours and 30 percent were malignant tumours. Sex wise analysis revealed that 60 percent of the affected animals were males and 40 percent were females. Fifty five percent of the animals were in 5 to 8 year group and 35 percent of the dogs affected were Mongrels.

The tumours encountered in this group were sebaceous adenoma, sebaceous epithelioma, hepatoid gland adenoma, hepatoid gland carcinoma, eccrine carcinoma, melanocytoma, fibroma, neurofibroma, benign peripheral nerve sheath tumor, liposarcoma, malignant fibrous histiocytoma and hemangiosarcoma.

Osteosarcoma was the only reported bone tumor. Lymphosarcoma, Acute Lymphoblastic Leukemia and canine multicentric lymphoma were the tumours reported in the haemolymphatic system. Tumours of the alimentary tract included ossifying epulis, pancreatic carcinoma and hepatic tumours such as hepatocellular carcinoma and cholangiocellular carcinoma. Tumours of the eye and ear included adenoma of the nictitans gland and ceruminous carcinoma. Sertoli cell tumor and Transmissible Venereal Tumour were the tumours encountered in the genital system. Seventy three percentage of the TVT were found in females. Among the TVT affected animals, maximum cases were reported in GSD and in the 5 to 8 year group.

Mammary tumours of the dogs comprised benign (61.1 percent), malignant (11.1 percent) and carcinosarcomas (27.8 percent). Maximum numbers of cases were reported in 5 to 8 year group females and in GSD. Caudal abdominal mammary glands were the most affected pair. Mixed mammary tumours, simple adenoma, myoepithelioma, complex adenoma and fibroadenoma were the benign tumours encountered. Malignant mammary tumours observed were simple tubulopapillary type and solid carcinoma. Osteosarcoma, fibrosarcoma and carcinosarcomas were the mammary sarcomas encountered.

The means of MI and AgNOR counts of benign and malignant tumours showed a significant difference ($p>0.05$). But the mean AI of benign and malignant group showed no significant difference ($p<0.05$). Also MI and AgNOR counts of the tumours were highly correlated ($r =0.949(**)$). But no correlation was found to exist between MI and AI and also between AI and AgNOR counts.

From the present study it was concluded that there exists a high incidence of tumours among canines in the area of study, including even the rare tumours like eccrine carcinoma, liposarcoma, neurofibroma, adenoma of the nictitans gland of the third eyelid and carcinosarcoma of the mammary glands. Mean while tumours of the skin and soft tissues, mammary tumours and TVTs were the common tumours observed among the canines in the area of study. Mitotic index

and Argyrophilic nucleolar organizer region counts could be used as reliable markers of proliferation besides enabling to differentiate benign from malignant neoplasms.

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INCIDENCE AND HISTOMORPHOLOGICAL CHARACTERIZATION OF CANINE NEOPLASMS

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ABSTRACT

The present study entitled "Incidence and histomorphological characterization of canine neoplasms" was conducted to assess the incidence of neoplasms among canines. Canine cases presented to the Veterinary hospitals of Mannuthy and Kokkalai and carcass brought for post mortem examination in the Centre of Excellence in Pathology, COVAS, Mannuthy from December 2008 to December 2009 was utilized for the study. Besides age, sex, breed and location wise incidence of tumours, haematological parameters, gross and histopathological features along with apoptotic index, mitotic index and AgNOR counts were recorded.

A total of 61 tumour cases were recorded in 60 animals. Age, sex and breed wise analysis revealed that 55 percent of tumour bearing animals were in 5 to 8 year group, 58 percent of the affected animals were females while 42 percent were males and German shepherds, were the most affected - 28.33 percent.

Among the cases studied benign tumours comprised 66 percent and malignant tumours were 34 percent. Moreover tumours of the skin and soft tissues constituted 32.8 percent, mammary tumours 29.51 percent and tumours of the genital system 21.31 percent. Tumours of the alimentary tract contributed 6.56 percent, 4.92 percent of tumours were from the haemolymphatic system, 3.3 percent tumours were of eye and ear origin and one tumour case (1.64 percent) from bone.

Anaemia and leucocytosis are the haematological changes noticed in tumour bearing animals. The mean values of MI and AgNOR counts of benign and malignant tumours showed significant variation. Besides the MI and AgNOR counts were highly correlated.

The tumours such as sebaceous gland tumours, eccrine carcinoma, melanocytoma, fibroma, neurofibroma, malignant fibrous histiocytoma, liposarcoma, hemangiosarcoma, osteosarcoma, lymphosarcoma, acute lymphoblastic leukemia, canine multicentric lymphoma, epulis, hepatic tumours, pancreatic carcinoma, adenoma of the nictitans gland, ceruminous carcinoma, sertoli cell tumor, TVT and mammary tumours were recorded.