

Thesis Ref No \_\_\_\_\_

**CYTOLOGICAL AND PATHOLOGICAL CHARACTERIZATION OF  
TUMORS OF DOMESTIC ANIMALS IN AND AROUND BISHOFTU,  
ETHIOPIA**

**MVSc Thesis**



**By**

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**MVSc Program in Veterinary Pathology**

**June, 2018**

**Bishoftu, Ethiopia**

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**A thesis submitted to the College of Veterinary Medicine and Agriculture of  
Addis Ababa University in partial fulfillment of the requirements for the degree  
of Master of Science in Veterinary Pathology**

**By**

**Mesfin Mathewos**

**June, 2018**

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## Signature and Approval Sheet

Addis Ababa University  
College of Veterinary Medicine and Agriculture  
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## **DEDICATION**

This thesis is dedicated to my uncle, Temesgen Ayano, whom I lost him at early age with mysterious case of “Congestive heart failure” and to my beloved family, for their true natural love and practical influence upon my life.

## STATEMENT OF THE AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MVSc) degree at Addis Ababa University, College of Veterinary Medicine and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

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## LIST OF ABBREVIATIONS

BCC	Basal Cell Carcinoma
BHV	Bovine Herpes Virus
BPV	Bovine PapillomaVirus
CMCT	Cutaneous Mast Cell Tumors
FNAC	Fine Needle Aspiration Cytology
H&E	Haematoxylin and Eosin
HPCs	Hemangiopericytomas
ICT	Interstitial cell tumours
IV	Intravenous
MCT	Mast Cell Tumors
MFHs	Malignant fibrous histiocytoomas
OSCC	Ocular Squamous Cell Carcinoma
SCC	Squamous Cell Carcinoma
SCT	Sertoli (Sustentacular) Cell Tumors
SEM	Seminoma
SSCT	Sclerosing Sertoli Cell Tumors
TVT	Transmissible Venereal Tumor
U.S.A.	United States of America
WHO	World Health Organization

## **ABSTRACT**

Tumors are one of the disorders that affect domestic animals. The present study was conducted from October, 2017 to May 2018 with the objectives of determining the types and frequencies of tumors of domestic animals based on cytological and pathological characteristics. A cross-sectional study with purposive sampling of animals with swelling or masses was conducted for evaluation of masses by gross examination, cytology and histopathology. A total of sixty neoplastic swelling were identified. Based on cellular origin, the overall frequencies of tumors were found to be 46.7% epithelial, 36.7% unclassified, 13.3% mesenchymal and 3.3% were round cell tumors. Gross lesions observed include firm consistency, cauliflower like lesions, nodular mass, ulcerated, inflamed and haemorrhagic surfaces, pediculate, necrosis, and lobulated mass with irregular margins. Cytologically, most of the epithelial cell tumor and equine sarcoid revealed clusters of cells with different arrangements, deep nuclear chromatin, anisocytosis and anisokaryosis. Where as, all tumors of mesenchymal origin including fibropapilloma showed pleomorphic spindle cells. Mast cell tumors was characterized by its cytoplasmic granules and transmissible venereal tumors by clear punctuate intracytoplasmic vacuoles, cell shape and prominent central nucleoli. Histopathologically, equine sarcoid and papillomatosis were characterized by dermal keratinocytes proliferation, long rete pegs that extend deep into the dermis. Ocular squamous cell carcinoma was characterized by keratin tonofilaments and keratin pearls while, leiomyoma, cutaneous fibrosarcoma, basal cell carcinoma and hemangiopericytomas were characterized plump smooth muscle-like cells, herring bone pattern, cluster of neoplastic basal cells and perivascular whorls of pericyte nuclei, in the respective order. Mammary tumors were characterized by severe cellular and nuclear pleomorphism, and highly infiltrative. Sertoli cell tumor, fibrothecoma, cutaneous mast cell tumor, transmissible venereal tumor and malignant fibrous histiocytoma were chracterized by sheets of sertoli cells, spindle cells with plump thecoma cells, granulated mast cells, round cells with vesicular nuclei and pleomorphic histiocytic cells, respectively. Based on the result it could be concluded that domestic animals were widely suffering from tumors of different origin. Thus, studies involving large sample size, and wider diagnostic tools should be conducted.

**Key words:** Cytology, Domestic animals, Ethiopia, Histopathology, Tumors

## 1. INTRODUCTION

The term tumor is derived from the Latin word tumor, which means a pathological swelling, whether of neoplastic, non-neoplastic, inflammatory, cystic, edematous or other nature (Meuten and Rebar, 1998). A tumor is an abnormal mass of tissue resulting from autonomous, progressive, excessive proliferation of body cells not integrated into normal tissue and exhibit varying degrees of fidelity to their precursors (Rubin *et al.*, 2008). In oncology, the expression tumor disease, cancer disease and the term neoplasm are used. The term neoplasm is composed of the prefix neo, which comes from the Greek neos (new) and plasis (formation) (Baba and Catoi, 2007). Non-neoplastic tumors are defined as neoplastic-like lesions without an inflammatory component whereas masses with inflammatory infiltrates with/or without an intralesional etiologic agent were defined as inflammatory lesions (Morrison, 2012).

Scientific research, at first of spontaneous tumors, has correlated the conditions of occurrence of tumors in various species with elements common to animals, such as the geographic area, nutrition, age, neurohormonal impact, pollution factors and also intrinsic local (tissue) and general factors (organism) (Milltaru *et al.*, 1999).

Tumor may cause major damage to the cattle industry and is responsible for significant economic losses (Kimura *et al.*, 2012). The review of Reisinger showed main causes of condemnation of whole and part of carcasses in the U.S.A. and that estimated financial loss from the condemnation of carcasses affected with all types of tumors was 2.2 million dollars (Bastianello, 1982). Other report indicated, neoplasms may result for decreased aesthetic value, cause discomfort and can result in ulceration, infertility, infection and occasionally lameness associated with lesion (Hewes and Sullins, 2009). Apart from their economic significance tumours in domestic animals are also of importance for cancer research (Bastianello, 1982).

The classification of tumors was and is both a practical and theoretic necessity and requirement. Difficulties are numberless, starting with the structural variability and the high number of factors, some of which are suspected, others still unknown, which are

involved in the onset and evolution of a tumor, then the response of the organism or even the organ and tissue involved, not to mention the accumulation of a large amount of facts and bibliography, difficult to systematize (Baba and Catoi, 2007). The World Health Organization has been supported by international bodies and commissions, within which reputed specialists in narrow oncologic areas have elaborated classifications based on clinicomorphological data with predictive support for both humans and animals (Joshi and Jadon, 2012).

Tumor may be classified by their primary site of origin, grade, stage (tumor size (T), the degree of regional spread or node involvement (N), distant metastasis (M) (TNM staging) and their histological or tissue types. However, the commonly used and most useful classification of tumors is histogenetic, that is, the tumors are named according to the tissues from which they arise and of which they consist (Joshi and Jadon, 2012). According to their histological structure, tumors can be grouped in the following main categories: epithelial tumors, of ectodermal and endodermal embryonic origin; mesenchymal or connective tumors, of mesodermal origin; neuroectodermal tumors, hematopoietic tissue tumors, multiple tissue tumors and pseudotumors or hyperplastic lesions (Ghilezan, 1992; Baba and Catoi, 2007; Birbrair *et al.*, 2014).

The most frequent tumors in domestic animals include: squamous cell carcinoma, papilloma, equine sarcoid, fibroma, fibrosarcoma, leiomyoma, leiomyosarcoma, lipoma, hematopoietic /lymphopoietic tumor (lymphoma, leukemia, hemangioma, haemangiopericytoma and hemangiosarcoma), melanoma, osteosarcoma, basal cell carcinoma, mammary adenocarcinoma, mammary adenoma, cutaneous mast cell tumor, transmissible venereal tumor, histiocytoma, malignant fibrous histocytoma, sertoli cell tumor, seminoma, leydig cell tumor, thecoma, fibrothecoma, hepatoid gland adenoma and malignant peripheral nerve sheath tumor (Bastianello, 1982; Mukaratirwa *et al.*, 2005; Hewes and Sullins, 2009; Kimura *et al.*, 2016).

Accurate identification of the source of tumor depends not only on the location but also on a morphologic resemblance to a normal tissue despite the histologic divergence of tissue they are originated from (Rubin *et al.*, 2008). Some of the histologic features of the

tumor cell include: high cellularity, cellular enlargement, increased nuclear/cytoplasmic ratio, nuclear hyperchromasia, discohesiveness of cells, prominent and large nucleoli, abnormal distribution of nuclear chromatin, increased mitotic activity and specially the presence of abnormal ones, nuclear membrane abnormalities, cellular and nuclear pleomorphism, and background tumor necrosis (also known as tumor diathesis) (Baba and Catoi, 2007; Rubin *et al.*, 2008).

Cytology unlike histopathology poorly differentiates some tumors. Cytological examination may not always identify the tissue of origin and differentiation between sarcomas and carcinomas can even sometimes be extremely difficult. Cytology, therefore, should not be regarded as a substitute for histopathological examination of biopsy specimens. Histology is more likely to provide a definitive diagnosis and, since biopsies preserve tissue architecture, grading and classification of tumor is usually possible (Harrison and Jayasundera, 1999). The advantages of diagnostic cytology whether exfoliative or fine needle aspirate (FNA) is that it is a non-invasive, simple procedure, helps in faster reporting, relatively inexpensive, has high population acceptance and facilitates cancer screening in the field (Dajani and Kilani, 1998).

There are no or very few reliable molecular indicators of tumors and the “gold standard” for diagnosis of tumor remains routine microscopy. However, there remain tumors that defy the diagnostic skills and experience of any pathologist. In effect, the criteria used to assess the true biological nature of any tumor are based not on scientific principles but rather on a historical correlation of cytologic and histologic patterns with clinical outcomes (Rubin *et al.*, 2008).

There is no publication on cytological and pathological characterization of tumors of domestic animals in Ethiopia. Therefore, the present study is initiated with the following specific objectives:-

- To study on cytological and pathological characteristics of tumors of domestic animals
- To determine the types and frequency of tumors of domestic animals in the study area

## 2. LITERATURE REVIEW

### 2. 1. Equine Sarcoid

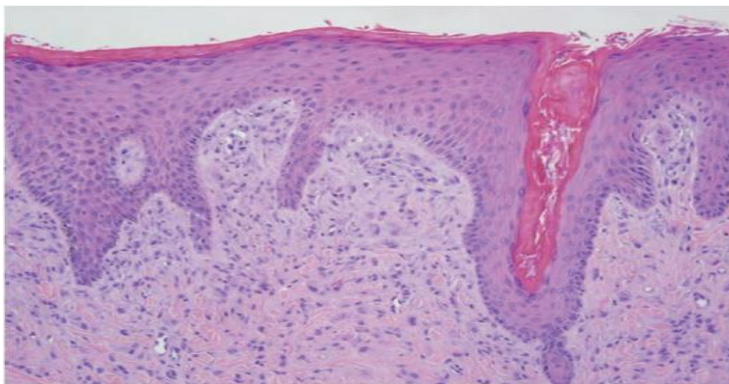
Sarcoids are locally invasive, fibroblastic skin tumours and represent the most common tumor in equidae worldwide (Martano *et al.*, 2016) has been reported in horses, donkeys and mules (Shah, 2015) with an incidence ranging from 12.5 to 67% of all tumors (Taylor and Haldorson, 2012). In combination with trauma and genetics (Shah, 2015) Bovine papillomavirus (BPV) *types 1* and *2* are casually associated with the development and pathogenesis of equine sarcoids (Taylor and Haldorson, 2012).

Sarcoids are often classified in six different forms: occult sarcoid, verrucous sarcoid, nodular sarcoid, fibroblastic sarcoid, mixed sarcoid and malevolent form (Taylor and Haldorson, 2012). Occult sarcoids are flat and alopecic lesions with mild scaling that is limited to the superficial epidermal layers; usually occurring in the medial thigh, sheath, neck and face. Verrucous sarcoids appear as small or large wart-like lesions having a raised, scaly, lichenified appearance with epidermal thickening; usually occurring in the axillary or groin regions (Knottenbelt and Kelly, 2000; Taylor and Haldorson, 2012).

Nodular sarcoids usually occur subcutaneously under grossly normal intact skin having a spherical shape and a fairly smooth surface; usually occurring in the groin or in the eyelid margins. Nodular sarcoids may ulcerate and progress to the fibroblastic form. Fibroblastic sarcoids are the most aggressive type of sarcoids resembling true neoplasms. They are large lobulated masses with irregular margins, fleshy and ulcerated surfaces with local infiltration and may or may not be pedunculated. Mixed sarcoids may include any, or all, of the aforementioned types and usually found in the axillary and groin region. Malevolent sarcoids are the most infrequent form and are aggressive, invasive tumors that proliferate rapidly and usually invade local lymphatics and may spread to regional lymph nodes (Knottenbelt and Matthews, 2001; Meuten, 2002; Gomes, 2011; Corteggio *et al.*, 2012).

Sarcoids can develop in any location, either as a single tumor or as multiple tumors of different types. The most common locations for sarcoid development include the head (periorbital region, ear pinnae and lips) and neck (39%), extremities (35%) and ventrum (26%) (Taylor and Haldorson, 2012). Inactive sarcoids may become aggressive if disrupted by injury, biopsy or inappropriate treatment. Although sarcoid tumors do not metastasize they can significantly impact the function and aesthetics of affected equids based on tumor location, size and frequency. Thus, the value of sarcoid-bearing equids is often dramatically decreased. Sarcoid tumors may cause discomfort and can result in ulceration, infection and occasionally lameness associated with lesion location (Knottenbelt and Kelly, 2000).

Histopathologically, sarcoids typically present as diphasic lesions having pathology in the epidermis and dermis (Shah, 2015). The epidermis usually shows acanthosis, hyperkeratosis and hyperplasia and forms numerous elongated rete pegs into the dermis, however, in some cases it may be thin or ulcerated (fig. 1) (Taylor and Haldorson, 2012). Within the dermis, bundles of fibroblasts are arranged in whorls, tangled bundles or herringbone patterns; with a variable amount of collagen. The fibroblasts are oval to spindle-shaped, with elongated nuclei and are well differentiated thus showing a low level of anaplasia. The rate of mitosis varies with higher rates in aggressively growing tumors or in areas where ulcerations are present. The cells are locally infiltrating and hence the lesion is not well demarcated microscopically (Knottenbelt and Matthews, 2001; Meuten, 2002; Gomes, 2011; Corteggio *et al.*, 2012).



**Figure 1:** Photomicrograph of equine sarcoid (Taylor and Haldorson, 2012).

## 2. 2. Ocular Squamous Cell Carcinoma

Ocular squamous cell carcinoma (OSCC) is a general designation for a group of primary neoplasms of keratinocytes emerging from ocular tissues, especially the eyelids and particularly the third eyelid (Carvalho *et al.*, 2005). It has been reported in all species of domestic animals (Bussanich *et al.*, 1987; Goldschmidt and Hendrick, 2008) with a higher frequency in cattle, especially in adult and old animals (Carvalho *et al.*, 2005; Pugliese *et al.*, 2014).

Bovine ocular squamous cell carcinoma is the most common bovine tumor and the one that causes the most significant economic losses (Carvalho *et al.*, 2005) to the owner, unnecessary suffering for the animal and negative public perceptions. The consequences of OSCC include: decline in body condition, lower calf birth weight, reduced growth rate in calf, reduced milk production, lower fertility in bulls, and, subsequently, a lack of animal welfare (Pugliese *et al.*, 2014).

Hereditary factors, environmental factors (e.g., latitude, altitude, exposure to sunlight), lack of eyelid pigmentation, age and dietary habits have all been recognized to play a role in the aetiopathogenesis of bovine ocular squamous cell carcinoma. In addition, in cattle the aetiology has been linked to a number of viral agents, especially bovine papillomavirus (BPV) and bovine herpes virus type 1 (BHV-1) and 5 (BHV-5). Nevertheless, ultraviolet light, viruses and circumocular pigmentation are the major epidemiologic risk factors for the development of the tumor (Carvalho *et al.*, 2005; Seite *et al.*, 2010; Pugliese *et al.*, 2014).

Tumors related to squamous-cell carcinoma (SCC) can appear anywhere on the body, but they are most often located in non-pigmented skin near mucocutaneous junctions such as on the eyelids, around the nostrils, lips, vulva, prepuce, penis or anus (Valentine, 2006). Rarely, primary SCC develops in the esophagus, stomach (non-glandular portion), nasal passages and sinuses, the hard palate, gums, guttural pouches and lung. The eyelid is the

most common site, accounting for 40-50% of cases, followed by male (25-10% of cases) and female (10% of cases) genitalia (Knottenbelt, 2003).

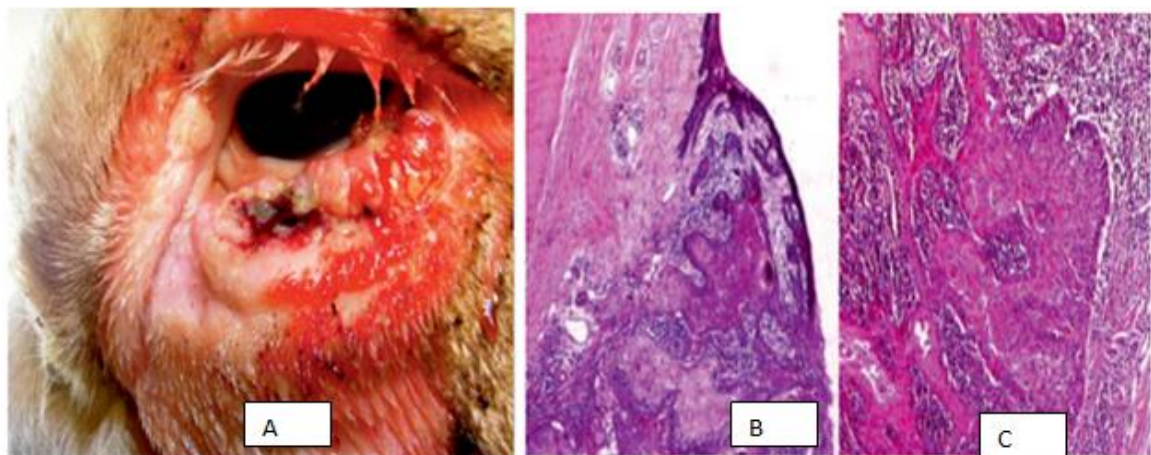
Grossly, the mass appear as oedematous, thickened raised lesions of different shapes, with irregular surfaces, firm in consistency, distributed over the palpebral conjunctiva, nictitating membrane, and eyelid skin (Pugliese *et al.*, 2014).

Cytologically, SCC is often composed of numerous keratinocytes that may or may not exhibit a cohesive nature. Keratinocytes can display notable malignant features to include retention of large nuclei, nuclear fragmentation, binucleation or multinucleation, anisokaryosis, and nuclear to cytoplasmic asynchrony. Atypical parabasal or basal epithelial cells can also be observed as well as variable numbers of neutrophils (Johnson and Myers, 2017).

Histologically, OSCC may range from well-differentiated to undifferentiated anaplastic carcinoma. The degree of invasiveness of these tumors is variable, and occasionally metastases occur, especially to the parotid lymph node (Carvalho *et al.*, 2005). Actinic keratosis (squamous cell carcinoma) shows epidermal hyperplasia, hyperkeratosis, parakeratosis, acanthosis, accentuation of the epidermal rete, and keratinocyte dysplasia. The affected keratinocytes, which are mostly found in the basal and spinous layer, show loss of polarity, karyomegaly, nuclear hyperchromatism, enlarged and prominent nucleoli, and mitotic figures of basal and suprabasal keratinocytes. At this stage there is no invasion through the basement membrane by the dysplastic keratinocytes, such as occurs with squamous cell carcinoma, extending into the dermis, with or without an association to the overlying epidermis, are islands, cords, and trabeculae of neoplastic epithelial cells showing a variable degree of squamous differentiation (Goldschmidt *et al.*, 1998).

The amount of keratin, seen as intracytoplasmic, eosinophilic fibrillar material (keratin tonofibers) produced by the neoplastic cells is quite variable; there is extensive keratinization, and in well-differentiated tumors there is formation of distinct keratin

“pearls”. In poorly differentiated tumors only a few cells have intracytoplasmic eosinophilic keratin tonofibers. Individual tumor cells have large, ovoid, often vesicular nuclei with a single, central, prominent nucleolus, abundant cytoplasm that varies from pale to brightly eosinophilic, and distinct cell borders. In more differentiated tumors it is also possible to recognize intercellular desmosomes, especially in areas where intercellular edema allows them to be more readily identified (Campbell *et al.*, 1987). The number of mitotic figures is variable, but they are more frequent in less well differentiated tumors. Invasion of the dermis and subcutaneous tissue may evoke a desmoplastic response. Ulceration is accompanied by an infiltrate of neutrophils into the superficial part of the tumor, while plasma cells and lymphocytes are found in the deeper parts of the tumor. The invasive margins of the tumor may show neurotropism as well as invasion of dermal and subcutaneous lymphatics (Goldschmidt and Hendrick, 2008).



**Figure 2:** Macroscopic appearance of OSCC involved periocular tissues (A) histopathological features of the dermic neoplastic growth (B) and Pseudo-cordonal growth pattern of invasive squamous cells (C) (Pugliese *et al.*, 2014).

### 2. 3. Cutaneous Fibrosarcoma

Fibrosarcoma is a common malignant neoplasm derived from fibrous connective tissue and characterized by immature proliferating fibroblasts or undifferentiated anaplastic spindle cells (Hesaraki *et al.*, 2010) that occurs in dogs, cats and other domestic animals (Goldschmidt and Hendrick, 2008). However, the reports of cutaneous fibrosarcomas in bovines compared with other tumors of cattle are really few. The cases reported in cattle

are scarce; its greater presentation is at the level of the vagina (Timaran *et al.*, 2015). This tumor has variable presentations depending on species, age, site, and etiopathogenesis (Ozmen *et al.*, 2016).

The skin and subcutis are the primary sites of occurrence of fibrosarcomas in domestic animals (Goldschmidt and Hendrick, 2008) but these can develop anywhere in the body such as the heart (Speltz *et al.*, 2007), liver, kidney (Avci *et al.*, 2012), urinary bladder (Olausson *et al.*, 2005), uterus (Govaere *et al.*, 2011), omentum (Rayner *et al.*, 2010), trachea (Mahler *et al.*, 2006) and, on rare occasions, in the mammary gland (Avci *et al.*, 2012), genital organs such as the vagina and penis (Hesaraki *et al.*, 2010). The most important effect of these tumors on the cattle industry is the increased culling rate due to metastases to the other critical organs, such as the lungs, liver and draining lymph nodes, which in turn causes severe complications (Kuru, 2016).

Macroscopically, its growth can be circumscribed or infiltrative, soft or firm, where the capsule is usually not present. The cut surface appears to be white to gray and bright coloration (Timaran *et al.*, 2015). Cytologically, fibrosarcomas consist of plump, spindle cells arranged individually or in aggregates associated with extracellular, pink, collagenous material. Nuclei are typically elongated and display marked pleomorphism (Raskin, 2016).

The tumor may appear with varying degrees of differentiation including low grade (differentiated), intermediate malignancy and high malignancy (anaplastic). Depending on this differentiation, tumor cells may resemble mature fibroblasts (spindle-shaped), secreting collagen, with rare mitoses. These cells are arranged in short fascicles giving the appearance of a herring bone pattern. The cytoplasm is sparse, and the nuclei are elongated or ovals with few nucleoli. Poorly differentiated tumors consist of more atypical cells, pleomorphic, multinucleated giant cells, and numerous atypical mitoses and reduced collagen production (Fletcher *et al.*, 2002; Pandit *et al.*, 2004; Top *et al.*, 2008; Timaran *et al.*, 2015). In the epidermis, orthokeratotic hyperkeratosis, many koilocytes may be present (Khodakaram-Tafti and Kargar, 2009).

## 2.4. Leiomyoma

Leiomyomas are benign tumors of smooth muscle cells arising predominantly from the musculature of tubular and hollow organs, such as, the gastrointestinal, urinary, and genital tracts (Lopez *et al.*, 1997; Esmailzadeh *et al.*, 2015). Such tumors constitute 1% to 2% of neoplasms found in cattle, dogs, humans, horses, sheep, goat and pigs, and they account for 10% to 50% of all tumors found in the reproductive tract of domestic animals (Kang and Holmberg, 1983; Lopez *et al.*, 1997; Cooper and Valentine, 2002; Esmailzadeh *et al.*, 2015).

The pathogenesis of the tumor is not understood, except that the tumours arise from the muscularis mucosa of the intestinal tract or from the outer smooth muscle coats of the oesophagus, gut, gall bladder, urinary bladder or uterus, and only rarely from scattered smooth muscle fibres in organ capsules or trabeculae (Esmailzadeh *et al.*, 2015).

Grossly, leiomyoma is characterized by sharply circumscribed, smooth, encapsulated, firm, and pale tan. The cut surface has a lobulated appearance (Ramadan *et al.*, 1993; Lopez *et al.*, 1997). Cytologic evaluation of tumors of smooth muscle origin typically reveals a spindle cell population rendering a diagnosis of mesenchymal tumor, but cytologic features do not allow for a more definitive diagnosis (Goldschmidt and Hendrick, 2008).

Histopathologically, leiomyomas are characterized by relatively homogeneous population of densely packed spindle cells with indistinguishable cytoplasmic borders and elongate, blunt-ended (cigar shaped) nuclei, arranged in broad interlacing fascicles that mimic normal smooth muscle tissue. Fascicles are often described as intersecting at 90 degree angles, forming a herringbone pattern. There may be alternating “bands” created by cutting cells longitudinally or transversely. The cytoplasm may be strongly eosinophilic. Vacuolization, similar to that seen in hypertrophic smooth muscle, may also be seen. Variation from this “typical” histological pattern occurs, however, particularly in cutaneous leiomyomas, in which cells may be more heterogeneous, with scattered binucleate or multinucleate cells and moderate anisokaryosis. Mitoses are uncommon (Johnson *et al.*, 1995; LaRock and Ginn, 1997; Goldschmidt and Hendrick, 2008; Sendag *et al.*, 2008; Esmailzadeh *et al.*, 2015).

## 2.5. Papillomatosis (Warts, Fibropapillomas)

Papillomatosis is a benign, exophytic, neoplastic proliferation of cutaneous and mucosal epithelia that is commonly found in cattle, horse and dog (Goldschmidt *et al.*, 1998; Jelinek and Tachezy, 2005; Claus *et al.*, 2009; Hatama, 2012), but uncommon in cat, sheep, and goat (Desrochers *et al.*, 1994). Bovine papilloma may be occur in all ages, but it commonly occurs in young animals (Ozsoy *et al.*, 2011).

Bovine papillomaviruses (BPV) are the causative agent of papillomatosis tumors in skin, upper gastrointestinal tract and urinary bladder (Ozsoy *et al.*, 2011). Currently, the genomes of thirteen BPVs (BPV1 to 13) have been fully characterized. They are classified into four genera based on their genome relatedness and biological properties. These genera are *Deltapapillomavirus* (BPV1, 2, and 13), *Xipapillomavirus* (BPV3, 4, 6, 9, 10, 11, and 12), *Epsilonpapillomavirus* (BPV5 and 8), and a yet unnamed PV genus (BPV7) (Tozato *et al.*, 2013), but BPV-1 and BPV-2 are outlined as main agents of fibropapillomatosis in cattle due to their affinity to epithelial tissue and skin and then results cutaneous and genital lesions (Terziev *et al.*, 2015). These (BPV) induced lesions lead to hyperplastic lesions defined as warts, papillomas are initially benign in nature and self-limiting spontaneously but can developed to cancer under the effect of the environmental co-factors (Mansour, 2017).

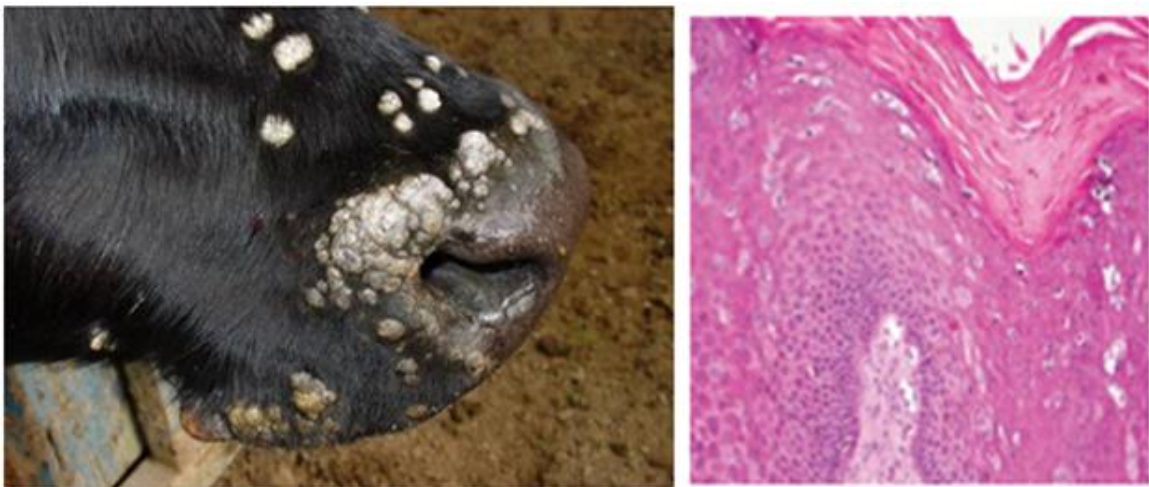
Cutaneous fibropapilloma are usually located in the head, neck, shoulders, neck folds, limbs, udder, perianal region, around eyes, ears and may spread to other areas of the body (Constable *et al.*, 2016; Mansour, 2017). Tumor formations are multiple; they can be generalized in almost all body areas, having a typical papilloma appearance of variable sizes, from 1–2 cm to large structures, which are rough, dense, and cauliflower-like. Tumors are exophytic, sometimes with a large attachment base or pediculate (Constable *et al.*, 2016).

Grossly, fibropapillomas are elevated, fleshy, and multi-nodular proliferations that emanate from the affected mucosa and may be ulcerated associated with haemorrhage

and secondary infection following spontaneous necrosis or trauma (Khodakaram-Tafti and Kargar, 2009).

Cytologic evaluation of masses can reveal large numbers of nucleated keratinocytes that display some nuclear atypia, such as retention of a large nucleus and increased nuclear to cytoplasmic ratio (Johnson and Myers, 2017) and some degenerate neutrophils (Fantini *et al.*, 2015). Intranuclear viral inclusions are rarely observed in keratinocytes in cytology samples of papillomas (Johnson and Myers, 2017).

Histopathological features of cutaneous papillomas were subdivided the naturally developing lesions into three phases: a growing phase, a developing phase, and a regressing phase (Hamada *et al.*, 1990; Hamada and Itakura, 1990). Common histopathologic characteristics of the spontaneous tumors are reported as proliferation of fibroblasts in the superficial dermis, epidermal orthokeratotic hyperkeratosis, acanthosis, presence of koilocytes which contain variably sized keratohyalin granules, and the occasional observation of intranuclear inclusion bodies. Abundant connective tissue covered by thickened hyperplastic stratified squamous epithelium and elongated epidermal–dermal interdigitation penetrates deeply into the subjacent connective tissue and is remarkable (Schulman *et al.*, 2001; Hanna and Dunn, 2003; Goldschmidt and Hendrick, 2008; Fantini *et al.*, 2015).



**Figure 3:** Papilloma (Constable *et al.*, 2016).

## 2.6. Basal Cell Carcinoma

Basal cell carcinoma is a malignant epithelial tumor of the skin which is entirely composed of basal cells and frequently seen in cats than in dogs and rare in other domestic animals (Kennedy *et al.*, 1998). The tumour is caused by malignant change in cells in the basal layer of the epidermis (Agner *et al.*, 2013), and is mainly occurs on sun exposed areas provides indirect evidence for the role of ambient solar radiation (Madan *et al.*, 2010; Agner *et al.*, 2013).

Many basal cell tumors are well circumscribed intradermal masses that may extend into the subcutaneous adipose tissue as the tumor enlarges. There is often an association with the overlying epidermis, even in tumors that are ulcerated. The tumor is multilobulated with the individual lobules separated by a fibrous stroma. Central cystic degeneration of the tumor lobules is common with an accumulation of brown/black necrotic debris in the center of the cysts and a zone of viable tumor cells at the periphery (Slovis *et al.*, 2001; Goldschmidt and Hendrick, 2008; Kumar *et al.*, 2016).

The cytological features of BCC include: tightly cohesive cellular fragments, small size of the tumor cells, morphologically uniform tumor cells, oval or fusiform and sometimes round nuclei with blurred chromatin structures, high nuclear-cytoplasmic ratio with a narrow basophilic rim of cytoplasm, nucleoli usually not evident and some fragments with distinct sharp borders (Naraghi *et al.*, 2005; Kate *et al.*, 2013).

Microscopically, BCC is composed of cells with large, elongated nuclei that display variably prominent palisading at the edge of tumor nodules. Cytoplasm may be inconspicuous, pale, or lightly eosinophilic. Mitoses and single-cell apoptoses are usually present and may be prominent. Intratumoral mucin may form large pools or cystic spaces. The BCCs display characteristic clefting between the stroma and edges of tumor nodules, which may be extensive or focal (Sexton *et al.*, 1990; Russell *et al.*, 1999; Vantuchova and Curik, 2006; Stanoszek *et al.*, 2017).

## 2. 7. Hemangiopericytomas

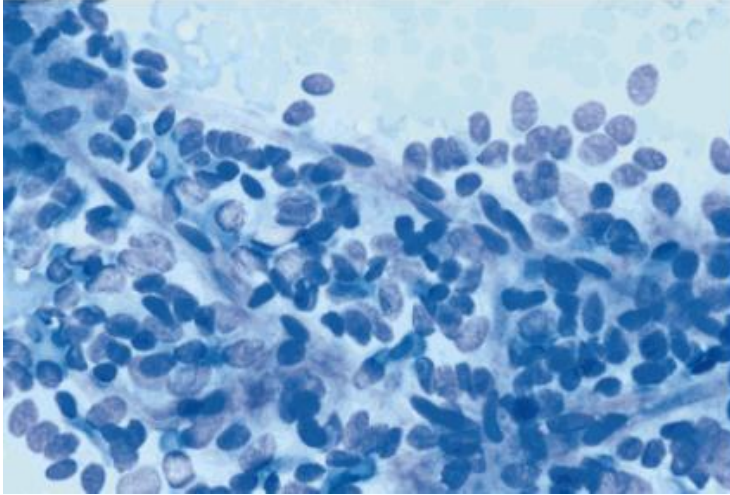
Hemangiopericytomas (HPCs) is a rare, vascularized mesenchymal tumor that develops from malignant transformation of pericytes, cells that comprise the walls of capillaries and postcapillary venules (Chhieng *et al.*, 1999; Goldschmidt and Hendrick, 2008; Gross *et al.*, 2008; Blutke *et al.*, 2012; Rutkowski *et al.*, 2012; Michi *et al.*, 2013).

Hemangiopericytomas may arise in any part of the body but occur most commonly in the lower extremities especially in the thigh, retroperitoneum, the head and neck region (Chhieng *et al.*, 1999). Grossly, they usually appear as subcutaneous, lobulated, firm, and white to grey colored tumors, occasionally reaching considerable dimensions (Gross *et al.*, 2008; Goldschmidt and Hendrick, 2008; Blutke *et al.*, 2012). They frequently display a locally infiltrative growth and tend towards recurrence after surgical excision; however, metastatic spread is uncommon (Handharyani *et al.*, 1999; Goldschmidt and Hendrick, 2008; Gross *et al.*, 2008).

Hemangiopericytomas, in cytologic preparations, comprised of single and tightly packed clusters of oval to spindle-shaped cells aggregated around branched capillaries, uniform and oval nuclei with finely granular chromatin, inconspicuous nucleoli; scant, pale, and delicate cytoplasm, cytoplasmic vacuoles, high nuclear to cytoplasmic ratio and indistinct cellular borders (Chhieng *et al.*, 1999). Aspirates of HP (HPCs) are usually hypercellular, with tightly packed cells surrounding small blood vessels. The presence of pleomorphism, clumped chromatin, prominent nucleoli, and increased mitoses suggests malignant behavior (Sawh *et al.*, 2000; Ghisleni *et al.*, 2006).

Histopathologically, HP (HPCs) are characterized by the presence of perivascular whorls, interlacing bundles and sheets of mostly spindle-shaped to fusiform cells with poorly defined cytoplasm borders, elongated pale nuclei with small central nucleoli, and an usually low mitotic activity (Chhieng *et al.*, 1999; Avallone *et al.*, 2007; Goldschmidt and Hendrick, 2008; Gross *et al.*, 2008; Blutke *et al.*, 2012). HPCs with large numbers of whorls around central vessels appear in a typical ‘fingerprint’ pattern, whereas tumors

with abundant stromal mucin often exhibit a more myxoid growth pattern. Areas of necrosis, fibrosis and haemorrhage are frequently observed within the tumor tissue (Handharyani *et al.*, 1999; Gross *et al.*, 2008).



**Figure 4:** Cytology of hemangiopericytoma (Chhieng *et al.*, 1999).

## 2. 8. Mammary Gland Tumors

Mammary gland tumors are the most common type of tumor in the unspayed female dog and cat (Munson and Moresco, 2007; Beauvais *et al.*, 2012; Dolka *et al.*, 2018) which represent over 50% of all neoplasms in female dogs over 8 years of age (Merlo *et al.*, 2008). In contrast to pets, mammary tumors rarely occur in mares, cows, ewes, goats, or sows (Munson and Moresco, 2007). Of the mammary gland tumors diagnosed in female dogs, 40-50% are malignant (Beauvais *et al.*, 2012). The majority of malignant mammary tumors in dogs are carcinomas, and <5% are sarcomas (Saeki *et al.*, 2012) which arises from epithelial tissue and metastasizes to the lungs, heart, spleen, liver, inguinal lymph nodes, brain and bones (Kim *et al.*, 2011). Breeds at risk for developing mammary gland tumors include toy and miniature Poodles, Spaniels, and German Shepherds (Saeki *et al.*, 2012; Im *et al.*, 2013).

The most important risk factors for the development of mammary gland tumors are age, breed, genetic predisposition, exposure to the female sex hormones estrogen and

progesterone in the early years of development, growth factors and diet (Sontas *et al.*, 2012). Mammary gland tumors also have been observed in male dogs, but the incidence is 1% or less. Affected male dogs usually have a hormonal imbalance such as an estrogen-secreting sertoli cell tumor of the testis (Meyer and Raskin, 2001; Arjanbhai, 2009).

Mammary gland tumors can be either malignant (cancerous) or benign (non-cancerous) and arise from the different types of tissues (epithelial or glandular tissues, and mesenchymal or connective tissues) in the mammary gland. The most common types are tumors from the glandular tissues and include adenoma, carcinoma, and adenocarcinoma (Arjanbhai, 2009).

Dogs affected with mammary tumors usually present with single or multiple nodules within a mammary gland or chain, with associated lymphadenopathy (Arjanbhai, 2009). The majority of mammary gland tumors occur in glands 4 and 5 (60-70%), possibly due to the fact that the two most caudal pairs of glands contain the most mammary tissue (Arjanbhai, 2009; Saeki *et al.*, 2012). Secondary dermal lesions may occur at the interface between tumor and skin, such as dermatitis, pyoderma and abscess formation (Arjanbhai, 2009).

Grossly, this type of tumors can appear as firm or soft, well-defined lumps or diffuse swellings that can be attached to underlying tissues or moveable, skin-covered or ulcerated. They can be different sizes, and they may grow slowly or quite fast (Arjanbhai, 2009; Information for Oncology Clients, 2018).

It is very difficult to determine the malignant potential of mammary neoplasms cytologically, and histological evidence of malignancy does not always imply an aggressive clinical course of disease (Arjanbhai, 2009; Saeki *et al.*, 2012). Cytologically, cells from adenocarcinomas may exfoliate in sheets or clusters. Individual epithelial cells contain a round to oval, eccentrically-placed nucleus and a moderate amount of basophilic cytoplasm. The cytoplasm may contain amorphous basophilic secretory

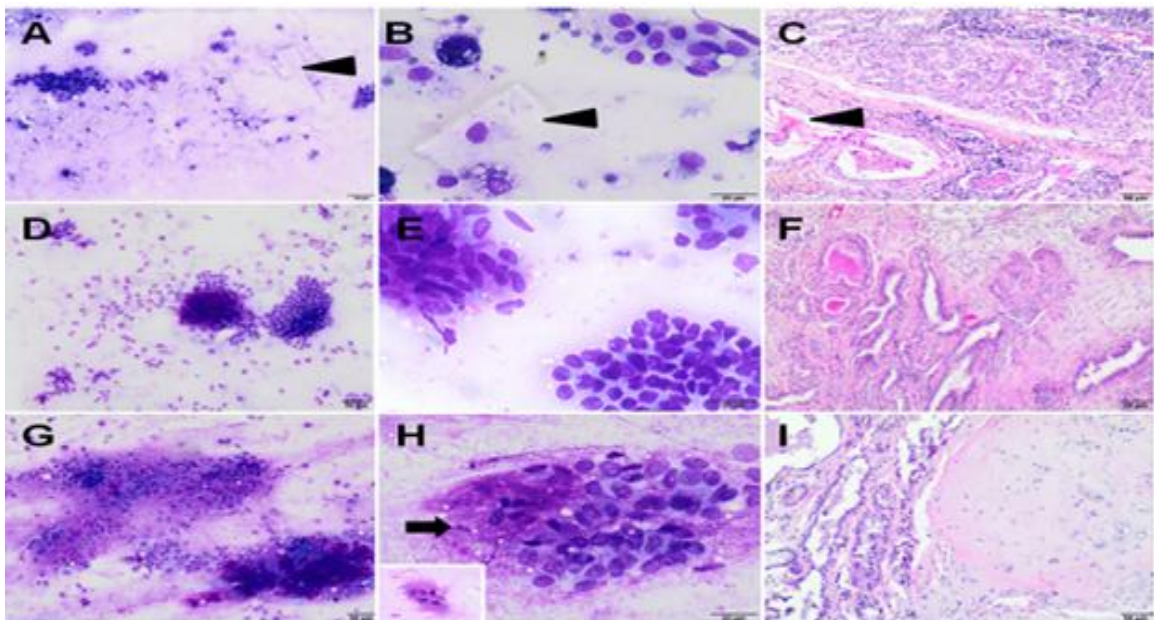
product and/or vacuoles. Acinar arrangements of epithelial cells also may be observed. Anaplastic carcinoma-these neoplasms yield very large, extremely pleomorphic epithelial cells that occur as single cells or as small clusters of cells. Unusual nuclear and nucleolar shapes may be seen. Multinucleated tumor cells and atypical mitotic figures may also be observed (Rutteman *et al.*, 2000; McCarthy *et al.*, 2001).

Cytological specimens from inflammatory carcinoma contain large, pleomorphic, epithelial cells, occasionally accompanied by stromal collagen, many non-degenerate neutrophils and macrophages. Squamous cell carcinoma of the mammary gland has a similar appearance to similar neoplasms that arise in the skin and other sites of the body. The nuclei of individual neoplastic cells range from small and pyknotic to large and round. Nucleoli are prominent and may be large and round to angular. The nuclear to cytoplasmic (N: C) ratio is variable. Binucleated and multinucleated tumor cells may be observed occasionally. The cytoplasm appears moderate to deep blue and has a smooth texture with occasional vacuoles. These changes are associated with keratinization (McCarthy *et al.*, 2001).

In malignant mixed mammary tumor, epithelial cells and individual spindleoid cells of mesenchymal origin can be observed in cytologic preparations. One of these cell populations usually will display nuclear and/or cellular criteria of malignancy. Occasionally, pink chondroid or osteoid matrix may be observed. Cytologic preparations from carcinosarcoma indicate epithelial and mesenchymal populations of cells with criteria of malignancy. In case of sarcoma, cells exfoliate poorly, yielding samples of low cellularity. In general, the cells are spindle to irregularly shaped, and are scattered individually or in small clumps. The cytoplasm is moderately to intensely basophilic with attenuated to indistinct cellular borders. Mammary sarcomas are rarely diagnosed cytologically in the dog (Rutteman *et al.*, 2000; McCarthy *et al.*, 2001).

Histopathologically, encapsulated and heterogeneous neoplasm composed by epithelial cells arranged in an acinar pattern presenting indistinct eosinophilic homogeneous

cytoplasm, with clear vacuoles of various sizes and discrete anisocytosis. Nuclei were oval in shape with a loose chromatin pattern, a distinct small nucleolus and mild anisokaryosis. The myoepithelial component was composed by cells immersed in a myxoid matrix scattered between the acini, with distinct eosinophilic star-like cytoplasm. Nuclei were elongated and hyperchromatic with no distinct nucleoli. The mesenchymal component was composed by cartilage, bone and fat differentiation. The active bone marrow was composed by trilineage hematopoietic cells, i.e. myeloid, erytroid and megakaryocytic precursors (Grandi *et al.*, 2010).



**Figure 5:** Cytological and histopathological samples of benign CMTs: Cytological smears were stained with Giemsa, while histopathological samples with hematoxylin and eosin method. Lobular hyperplasia (A, B-cytology; C-histopathology), arrows indicate cholesterol clefts; Adenoma complex (D, E-cytology; F-histopathology); Benign mixed tumor (G, H- cytology; I-histopathology); an arrow indicates the extracellular matrix, an insert in the bottom left corner of fig 5 H shows osteoclast. Original magnification: (A, C, D, F, G, I) 100x; (B,E,H) 400x (Dolka *et al.*, 2018).

## 2. 9. Testicular Tumors

Testicular tumors are the most common genital neoplasms in male dogs (Hohsteter *et al.*, 2014) and are also described in other species such as horses, cattle, cats, and man (MacLachlan and Kennedy, 2008; Togni *et al.*, 2015). Testicular tumors represent more than 90% of all canine male genital tumors and dogs have the highest incidence of testicular tumor than all other animal species (Hohsteter *et al.*, 2014). These tumors have different embryonic origins: Sertoli cells are of epithelial origin, Leydig cells are of mesenchymal origin and seminomas are derived from spermatogenic cells of the seminiferous epithelium (Valente *et al.*, 2017).

According to the World Health Organization's (WHO) classification of tumors of domestic animals, the major types of testicular tumors are sex cord-stromal (gonadostromal) tumors, germ cell tumors and mixed germ cell-sex cord stromal tumors. Interstitial (Leydig) cell tumors (ICT) and Sertoli (sustentacular) cell tumors (SCT) are the most common types of testicular tumors, derived from the sex cord-stromal tumors, whereas seminoma (SEM) is the most common type which is derived from the germ cells (Hohsteter *et al.*, 2014; Svara *et al.*, 2014). Testicular tumors are often classified as mixed tumors, although they actually result from two different tumor types occurring in the same testis ( Masserdotti *et al.*, 2005; Hohsteter *et al.*, 2014).

Several factors may influence the development of dog testicular tumors, including age, breed, cryptorchidism, and exposure to environmental carcinogens ( Liao *et al.*, 2009; D'Angelo *et al.*, 2012; Hohsteter *et al.*, 2014). Among these risk factors cryptorchid testes were approximately 13.6 times more likely to develop a tumor than scrotal testes (Liao *et al.*, 2009). However, tumor-cell types associated with cryptorchidism are different in each species. Seminoma and sertoli cell tumors are more common in cryptorchid testes than in scrotal testes (Nodtvedt *et al.*, 2011). Leydig cell tumors are generally considered benign although malignant forms have been described. An irregular cell form and an increased mitotic rate histologically characterize these. The distinction between benign

and malignant forms is based on histological criteria such as infiltrative growth and can be difficult to diagnose (MacLachlan and Kennedy, 2008; Togni *et al.*, 2015).

Sertoli cell tumors are very rare, slow growing, non-invasive testicular tumors with low malignancy features, representing 0.4-1.5% of all testicular malignancies (Hohsteter *et al.*, 2014). They are sub-classified as classic, large-cell calcifying, and sclerosing sertoli cell tumors (SSCT) based on distinct clinical features (Coleman *et al.*, 2014). SSCTs are typically small (0.4–1.5cm, maximum 4cm) (Giglio *et al.*, 2003) whereas the other subtypes can be significantly larger (Coleman *et al.*, 2014). Metastasis are rare, occurring in approximately 10% of cases, but when present is often located in the spermatic cord and lymph nodes. Metastasis can also occur in mesenteric and periaortic lymph nodes, liver, lungs, kidneys, spleen and adrenal glands (Kang *et al.*, 2011; Svara *et al.*, 2014; Castro *et al.*, 2016).

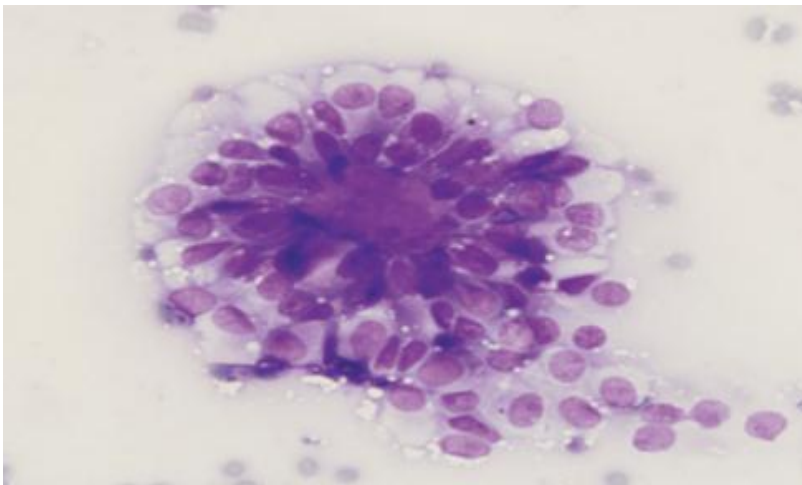
Dogs with a Sertoli cell tumor may show clinical signs of feminization attributable to paraneoplastic hyperestrogenism (Valente *et al.*, 2017). Feminization is a frequent paraneoplastic syndrome associated with SCT, showing signs of hyperestrogenism, contralateral testicular atrophy, symmetrical alopecia, prostate gland and bone marrow disorders (Peters *et al.*, 2000). However, one report indicated that dogs with malignant SCT did not show feminization syndrome (Kang *et al.*, 2011; Castro *et al.*, 2016).

Macroscopical examination revealed a grossly unifocal, well-circumscribed, white, nodular, firm mass markedly enlarged to 8cm in diameter that is almost totally replaced the testicular tissue (Masserdotti *et al.*, 2008; Coleman *et al.*, 2014). On cut surface, lobulated and well demarcated milk-white neoplastic areas were irregularly separated by white fibrous bands (Kang *et al.*, 2011; Castro *et al.*, 2016).

Cytological specimens from fine-needle aspirates of SCT is highly cellular and consists of a population of medium to large (approximately 21–30 mm), round to elongated cells arranged in large sheets and frequently in palisades. The cells have lightly basophilic cytoplasm, sometimes with indistinct borders, which often contain many small to large

clear vacuoles. Nuclei are round to oval, with fine granular chromatin and single prominent nucleoli. Mitotic figures are observed occasionally. A variable number of structures are observed that consists of a central round area of amorphous, deeply eosinophilic, hyaline material surrounded by a peripheral, rosette-like arrangement of single or multiple rows of the nucleated cells (Fig. 6). The specimen collected from one dog has shown a few individual cells contained eosinophilic intracytoplasmic inclusions (Bazzo *et al.*, 2002; Masserdotti *et al.*, 2008).

A histopathological characteristic of Sertoli cell tumors consists of neoplastic cell populations that are arranged in irregular tubular structures surrounded by thick fibrous bands, severe anisocytosis and anisokaryosis, one or two evident nucleoli, high mitotic rate and significant presence of stroma. Neoplastic cells are columnar, have vacuolated cytoplasm, and have round to oval nuclei with irregularly clumped chromatin. The cells are arranged in multilayered palisading structures, with their long axes perpendicular to the basement membrane. In some areas, concentric structures consisting of peripheral rows of neoplastic Sertoli cells arranged in pseudorosettes around an intensely eosinophilic region and a brighter central lamellated area are observed, consistent with Call-Exner bodies (Masserdotti *et al.*, 2008; Castro *et al.*, 2016).



**Figure 6:** Fine-needle aspirate of a testicular mass from a dog: A large cluster of round to elongated cells is arranged in a rosette around deeply eosinophilic, hyaline material, consistent with a Call-Exner body. May-Grunwald-Giemsa, 40 objective (Masserdotti *et al.*, 2008).

## 2. 10. Ovarian Tumors

Ovarian diseases in female dogs are uncommon (Arlt and Haimerl, 2016) but may affect health, fertility or even the life of the animal. One reason for the rarity of the diagnosis of ovarian disease presumably is that many female dogs are neutered at an early age (McEntee, 2002; Arlt and Haimerl, 2016). Several authors, however, also reported ovarian diseases that arose from incompletely resected ovarian tissue (McEntee, 2002). Most common ovarian diseases appear to be cystic ovaries and ovarian tumors (Johnston *et al.*, 2001).

Ovarian tumors are classified as epithelial tumors (Papillary adenoma, Cystadenoma, Papillary adenocarcinoma and other (rete adenoma, undifferentiated carcinomas), sex cord-stromal tumors (granulosa cell tumor, thecoma (theca cell tumor), interstitial cell tumor and Sertoli cell tumor), germ cell tumors (dysgerminoma, teratoma and embryonal carcinoma) and other tumors (Arlt and Haimerl, 2016). The majority of primary ovarian neoplasia belongs to the categories epithelial tumors, sex cord-stromal tumors and germ cell tumors (McEntee, 2002). Other tumors that are of less significance are as follows: connective tissue tumors, secondary (metastatic) tumors and tumors that cannot be classified (Arlt and Haimerl, 2016).

Epithelial tumors and sex cord stromal tumors together in bitches account for 80-90% of all canine ovarian tumors (Diez-Bru *et al.*, 1998). Mesenchymal tumors (originate from cells surrounding the skin, like fat, connective tissues, blood vessels and nerves) like primary ovarian hemangiosarcoma and fibromas have also been reported (Diez-Bru *et al.*, 1998; Arlt and Haimerl, 2016). Among ovarian tumors granulosa cell tumors are described to be the most common neoplasia in the bitch accounting for 50% of all ovarian neoplasias (Arlt and Haimerl, 2016). They often occur unilaterally and are seen as a huge, lobulated, firm mass with a yellow- white surface. Also, they often show cysts, haemorrhagic areas and necrosis (Arlt and Haimerl, 2016). Also thecomas and luteomas have been reported in bitches. Usually, they are considered benign but may show expansive growth (Kennedy *et al.*, 1998). The thecoma cells are less spindly, ill-defined,

and oval or rounded, showing abundant, pale, and vacuolated cytoplasm containing lipid (Yang and Mesia, 1999; Goldschmidt and Hendrick, 2008).

The fibrothecoma is a rare tumor, occurring at a rate of approximately 1%-4.7% of all benign ovarian tumors, and arising in stroma and exhibit a morphological spectrum from those composed of entirely fibroblasts and producing collagen (fibromas) to those containing more plump spindle cells with lipid droplets (thecomas) (Sharma *et al.*, 2013; Danendran Krishnan and Anitha Ann Thomas, 2014).

Grossly, ovarian fibrothecomas are unilateral in about 90% of all cases and are usually solid, circular or slightly lobulated, or oval tumors with smooth contours and a regular echogenicity (Kievitsbosch *et al.*, 2013; Sharma *et al.*, 2013).

Histopathologically, ovarian fibrothecomas frequently show interlacing bands and storiform pattern of elongated spindly cells containing scanty to moderate amount of eosinophilic to clear cytoplasm were seen. Collagenized and hyalinized stroma and focal collections of plump cells with clear cytoplasm were seen. Areas of hemorrhage and edema were present (Sharma *et al.*, 2013; Danendran Krishnan and Anitha Ann Thomas, 2014).

## **2. 11. Mast Cell Tumors**

Mast cell tumors (MCTs) are highly invasive, metastatic and are the most frequent round cell tumors in dogs comprising 16-21% of all cutaneous tumors diagnosed (Misdorp, 2004; Newman *et al.*, 2007) but are less common in cats, and uncommon to rare in other species (Meuten, 2017).

Canine MCTs have variable biologic behaviors, ranging from solitary benign masses that can be cured with surgery alone to systemic and potentially fatal metastatic disease (Welle *et al.*, 2008), and are always considered potentially malignant, but their true metastatic potential is not entirely known (Webster *et al.*, 2007).

Breeds found to be predisposed to develop MCTs include boxers, Boston terriers, bull terriers, bullmastiffs, cocker spaniels, Staffordshire terriers, fox terriers, English bulldogs, dachshunds, Labrador retrievers, golden retrievers, beagles, pugs, schnauzers, sharpeis, Rhodesian ridgebacks, Weimaraners and Australian cattle dogs (Goldschmidt and Hendrick, 2008; Welle *et al.*, 2008). Predilection sites have also been reported for various breeds: hind limbs and multiple lesions in boxers and pugs, hind limbs in Boston terriers and American Staffordshire terriers, the tail in Rhodesian ridgebacks, multiple lesions in Weimaraners and golden retrievers, and the head and hind limbs in English setters (Welle *et al.*, 2008).

Mast cell tumor in dogs can occur in two different forms: common as cutaneous tumor, or less common as a systemic form of mast cell proliferation tumors; systemic mastocytosis (Marinkovic *et al.*, 2015). Systemic mastocytosis (systemic mast cell tumor) is a term used to describe the proliferation and invasion of mast cells in several tissues, such as subcutis, lymph nodes, internal organs and bone marrow, with or without concurrent mast cell leukemia (Marinkovic *et al.*, 2015; Meuten, 2017). This type of mast cell tumor is less frequent comparing to cutaneous mast cell tumor, which represents one of the commonest skin tumors in dogs (Welle *et al.*, 2008). Cutaneous MCTs are most common on the trunk (50–60%), followed by the extremities (25–40%), and the head and neck (10%). The scrotum, perineum, back and tail are less commonly affected. In general, a greater number of tumors affect the posterior part of the body (hind limbs, perineum and prepuce) (Welle *et al.*, 2008).

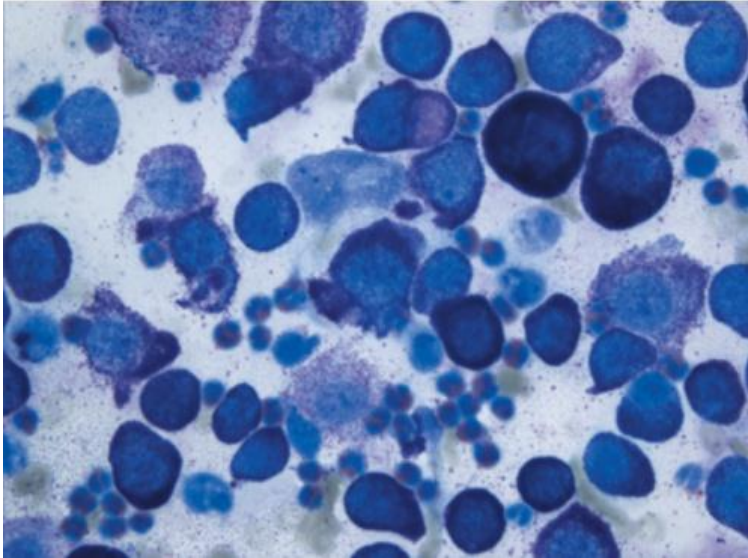
MCTs are most often graded histologically by the scheme described by Patnaik, and nowadays more often by the grading system proposed by Kiupel *et al.* (2011). Patnaik grading system is based on level of differentiation, cellularity, cellular morphology, mitotic index and tissue reaction and it has been widely used (Hosseini *et al.*, 2014). Tumors of grade I are circumscribed, mainly dermal in location, and consist of well differentiated mast cells with prominent metachromatic cytoplasmic granules. Poor cellular differentiation, aggressive growth pattern, moderate to high mitotic activity, mitotic atypia and sparse to absent cytoplasmic granulation are the major features of

grade III tumors. Grade II MCTs constitute an intermediary form between the two other grades, but they tend to infiltrate more deeply than grade I tumors (Gross *et al.*, 2008).

According to the Kiupel grading system, high-grade MCTs include tumors with at least one of the following features: at least 7 mitotic figures in 10 high power fields (hpf); at least 3 multinucleated cells in 10 hpf; at least 3 bizarre nuclei in 10 hpf; or at least 10% karyomegalic neoplastic cells. All other tumors are considered low grade (Kiupel *et al.*, 2011).

MCTs can generally be diagnosed by cytological examination of stained smears of FNAs (Welle *et al.*, 2008; Withrow *et al.*, 2013). Cytology of MCTs reveals a discrete round-cell population with moderate amounts of cytoplasm containing purplish red cytoplasmic granules of variable number and size. The cells have a round to oval nucleus that may be masked by intense staining in highly granulated cells (fig. 7). Other cells found on cytology are variable numbers of eosinophils and/or plump spindle cells, presumably fibroblasts (Webster *et al.*, 2007; Welle *et al.*, 2008).

Histopathologically, MCT cells were less well circumscribed by connective tissue and tumors cells often exhibited an aggressive behavior, high cellularity, cellular pleomorphism, and various morphologic patterns. However, in some regions of the tumor tissue shows tumors' cells extending into the subcutis. Tumor cells had distinct cell borders and widely variable amounts of pale to brightly eosinophilic cytoplasm. Furthermore, tumor had tumors cells containing numerous fine eosinophilic to basophilic cytoplasmic granules consistent with mast cell granules and also highly pleomorphic tumor cells with a high mitotic index, anisokaryosis and infiltration of the surrounding dermis and subcutis were observed (Hosseini *et al.*, 2014).



**Figure 7:** Fine-needle aspirate smear of a mast cell tumor; note highly granular mast cells, staining is so intense that cellular morphology is often obscured (Welle *et al.*, 2008).

## **2. 12. Canine Transmissible Venereal Tumor**

Canine transmissible venereal tumor (CTVT), also called transmissible venereal sarcoma, Sticker's sarcoma, venereal granuloma and infectious sarcoma, is a relatively common contagious venereal tumor of young, sexually active, free-roaming dogs (Ganguly *et al.*, 2013). It commonly affects the mucosa of the penis and vagina and is usually transmitted via coitus. Transmission may also occur through mucosal contact with neoplastic cells, biting, licking, scratching, and sniffing lesions (Kabuusu *et al.*, 2010; Bautista-Gomez *et al.*, 2011; Ganguly *et al.*, 2013; Strakova and Murchison, 2014). Occasionally, lesions may be present in other parts of the body without any genital involvement (Ganguly *et al.*, 2013).

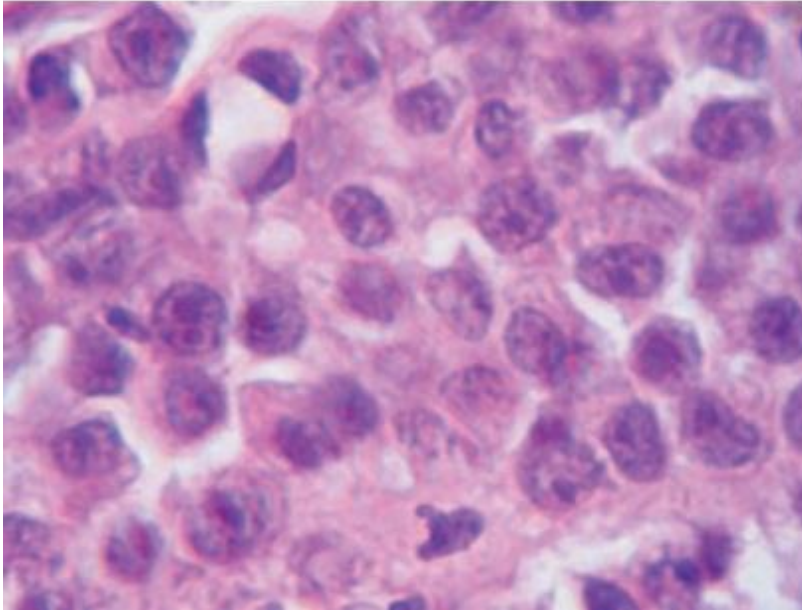
Canine transmissible venereal tumors may be classified based on cytomorphological characteristics as lymphocytic, plasmacytic or mixed (Florez *et al.*, 2012; Nalubamba, 2015). The lymphocytoid subtype is characterized by > 60% round cells with finely granular cytoplasm containing few clear vacuoles; nuclei are centrally located and are round with a coarse chromatin pattern and 1–2 distinct nucleoli. The plasmocytoid

subtype is composed of > 60% ovoid cells with more abundant cytoplasm, several clear vacuoles, and an eccentrically located nucleus. The mixed type contains both lymphocytoid and plasmacytoid cells, with neither type exceeding 59% of total cells (Florez *et al.*, 2012).

The tumor is cauliflower-like, pedunculated, nodular, papillary or multilobulated in appearance. It ranges from a small nodule (5µm) to a large mass (up to 15cm) and is firm, though friable. The surface is often ulcerated and inflamed and may be haemorrhagic and infected. The tumor may be solitary or multiple and is almost always located on the external genitalia, although it may occur in adjacent skin and oral, nasal and conjunctival mucosae (Goldschmidt and Hendrick, 2008; Ganguly *et al.*, 2013).

Cytology provides a better evidence of CTVT as compared with histology. Cytologically, CTVT cells have a very distinct appearance (fig. 8). They are round to oval in shape and often contain mitotic figures, with chromatin clumping and one or two prominent nucleoli. The most striking cytological finding is the presence of multiple clear cytoplasmic vacuoles. Vacuolation increases during early stages of regression as CTVT cells undergo degeneration. During degeneration, amounts of endoplasmic reticulum and ribosomes also increase, as does swelling and vacuolation of mitochondria. Degenerating cells often contain numerous membrane-bound granules and clusters (Gonzalez *et al.*, 2000; Ganguly *et al.*, 2013).

The histologic appearance of transmissible venereal tumor may be difficult to distinguish from other round cell tumors (Das *et al.*, 1990; Ganguly *et al.*, 2013). Histopathologically, the tumor is composed of loose sheets, rows and cords of relatively uniform round to ovoid cells. Cell margins are generally indistinct. Nuclei are large, round, with a single centrally placed nucleolus surrounded by marginated chromatin. There is a moderate amount of light pink to clear cytoplasm. The mitotic index is high. Variable numbers of lymphocytes, plasma cells and macrophages infiltrate the tumor. In regressing tumors, increased inflammation and zones of necrosis and fibrosis are often present (Marchal *et al.*, 1997; Gonzalez *et al.*, 2000; Goldschmidt and Hendrick, 2008).



**Figure 8:** Cytology of canine transmissible venereal tumor: numerous round to oval cells are present with multivacuolated cytoplasm. Many cells show more than one nucleoli; the granular appearance of the karyoplasm due to chromatin clumping and increased nucleus: cytoplasm ratio are other cytological features of CTVT (Ganguly *et al.*, 2013).

### **2. 13. Malignant Fibrous Histiocytoma**

Malignant fibrous histiocytoma (MFH) is a pleomorphic sarcoma of unknown aetiology that are occasionally reported in horses and mules, and rarely in the skin of other domestic species, including dogs ( Perez-Martinez *et al.*, 2000; Geburek *et al.*, 2007; Goldschmidt and Hendrick, 2008). This neoplasm is composed of both histiocytic and fibroblastic-like cell types (Fulmer and Mauldin, 2007; Ozyigit *et al.*, 2011) and was previously grouped with canine histiocytic diseases but is now more appropriately considered a soft tissue sarcoma (Risbon *et al.*, 2007). No sex or breed predilection has been reported and the tumor is most commonly seen in middle-aged or older animals (Bush and Powers, 2008; Goldschmidt and Hendrick, 2008).

Malignant fibrous histiocytomas may occur in all body regions (Geburek *et al.*, 2007) although predominantly in dermal or subcutaneous extraskeletal areas including dorsal

thoracic and scapular area, neck, flank, abdomen, muzzle, and hind (Aydin *et al.*, 2003; Bush and Powers, 2008; Goldschmidt and Hendrick, 2008), forelimbs (Bush and Powers, 2008; Ozyigit *et al.*, 2011), and costal abdominal regions (Ozyigit *et al.*, 2011).

The neoplasm generally appears as a single, solid to soft tissue mass with a diameter from one up to several centimeters. In general, it is poorly demarcated from the surrounding tissue, and characterised by invasive growth. In horses metastases either do not occur or develop very slowly (Marryatt, 2003; Geburek *et al.*, 2007).

Grossly, the tumor is well circumscribed, small, firm, solitary, nodular, and encapsulated or unencapsulated with ulcerated overlying skin (Kiran *et al.*, 2005; Bush and Powers, 2008; Goldschmidt and Hendrick, 2008; Boneschi *et al.*, 2009). On the cut surface, the tumor consists of multiple nodules and is often gray/white and firm with small red mottled areas of hemorrhage and necrosis (Bush and Powers, 2008; Goldschmidt and Hendrick, 2008; Boneschi *et al.*, 2009).

Cytologic features seen in smears obtained by thin needle aspiration included loose aggregates of markedly pleomorphic, elongated cells, histiocytelike cells and isolated bizarre giant cells (Kim and Goldblatt, 1982; Berardo *et al.*, 1997).

The classic histologic presentation of MFH in case of human has been divided into five types: pleomorphic, angiomatoid, myxoid, giant cell, and inflammatory (Kiran *et al.*, 2005). However, in domestic animals three subtypes have been reported, depending on the predominant cell types and the amount of intercellular materials. These include storiform-pleomorphic, xanthogranulomatous (or inflammatory) and giant cells (Kiran *et al.*, 2005; Geburek *et al.*, 2007).

Histopathologically, a tumor cell consists of a mixture of fibroblast-like spindle and histiocytic-like cells with a pale pink cytoplasm, oval or elongated with a round, large nucleus with small and single nucleoli. Besides these cells, abundant, randomly scattered, eosinophilic, multinucleated giant cells with a finely granular cytoplasm and shapes that varied from round to oval or irregular were described (Ozyigit *et al.*, 2011).

## 2.14. Perianal Gland Tumor

The perianal region of dogs contains multiple glands, some of which are unique to the area. These are the perianal or circumanal gland (hepatoid) glands, the anal sac glands and the anal glands (Polton *et al.*, 2006; Javanbakht *et al.*, 2013). The perianal glands, unique into the dog and marsupials, are non-secretory abortive sebaceous glands situated around the anus in a uniform circle up to 2 cm from the anal orifice, and scattered areas on the prepuce, tail, hind legs, and trunk (Javanbakht *et al.*, 2013). Three types of glandular tumors, namely perianal gland (or circumanal or hepatoid ) tumor, apocrine gland tumor of the anal sac, and apocrine gland tumor commonly occur in the perianal area of the dog (Parry, 2006; Javanbakht *et al.*, 2013).

A perianal gland tumor is a type of tumor found near the anus in intact male dogs that arises from specialized glandular tissue found in the perineum (Petterino *et al.*, 2004). Most perianal gland swellings are focal hyperplasia and the benign proliferative form (adenoma), while their malignant counterparts (adenocarcinoma) are uncommon (Javanbakht *et al.*, 2013). Perianal gland adenomas are three times more likely to be found in intact male dogs than females, and perianal gland adenocarcinomas are ten times more common in male dogs than females. The development of adenomas is largely dependent on the influence of testosterone and other androgens (DACVP and Vandis, 2010).

The most commonly affected breeds for adenomas are the Siberian Husky, Cocker Spaniel, Pekingese, and Samoyed; for adenocarcinomas the most commonly affected breeds are the Siberian Husky, Bulldog, and Alaskan Malamute (DACVP and Vandis, 2010; Yumusak *et al.*, 2016).

These tumors are typically nodular but may vary in size and diameter. The tumors may present as a single mass or as multiple masses. Depending on where the tumor is located, the masses may be variably raised, ulcerated, or hairless (DACVP and Vandis, 2010).

Cytologically, anal sac adenocarcinoma is characterized by variably-sized clusters of neoplastic cells with indistinct cell borders, a prominent population of round-to-polygonal nucleated cells arranged in rosette-like patterns, pale to basophilic cytoplasm, a mild degree of anisocytosis, anisokaryosis, pleomorphism, smaller reserve type cells, with darker cytoplasm and a higher nucleocytoplasmic ratio. Ulceration, hemorrhage, necrosis and a small number of erythrocytes and occasional nondegenerate neutrophils and large mononuclear cells are not uncommon (Sakai *et al.*, 2012; Javanbakht *et al.*, 2013).

Histologically, canine anal sac adenocarcinoma is classified into solid, rosette, and tubular subtypes. The solid subtype consists of sheets of neoplastic cells sub-divided into thin bands of fibrous tissue, but lacks glandular structures. In the rosette subtype, neoplastic cells with peripherally located nuclei and eosinophilic cytoplasm are radially arranged around a small central focus of an eosinophilic secretory substance. This subtype is bimorphic, featuring glandular areas that contain acini inter-spersed with solid lobules that may contain rosette-like structures. The tubular subtype has accumulations of eosinophilic secretory material within the tubular lumina (Sakai *et al.*, 2012).

### **3. MATERIALS AND METHODS**

#### **3. 1. Study Areas**

The study was conducted from October, 2017 to May 2018 to study cytological and pathological characteristics of tumors of domestic animals from veterinary teaching hospitals of college of veterinary medicine of Addis Ababa University and some from around Bishoftu. Bishoftu is located 45 km South East of Addis Ababa. The area is located at 9°N latitude and 40°E longitude at an altitude of 1850 meters above sea level (masl) with annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26°C and 4°C, respectively, with mean relative humidity of 61.3% (Belay and Muktar, 2015). The domestic animals raised in the area are 91,040 cattle population, 39,055 goats, 39,048 sheep, 22,676 donkeys, 6,136 horses, and 2,015 mules. However, the total number of pet animals is not yet known (Genzebu *et al.*, 2016).

#### **3. 2. Study Population**

The study populations were domestic animals brought to clinics and/or reported by assigned technician for complaint of grossly visible growth or swellings. Those animals of all age and breed reared under intensive, semi-intensive and extensive production and management system were included in the study for assessing the neoplastic and non-neoplastic swellings.

#### **3. 3. Study Design**

A cross sectional study design with purposive sampling of animals showing with any type of swelling or mass was conducted and then the neoplastic masses were identified.

### **3. 4. Methods of Sampling and Sample Processing**

#### *3.4.1. Gross examination*

All suspected lesions were first visually observed carefully and then palpated for consistency, texture and attachments. Then either biopsy samples from live animals or necropsy from dead or euthanized animals were collected for further characterization of the lesions. In all biopsy sampling based on lesion type, animal species and sample size either lidocaine (ring block infiltration) or intramuscular injections of 0.5- 3ml ketamine were used.

#### *3. 4. 2. Cytologic sample collection and processing*

The masses were first washed with soap and water, shaved, and disinfected with alcohol just before biopsy samples. The FNA biopsies were collected either by aspiration or non-aspiration techniques based on firmness of lesions. Briefly, 21-25 gauge needles (Annex 1) attached to a 3-5 ml syringes were inserted into the mass and samples were collected by aspiration, if the mass is hard or firm and by non-aspiration, if the mass is soft or cystic. In FNA, to collect maximum cells the needles were redirected several times in the mass. Then the syringes were removed and the contents were expelled onto a new grease free slide and smear was prepared (Annex 2). Several smears were made when possible from a single mass (John, 2014).

Impressions were made from ulcerated lesions, from surgically removed tissues or after necropsy. Ulcers were imprinted before cleaned and then cleaned with saline moistened sponges and reimprinted to compare as ulcerated lesions may yield inflammatory cells. Similarly, squash smears were also prepared from biopsy and necropsy masses. All biopsied and necropsy collected tissue were also subjected to FNA in addition to impression and squash to increase chance of diagnosis (Annex 2 and Annex 3) (Meinkoth *et al.*, 2008).

After the smears were made by any of these methods then the smears were air dried, fixed with methanol and stained with Giemsa (Annex 4). Finally, the stained smears were scanned microscopically starting from lower magnifications to 100X (Annex 5) (Meinkoth *et al.*, 2008).

### *3. 4. 3. Histopathology techniques*

Both biopsy and necropsy samples were taken for histopathology. Biopsy tissues were either incised from lesions using a sharp surgical blade or excised from surgical cases. Whereas for necropsy, animals were euthanized by fully anaesthetizing with 20ml thiopental sodium IV injection and then killed by IV injection of potassium chloride. Necropsies of euthanized animals were undertaken according to the procedure by Dennis and Joanna (2006).

Samples were immediately fixed by 10% neutral buffered formalin. Formalin fixed tissues were trimmed and put in to plastic tissue cassettes and then processed using an automatic tissue processor. Within the automatic tissue processor tissues were dehydrated in graded concentrations of absolute alcohol (70%, 95% and 100%), cleared by xylene and impregnated/infiltrated by molten paraffin. Impregnated tissues were made into a mould and solidify and then it was embedded. Tissues were sectioned to 4-5 mm thickness using a semi-automatic microtome machine, tissue ribbons were floated on warm water bath at 45<sup>o</sup>c and they were adhered to albumenized glass slides. The sectioned tissues were dewaxed (by heat and xylene), hydrated (by descending grades of alcohols (100%, 95%, 70%), and then stained by Haematoxylin and Eosin (H&E). The stained slides were dehydrated in ascending grades of alcohols (70%, 95% and 100%) and cleared by xylene and then mounted by Canada balsam and examined under the microscope and finally photographs of the slides were taken (Takulder, 2007) (Annex 6).

### **3. 6. Ethical Consideration**

Before starting the research a nine page request that explain the purpose of this study and the possible management planned to reduce pain and suffering of animal during sampling was submitted to Addis Ababa University College of Veterinary Medicine and Agriculture Minutes of Animal Research Ethics and Review committee. After the committee evaluated the importance of this study through different aspect, approval was given on minute number (VM/ERC/05/10/018) with the reference number (VM/ERC/10/05/10/2018) (Annex 7).

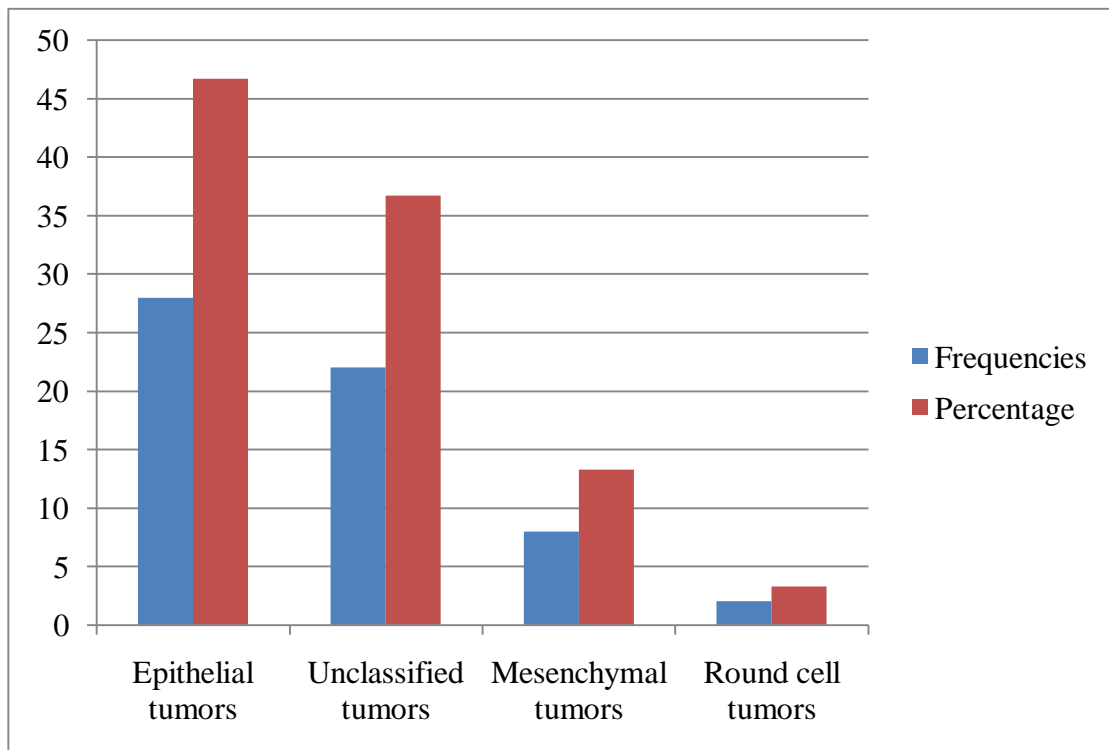
### **3. 7. Data Analysis**

Data generated from field and laboratory investigations were recorded, screened and coded using Microsoft Excel spreadsheet and the cytological, gross as well as histopathological lesions and findings were described using qualitative methods. Descriptive statistics was used for summarizing data using Microsoft Excel.

## 4. RESULTS

### 4.1. Cellular Origin, Types and Frequencies of Tumors

Different tumors from different domestic animals were examined and differentiated. Out of 60 masses diagnosed as tumors; epithelial tumors represented 46.7% (n=28), unclassified tumors represented 36.7% (n=22), mesenchymal tumors represented 13.3% (n=8) and round cell tumors represented 3.3% (n=2) (fig. 10).



**Figure 9:** Over all frequencies and proportions of tumor type.

In this study cattle, dogs and pigs were affected by tumors of epithelial origin but these tumors were not observed in equine species. Of tumors of epithelial origin, squamous cell papilloma were the most frequent tumors occurred in cattle represented 33.3% (n=20) as compared with other animals. However, mammary gland tumors were frequently characterized in dog represented 6.7% (n= 4). Whereas Basal cell carcinoma and Sertoli cell tumor were encountered in pig and dog, respectively (table 1).

**Table 1:** Frequencies of tumors of epithelial origin

<b>Serial No</b>	<b>Tumor type</b>	<b>Cattle</b>	<b>%</b>	<b>Dog</b>	<b>%</b>	<b>pig</b>	<b>%</b>
<b>1</b>	Squamous cell papilloma	20	33.3	-	-	-	-
<b>3</b>	Ocular squamous cell carcinoma	1	1.7	-	-	-	-
<b>4</b>	Basal cell carcinoma	-	-	-	-	1	1.7
<b>5</b>	Mammary gland tumor	-	-	4	6.7	-	-
	<i>Papillary mammary adenocarcinoma</i>	-	-	1	1.7	-	-
	<i>Carcinoma–mixed type</i>	-	-	2	3.3	-	-
	<i>Mammary adenocarcinoma</i>	-	-	1	1.7	-	-
<b>6</b>	Sertoli cell tumor	-	-	1	1.7	-	-
<b>7</b>	Atypical anal sac adenocarcinoma	-	-	1	1.7	-	-
<b>Total</b>		<b>21</b>	<b>35</b>	<b>6</b>	<b>10.1</b>	<b>1</b>	<b>1.7</b>

From tumors of unclassified origin, cattle were most frequently affected by fibropapilloma representing 23.3% (n=14). However, horses and donkeys were frequently affected by verrucous 5% (n=3) and fibroblastic sarcoïd 5% (n=3), respectively. Tumors of unclassified origin were not observed in pig and dog (table 2).

**Table 2:** Frequencies of tumors of unclassified origin

<b>Serial No</b>	<b>Tumor type</b>	<b>Cattle</b>	<b>%</b>	<b>Horse</b>	<b>%</b>	<b>Donkey</b>	<b>%</b>
<b>1</b>	Fibropapilloma	14	23.3	-	-	-	-
<b>2</b>	Equine sarcoid						
	a) Fibroblastic sarcoid	-	-	1	1.7	3	5
	b) Verrucous sarcoid	-	-	3	5	1	1.7
<b>Total</b>		<b>14</b>	<b>23.3</b>	<b>4</b>	<b>6.7</b>	<b>4</b>	<b>6.7</b>

Of tumors of mesenchymal origin, 3.3% cutaneous fibrosarcoma and 3.3% hemangiopericytoma were diagnosed in cattle. Fibrothecoma and malignant fibrous histiocytoma were another tumors of mesenchymal origin characterized in dog and mule with 1.7% (n=1) in each case. However, no mesenchymal tumors were encountered in horse and pig (table 3).

**Table 3:** Frequencies of tumors of mesenchymal origin

<b>Serial No</b>	<b>Tumor type</b>	<b>Cattle</b>	<b>%</b>	<b>Donkey</b>	<b>%</b>	<b>Mule</b>	<b>%</b>	<b>Dog</b>	<b>%</b>
<b>1</b>	Cutaneous fibrosarcoma	2	3.3	1	1.7	-	-	-	-
<b>2</b>	Vaginal leiomyoma	1	1.7	-	-	-	-	-	-
<b>3</b>	Hemangiopericytoma	2	3.3	-	-	-	-	-	-
<b>4</b>	Malignant fibrous histiocytoma	-	-	-	-	1	1.7	-	-
<b>5</b>	Fibrothecoma	-	-	-	-	-	-	1	1.7
<b>Total</b>		<b>5</b>	<b>8.3</b>	<b>1</b>	<b>1.7</b>	<b>1</b>	<b>1.7</b>	<b>1</b>	<b>1.7</b>

Cutaneous mast cell tumor and transmissible venereal tumor were the only round cells tumors encountered, and both were in dogs. However, in this study no round cell tumors were found in other species of animals (table 4).

**Table 4:** Frequencies of round cell tumors

<b>Serial No</b>	<b>Tumor type</b>	<b>Dog</b>	<b>%</b>
Round cell tumors			
<b>1</b>	Cutaneous mast cell tumor	1	1.7
<b>2</b>	Transmissible venereal tumor	1	1.7
<b>Total</b>		<b>2</b>	<b>3.4</b>

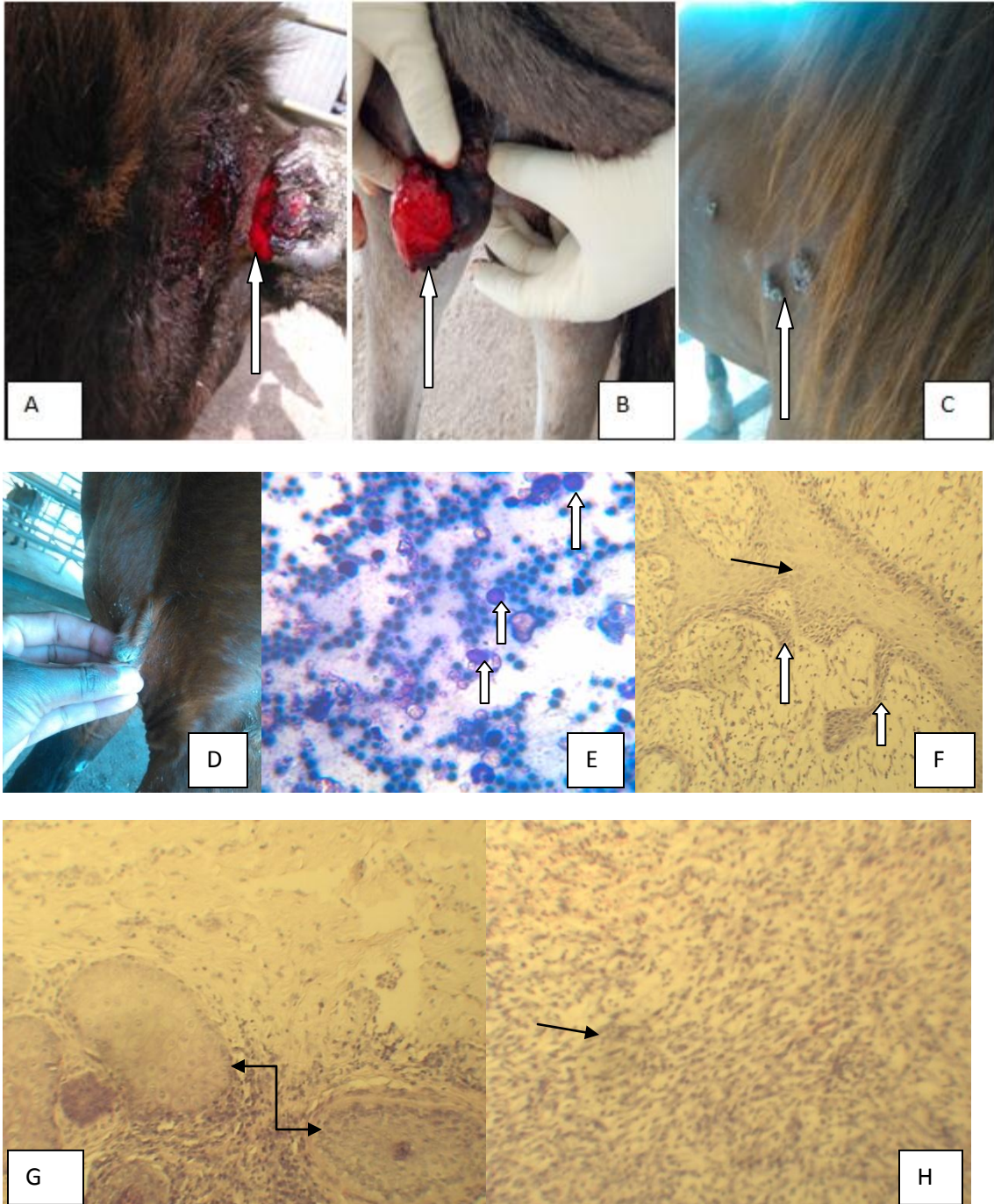
## **4. 2. Gross, Cytological and Histopathological Characterization of Tumors**

### *4. 2. 1. Equine sarcoid*

Equine sarcoids were frequently located on ear, neck, chest, legs, and perineum. The gross characteristic of equine sarcoids varies according to type; with the fibroblastic sarcoids have a characteristic fleshy, ulcerated appearance on the ear (fig. 10 A) and anal region of donkeys (fig. 10 B). Verrucous sarcoids were characterized by presence of small wart-like lesions, a raised, scaly, lichenified appearance with epidermal thickening and alopecia on chest (fig. 10 C) and around sternum (fig. 10 D) of horse.

Fine needle aspirate of equine sarcoid yield high cellularity perfield with deep nuclear chromatin and prominent nucleus. The cells arranged either in trabecular clusters or in a cord like arrangements (fig. 10 E). Anisocytosis was so prominent with some extremely large cells with large nucleus compared to the neighbouring cells (fig. 10 E). These cells have eccentric large nucleus with prominent chromatins (arrows in fig. 10 E). Anisokaryosis, indistinct cell borders, mitotic figures, high nuclear to cytoplasmic ratio were another characteristic (fig. 10 E), (Geimsa, original magnification 100X).

Histopathologically, equine sarcoids were characterized by epidermal spinous and squamous keratinocytes differentiation (hyperplasia) and infiltration into dermal layer (black arrow in fig. 10 F) forming a long rete pegs extending deep into the proliferating dermis and dermal proliferation of transformed fibroblasts (white arrow in fig. 10 F). Epidermal keratinocytes (spinous and/or squamous) were detached and infiltrated into dermal layers forming islands (double arrow connector in fig. 10 G). In deeper layers of the dermis there was proliferation of fibroblasts which were spindle in shape. The proliferated fibroblasts were arranged in tangles or interlacing bundles running in a wavy pattern, forming a whorl like appearance (arrow in fig. 10 H). H & E, X10.



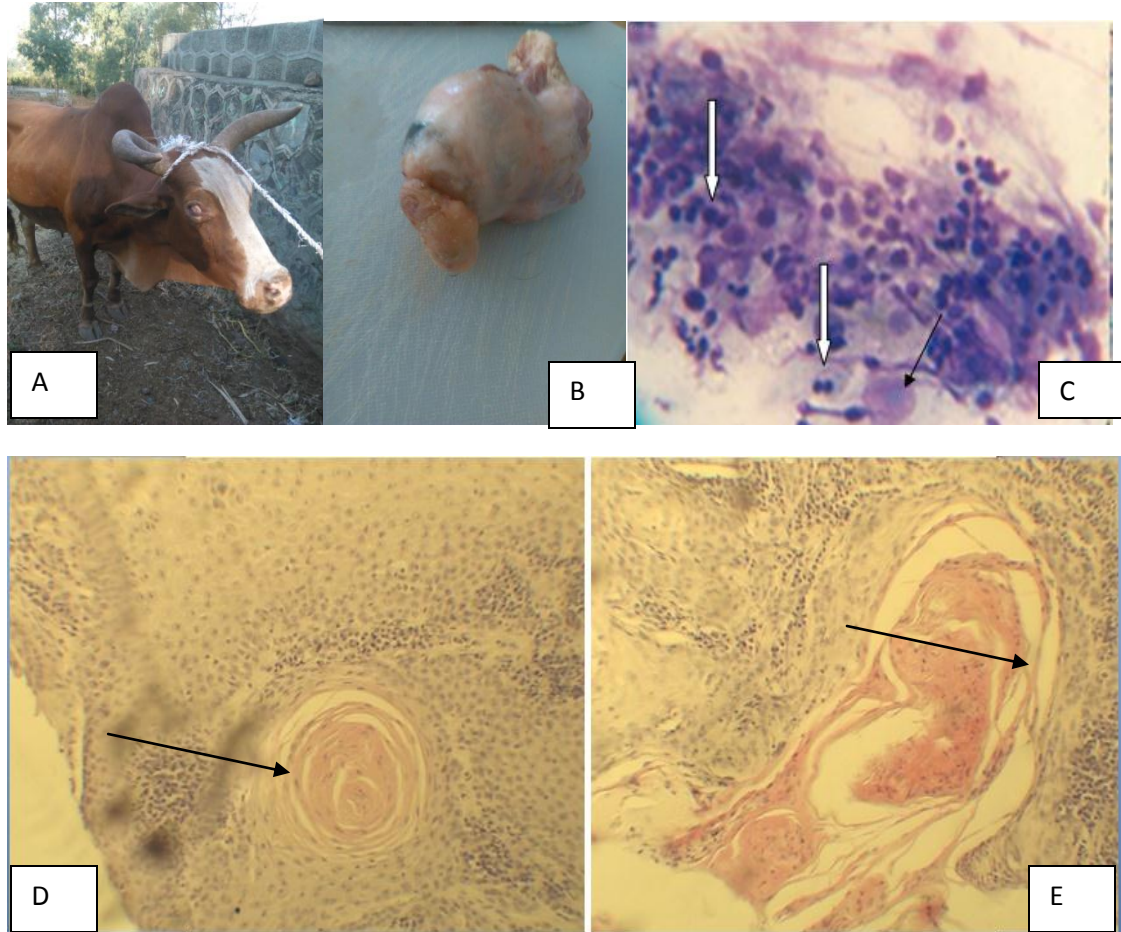
**Figure 10:** Photomicrograph of fibroblastic (A, B & H) and verrucous (C, D, F & G) sarcoid and their cytology (E)

#### 4. 2. 2. *Ocular squamous cell carcinoma*

The ocular squamous cell carcinoma was located on the third eyelid of 12 years old age ox and grossly it was raised with irregular surfaces, some portion friable, and some soft and other part hard in consistency, ovoid in shape and white to grey in color (fig. 11 A & B).

Impression smear of ocular squamous cell carcinoma yield high cellularity perfield in this study. Anisokaryosis was intense with some extremely large cells compared to others (black arrow in fig. 11 C) and such cells were arranged with cluster having deep stained nucleus, apoptotic (degenerated neutrophils) and prominent nucleoli. Mitotic figures were significant (white arrows in fig. 11 C). Generally, nuclei were extremely dark, round to oval with irregular profiles. Moreover, there was a high nuclear to cytoplasm ratio, thick nuclear membranes with fine nuclear chromatin (Geimsa, original magnification 100X) (fig. 11 C).

The neoplastic squamous epithelial cells were arranged in islands, cords, and trabecular form and invade the dermis and subcutis (fig. 11 D). The neoplastic cells were well differentiated with a formation of distinct keratin “pearls” (arrows in fig. 11 D & E) which is characteristic of squamous cell carcinoma. On higher magnification keratin tonofilaments were visible as intracytoplasmic eosinophilic fibrillar material. Mitotic figures, pleomorphism, hyperchromatism of the neoplastic cells, variation in size of nuclei, enlarged and prominent nucleoli and vacuolation of neoplastic cells were also evident. Nuclear to cytoplasm ratio (N/C) was markedly increased (fig. 11 D & E). H & E, X10



**Figure 11:** Macroscopic (A & B), cytological (C) and microscopic (D&E) appearance of ocular squamous cell carcinoma from third eyelid in bull.

#### 4. 2. 3. *Cutaneous fibrosarcoma*

Fibrosarcoma was encountered on the dewlap of bovine (fig.12 A) and perineal region of donkey (fig.12 B). Grossly, it revealed a hard, solid consistency, small to large circumscribed or irregular firm mass (fig. 12 A & B) with pediculated, ulcerated and haemorrhagic area on the perineal region of a donkey (fig. 12 B). The cut section was homogenously white in color (fig. 12 A).

Fibrosarcoma was of tumors of a poor cellular yields on fine needle aspiration while squash smears of biopsy yielded significant number of cells. Cytologically cutaneous

fibrosarcoma was characterized by elongated neoplastic spindle cells with nuclear and cytoplasmic variations (double arrow head in fig. 12 C), binucleation and multinucleation (arrow head in fig. 12 C) which were situated individually and displayed characteristics of malignancy, by variably sized anisocytosis and anisokaryosis (Geimsa, original magnification 100X) (fig. 12 C).

Fibrosarcoma was microscopically characterized by spindle-shaped fibroblastic cells with repetitive collagenous fibers arranged in interlacing or interwoven bundles (Herring bone appearance) (fig. 12 D). The neoplastic cells were composed of fibroblast-type cells that showed hypercellularity, anisocytosis, anisokaryosis and nuclear hyperchromasia (fig. 12 E). Most of nuclei were elongated to oval shapes and contained one or more prominent nucleoli. The tumor cells had a scant amount of cytoplasm. The cell boundaries were ill-defined. The nuclear to cytoplasm ratio (N/C) was increased. The frequency of mitotic figures was high and no areas of necrosis were observed (fig. 12 E). H & E, X10 (D) & X100 (E).

#### *4. 2. 4. Vaginal leiomyoma*

Microscopically, leiomyoma was characterized by proliferation of a relatively homogeneous population of densely packed spindle-shaped to plump smooth muscle-like cells with indistinguishable cytoplasmic borders and elongate, blunt-ended (cigar shaped) nuclei. The bundles within lobules of neoplastic smooth muscle were arranged in a very irregular pattern, traversing at intersecting and obtuse angles from one another. They were subdivided by sparse, thin, fibrovascular trabeculae. Mitotic figures were scarce (fig. 13).

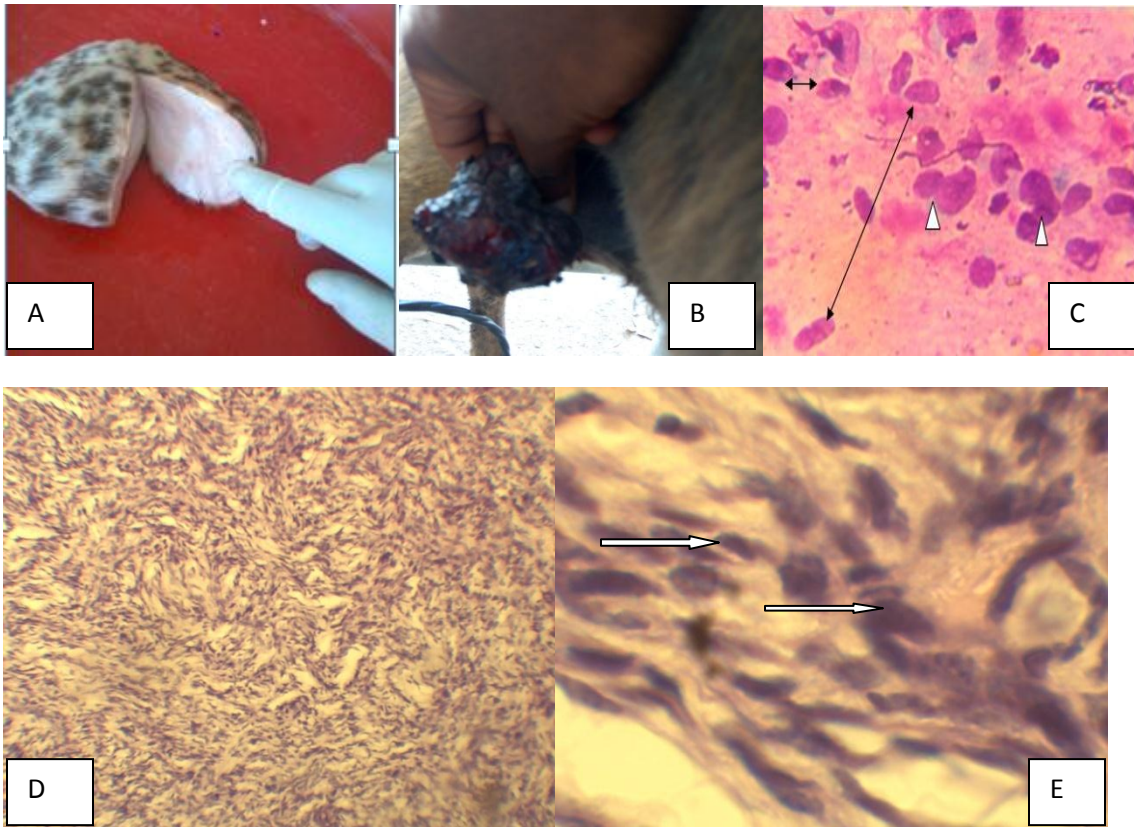
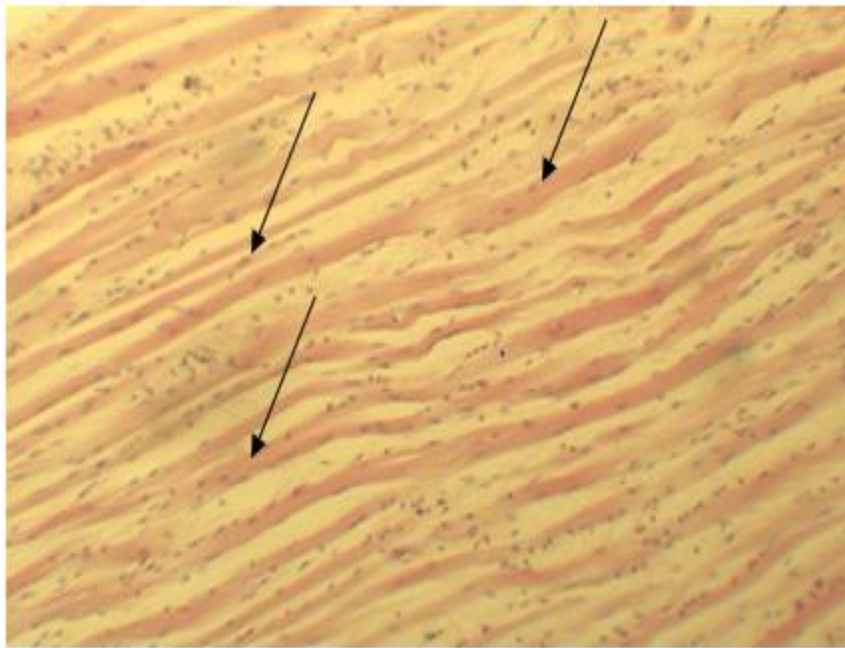


Figure 12: Photomicrograph of cutaneous fibrosarcoma



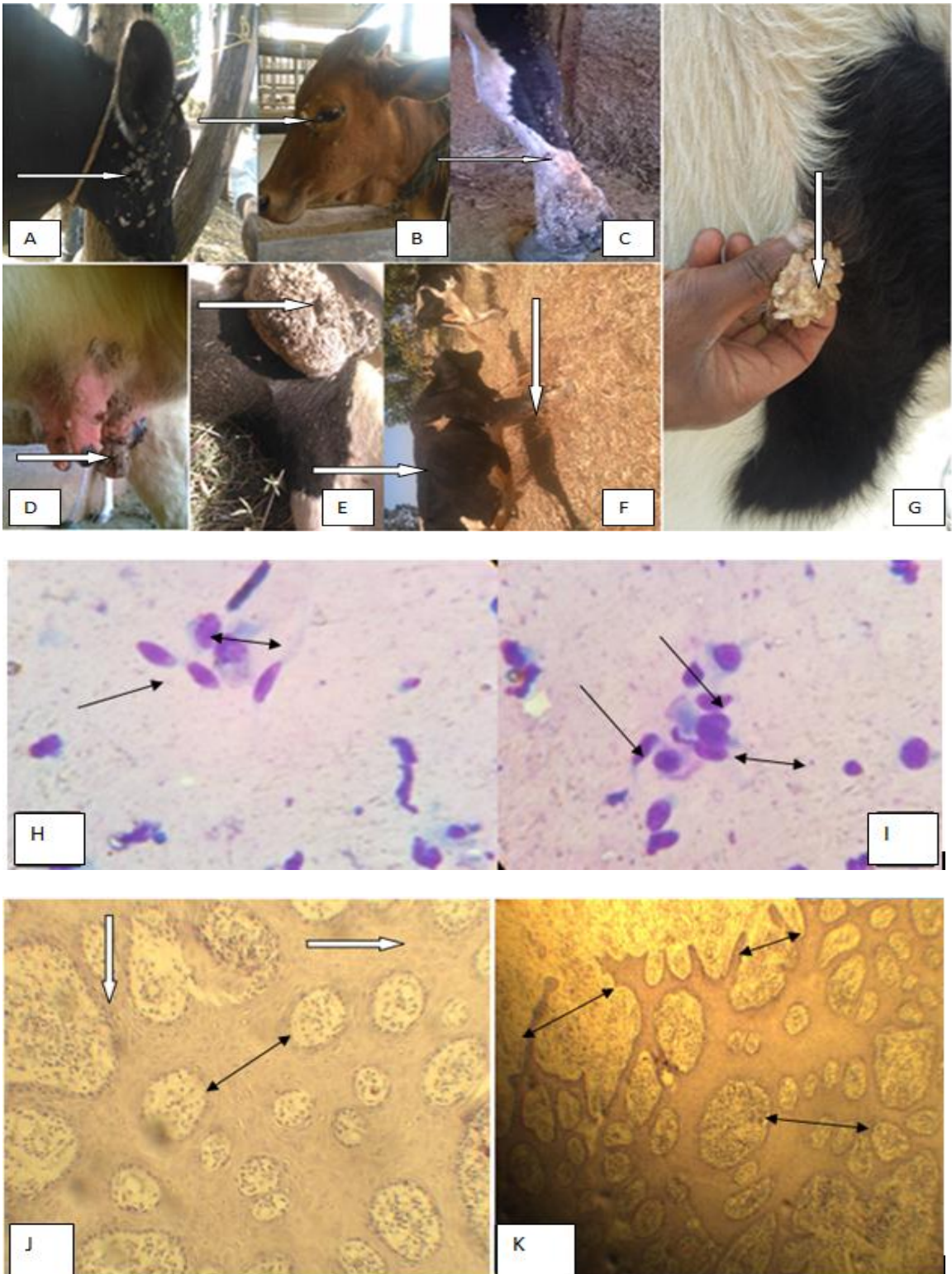
**Figure 13:** Photomicrograph of a section of tissue from vaginal leiomyoma of cow; note interlacing, whorls of smooth muscle and increased nuclei (arrow). H&E; X10.

#### 4. 2. 5. Papillomatosis

Papillomatosis was observed in different parts of the body including head, neck, around eyes, shoulders, limbs, udder, teat, perineum, ears and on the entire surface of the body. The most frequent gross characteristic of papillomatosis in the study period were elevated and multi-nodular proliferations; pediculated and firm mass; rough and dense surface with characteristic cauliflower-like lesions, lichenified appearance with epidermal thickening, alopecia, ulcerated and inflamed surface with local to general infiltration. Note firm, raised and pediculated mass around head region, ear, eye and neck (fig. 14 A&B), ulcerated and inflamed area together with diffuse multi-nodular lesion and lichenified appearance with thickened epidermis over foreleg (fig. 14 C), pediculated mass over the teat (fig. 14 D), diffuse multi-nodular proliferations on testis (fig. 14 E), wart like lesions over the entire surface of the bull (fig. 14 F), rough and dense surface with characteristic cauliflower-like appearance on the shoulder (fig. 14 G).

Impression smear of papillomatosis from neck (H) and teat (I) of cattle yielded mixed epithelial cells (squamous) (fig. 14 I) and dermal fibroblast cells proliferation (fig. 14 H). It was also one of the poorly yielding neoplastic growths in fine needle aspiration. The cytologic characteristics included, the spindle cells appeared individually (arrows) with scant amount of bluish cytoplasm, high nuclear to cytoplasmic ratio, anisocytosis while the squamous epithelium were arranged in group (double head arrow) (fig. 14 H & I).

Microscopically, in both squamous cell papilloma and fibropapillomas the squamous keratinocytes were severely proliferated and invade into the dermis either as long rete-pegs (hyperplastic epidermis) (arrow in fig. 14 K) or infiltrate as fibrovascular core (double head arrow in fig. 14 J & K). In fibropapilloma in addition to epidermal keratinocytes proliferation, there was excessive proliferation of spindle to plump shaped fibroblasts (white arrow in fig. 14 J). Cell nuclei were elongated to fusiform in shape, with scanty cytoplasm (fig. 14 J & K). H&E; X10.



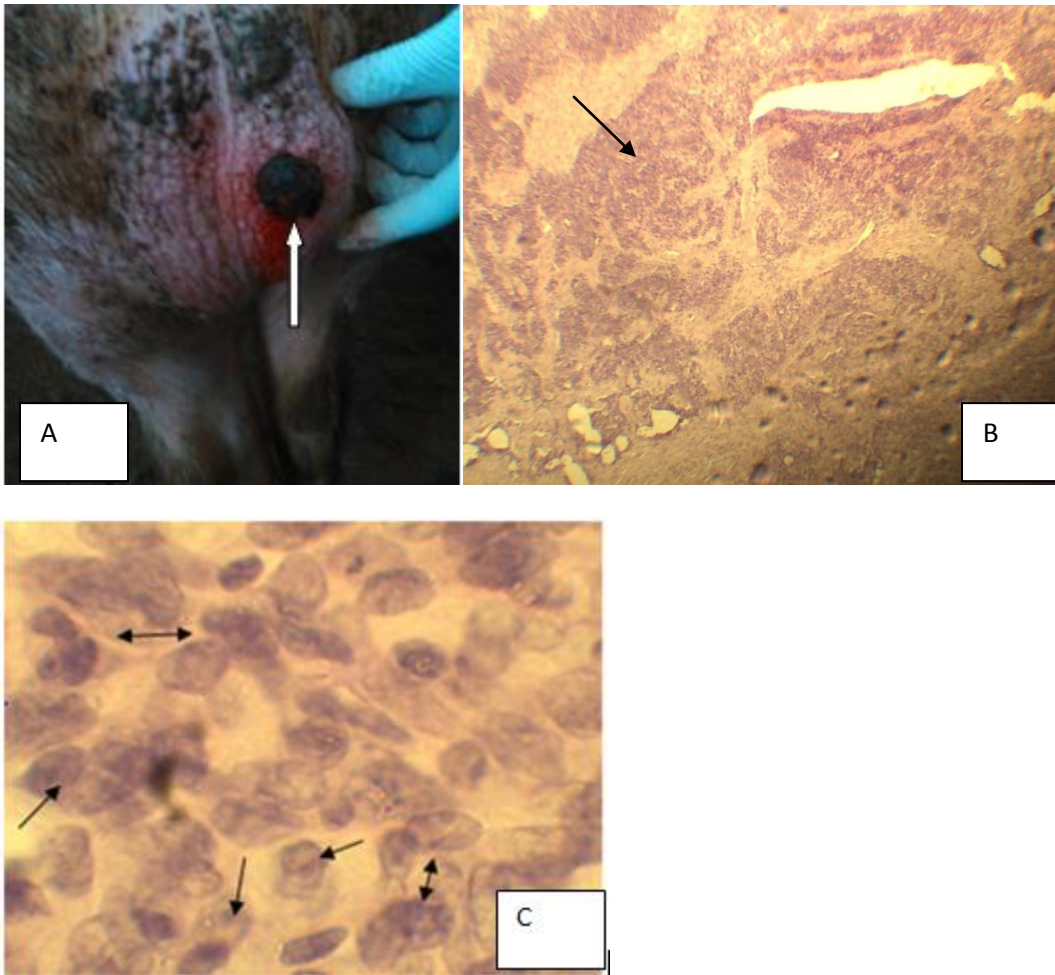
**Figure 14:** Photomicrograph of papillomatosis

#### 4. 2. 6. *Basal cell carcinoma*

The swelling was located on the scrotal skin of a pig and was characterized by firm consistency, slightly raised, with epidermal ulceration and covering large area on scrotal skin (fig. 15).

Cytologically, the basal cell carcinoma was characterized by clusters of deep stained or plump epithelial cells that were arranged in a trabecular manner. Unfortunately on preparing the slide under higher magnification for photomicrograph it was broken, and our second slides was mixed with large number of neutrophils and red blood cells. Hence, the figure was not used.

Microscopically, basal cell carcinoma was characterized by severe proliferation of neoplastic basal cell in situ and massively invades the whole dermis (fig. 15 B) in the form of cords or trabeculae (arrow in fig. 15 B). At high magnification there were severe anisokaryosis (double head arrows fig. 15 C) with some large elongated nuclei (double head arrows fig. 15 C) and medium sized nuclei with prominent nucleoli (arrows in fig. 15 C). The nuclei had deep chromatin, cytoplasm were inconspicuous which is characteristic for basal cell carcinoma. The nuclei exhibit intense pleomorphism with high mitotic figures (fig. 15 C).



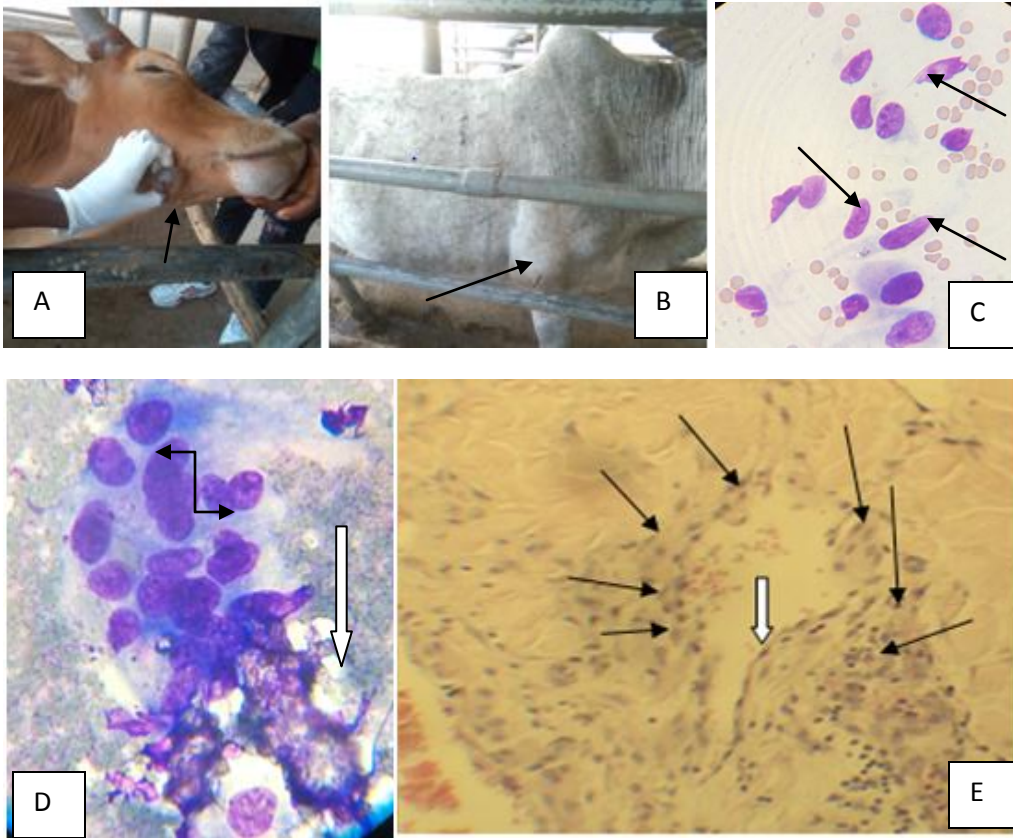
**Figure 15:** Basal cell carcinoma from scrotal skin of pig; note the ulcerated area with slightly raised mass (white arrow) (A), the cords of neoplastic cells invading dermis (arrow) (B) and nuclear pleomorphism with hyperchromatic prominent nucleoli (arrow) (C). H&E; X10 (B) & X100 (C).

#### 4. 2. 7. *Hemangiopericytomas*

Hemangiopericytomas was located on the lower jaw of cow (fig. 16 A) and shoulder region (fig. 16 B) of bull. Grossly, the mass revealed well demarcated, ulcerated, and round to irregular in shape. The consistency was soft in one case and firm on the other case. The outer surface of the mass was relatively smooth and on cut surfaces there was coagulated blood together with areas of necrosis, congestion and fibrous capsule (fig. 16 B).

Fine needle aspirates of hemangiopericytoma yielded less cellular. However, the individual cells were typically comprised of pericytes (arrows in fig. 16 C). Some clusters of pericytes with binucleation and multinucleation were observed (elbow arrow in fig. 16 D) together with fat droplets (white arrow in fig. 16 D). The tumor cells were oval or spindle-shaped with a high nuclear to cytoplasmic ratio and indistinct cellular borders. The nuclei had finely granular, evenly distributed chromatin with indistinct nucleoli (arrows in fig. 16 C). The nuclear contour generally was smooth and regular (fig. 16 C & D), (Geimsa, original magnification 100X).

Microscopically, hemangiopericytoma was characterized by highly cellular pericyte nuclei in situ forming perivascular whorls of cells with poorly defined cytoplasm, elongated nuclei (black arrows in fig. 16 E). Hemorrhagic and necrotic areas were also evident (fig. 16 E). Note also capillary endothelium nuclei are squamous bulged in and at their position (white arrow in fig. 16 E). H&E; 10X.



**Figure 16:** Macroscopic features of hemangiopericytomas; note ulcerated and soft mass on the lower jaw of a cow (A), hard large mass on the shoulder region in a bull (B).

#### 4. 2. 8. Mammary gland tumors

The mammary gland tumors in this study were located on the caudal abdominal and caudal thoracic pairs of mammary glands. The gross characteristics of this tumor showed great variations, which ranges from well circumscribed masses to diffuse swellings. Some were moveable while others were well attached, skin-covered to ulcerate in some cases (fig. 17 A & C). Besides, in some the mass have had necrotic material, some firm while some soft in consistency and irregular in shape. The cut surface appeared multilobulated, papillary like projections and white to grey in color (fig. 17 B). In fig 17 D, the cut surface appeared with oedematous, multilobulated, papillary like projections and pale to grey in color.

Mammary gland tumors was one of the tumor which yielded high cellularity on fine needle biopsy but the cytologic evaluation of aspirates from mammary gland tumor revealed great variations among the cases. FNA of mammary adenocarcinoma (fig. 17 E) and carcinoma-mixed type (fig. 17 F) in dog showed a large sheet of epithelial cells with nuclear and cellular pleomorphism, cellular and nuclear atypia (fig. 17 E) but regular cellular shape, prominent nucleoli variation and significant mitoses (fig. 17 F). Also note some neutrophils on the upper top of (fig. 17 F). In some case, the tumor cells were arranged haphazardly with irregularly shaped epithelial cells that had high nuclear to cytoplasmic ratio and indistinct cellular borders (fig. 17 E). In such tumors anisokaryosis were so severe with some giant nuclei (double headed arrows in fig. 17 E). Such nuclei were coarsely granular, evenly distributed chromatin with indistinct nucleoli. Some cells have medium sized nucleus with prominent nucleoli (arrow head in fig. 17 E). Mitotic figures were abundant (elbow arrows in fig. 17 E). In some other cases (fig. 17 F) the epithelial cells were regularly arranged but the nuclear contours generally were rough and irregular. The cytoplasm were scant and were accompanied by cytoplasmic vacuoles. Cytologic atypia, mitotic figures (white arrows in fig. 17 F), anisocytosis and anisokaryosis were abundant (Geimsa, original magnification 100X) (fig. 17 F).

### *Papillary mammary adenocarcinoma*

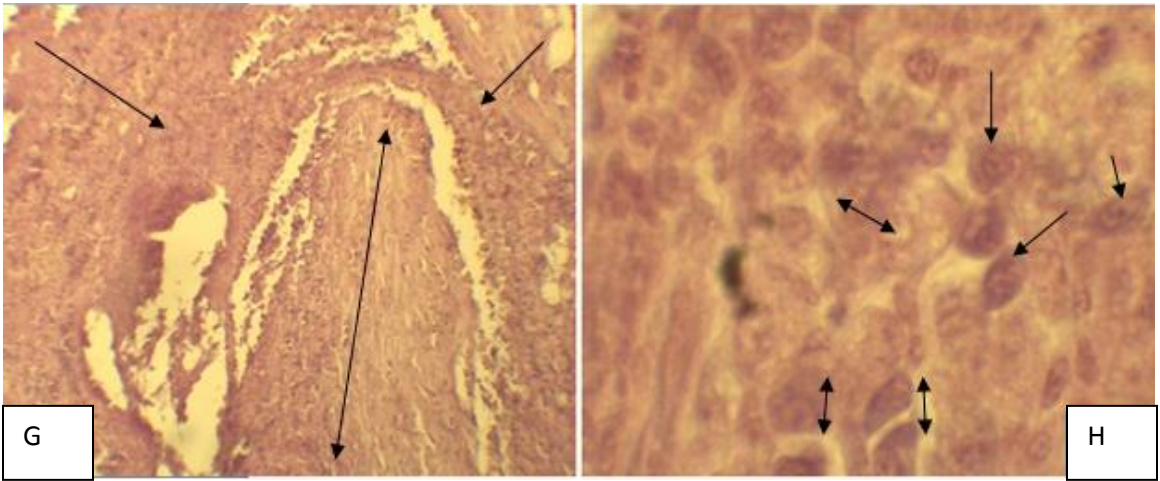
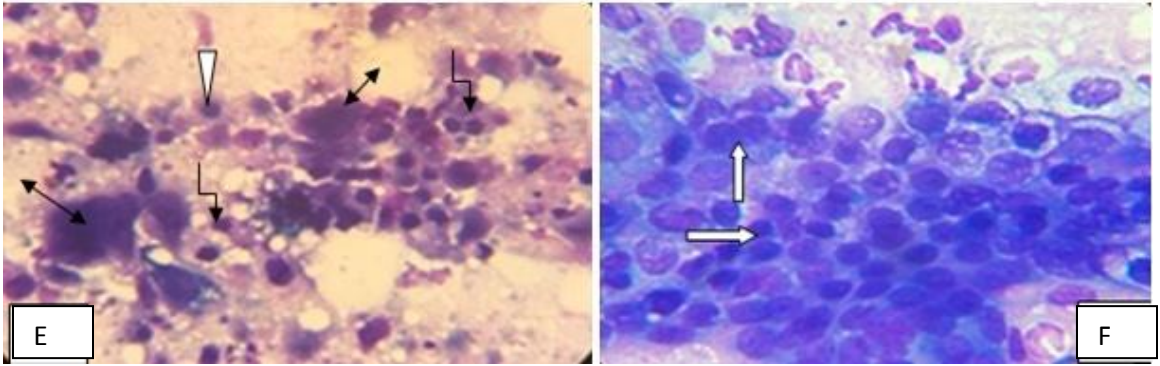
At low power magnification the neoplastic ductal epithelial cells were proliferated into the ducts of the mammary gland in a papillary pattern (double headed arrow fig. 42 A). However, since the tumor was aggressive it was infiltrated and invades into the surrounding mammary stroma (arrows fig. 17 G). This tumor revealed severe hypercellularity and severe papillary projection. At higher magnification the neoplastic epithelial cells showed higher nuclear to cytoplasm ratio, cellular pleomorphism, and prominent nucleoli (arrows in fig. 17 H) with increased numbers of mitotic figures (double nucleus and nuclear fragmentation) (double head arrows fig. 17 H). H&E; X10.

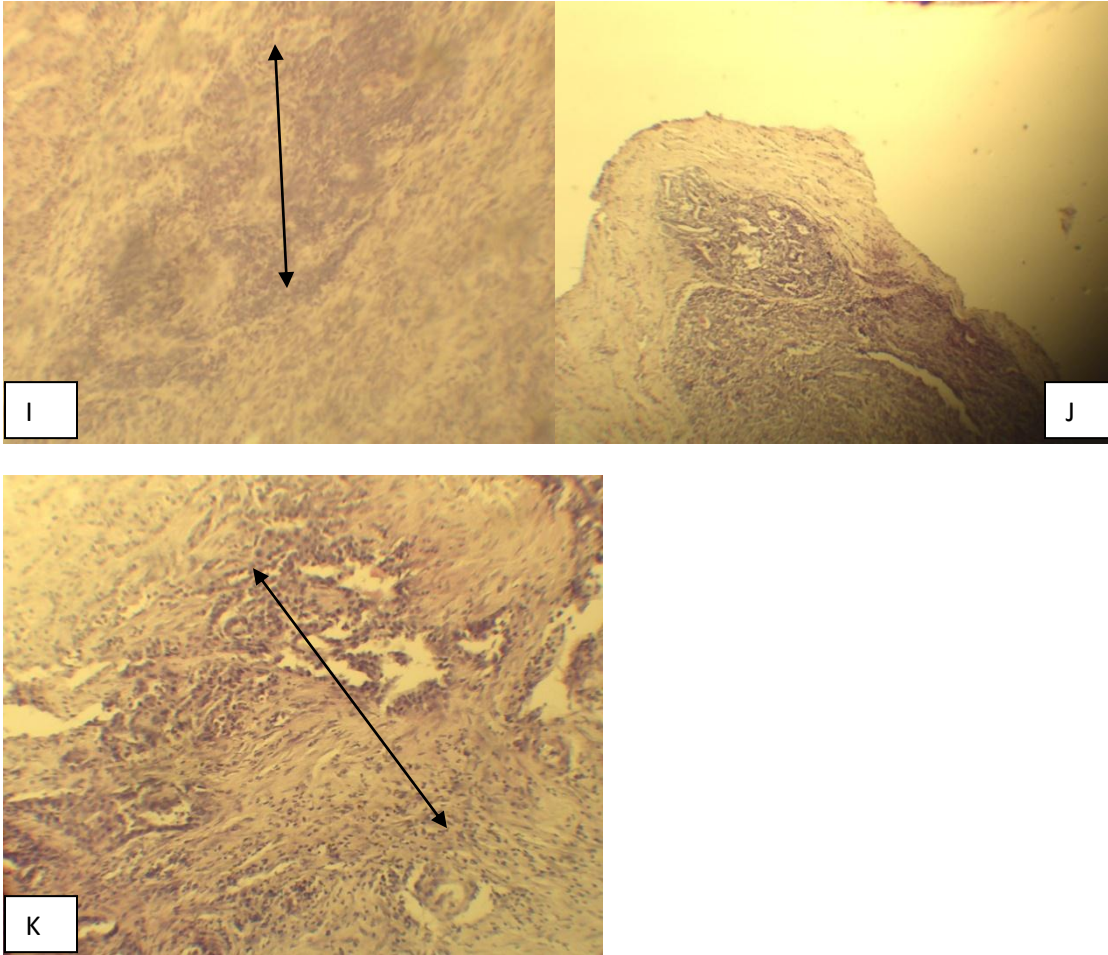
### *Carcinoma –mixed type*

Both the epithelial cells and the myoepithelial cells were pleomorphic. The cells were fusiform with severe nuclear and cellular pleomorphism. The epithelial cells occupying the whole duct were revealed severe hyperplasia. The cells had poorly demarcated cell borders, high N: C ratio, a small amount of fibrillar cytoplasm, and a round nucleus with stippled chromatin. Cells were exhibited high mitotic figures (fig. 17 I). H&E; X10.

### *Mammary adenocarcinoma*

Microscopically this tumor was infiltrating tubular adenocarcinomas but was invasive as proliferations extend beyond the mammary ducts, invading the adjacent mammary stroma (large double head arrow fig. 17 K). The neoplastic cells were hypercellular with significant pleomorphism of epithelial component and arranged in a cord like pattern deep into the mammary stroma (fig. 17 J). The cell border was indistinct with a small amount of cytoplasm, and oval to elongate nuclei that contain finely stippled chromatin and variably distinct nucleoli. Anisokaryosis and anisocytosis were moderate with some mitotic figures (fig. 17 K & J). H&E, X10.





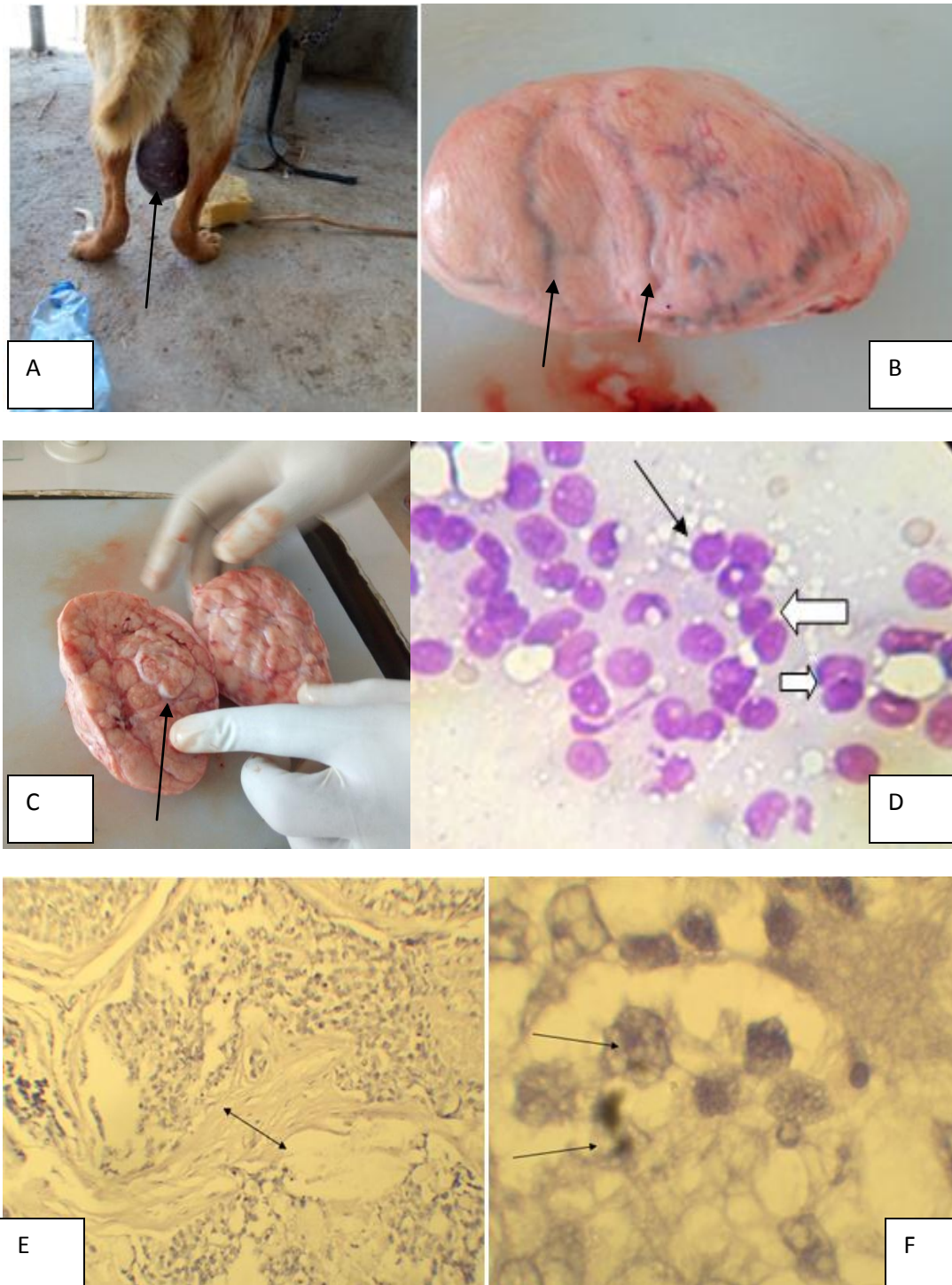
**Figure 17:** Photomicrograph of gross, cytological and histopathological characteristics of Mammary gland tumors from bitches.

#### 4. 2. 9. Sertoli cell tumor

The tumor mass was located on the right testicle and grossly it was unifocal, well-circumscribed, nodular, and moveable, enlarged, pendulous with no evidence of ulcer (fig. 18 A). The surgically removed mass appeared with numerous dark blood vessels supplying the mass (fig. 18 B). On cut it revealed firm, round and pale to grey islands in the testis. Additionally, the neoplastic islands were well separated in some part and irregular in other parts (arrow in fig. 18 C).

The cytological features of a case of Sertoli cell tumor showed large cells with less visible cytoplasmic borders with macrovacuolation in the cytoplasm (characteristic of Sertoli cell tumor) (arrows in fig. 18 D). The nuclei showed only occasional nucleoli but significant anisokaryosis, fine granular chromatin, and intranuclear vacuoles. In addition, the neoplastic cells were binucleated and multinucleated with low number of typical mitoses were observed (white arrows in fig. 18 D), (Geimsa, original magnification 100X).

Microscopically, some intratubular but severely coalescing sheets of neoplastic Sertoli cells were evident in the affected testicle. The neoplastic cells had almost replaced and compressed the adjacent cells. Neoplastic Sertoli cells had irregularly arranged. There was abundant hypercellular stroma in between the proliferating Sertoli cells (double headed arrow in fig. 17 E) which is characteristic of Sertoli cell tumor. The Sertoli nuclei were elongated to oval with scant cytoplasm. Anisocytosis, anisokaryosis, hyperchromasia, prominent nucleoli and high mitotic figures were numerous (arrows in fig. 17 F).



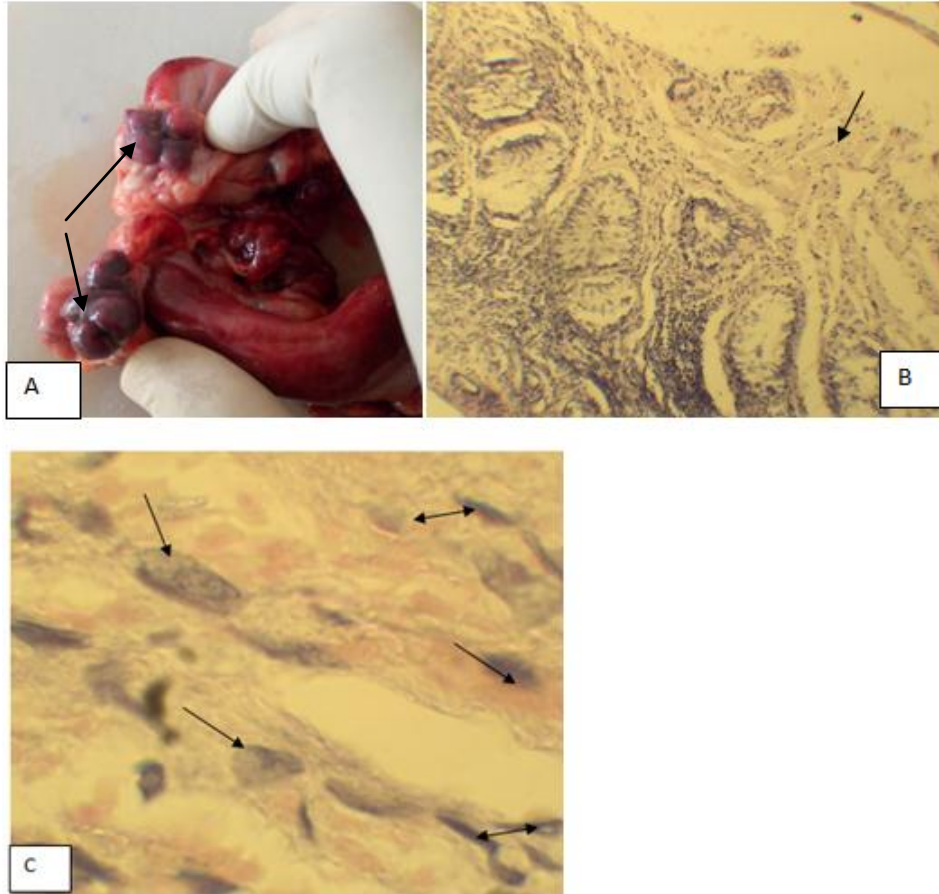
**Figure 18:** Macroscopic, cytologic and histologic view of Sertoli cell tumor in male dog.

#### *4. 2. 10. Fibrothecoma*

The tumor was located on both right and left ovaries of a bitch. Grossly, the mass was aggressive and covering the entire ovaries, lobulated, and firm in consistency, bilateral, irregular in shape, deep-red to light red in color (fig. 19 A). On cut the surface it was congested, largely solid but very few cystic structures were also evident.

The smears were less cellular and consisted of a few population of oval nuclei of theca cells arranged in sheets and some undefined epithelial cells intermingled among the oval nuclei. Since the cytologic characteristic was not definitive we did not use the figure and the tumor was confirmed by histopathology.

Microscopically, fibrothecoma was characterized by proliferation of spindly fibroblastic cells (arrow in fig. 19 B) with collagen fibrils, and plump, oval thecoma cells with nuclear anaplasia (double headed arrow fig.19 B). At higher magnification the theca cell nuclei were plump and oval (arrows in fig. 19 B) and that of fibroblastic cell was typical spindle nuclei (double headed arrows fig. 19 C). H&E, X10 (B) & X100 (C).



**Figure 19:** Gross and microscopic appearance of ovarian fibrothecoma

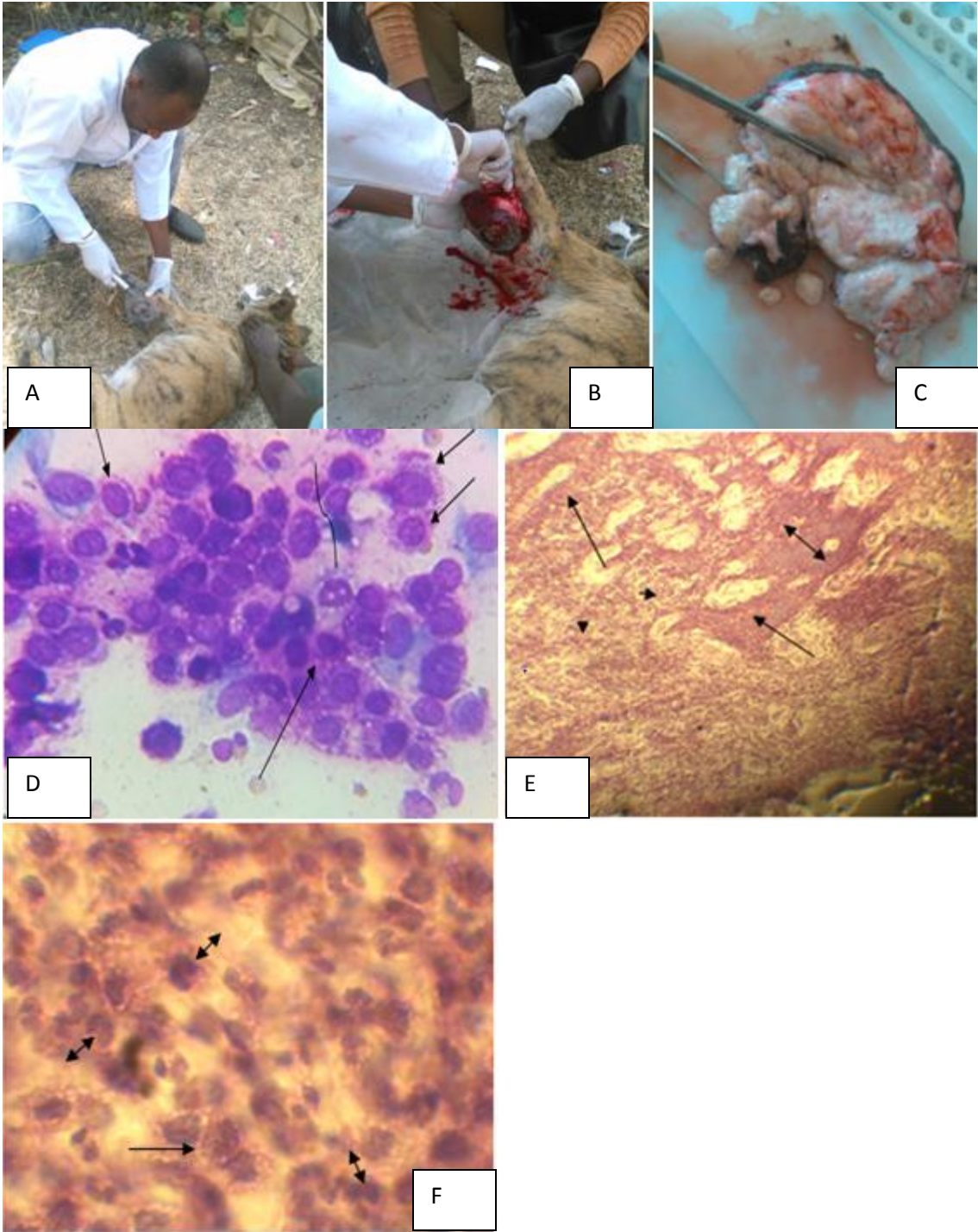
#### 4. 2. 11. Cutaneous mast cell tumor

The mass was located on the forelimb of a male dog. Grossly it was well demarcated extremely large mass, circular in shape and firm in consistency. The outer surface was ulcerated at the center with mild abscessation. The cut surface was gray-white in color (fig. 20 A & B).

On fine needle biopsy the cellular yield of mast cell tumor was high and the cells were irregularly distributed. In this case the neoplastic mast cells have moderate amounts of cytoplasm when the nuclei were relatively smaller and scanty cytoplasm when the nuclei were larger. All cells contain variable numbers of cytoplasmic granules (arrows in fig. 20 D). In some parts the tumor cells did have mast cell granules in cytoplasm with several,

clear; punctuate cytoplasmic vacuoles (characteristics of mast cell) with some eosinophils (curved connector in fig. 20 D) and distinct cell borders. Cytologic atypia, anisocytosis, and anisokaryosis were also noted (Geimsa, original magnification 100X).

Histopathological examination of cutaneous mast cell tumor at low magnification revealed hypercellularity, sheets, cords (arrows in fig. 20 E) and diffuse (arrow heads fig. 20 E) arrangement of neoplastic mast cells which infiltrated into dermis and the subcutis. There was a lymphocytic nodule at one focus (double head arrow in fig. 20 E). At high magnification, mast cells were poorly differentiated with numerous eosinophils and hence the granules were not clearly visible (arrow in fig. 20 F). In poorly differentiated mast cells tumors, eosinophils are characteristic and in this mast cell tumor they were abundant (double headed arrows in fig. 20 F). Additionally, there were severe anisocytosis and anisokaryosis. The nucleus was large and irregular in shape and had several nucleoli. Neoplastic cells with high mitotic figure and hyperchromasia were discerned (fig. 20 F). H&E, X10 and X100.



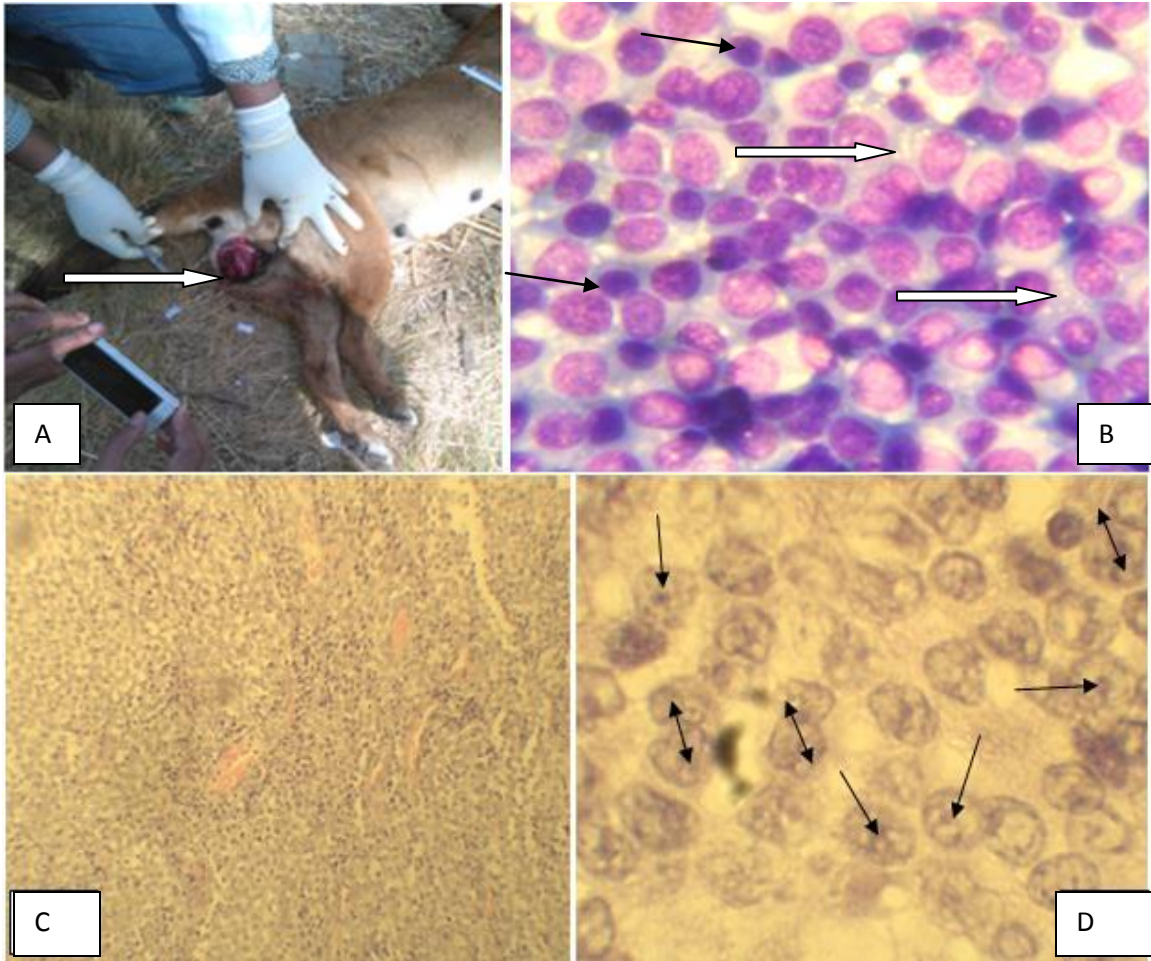
**Figure 20:** Macroscopic, cytological and microscopic appearance of cutaneous mast cell tumor

#### *4. 2. 12. Transmissible venereal tumor*

The mass was grown in the vagina of a bitch. Macroscopically, it was attached to the underlying mucosa, friable in consistency, irregular in shape, and cherry in color. The surface was smooth with a multilobular verrucous ulcerated and haemorrhagic area (fig. 21 A).

Cytologically, the smear were highly cellular and the cells were evenly spread throughout the slide. The tumor cells did have discrete cell pattern having moderate amount of light to medium blue cytoplasm with several, clear, punctate, intracytoplasmic vacuoles (white arrow), nuclear pleomorphism and distinct cell borders. Nuclei were round, with fine granular chromatin and prominent nucleoli (black arrow). Numerous mitotic figures were observed (fig. 21 B) (Geimsa, original magnification 100X).

At low power magnification tumor cells revealed hypercellularity into the mucosa, submucosa and muscularis layer of the vagina (fig. 21 C). But at high magnification neoplastic TVT cells were round with scant but well-defined cytoplasm. The nuclear chromatin was fine and diffusely distributed, and the nuclei, which were ovoid and large, contained prominent, centrally placed nucleoli (arrows in fig. 21 D). Anisocytosis, anisokaryosis and mitoses were abundant (double head arrows in fig. 21 D). Histologic section of TVT showing sheet of round cells with vesicular nuclei (fig. 21 D) and arborizing fibrovascular network and scanty stroma (fig. 21 C). H&E X10 (C) & X100 (D).



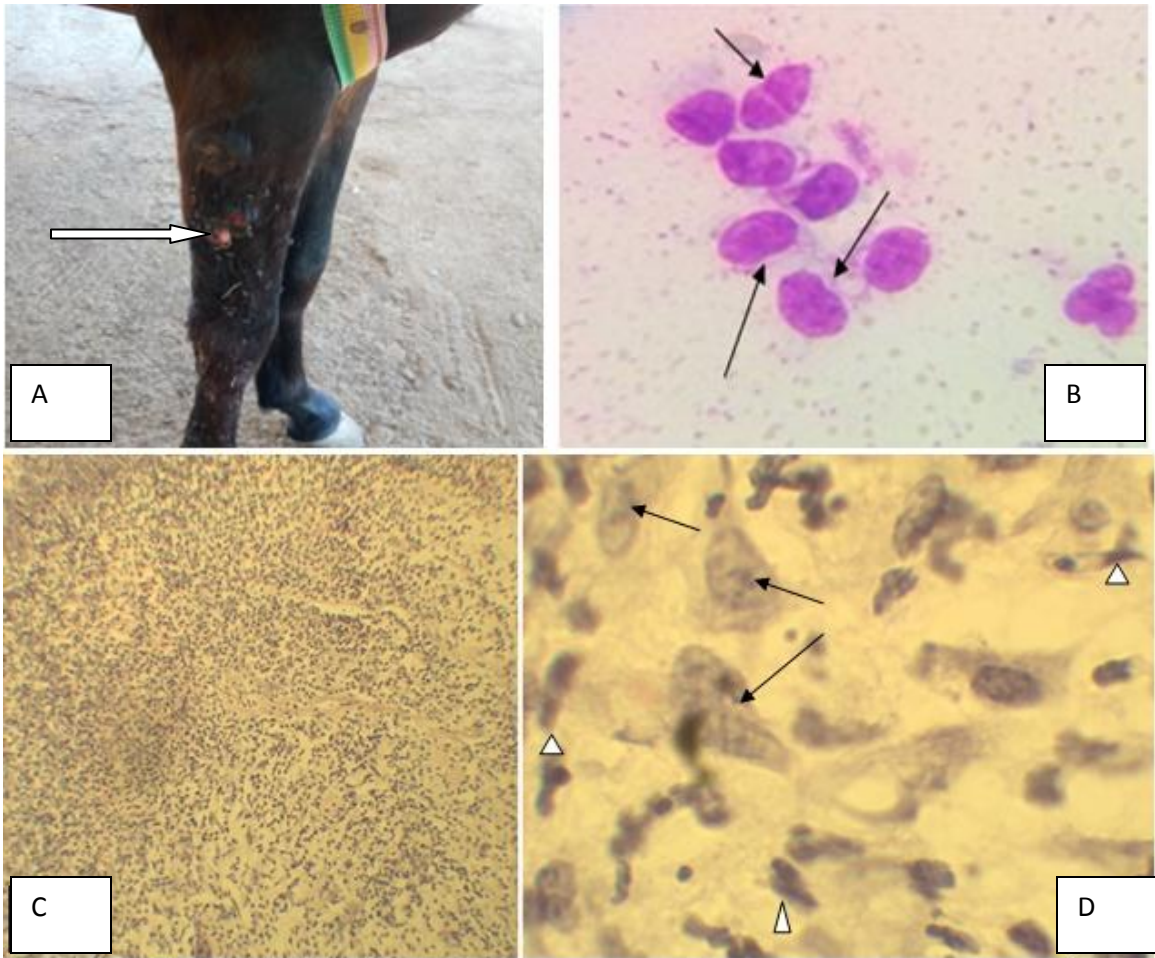
**Figure 21:** Macroscopic, cytological and microscopic appearance of transmissible venereal tumor

#### *4. 2. 13. Malignant fibrous histiocytoma*

The tumor was located on the forelimb of a mule. Grossly it was a medium sized firm nodule, with ulcerated surface. The cut surface was grey to white in color (fig. 22 A).

Cytologically, smear revealed less cellular with round to oval cells that had generally non-vacuolated and indistinct cytoplasm. The nuclei were typically indented, bean shaped and irregular with some multinucleation (arrows in fig. 22 B). The cells were uniform in appearance with minimal cytologic characteristics of malignancy (Geimsa, original magnification 100X).

Histologic section of malignant fibrous histiocytoma showed hypercellularity (fig. 22 C). At lower magnification, the neoplastic histiocytic cells were uniformly infiltrated into dermis. This tumor was histologically characterized by highly pleomorphic histiocytic cells (arrows in fig. 22 D), elongated spindle-shaped cells with hyperchromasia (arrow head), indistinct cytoplasmic borders and indented nuclei with prominent nucleoli. The nuclei were vesicular. Anisokaryosis, anisocytosis and mitotic figures were noted (fig. 22 D). H & E, X10 (A) & X100 (B).

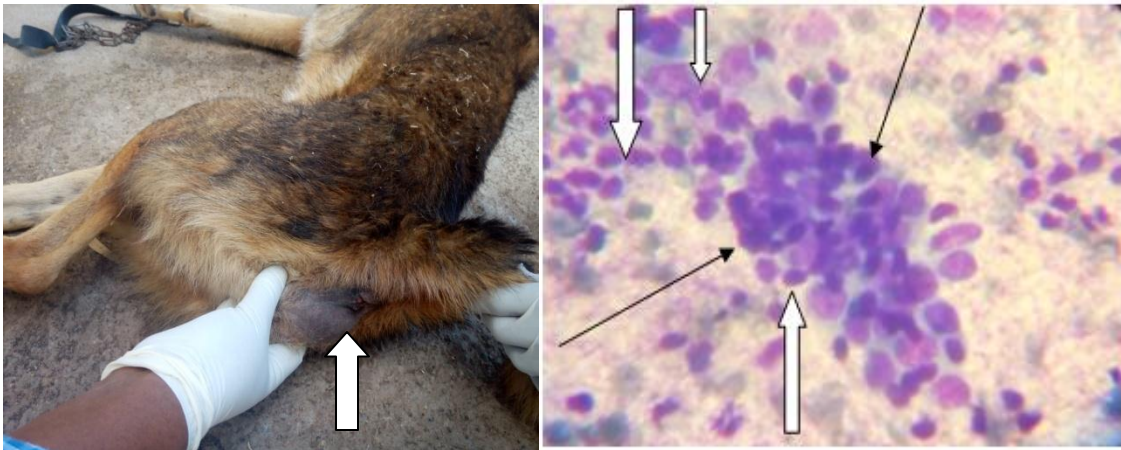


**Figure 22:** Gross, cytologic and histologic section of malignant fibrous histiocytoma

#### 4. 2. 14. Atypical anal sac adenocarcinoma

The mass is located on the ventrolateral aspect of the dog. Grossly only a part of the mass was easily visualized and on grasping the remaining half part will be protruded. It was unilateral and was firm nodular swelling (fig. 23 A).

Cytologically, the fine-needle aspirates were markedly cellular. The epithelial cells arranged in clusters or sheets some have round hyperchromatic nuclei, a prominent nucleoli, and very scanty cytoplasm (arrows in fig. 23 B). Most of the neoplastic cells were poorly differentiated with lack of distinct cellular differentiation with in large portions of neoplastic cells and was consistent with atypical anal sac adenocarcinoma (white arrows in fig. 23 B).



**Figure 23:** Gross and cytologic characteristics of atypical anal sac adenocarcinoma

## 5. DISCUSSION

Tumors have been gaining relevance in veterinary medicine and represent a continuous challenge for veterinarians (D'Angelo *et al.*, 2012). Domestic animals may serve as useful environmental sentinels of cancer risks and adds further value to the study of naturally occurring tumors in domestic animals which can provide a useful (sometimes irreplaceable) contribution in comparative oncology (Hayes *et al.*, 1990; Reif, 2011; Pinho *et al.*, 2012).

It is always difficult to estimate the true frequency as well as the biological pattern and clinical outcome of spontaneous tumors in domestic animals (D'Angelo *et al.*, 2012) despite the fact that numerous surveys were conducted in countries throughout the world (Bastianello, 1982). In general, frequencies of tumors in domestic animals are to a large extent epithelial followed by mesenchymal and round cell tumors based on cytological and histological types (Bastianello, 1982; Meirelles *et al.*, 2010; Kimura *et al.*, 2012).

Likewise, the present study characterized epithelial tumors as the most frequent outcome followed by tumors of unclassified origin, mesenchymal and round cell tumors which were represented by 46.7%, 36.7%, 13.3% and 3.3%, respectively. Of tumors of epithelial origin, cutaneous papilloma (pure papilloma) was the most frequent followed by mammary gland tumors which were represented by 33.3% and 6.7%, respectively. However, of tumors of unclassified origin fibropapilloma was the most frequent tumor represented 23.3%, and from mesenchymal tumors cutaneous fibrosarcoma and hemangiopericytomas were the frequent ones indicated 3.3% and 3.3 %, respectively. In addition, mast cell tumors and transmissible venereal tumors were diagnosed from round cell tumors represented by 1.7% in each case.

Equine sarcoids are the most common skin tumors that have been characterized frequently as biphasic tumors with a dermal and epidermal components (Martens *et al.*, 2000; Martano *et al.*, 2016). However, several authors have remarked that the epidermal

changes are variable and can range from typical hyperplasia with rete peg formation to a normal or even atrophic epidermis with marked hyperkeratosis (Marti *et al.*, 1993; Martens *et al.*, 2000).

It was obvious in the present study is that equine sarcoids were considered as the most common cutaneous tumors in equine species mainly in horses and donkeys with different body location. This finding is in agreement with that of Shokry *et al.* (2015) who stated the tumor may occur in any part of the body either as solitary or multiple lesions and affect the equidae of all ages, types and breeds. Only two types of equine sarcoids namely verrucous and fibroblastic were documented in the current finding from six different clinical forms of sarcoids that are known globally. This finding is in disagreement to the one reported by Shokry *et al.* (2015) who observed all clinical forms of equine sarcoids.

Grossly, the tumor was characterized as verrucous and fibroblastic sarcoids with presence of small wart-like lesions, a raised, scaly, lichenified appearance with epidermal thickening, alopecia, fleshy ulcerated and inflamed surface with local infiltration, pediculate and firm mass. These gross findings were consistent with previous reports by Knottenbelt and Matthews (2001), Meuten (2002), Gomes (2011) and Corteggio *et al.* (2012) who observed similar gross lesions.

Equine sarcoid showed high cellularity with deep nuclear chromatin and prominent nucleus that appeared in clusters with trabecular and cord like arrangements on the basis of cytological characterization. Anisocytosis, anisokaryosis, indistinct cell borders, prominent eccentric large nucleus, mitotic figures and high nuclear to cytoplasmic ratio were also discerned. No study was conducted to compare the cytological characterization of equine sarcoid to the knowledge of an author. Therefore, the present finding is the first of its kind to document the cytological report.

Histopathology of verrucous and fibroblastic sarcoids revealed the typical histological changes in their epidermal and dermal components such as spinous and squamous

differentiation of keratinocytes together with long rete pegs extending deep into the proliferating dermal connective tissue. In deeper layers of the dermis there was proliferation of fibroblasts which were spindle in shape. The proliferated fibroblasts were arranged in tangles or interlacing bundles running in a wavy pattern forming a whorl like appearance. Similar findings of histopathological characterization of sarcoids were indicated by Goodrich *et al.* (1998), Scott and Miller (2003), Sala *et al.* (2010), and Shokry *et al.* (2015). Picket fence pattern, cystic hair follicles or erosion of epidermal surfaces in these types of sarcoids reported by Martens *et al.* (2000) was not noticed in the current study.

Bovine ocular squamous cell carcinoma is the most common malignant neoplasm of epithelial origin affecting cattle and is responsible for significant economic losses (Carvalho *et al.*, 2005; Tsujita and Plummer, 2010).

According to the present study, the main site for OSCC was third eyelid. This finding was in line with the one reported by Gharagozlou *et al.* (2007). In contrast, the current results are different from Anderson and Badzioch (1991), and Pugliese *et al.* (2014) who indicated roughly 75% of ocular carcinomas were found on the limbus and cornea. OSCC occurs with high frequency in cattle all over the world (Carvalho *et al.*, 2005), and it is the leading cause of enucleation (Schulz and Anderson, 2010) and rejection from consumption of large numbers of carcasses in affected cattle (Heeney and Valli, 1985). Conversely, the lower frequency to the extent of 1.7% of BOSCC from all tumors of domestic animals were consistent with previous reports by Heeney and Valli (1985). The reason for this lower frequency of OSCC in this study might be due to a lower ultraviolet index and other factors including genetic resistance or a lower rate of infection with oncogenic viruses. This was supported by Fornazari *et al.* (2017).

In the present study, OSCC was grossly characterized by formation of friable to firm white nodular mass with irregular surfaces distributed over the entire third eyelid. The growths of these nodular lesions were led to erosion and superficial ulceration. This gross finding was consistent with the one reported by Pugliese *et al.* (2014).

Cytological characterization of OSSC in this study revealed neoplastic cells with high cellularity, extremely dark nuclei with prominent nucleoli, round to oval in shape exhibiting intense anisokaryosis and anisocytosis, high nuclear to cytoplasmic ratio and significant mitotic figure. Such observations were in line with the previous studies by Garma-Avina, (1994), and Manesh *et al.* (2014) that characterized SCC in the prepuce of a dog.

Histological characterization of OSSC of the third eyelid revealed small aggregates, irregular islands, nests or cords of neoplastic keratinocytes that invade the dermis and subcutis. Frequent findings include keratin pearls, hyperchromatism of the neoplastic cells, enlarged and prominent nucleoli, high N: C ratio, severe mitoses and cellular atypia. Keratin tonofilaments were visible as intracytoplasmic eosinophilic fibrillar material. This histopathological finding was consistent with previous study of SCC in the prepuce of a dog by Manesh *et al.* (2014) and on the third eyelid of a bovine by Carvalho *et al.* (2005), and Pugliese *et al.* (2014).

Fibrosarcomas are tumors of varying frequency in domestic animals with cats being the most affected by them. The cases reported in bovines are few and its description is mainly centered on the female reproductive system, at the level of the vulva and vagina (Hamali and Ashrafihelan, 2010; Avci *et al.*, 2012; Hemida *et al.*, 2017). However, in the present study cutaneous fibrosarcomas were discerned in the skin of cattle and donkey with frequencies of 3.3% and 1.7%, respectively. This finding was comparable with that of Timaran *et al.* (2015) who reported dermal fibrosarcoma in cattle.

Cutaneous fibrosarcoma was characterized macroscopically as hard circumscribed mass without presence of capsule. The cut section was homogenously white in color with no foci of necrosis or haemorrhage which was reported by Timaran *et al.* (2015) in bovine agreed to the present gross finding of dewlap fibrosarcoma of bovine. Conversely, gross findings of fibrosarcomas in donkey revealed fleshy ulcerated and haemorrhagic surface with dark color.

Cytological study revealed large, elongated neoplastic spindle cells arranged individually and displayed characteristics of malignancy including multiple and variable sized nucleoli, binucleation and multinucleation, anisocytosis and anisokaryosis. The present cytological findings of fibrosarcoma were in line with Johnson and Myers (2017) who reported similar cytological characterization of fibrosarcoma in a dog.

The tumor at the level of histopathological examination was consisted of spindle-shaped fibroblastic cells with repetitive collagenous fibers arranged in interlacing or interwoven bundles in typical herringbone pattern. The neoplastic cells were composed of fibroblast-type cells that exhibited hypercellularity, anisocytosis, anisokaryosis and nuclear hyperchromasia. The nuclei were nuclei were elongated to oval shaped and contained one or more prominent nucleoli. The tumor cells had a scant amount of cytoplasm, ill-defined cell boundaries, high nuclear to cytoplasm ratio (N/C) and high mitotic figures. Similar findings of histopathological characterization of fibrosarcomas were pointed out by Goldschmidt and Hendrick (2008), Hamali and Ashrafihelan (2010), Avci *et al.* (2012), Kuru (2016), Meuten, (2017), and Hemida *et al.*(2017).

It has been suggested that leiomyomas of the tubular genital organs can arise from either normal smooth muscle cells in the wall of uterus and vagina (Cooper and Valentine, 2002). In the present study leiomyomas observed in the vagina of a cow with frequency of 1.7% were consistent with previous findings reported by Ramadan *et al.* (1993), and Cooper and Valentine (2002).

The histopathological features of leiomyoma in the present study were similar to those described in the genital system and also in other organ systems of different species with the highest frequency in dogs and cats. In these species, the tumor was composed of proliferating elongated cells forming interlacing bundles and streams (Kennedy *et al.*, 1998; Cooper and Valentine, 2002; Meuten, 2002; Sato *et al.*, 2003; Firat *et al.*, 2007). Liptak and Forrest (2007), and Skoric *et al.* (2010) indicated that neoplastic cells contained cigar-shaped nuclei with rounded ends and indistinguishable cytoplasmic borders and exhibited low mitotic figure. These findings supported the diagnosis of a

leiomyoma in the current study. In contrast, marked cellular pleomorphism including hyperchromasia, anisokaryosis, multinucleated cells, anisocytosis and bizarre mitoses were reported by Liptak and Forrest (2007), Skoric *et al.* (2010) were not observed in the present study.

Papillomatosis is a benign proliferative tumor of cutaneous and mucosal epithelia that is commonly found in cattle. The disease affects mainly young animals up to two years of age; however, cattle of all ages can develop such lesions (Campo and Jarrett, 1994; Jelinek and Tachezy, 2005; Claus *et al.*, 2009). In this study, papillomatosis was detected in animals of all age groups which agreed with previous study by Claus *et al.* (2009). In the present study, bovine papillomatosis that were found in almost all body parts (the skin, head, neck, eye, perinal region, shoulder, limbs, teats and mucosal surfaces) were in line with the report described by Ozsoy *et al.* (2011), Terziev *et al.* (2015), and Mansour (2017).

Papillomatosis may become a significant herd problem when a large group of young and susceptible cattle become infected. The percentage of disease may be increased approximately up to 20-25% (Ozsoy *et al.*, 2011). In this study, the frequency of bovine papillomatosis was represented by 56.6 %. Of this squamous cell papilloma and fibropapilloma were represented by 33.3% and 23.3%, respectively, which agreed with previous report by Ozsoy *et al.* (2011).

Macroscopically elevated, fleshy, multi-nodular proliferations and cauliflower-like shaped tumoral masses were observed. These gross findings of tumor in the present study were consistent with previous reports by Jelinek and Tachezy (2005), Atasever *et al.* (2005), Goldschmidt and Hendrick (2008), and Ozsoy *et al.* (2011).

Cytological characterization of papillomatosis (fibropapilloma and squamous cell papilloma) in this study revealed spindle cells that appeared in cluster and individually with scant amount of bluish cytoplasm, high nuclear to cytoplasmic ratio, anisocytosis

and anisokaryosis with atypical mitotic figures. This cytological characterization agreed with previous findings by Johnson and Myers (2017) in skin of a dog.

Histopathological characteristics of papillomatosis showed varying degrees of epidermal proliferation with a hyperplastic epidermis / rete pegs. In the epidermis, squamous differentiation of keratinocytes together with many koilocytes was noted. But, in the dermis, excessive proliferation of spindle to plump shaped fibroblasts and fibrovascular core were presented. Moreover, similar histopathologic features in papillomatosis have been previously reported in cattle by Khodakaram-Tafti and Kargar (2009), Tozato *et al.* (2013), Ataseven *et al.* (2016), Griffiths *et al.* (2016), and Mansour (2017).

Basal cell carcinoma is a malignant epithelial tumor of the skin which is entirely composed of basal cells and frequently seen in cats than in dogs and rare in other domestic animals (Kennedy *et al.*, 1998; Kumar *et al.*, 2016). However, in this study basal cell tumor was only noticed in a pig with a frequency of 1.7% from all tumors discerned.

The usual locations of basal cell tumor in dogs is head and neck where it is majorly exposed to sun light (Goldschmidt and Hendrick, 2008; Kumar *et al.*, 2016). However, Gross *et al.* (1992) observed basal cell carcinomas in cats at non sun exposed locations of the body which coincided with the present study in which the mass was located on the scrotal skin of pig. The tumors were appeared as slightly raised, ulcerated, haemorrhagic and firm in consistency reported by Kumar *et al.* (2016) in dog agreed with the present gross finding.

The cytological characteristics of basal cell carcinoma indicated clusters of deep stained or plump epithelial cells that were arranged in a trabecular manner, and mixed with inflammatory neutrophils and erythrocytes. This finding is in agreement with Johnson and Myers (2017) who observed numerous clusters of basilar-type epithelial cells which were characterized by small size and a high nuclear to cytoplasmic ratio.

The neoplastic basal cells on histological section were infiltrated into the dermis, and appeared as cords or sheets, which was characterized by medium to large size elongated nuclei with prominent nucleoli reported by Vantuchova and Curik (2006) was in consistent with the present study. Moreover, the basal cells had deep nuclear chromatin with inconspicuous cytoplasm and exhibited nuclear pleomorphism with high mitotic figure.

Hemangiopericytomas are rare and potentially aggressive lesions (Ramakrishna *et al.*, 2014). The tumor may arise in any location but has a predilection for the lower extremities, limbs, pelvis, retroperitoneum, and head and neck region (Chhieng *et al.*, 1999; Chou *et al.*, 2009; Blutke *et al.*, 2012). In the present study, hemangiopericytoma was located in submandibular and shoulder regions were coincided with previous reports by Chhieng *et al.* (1999), Sawh *et al.* (2000), and Marec-Berard (2004) in human. To the author's knowledge, no report was documented regarding the frequencies of hemangiopericytomas in bovine species. However, the report by previous study in human indicated that the frequencies of hemangiopericytomas was approximately 3% of all soft tissue sarcomas (Marec-Berard, 2004). Moreover, 7% was reported in dogs (Blutke *et al.*, 2012). Based on the above facts, the results of this study showed 3.4% of all tumors of domestic animal species.

Macroscopically, the tumors was entirely circumscribed with smooth surface and appeared firm in consistency and round in shape as reported by Goldschmidt and Hendrick (2002), and Gross *et al.* (2005) in pets as well as Maresi *et al.* (2007), and Vetorazzo Filho *et al.* (2015) in man was in agreement with this study. Furthermore, a cut-surface revealed a fibrous capsule associated with complex network of vessels, coagulated blood, and small nodules within the main mass was observed in the present study agreed with previous reports by Goldschmidt and Hendrick (2008), Gross *et al.* (2008), and Blutke *et al.* (2012) in dog and cat.

Cytologic features of hemangiopericytomas in this study revealed low cellularity which typically comprised of pericytes that differed from the previous report by Chhieng *et al.*

(1999), and Sawh *et al.* (2000) who found hypercellular neoplastic cells. The reason for this low cellularity in the present study might be due to insufficient specimen during fine needle aspirate. Moreover, some clusters of pericytes with binucleation and multinucleation were also observed in the present study agreed with reports by Goldschmidt and Hendrick (2008), and Gross *et al.* (2008) in dog. The tumor cells were oval or spindle-shaped with a high nuclear to cytoplasmic ratio, indistinct cellular borders, finely granular nuclei with evenly distributed chromatin and indistinct nucleoli. The nuclear contour generally was smooth and regular. The cytoplasm was scant, pale, and delicate reported by Chhieng *et al.* (1999) in human agreed with this study.

Microscopically, hemangiopericytoma was highly cellular characterized by the presence of perivascular whorls, interlacing bundles and sheets with poorly defined cytoplasm, elongated pale nuclei and mitotic activity reported by Blutke *et al.* (2012) in cat and Handharyani *et al.* (1999), Goldschmidt and Hendrick (2008), and Gross *et al.* (2008) in dog were in agreement with the present study. Areas of haemorrhage and necrosis observed by Gross *et al.* (2008) in dog within the neoplastic tissue were also observed in the present study. Histopathologically, however the so-called 'stag-horn' sign, formed by proliferation of fusiform to roundish undifferentiated tumor cells in dendritic branches around the capillary vessels, was formerly regarded as useful in the diagnosis of hemangiopericytoma reported by Chhieng *et al.* (1999) was not noted in this study.

Mammary neoplasms are the most common neoplasms in female dogs (Goldschmidt *et al.*, 2011) comprising up to 25-30% of all neoplasms in dogs (Hazirolu *et al.*, 2010), and mixed tumors and carcinoma are diagnosed in 50-65% and 25-40% of cases of mammary tumors, respectively (Allen *et al.*, 1986; Hazirolu *et al.*, 2010). However, the frequency of mammary gland tumor in the present study was represented by 6.7%. Of this, carcinoma-mixed type, papillary mammary adenocarcinoma and mammary adenocarcinoma were frequently examined and represented 3.3%, 1.7% and 1.7%, respectively of all tumors diagnosed in the study which were in agreement with previous report by Allen *et al.* (1986), and Hazirolu *et al.* (2010).

Majority of the mammary gland tumors macroscopically showed a hard or an elastic consistency but some of them appeared fluctuant with edema or necrotic areas. Moreover, in the great majority of cases, the aspect of the tumor on the cut surface was white to grey and lobed which was reported by Allen *et al.* (1986) that agreed with this study.

Cytological examination has important benefits in clarifying some aspects in early diagnosis of mammary lesions (Hazroglu *et al.*, 2011). However, in dog, few data are available on the value of cytologic examination of mammary tumors due to less favorable accuracy of cytologic diagnosis for mammary tumors as a result of the heterogeneous composition of mammary tumors (Simon *et al.*, 2009). Accordingly, to exclude such inconvenience, the present report described cytological examination for the diagnosis of mammary tumors in comparison to histopathological results. In this study, impression smears and fine needle aspirates of mammary gland tumor in all cases revealed characteristics of malignancy including clusters of cells with indistinct borders, high nuclear to cytoplasmic ratio, cytoplasmic vacuoles, anisocytosis, anisokaryosis and hyperchromasia that agreed with previous studies by Hazroglu *et al.* (2011).

Histologically, the general features of mammary gland tumors that shared in common with this study agreed to the report by Goldschmidt *et al.* (2011) who described similar histological characteristics including hypercellularity, hyperplasia, hyperchromasia, nuclear and cellular pleomorphism, high nuclear to cytoplasmic ratio and moderate to severe mitotic figures with prominent nucleoli in canine. More specifically, papillary mammary adenocarcinoma revealed severe papillae of epithelial cells projecting into the surrounding mammary stroma were demonstrated in the present study which agreed with Hazroglu *et al.* (2011), and Goldschmidt *et al.* (2011). Furthermore, epithelial cells and the myoepithelial cells were pleomorphic with fusiform neoplastic cells and a small amount of fibrillar cytoplasm was observed in carcinoma-mixed type by Allen *et al.* (1986), Cassali *et al.* (2007), Hazroglu *et al.* (2011), and Goldschmidt *et al.* (2011) which similarly documented in this study. On the other hand, mammary adenocarcinoma was characterized by neoplastic cells that were composed of pleomorphic epithelial component and arranged in a cord like pattern deep into the mammary stroma. The cell

border was indistinct with a small amount of cytoplasm, and oval to elongate nuclei that contain finely stippled chromatin were mentioned by Cassali *et al.* (2007), and Goldschmidt *et al.* (2011) agreed with the present study.

Sertoli cell tumors are the most common testicular tumors (Masserdotti *et al.*, 2005; Nodtvedt *et al.*, 2011) representing 8-20.2% of all testicular malignancies in dog (Liao *et al.*, 2009; D'Angelo *et al.*, 2012). In this study, dogs affected by SCT represented 1.7% of all tumors that occurred in domestic animals were in agreement with the previous report by Grieco *et al.* (2004), and Liao *et al.* (2009).

The enlarged testis upon macroscopic examination described as well-circumscribed, nodular, composed of unifocal, moveable and pendulous lumps with no evidence of ulcer reported by Castro *et al.* (2016) agreed with the present study. Moreover, lobulated neoplastic areas separated by white fibrous bands on cut section were coincided with that of Valente *et al.* (2017). Furthermore, the outer surfaces of the neoplasms revealed smooth and accompanied by large dark blood vessels innervating to the neoplastic mass were reported by Castro *et al.* (2016), and Valente *et al.* (2017) in which were accordance with the present study.

Previous descriptions of the cytology of testicular tumors in the dog have been published (Masserdotti *et al.*, 2005; Masserdotti *et al.*, 2008). The features described for Sertoli cell tumors were highly diagnostic including large sheets and cords of round to columnar cells with poorly defined cytoplasmic borders, and macrovacuolation in the cytoplasm were in agreement with the present study. Moreover, the nuclei showed only occasional nucleoli but significant anisokaryosis, fine granular chromatin which was irregularly clumped, intranuclear vacuoles and typical mitoses were noted in this study. Similar finding was indicated by Masserdotti *et al.* (2005), Masserdotti *et al.* (2008), and Valente *et al.* (2017).

Histopathological characterization of SCT in the present study showed intratubular but severely coalescing sheets of neoplastic sertoli cells, elongated to oval sertoli cell nuclei

with scant cytoplasm, severe anisocytosis, anisokaryosis, prominent nucleoli, hyperchromasia, high mitotic rate and abundant hypercellular stroma in between the proliferating sertoli cells. Similar findings were reported by Castro *et al.* (2016) from left testicle and abdominal mass of dog.

The majority of primary ovarian neoplasia belongs to the categories of epithelial tumors, sex cord- stromal tumors and germ cell tumors. In bitches, epithelial tumors represent 40%–50% of all ovarian neoplasms (Arlt and Haimerl, 2016) and are often bilateral (Diez-Bru *et al.*, 1998). Granulosa cell tumors are described to be the most common sex cord-stromal ovarian neoplasia in the bitch accounting for 50% of all ovarian neoplasia (Herron, 1983). Conversely, the present study found the rare type amongst the sex cord-stromal tumor namely fibrothecoma with frequency of 1.7% of all tumors of domestic animals.

In the present study, gross characterization of fibrothecoma in a bitch showed the tumor mass was well demarcated with firm consistency, bilateral, irregular in shape, deep-red to light red in color, congested and lobulated. However, previous reports by Kennedy *et al.* (1998), Goldschmidt and Hendrick (2008) disagreed with the present finding who observed unilateral mass located on the ovary.

Cytological examination of impression smear from dog showed less cellular and consisted of a few population of oval nuclei of theca cells arranged in sheets and some undefined epithelial cells intermingled among the oval nuclei observed in the current study was in disagreement with Yang and Mesia (1999) who reported fragments of tightly packed, uniform, small oval neoplastic cell appeared in large sheets with seldom rosette-like arrangement in 70-yr old woman. The reason for this might be due to insufficient cell population during imprint because of the mass is too firm and complicated by blood.

Histological section of ovarian fibrothecoma showed cells with spindly fibroblasts with collagen fibrils, and plump, oval thecoma cells with prominent central nucleoli and

abundant, pale, and vacuolated cytoplasm that was reported by Yang and Mesia (1999), and Young (2011) in human which agreed with the present finding.

Cutaneous MCTs are the most frequent types of dog neoplasms representing approximately 21% of the skin tumors (Hosseini *et al.*, 2014; Bahrami *et al.*, 2014) which is in agreement with the present study that observed 1.7% frequency of CMCTs of all tumors of domestic animals. MCTs can develop anywhere on the body, but there is a higher incidence of MCTs on the forelimb of dogs was reported by Welle *et al.* (2008), and Meuten (2017) who supported the present finding.

The gross morphology of cutaneous CMCTs was highly variable ranging from nodular rashes to diffuse swellings, large-sized masses, hairless, raised and erythematous tumors which were reported by Meuten (2017) that agreed with the present findings. Moreover, the mass was poorly circumscribed, ulcerated and pruritic.

Tumor cells by cytological examination revealed poorly granulated mast cell in cytoplasm with several, clear, punctuate cytoplasmic vacuoles and distinct cell borders, nuclear and cellular pleomorphism, high N:C ratio, numerous eosinophils, prominent nucleoli with marked mitotic figures which were reported by Hosseini *et al.* (2014), and Meuten (2017) that were consistent with present findings.

Histopathologically, CMCTs were less well circumscribed by connective tissue and neoplastic cells often exhibited an aggressive behavior, high cellularity and cellular pleomorphism which were reported by Welle *et al.* (2008), and Hosseini *et al.* (2014) that coincided with the present study. Furthermore, neoplastic cells had distinct cell borders and widely variable amounts of fine eosinophilic cytoplasmic granules consistent with mast cell granules and also highly pleomorphic tumour cells with a high mitotic index, anisokaryosis and infiltration of the surrounding dermis and subcutis which were observed in the present study were in agreement with Welle *et al.* (2008), and Hosseini *et al.* (2014).

Canine transmissible venereal tumor is one of the most commonly occurring round cell tumors that affects the external genitalia of dogs (Thangathurai *et al.*, 2008) with a frequency ranging from 2 to 43% all tumors (Purohit, 2009). Although this study found relatively same frequency represented by 1.7% of all tumors of domestic animals as reported by Purohit (2009).

Grossly, the multiple, friable, reddish, ulcerated, inflamed and verrucous like masses observed on the vagina of a dog in the present study were in agreement with earlier observations by Goldschmidt and Hendrick (2008), MacLachlan and Kennedy (2008), and Thangathurai *et al.* (2008).

Cytological characterization of TVT in the present study revealed presence of distinct, clear, cytoplasmic vacuoles often referred to as punctate vacuoles with a delineated outline, high nuclear to cytoplasmic ratio of tumor cells, anisocytosis, anisokaryosis and numerous mitotic figures. Similar cytological features were reported by many researchers (Alleman and Bain, 2000; Thangathurai *et al.*, 2008; Purohit, 2009; Stockmann *et al.*, 2011; Johnson and Myers, 2017).

Histopathological studies of TVT in the present study revealed hypercellularity into the mucosa, submucosa and muscularis layer of the vagina with a sheet of round individual cells containing round vesicular nuclei. The cells were situated in an arborizing fibrovascular network with distinct single, centrally placed nucleolus having dispersed chromatin, scant stroma and high mitotic figures. These observations agreed with those of earlier studies (Krithiga *et al.*, 2005; Thangathurai *et al.*, 2008; Ghaffari *et al.*, 2009; Stockmann *et al.*, 2011).

Malignant fibrous histiocytoma is a rare extraskeletal neoplasm of the superficial and deep connective tissues occurring in animals and humans. The tumors in equines have all been of the superficial type with a predilection for the limb (Booth *et al.*, 1998). It is interesting that in one report 3 out of 6 horses were quarter horses (Geburek *et al.*, 2007). The occurrence of MFH on the upper limb of a mule in the present study corresponds

with previous reports by Hamir, (1989), Marryatt (2003), and Geburek *et al.* (2007). Reported frequencies of malignant fibrous histiocytoma in areas of Pacific North West represented 1.5%. Similarly, the present study indicated 1.7% of all tumors of domestic animals.

The gross presentations of MFH in the present study was well circumscribed, small, firm in consistency, nodular, and encapsulated with ulcerated overlying skin. The cut surface was grey to white in color. The surface of the mass was rough and alopecic accompanied by haemorrhage. Similar gross findings were reported by Geburek *et al.* (2007).

The cytological smear of MFH revealed less cellular with round to oval cells that had generally non-vacuolated and indistinct cytoplasm. The nuclei were typically indented, bean shaped and irregular that showed minimal cytologic characteristics of malignancy reported by Ozyigit *et al.* (2011) that supported the present finding.

Histological characterization of malignant fibrous histiocytoma was revealed highly pleomorphic histiocytic cells with indistinct cytoplasmic borders and indented nuclei with prominent nucleoli. Moreover, the nuclei were vesicular with anisokaryosis, anisocytosis and mitotic figures. This finding was supported by the reports of Walaas *et al.* (1986), Fulmer and Mauldin (2007), and Ozyigit *et al.* (2011).

Atypical anal sac adenocarcinoma is reported to be the most common malignant perianal neoplasm in dogs accounting for approximately 2% of canine skin tumors reported by Parry (2006) which supported the present study representing 1.7% of all tumors of domestic animals.

The gross characterization of the atypical anal sac adenocarcinoma showed a unilateral and firm nodular swelling reported by Parry (2006), Goldschmidt and Hendrick (2008) in dog and cat agreed with the present finding. Cytologically, atypical anal sac adenocarcinoma showed hypercellularity, and arranged with clusters or sheets epithelial cells having round hyperchromatic nuclei, a prominent nucleoli, and very scanty cytoplasm. Most of the neoplastic cells were poorly differentiated in the present study concurred with that of Sakai *et al.* (2012), and Johnson and Myers (2017).

## 6. CONCLUSION AND RECOMMENDATIONS

This study was a preliminary type on tumors of domestic animals in the study area. In this study tumors were encountered in wide species of domestic animals. Based on cellular origin, tumor of epithelial origin, mesenchymal origin, and round cell were diagnosed. It was difficult to classify some of the tumors based on cells involved and these were taken as unclassified. Papillomatosis, equine sarcoid and mammary gland tumors were the most frequently encountered tumors followed by fibrosarcoma, and hemangiopericytomas. Frequent microscopic lesions were hypercellularity, high nuclear to cytoplasmic ratio, nuclear and cellular pleomorphism, binucleation, prominent nucleoli and hyperchromasia.

Based on the findings, the following recommendations were forwarded:

- Further study that uses large sample size with wide area coverage should be conducted on the neoplastic disorders.
- Study that include, hormonal, hematological, molecular, tumor biomarkers and immunohistopathology should be conducted to have complete data on tumors of domestic animals in Ethiopia.

## 7. REFERENCES

- Agner T., Ebbelohj N. E., Wulf H. C., Bonde J. P. (2013): A scientific review addressing occupational skin cancer. Pp. 1-75.
- Alleman A. R., Bain P. J. (2000): Diagnosing neoplasia: the cytologic criteria for malignancy. *Veterinary medicine* **95(204)**: 223.
- Allen S. W., Prasse K. W., Mahaffey E. A. (1986): Cytologic differentiation of benign from malignant canine mammary tumors. *Veterinary pathology* **23(6)**: 649-655.
- Anderson D. E., Badzioch M. (1991): Association between solar radiation and ocular squamous cell carcinoma in cattle. *American journal of veterinary research* **52(5)**: 784-788.
- Arjanbhai, D.M. (2009). Studies on Canine Mammary Gland Tumours. *Anand Agricultural University*. Pp. 1-15.
- Arlt S. P., Haimerl P. (2016): Cystic ovaries and ovarian neoplasia in the female dog-a systematic review. *Reproduction in Domestic Animals* **51**: 3-11.
- Ataseven V. S., Kanat O., Ergun Y. A. (2016): Molecular identification of bovine papillomaviruses in dairy and beef cattle: first description of Xi-and Epsilonpapillomavirus in Turkey. *Turkish Journal of Veterinary and Animal Sciences* **40(6)**: 757-763.
- Atasever A., Cam Y., Atalay O. (2005): Bir sigir sürüsünde deri papillomatosis olgulari. *Ankara Univ Vet Fak Derg* **52**: 197-200.
- Avallone G., Helmbold P., Caniatti M., Stefanello D., Nayak R. C., Roccabianca P. (2007): The spectrum of canine cutaneous perivascular wall tumors: morphologic, phenotypic and clinical characterization. *Veterinary pathology* **44(5)**: 607-620.
- Avci H., Yaygingul R., Gultekin M., Epikmen E. T., Ural K., Belge A., Birincioglu S. S. (2012): Primary intestinal fibrosarcoma caused by intestinal perforation in a dog: a case report. *Veterinarni Medicina* **57(6)**:314-319.
- Aydin Y., Atalay Vural S., Oznur N. (2003): Malignant fibrous histiocytoma of the giant-cell type in a cat. *Ankara Eniv Vet Fak Derg* **50**: 247-249.

- Baba A. I., Catoi C. (2007): Epithelial and melanocytic tumors of the skin. *Comparative Oncology*. Pp. 1-113.
- Bahrami A. M., Khaki F., Zehtabian S., Cheraghi J., Rashnavadi M., Ahmadi M. R. H., Naderafif M., Javaherypour S., Mohsenzadeh S., Hosseini E. (2014): Retracted Article: Uterine mast cell tumor: a clinical and cytohistopathological study. *Journal of ovarian research* **7(1)**: 105.
- Bastianello S. S. (1982): A survey on neoplasia in domestic species over a 40-year period from 1935 to 1974 in the Republic of South Africa. I. Tumours occurring in cattle. *The Onderstepoort journal of veterinary research* **49(4)**: 195-204.
- Bautista-Gomez L. G., Martinez-Castaneda E., Cordova-Alarcon E., Vazquez-Chagoyan J. C. (2011): Analysis of canine transmissible venereal tumor genotypes using the D-loop region of mitochondrial DNA. *Genes & genetic systems* **86(5)**: 351-355.
- Bazzo R., Sarli G., Mandrioli L., Marcato P. S. (2002): Sertoli Cell Tumour with Call-Exner-Like Bodies in a Dog. *Transboundary and Emerging Diseases* **49(10)**: 535-537.
- Beauvais W., Cardwell J. M. and Brodbelt D. C. (2012): The effect of neutering on the risk of mammary tumours in dogs-a systematic review. *Journal of Small Animal Practice* **53**: 314-322.
- Belay, H. and Muktar, Y. (2015): Isolation and Identification of Foot and Mouth Disease Virus from Clinically Infected Cattle in Ada Veterinary Clinic. *American-Eurasian Journal of Scientific Research* **10**:368-374.
- Berardo M. D., Powers C. N., Wakely P. E., Almeida M. O., Frable W. J. (1997): Fine-needle aspiration cytopathology of malignant fibrous histiocytoma. *Cancer Cytopathology* **81(4)**: 228-237.
- Birbrair A., Zhang T., Wang Z. M., Messi M. L., Olson J. D., Mintz A., Delbono O. (2014): Type-2 pericytes participate in normal and tumoral angiogenesis. *American Journal of Physiology-Cell Physiology* **307(1)**: 25-38.
- Blutke A., Knebel J., Bruhschwein A., Breuer W., Hermanns W. (2012): Hemangiopericytoma in a cat: a case report. *Veterinarni Medicina* **57(5)**: 263-269.

- Boneschi V., Parafioriti A., Armiraglio E., Gaiani F., Brambilla L. (2009): Primary giant cell tumor of soft tissue of the groin-a case of 46 years duration. *Journal of cutaneous pathology* **36(S1)**: 20-24.
- Booth M. J., Bastianello S. S., Jiminez M., Van Heerden A. (1998): Malignant fibrous histiocytoma of the deep peri-articular tissue of the stifle in a dog: case report. *Journal of the South African Veterinary Association* **69(4)**: 163-168.
- Bush J. M., Powers B. E. (2008): Equine giant cell tumor of soft parts: a series of 21 cases (2000-2007). *Journal of veterinary diagnostic investigation* **20(4)**: 513-516.
- Bussanich N. M., Dolman P. J., Rootman J., Dolman C. L. (1987): Canine uveal melanomas: series and literature review. *The Journal of the American Animal Hospital Association (USA)* **23**:415-422.
- Campbell G. A., Gross T. L., Adams R. (1987): Solar elastosis with squamous cell carcinoma in two horses. *Veterinary Pathology* **24(5)**: 463-464.
- Campo M. S., Jarrett W. F. (1994): Vaccination against cutaneous and mucosal papillomavirus in cattle. *Ciba Found Symp.* Pp. 61-73.
- Carvalho T., Vala H., Pinto C., Pinho M., Peleteiro M. C. (2005): Immunohistochemical studies of epithelial cell proliferation and p53 mutation in bovine ocular squamous cell carcinoma. *Veterinary pathology* **42(1)**: 66-73.
- Cassali G. D., Gobbi H., Malm C., Schmitt F. C. (2007): Evaluation of accuracy of fine needle aspiration cytology for diagnosis of canine mammary tumours: comparative features with human tumours. *Cytopathology* **18(3)**: 191-196.
- Castro J. L. C., Albernaz V. G. P., Domingues C. M. T., Ferreira A. A., Dittrich G., Moratelli C. B. S., Freitag F. V., Miara L. C. (2016): Malignant Sertoli Cell Tumor with Abdominal Infiltrative Metastasis in a Dog. *Pakistan Veterinary Journal* **36(3)**: 379-381.
- Chhieng D., Cohen J., Waisman J., Fernandez G., Cangiarella J. (1999): Fine-needle aspiration cytology of hemangiopericytoma. *Cancer Cytopathology* **87(4)**: 190-195.

- Chou C. W., Hsu S. P., Lin S. C., Chen M. H., Shih Y. H., Lee L. S., Lin C. F. (2009): Primary intradural hemangiopericytoma with intramedullary invasion. *Journal of the Chinese Medical Association* **72(10)**: 536-541.
- Claus M. P., Lunardi M., Alfieri A. A., Otonel R. A. A., Sartori D., Fungaro M. H. P., Alfieri A. F. (2009): Multiple bovine papillomavirus infections associated with cutaneous papillomatosis in Brazilian cattle herds. *Brazilian Archives of Biology and Technology* **52**: 93-98.
- Coleman S., Barazani Y., Roma A. A., Sabanegh Jr E. (2014): Testicular sclerosing Sertoli cell tumor: a case report and review of the literature. *Reviews in urology* **16(4)**: 191.
- Constable, P. D., Hinchcliff, K. W., Done, S. H., and Gruenberg, W. (2016): *Veterinary Medicine-E-BOOK: A textbook of the diseases of cattle, horses, sheep, pigs and goats*. Elsevier Health Sciences. Pp. 2065.
- Cooper, B. J. and Valentine, B. A. (2002): Tumors of muscle. In: *Tumors in domestic animals*, ed. Meuten, DJ, 4<sup>th</sup> ed. Iowa State University Press, Ames, IA . Pp. 319- 363.
- Corteggio A., Altamura G., Roperto F., Veneziano V., Traversa D., Mascioni A., Borzacchiello G. (2012): Equine sarcoid associated with cutaneous habronemosis. *Journal of equine veterinary science* **32(12)**: 831-834.
- DACVP J. S. K. V. P., Vandis M. (2010): Clinical Exposures: Canine circumanal gland adenoma: The cytologic clues . Pp. 1-4.
- Dajani Y. F., Kilani Z. (1998): Role of testicular fine needle aspiration in the diagnosis of azoospermia. *International journal of andrology* **21(5)**: 295-300.
- Danendran Krishnan MBBS B. M., Anitha Ann Thomas MD F. (2014): Unilateral ovarian fibrothecoma with menorrhagia. *The Malaysian journal of pathology* **36(1)**: 55-58.
- D'Angelo A. R., Vita S., Marruchella G., Di Francesco G. (2012): Canine testicular tumours: a retrospective investigation in Abruzzo and Molise, Italy. *Vet Ital* **48(3)**: 329-333.
- Das A. K., Das U., Das D., Sengupta J. (1990): Histopathological study of canine transmissible venereal tumour. *Indian Veterinary Journal* **67(5)**: 473-474.

- Dennis M. and Joanna M. (2006): *Clinical Textbook for Veterinary Technicians*. 5<sup>th</sup> ed., Elsevier pub. Pp. 102-108.
- Desrochers A., St-Jean G., Kennedy G. A. (1994): Congenital cutaneous papillomatosis in a one-year-old Holstein. *The Canadian Veterinary Journal* **35(10)**: 646.
- Diez-Bru N., Garcia-Real I., Martinez E. M., Rollan E., Mayenco A., Llorens P. (1998): Ultrasonographic appearance of ovarian tumors in 10 dogs. *Veterinary Radiology & Ultrasound* **39(3)**: 226-233.
- Dolka, I., Czopowicz, M., Gruk-Jurka, A., Wojtkowska, A., Sapieryński, R., Jurka, P. (2018). Diagnostic efficacy of smear cytology and Robinsons cytological grading of canine mammary tumors with respect to histopathology, cytomorphometry, metastases and overall survival. *PloS one* **13**, e0191595.
- Esmailzadeh S., Rezaei A., Mazaheri Y. (2015): Hepatic leiomyoma in a cow. *Veterinary Record* **2007(161)**: 423-424.
- Fantini O., Videmont E., Pin D. (2015): Successful treatment of florid papillomatosis in a dog using subcutaneous feline recombinant interferon-omega. *Revue de Medecine Veterinaire* **166**: 25-29.
- Firat I., Haktanir-Yatkin D., Sontas B. H., Ekici H. (2007): Vulvar leiomyosarcoma in a cat. *Journal of feline medicine and surgery* **9(5)**: 435-438.
- Fletcher, C. D., Unni, K. K., and Mertens, F. (2002): *Pathology and genetics of tumours of soft tissue and bone* 4<sup>th</sup> edition. Iarc . Pp. 47-125.
- Florez M. M., Pedraza F., Grandi F., Rocha N. S. (2012): Cytologic subtypes of canine transmissible venereal tumor. *Veterinary clinical pathology* **41(1)**: 3-5.
- Fornazari G. A., Kravetz J., Kiupel M., Sledge D., De Barros Filho I. R., Montiani-Ferreira F. (2017): Ocular squamous cell carcinoma in Holstein cows from the South of Brazil. *Veterinary world* **10(12)**: 1413.
- Fulmer A. K., Mauldin G. E. (2007): Canine histiocytic neoplasia: an overview. *The Canadian Veterinary Journal* **48(10)**: 1041.
- Ganguly B., Das U., Das A. K. (2013): Canine transmissible venereal tumour: a review. *Veterinary and comparative oncology* **14(1)**: 1-12.
- Garma-Avina A. (1994): The cytology of squamous cell carcinomas in domestic animals. *Journal of veterinary diagnostic investigation* **6(2)**: 238-246.

- Geburek F., Oppen T. v., Hewicker–Trautwein M., Ohnesorge B. (2007): Treatment of a malignant fibrous histiocytoma involving the forelimb of a Quarter Horse mare using conventional and laser surgery. *Equine Veterinary Education* **19(1)**: 19-22.
- Genzebu D., Tamir B. and Berhane G. (2016): Study of Reproductive and Production Performance of Cross Breed Dairy Cattle under Smallholders Management System in Bishoftu and Akaki Towns. *Int J Adv Res Biol Science* **3**: 118-123.
- Ghaffari M. S., Dezfoulian O., Aldavood S. J., Masoudifard M. (2009): Estrogen-related alopecia due to polycystic ovaries in a terrier dog. *Comparative Clinical Pathology* **18(3)**: 341-343.
- Gharagozlou M. J., Hekmati P., Ashrafihelan J. (2007): A clinical and histopathological study of ocular neoplasms in dairy cattle. *Veterinarski Arhiv* **77(5)**: 409-426.
- Ghilezan N. (1992): Oncologie generala Ed. *Medicala Bucuresti* . Pp. 15-18.
- Ghisleni G., Roccabianca P., Ceruti R., Stefanello D., Bertazzolo W., Bonfanti U., Caniatti M. (2006): Correlation between fine-needle aspiration cytology and histopathology in the evaluation of cutaneous and subcutaneous masses from dogs and cats. *Veterinary clinical pathology* **35(1)**: 24-30.
- Giglio M., Medica M., De Rose A. F., Germinale F., Ravetti J. L., Carmignani G. (2003): Testicular Sertoli cell tumours and relative sub-types. *Urologia internationalis* **70(3)**: 205-210.
- Goldschmidt M. H., Dunstan R. W., Stannard A. A., von Tscharner C., Walder E. J., Yager J. A. (1998): Melanocytic tumors and tumor-like lesions. *Histological Classification of Epithelial and Melanocytic Tumors of the Skin of Domestic Animals, ed Goldschmidt MH, Dunstan RW, Stannard AA, von Tscharner C, Walder EJ, and Yager JA* . Pp. 38-40.
- Goldschmidt M. H., Hendrick M. J. (2008): Tumors of the skin and soft tissues. Tumors in Domestic Animals, Fourth Edition . Pp. 45-117.
- Goldschmidt M., Pena L., Rasotto R., Zappulli V. (2011): Classification and grading of canine mammary tumors. *Veterinary pathology* **48(1)**: 117-131.
- Gomes D. G. L. d. M. (2011): Equine sarcoids: a therapeutic challenge. Master Thesis Dissertation on Veterinary Medicine. Lisbon, Portugal. Pp. 1-116.

- Gonzalez C. M., Griffey S. M., Naydan D. K., Flores E., Cepeda R., Cattaneo G., Madewell B. R. (2000): Canine transmissible venereal tumour: a morphological and immunohistochemical study of 11 tumours in growth phase and during regression after chemotherapy. *Journal of comparative pathology* **122(4)**: 241-248.
- Goodrich L., Gerber H., Marti E., Antczak D. F. (1998): Equine sarcoids. *Veterinary Clinics: Equine Practice* **14(3)**: 607-623.
- Govaere J., Maes S., Saey V., Blancke W., Hoogewijs M., Deschauer C., Smits K., Roels K., Vercauteren G., de Kruif A. (2011): Uterine fibrosarcoma in a warmblood mare. *Reproduction in Domestic Animals* **46(3)**: 564-566.
- Grandi F., Colodel M. M., Monteiro L. N., Leao J. R. V., Rocha N. S. (2010): Extramedullary hematopoiesis in a case of benign mixed mammary tumor in a female dog: cytological and histopathological assessment. *BMC veterinary research* . Pp. **6**: 45.
- Grieco V., Rondena M., Romussi S., Stefanello D., Finazzi M. (2004): Immunohistochemical characterization of the leucocytic infiltrate associated with canine seminomas. *Journal of comparative pathology* **130(4)**: 278-284.
- Griffiths, C., Barker, J., Bleiker, T., Chalmers, R., and Creamer, D. (2016): Rook's textbook of dermatology. John Wiley & Sons.
- Gross, T. L., Ihrke, P. J., and Walder, E. J. (1992): Basal cell tumor. In: *Veterinary Dermatopathology*. T.L Gross, P.J. Ihrke and E.J. Walder (edn.). Missouri, Mosby Year Book, Inc . Pp. 520.
- Gross, T. L., Ihrke, P. J., Walder, E. J., and Affolter, V. K. (2008): *Skin diseases of the dog and cat: clinical and histopathologic diagnosis* 2<sup>nd</sup> ed. John Wiley & Sons . Pp. 709-894.
- Hamada M., Itakura C. (1990): Ultrastructural morphology of hypomelanosis in equine cutaneous papilloma. *Journal of comparative pathology* **103(2)**: 199-213.
- Hamada M., Oyamada T., Yoshikawa H., Yoshikawa T., Itakura C. (1990): Keratin expression in equine normal epidermis and cutaneous papillomas using monoclonal antibodies. *Journal of comparative pathology* **102(4)**: 405-420.

- Hamali H., Ashrafihelan J. (2010): Vaginal fibrosarcoma in cow (A case report). *International Journal of Veterinary Research* **4(4)**: 225-228.
- Hamir A. N. (1989): Equine giant cell tumor of soft tissues. *The Cornell veterinarian* **79**: 173-177.
- Handharyani E., Ochiai K., Kadosawa T., Kimura T., Umemura T. (1999): Canine hemangiopericytoma: an evaluation of metastatic potential. *Journal of veterinary diagnostic investigation* **11(5)**: 474-478.
- Hanna P. E., Dunn D. (2003): Cutaneous fibropapilloma in a cat (feline sarcoid). *The Canadian Veterinary Journal* **44(7)**: 601-602.
- Harrison A. C., Jayasundera T. (1999): Mycobacterial cervical adenitis in Auckland: diagnosis by fine needle aspirate. *The New Zealand medical journal* **112(1080)**: 7-9.
- Hatama S. (2012): Cutaneous papillomatosis in cattle. *Journal of Disaster Research* **7(3)**: 319-323.
- Hayes H. M., Tarone R. E., Casey H. W., Huxsoll D. L. (1990): Excess of seminomas observed in Vietnam service US military working dogs. *JNCI: Journal of the National Cancer Institute* **82(12)**: 1042-1046.
- Haziroglu R., Yardimci B., Aslan S., Yildirim M. Z., Yumusak N., Beceriklisoy H., Agaoglu R., Kucukaslan I. (2010): Cytological Evaluation of canine mammary tumours with fine needle aspiration biopsy. *Revue Med Vet* **161(5)**: 212-218.
- Hazroglu R., Yardmc B., Aslan S., Yldrm M. Z., Yumusak N., Beceriklisoy H., Agaoglu R., Kucukaslan I., Coskan A. S. (2011): Cytological Evaluation Of Canine Mammary Tumours With Fine Needle Aspiration Biopsy Technique. *Cytopathology* **22**: 18.
- Heeney J. L., Valli V. E. (1985): Bovine ocular squamous cell carcinoma: an epidemiological perspective. *Canadian Journal of Comparative Medicine* **49(1)**: 21.
- Hemida H., Benchohra M., Kalbaza A. Y., Amara K. (2017): Vaginal Fibrosarcoma in an Algerian Crossbreed Cow. *Pakistan Veterinary Journal* **38(1)**: 119-120.
- Herron M. A. (1983): Tumors of the canine genital system. *Journal of the American Hospital Association* **19**: 981-994.

- Hesaraki S., Abedi G. H., Rismanchi S. (2010): Penile fibrosarcoma tumor in a bull. *Iranian Journal of Veterinary Research* **11(3)**: 283-286.
- Hewes C. A., Sullins K. E. (2009): Review of the treatment of equine cutaneous neoplasia. *Proc Am Ass Equine Practnrs* **55**: 386-393.
- Hohsteter M., Artukovic B., Severin K., Kurilj A. G., Beck A., sostaric-Zuckermann I. C., Grabarevic z. (2014): Canine testicular tumors: two types of seminomas can be differentiated by immunohistochemistry. *BMC veterinary research* **10(1)**: 169.
- Hosseini E., Pedram B., Bahrami A. M., Moghaddam M. H. J., Javanbakht J., Ghomi F. E., Moghaddam N. J., Koohestani M., Shafiee R. (2014): Retracted Article: Cutaneous mast cell tumor (Mastocytoma): Cyto-histopathological and haematological investigations. *Diagnostic pathology* **9(1)**: 9.
- Im K. S., Kim I. H., Kim N. H., Lim H. Y., Kim J. H. and Sur J. H. (2013): Breed-related differences in altered BRCA1 expression, phenotype and subtype in malignant canine mammary tumors. *The Veterinary Journal* **195**: 366-372.
- Information for Oncology Clients (2018): Mammary Tumors in Dogs Clinical Oncology Service Ryan Veterinary Hospital of the University of Pennsylvania. Available at [http://www.vet.upenn.edu/docs/default-source/ryan/oncology-handouts/mammary-tumors-in-dog\\_ek-ks.pdf?sfvrsn=4](http://www.vet.upenn.edu/docs/default-source/ryan/oncology-handouts/mammary-tumors-in-dog_ek-ks.pdf?sfvrsn=4)
- Javanbakht J., Tavassoli A., Sasani F., Sabbagh A., Hassan M. A., Samakkhah S. A., Shafiee R., Jani M., Alimohammadi S., Samani R. (2013): An overall assessment of circumanal gland adenoma in a terrier mix breed dog. *Asian Pacific journal of tropical biomedicine* **3(7)**: 580-583.
- Jelinek F., Tachezy R. (2005): Cutaneous papillomatosis in cattle. *Journal of comparative pathology* **132(1)**: 70-81.
- John D. (2014): Manual of Diagnostic Cytology of the Dog and Cat. Axiom Vet. John Wiley & Sons. Pp. 288.
- Johnson G. C., Miller M. A., Ramos-Vara J. A. (1995): Comparison of argyrophilic nucleolar organizer regions (AgNORs) and mitotic index in distinguishing benign from malignant canine smooth muscle tumors and in separating inflammatory hyperplasia from neoplastic lesions of the urinary bladder mucosa. *Journal of veterinary diagnostic investigation* **7(1)**: 127-136.

- Johnson M. C., Myers A. N. (2017): Cytology of Skin Neoplasms. *Veterinary Clinics: Small Animal Practice* **47(1)**: 85-110.
- Johnston S.D., Kustritz M.V, Olson P.N.S. (2001): Disorders of canine ovary. In: Johnston SD, Kustritz MV, Olson PNS (eds), *Canine and Feline Theriogenology*. Saunders, Philadelphia. Pp. 193–205.
- Joshi S. K., Jadon G. (2012): Introduction to neoplasm: Tumor classification a review article. *Int J Adv Res Pharm Biosci* **1(2)**: 227-263.
- Kabuusu R. M., Stroup D. F., Fernandez C. (2010): Risk factors and characteristics of canine transmissible venereal tumours in Grenada, West Indies. *Veterinary and comparative oncology* **8(1)**: 50-55.
- Kang S. C., Yang H. S., Jung J. Y., Jung E. H., Lee H. C., Hwang E. K., Kim J. H. (2011): Malignant Sertoli cell tumor in Shih Tzu dog. *Korean Journal of Veterinary Research* **51(2)**: 171-175.
- Kang T. B., Holmberg D. L. (1983): Vaginal leiomyoma in a dog. *The Canadian Veterinary Journal* **24(8)**: 258.
- Kate M. S., Jain P., Patne S. S. (2013): Basal cell carcinoma diagnosed on Fine-Needle Aspiration Cytology-A Pathological Case Report. *The Southeast Asian Journal of Case Report and Review* **2(4)**: 275-280.
- Kennedy, P. C., Cullen, J. M., Edwards, J. F., Goldschmidt, M. H., Larsen, S., Munson, L., and Nielsen, S. (1998): Histological classification of tumors of the genital system of domestic animals. Washington, D.C. Armed Forces Institute of Pathology. Pp. 17–19.
- Khodakaram-Tafti A., Kargar M. (2009): Gross and histopathologic characteristics of penile fibropapillomas in young bulls. *Comparative Clinical Pathology* **18(3)**: 261-263.
- Kievitsbosch T., Prestes N. C., Fabris V. E., Salgado L. z. F. S., Alvarenga M. A. n. (2013): Ovarian Fibrothecoma in Mare–Case Report. *Journal of equine veterinary science* **33(10)**: 813-819.
- Kim J. H., Im K. S., Kim N. H., Chon S. K., Doster A. R. and Sur J. H. (2011): Inflammatory mammary carcinoma with metastasis to the brain and distant organs

- in a spayed Shih Tzu dog. *Journal of Veterinary Diagnostic Investigation* **23**: 1079-1082.
- Kim K., Goldblatt P. J. (1982): Malignant fibrous histiocytoma. Cytologic, light microscopic and ultrastructural studies. *Acta cytologica* **26(4)**: 507-511.
- Kimura K. C., Garate A. P., Dagli M. L. Z. (2012): Retrospective study of neoplasms in domestic animals: A survey between 1993 and 2002 of the service of animal pathology, department of pathology, school of veterinary medicine and animal science, University of Sao Paulo, Southeast Brazil. *Brazilian Journal of Veterinary Pathology* **5(2)**: 60-69.
- Kiran M. M., Karaman M., Hatipoglu F., Koc Y. (2005): Malignant fibrous histiocytoma in a dog: a case report. *Veterinarni medicina-praha-* **50(12)**: 553.
- Kiupel M., Webster J. D., Bailey K. L., Best S., DeLay J., Detrisac C. J., Fitzgerald S. D., Gamble D., Ginn P. E., Goldschmidt M. H. (2011): Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. *Veterinary pathology* **48(1)**: 147-155.
- Knottenbelt D. C. (2003): Basic principles of diagnosis and management of neoplasia in horses. Proceedings of the Annual Meeting of the Italian Association of Equine Veterinarians, Pisa, Italy, XIV Congress [http://www ivis org/proceedings/SIVE/2003/lectures/knottenbelt1 pdf](http://www.ivis.org/proceedings/SIVE/2003/lectures/knottenbelt1.pdf).
- Knottenbelt D. C., Kelly D. F. (2000): The diagnosis and treatment of periorbital sarcoid in the horse: 445 cases from 1974 to 1999. *Veterinary ophthalmology* **3(23)**: 169-191.
- Knottenbelt D. C., Matthews J. B. (2001): A positive step forwards in the diagnosis of equine sarcoid. *Veterinary Journal* **161**: 224-226.
- Krithiga K., Manohar B. M., Balachandran C. (2005): Cytological and histopathological study on cutaneous round cell tumours in canines. *Indian Journal of Veterinary Pathology* **29(1)**: 38-41.
- Kumar P. R., Sailaja B., Prasad V. D., Chowdary P. S., Sreenu M. (2016): Basal Cell Carcinoma in Dogs- A Report of 2 Cases. *International Journal of Science, Environment and Technology* **5(4)**: 1980-1986.

- Kuru M. (2016): Vaginal fibrosarcoma in a brown swiss cow. *Ataturk universitesi Veteriner Bilimleri Dergisi* **11(3)**: 327-331.
- LaRock R. G., Ginn P. E. (1997): Immunohistochemical staining characteristics of canine gastrointestinal stromal tumors. *Veterinary pathology* **34(4)**: 303-311.
- Liao A. T., Chu P. Y., Yeh L. S., Lin C. T., Liu C. H. (2009): A 12-year retrospective study of canine testicular tumors. *Journal of Veterinary Medical Science* **71(7)**: 919-923.
- Liptak J. M., Forrest L. J. (2007): Soft tissue sarcomas. *Small animal clinical oncology* **4**: 425-454.
- Lopez M. J., Prichard M., Steinberg H. (1997): Anal leiomyoma in a Holstein heifer. *The Canadian Veterinary Journal* **38(6)**: 377.
- MacLachlan N. J., Kennedy P. C. (2008): Tumors of the genital systems. Tumors in Domestic Animals, Fourth Edition . Pp. 547-573.
- Madan V., Lear J. T., Szeimies R. M. (2010): Non-melanoma skin cancer. *The lancet* **375(9715)**: 673-685.
- Mahler S. P., Mootoo N. F. A., Reece J. L. M., Cooper J. E. (2006): Surgical resection of a primary tracheal fibrosarcoma in a dog. *Journal of small animal practice* **47(9)**: 537-540.
- Manesh J. Y. Y., Shafiee R., Bahrami A. M., Pourzaer M., Pourzaer M., Pedram B., Javanbakht J., Mokarizadeh A., Khadivar F. (2014): Retracted Article: Cyto-histopathological and outcome features of the prepuce squamous cell carcinoma of a mixed breed dog. *Diagnostic pathology* **9(1)**: 999.
- Mansour K. A. (2017): Clinical, Histopathological and Molecular detection of cutaneous papillomatosis associated with bovine papillomavirus types 1 and 2 in cattle from Al-Qadissiyia Province. *Kufa Journal For Veterinary Medical Sciences* **7**: 182-191.
- Marchal T., Chabanne L., Kaplanski C., Rigal D., Magnol J. (1997): Immunophenotype of the canine transmissible venereal tumour. *Veterinary immunology and immunopathology* **57**: 1-11.
- Marec-Berard P. (2004): Malignant hemangiopericytoma. *Orphanet Encyclopedia* . Pp. 1-2.

- Maresi E., Tortorici S., Campione M., Buzzanca M. L., Burruolo F., Mastrangelo F., Tete S. (2007): Hemangiopericytoma of the Oral Cavity after a Ten-year Follow-up. *Annals of Clinical & Laboratory Science* **37(3)**: 274-279.
- Marinkovic D., Milcic-Matic N., Jovanovic M., Vucevic I., Nestic S., Anicic M., Aleksic-Kovacevic S. (2015): Morphological Features and KIT Receptor Expression in Canine Cutaneous Mast Cell Tumor and Systemic Mastocytosis/Morfoloke Karakteristike I Ekspresija KIT Receptora Kod Kutanih Mastocitoma I Sistemske Mastocitose Pasa. *Acta Veterinaria* **65 (2)**: 226-237.
- Marryatt P. A. (2003): Malignant giant cell tumor of soft parts in a mare. *The Canadian Veterinary Journal* **44(9)**: 743.
- Martano M., Corteggio A., Restucci B., De Biase M. E., Borzacchiello G., Maiolino P. (2016): Extracellular matrix remodeling in equine sarcoid: an immunohistochemical and molecular study. *BMC veterinary research* **12(1)**: 24.
- Martens A., Moor A. D. E., Demeulemeester J., Ducatelle R. (2000): Histopathological characteristics of five clinical types of equine sarcoid. *Research in Veterinary Science* **69(3)**: 295-300.
- Marti E., Lazary S., Antczak D. F., Gerber H. (1993): Report of the first international workshop on equine sarcoid. *Equine veterinary journal* **25(5)**: 397-407.
- Masserdotti C., Bonfanti U., De Lorenzi D., Tranquillo M., Zanetti O. (2005): Cytologic features of testicular tumours in dog. *Transboundary and Emerging Diseases* **52(7)**: 339-346.
- Masserdotti C., De Lorenzi D., Gasparotto L. (2008): Cytologic detection of Call-Exner bodies in Sertoli cell tumors from 2 dogs. *Veterinary clinical pathology* **37(1)**: 112-114.
- McCarthy, A., Perry, J., Kenneth, S., 2001. Canine mammary carcinoma. Class of McCarthy, Department of Pathology, College of Veterinary Medicine, The University of Georgia, Athens. Pp. 1-8.
- McEntee M. C. (2002): Reproductive oncology. *Clinical techniques in small animal practice* **17(3)**: 133-149.

- Meinkoth J.H., Cowell R.L., Tyler R.D., Morton R.J. (2008): Sample collection and preparation. In: Diagnostic cytology and hematology of the dog and cat. 3<sup>rd</sup> ed. Mosby Elsevier. Pp. 1-18.
- Meirelles A. E. W. B., Oliveira E. C. d., Rodrigues B. A., Costa G. R. d., Sonne L., Tesser E. S., Driemeier D. (2010): Prevalencia de neoplasmas cutâneos em caes da Regiao Metropolitana de Porto Alegre, RS: 1017 casos (2002-2007). *Pesquisa veterinaria brasileira* **30(11)**: 968-973.
- Merlo D. F., Rossi L., Pellegrino C., Ceppi M., Cardellino U., Capurro C., Ratto A., Sambucco P. L., Sestito V. and Tanara G. (2008): Cancer incidence in pet dogs: findings of the Animal Tumor Registry of Genoa, Italy. *Journal of veterinary internal medicine* **22**: 976-984.
- Meuten D. J. (2002): Tumors of the urinary system. Tumors in Domestic Animals, Fourth Edition . Pp. 509-546.
- Meuten D. J., Rebar A. (1998): Cytopathology of skin and subcutaneous masses. Proceedings of the North American Veterinary Conference. Pp. 1-40.
- Meuten, D. J. (2017): Tumors in domestic animals, 5 ed. John Wiley & Sons. Pp. 202-340.
- Meyer D. J. and Raskin, R. (2001): Atlas of canine and feline cytology. wB Saunders. Pp. 236-245.
- Michi Y., Suzuki M., Kurohara K., Harada K. (2013): A case of hemangiopericytoma of the soft palate with articulate disorder and dysphagia. *International journal of oral science* **5(2)**: 111.
- Milltaru M., Ciobatoru E., Dinescu G. (1999): Differential anatomo-pathological diagnosis in benign and malignant tumors. *Rom Rev Comp One* **1**: 70-77.
- Misdorp W. (2004): Mast cells and canine mast cell tumours. A review. *Veterinary quarterly* **26**: 156-169.
- Morrison W. B. (2012): Inflammation and cancer: a comparative view. *Journal of veterinary internal medicine* **26(1)**: 18-31.
- Mukaratirwa S., Chipunza J., Chitanga S., Chimonyo M., Bhebhe E. (2005): Canine cutaneous neoplasms: prevalence and influence of age, sex and site on the

- presence and potential malignancy of cutaneous neoplasms in dogs from Zimbabwe. *Journal of the South African Veterinary Association* **76(2)**: 59-62.
- Munson L., Moresco A. (2007). Comparative pathology of mammary gland cancers in domestic and wild animals. *Breast disease* **28**:7-21.
- Nalubamba K. S. (2015): Unusual Presentation of Extra-Genital Canine Transmissible Venereal Tumor in an Adult Cross-Breed Dog-Palatine and Rectal Lesions without Primary Genital Lesions. *J Vet Sci Med Diagn* **4 (1)**: 2.
- Naraghi Z., Ghaninejad H., Akhyani M., Akbari D. (2005): Cytological diagnosis of cutaneous basal cell carcinoma. *Acta Medica Iranica* **43(1)**: 50-54.
- Newman S. J., Mrkonjich L., Walker K. K., Rohrbach B. W. (2007): Canine subcutaneous mast cell tumour: diagnosis and prognosis. *Journal of comparative pathology* **136**: 231-239.
- Nodtvedt A., Gamlem H., Gunnes G., Grotmol T., Indrebo A., Moe L. (2011): Breed differences in the proportional morbidity of testicular tumours and distribution of histopathologic types in a population-based canine cancer registry. *Veterinary and comparative oncology* **9(1)**: 45-54.
- Olausson A., Stieger S. M., Loefgren S., Gillingstam M. (2005): A urinary bladder fibrosarcoma in a young dog. *Veterinary Radiology & Ultrasound* **46(2)**: 135-138.
- Ozmen O., Sengoz O. S., çinar H., Dolu H. (2016): Retrobulbar fibrosarcoma in a sheep. *Acta Veterinaria* **66(2)**: 265-270.
- Ozsoy Y. S., Ozyildizz Z., Guzel M. (2011): Clinical, pathological and immunohistochemical findings of bovine cutaneous papillomatosis. *Ankara Üniv Vet Fak Derg* **58**: 161-165.
- Ozyigit M. O., Nak D., Yilmaz R., Nak Y., Simsek G. (2011): Malignant fibrous histiocytoma (giant cell type) of soft parts in the abdominal mammary region of a female Ankara (Angora) cat. *Turkish Journal of Veterinary and Animal Sciences* **34(5)**: 471-475.
- Pandit G. A., Kudrimoti J. K., Kokandakar H. R., Bhople K. S. (2004): Fibrosarcoma of penis--a case report. *Indian journal of pathology & microbiology* **47(3)**: 389-390.
- Parry N. M. A. (2006): Brief Communications and Case Reports. *Vet Pathol* **43**: 1008-1009.

- Perez-Martinez C., RA G. a. F., LE R. A., Perez-Perez V., Gonzalez N., Garcia-Iglesias M. J. (2000): Malignant fibrous histiocytoma (giant cell type) associated with a malignant mixed tumor in the salivary gland of a dog. *Veterinary pathology* **37(4)**: 350-353.
- Peters M. A., De Jong F. H., Teerds K. J., De Rooij D. G., Dieleman S. J., Van Sluijs F. J. (2000): Ageing, testicular tumours and the pituitary-testis axis in dogs. *Journal of endocrinology* **166(1)**: 153-161.
- Petterino C., Martini M., astagnaro M. (2004): Immunohistochemical detection of growth hormone (GH) in canine hepatoid gland tumors. *Journal of Veterinary Medical Science* **66(5)**: 569-572.
- Pinho S. S., Carvalho S., Cabral J., Reis C. A., Gartner F. (2012): Canine tumors: a spontaneous animal model of human carcinogenesis. *Translational Research* **159(3)**: 165-172.
- Polton G. A., Mowat V., Lee H. C., McKee K. A., Scase T. J. (2006): Breed, gender and neutering status of British dogs with anal sac gland carcinoma. *Veterinary and comparative oncology* **4(3)**: 125-131.
- Pugliese M., Mazzullo G., Niutta P. P., Passantino A. (2014): Bovine ocular squamous cellular carcinoma: a report of cases from the Caltagirone area, Italy. *Veterinarski Arhiv* **84(5)**: 449-457.
- Purohit G. N. (2009): Canine transmissible venereal tumor: a review. *Internet Journal of Veterinary Medicine* **6(1)**: 1-7.
- Ramadan R. O., Fadle W. S., Hassan A. M., Bindary M., Gomaa A. (1993): Vaginal leiomyoma in a cow. *Reproduction in Domestic Animals* **28(1)**: 39-43.
- Ramakrishna R., Rostomily R., Sekhar L., Rockhill J., Ferreira M. (2014): Hemangiopericytoma: radical resection remains the cornerstone of therapy. *Journal of Clinical Neuroscience* **21(4)**: 612-615.
- Raskin R.E. (2016): Skin and subcutaneous tissues. In: Raskin RE, Meyer DJ, editors. Canine and feline cytology: a color atlas and interpretation guide. 3rd edition. St Louis (MO): Elsevier. Pp. 34–90.

- Rayner E. L., Scudamore C. L., Francis I., Schoniger S. (2010): Abdominal fibrosarcoma associated with a retained surgical swab in a dog. *Journal of comparative pathology* **143(1)**: 81-85.
- Reif J. S. (2011): Animal sentinels for environmental and public health. *Public Health Reports* **126**: 50-57.
- Risbon R. E., Clifford C. A., Skorupski K. (2007): An overview of canine histiocytic disorders. *Veterinary medicine* **102(7)**: 464-480.
- Rubin, R., Strayer, D. S., and Rubin, E. (2008): Rubin's pathology: clinicopathologic foundations of medicine. Lippincott Williams & Wilkins.
- Russell E. B., Carrington P. R., Smoller B. R. (1999): Basal cell carcinoma: a comparison of shave biopsy versus punch biopsy techniques in subtype diagnosis. *Journal of the American Academy of Dermatology* **41(1)**: 69-71.
- Rutkowski M. J., Jian B. J., Bloch O., Chen C., Sughrue M. E., Tihan T., Barani I. J., Berger M. S., McDermott M. W., Parsa A. T. (2012): Intracranial hemangiopericytoma. *Cancer* **118(6)**: 1628-1636.
- Rutteman, G.R., Withrow, S.J., MacEwen, E.G. (2000). Tumors of the Mammary Gland. In: Winthrow SJ, MacEwen EG (eds): Small Animal Clinical Oncology, 3<sup>rd</sup> ed. Philadelphia, WB Saunders Co. Pp. 450-467.
- Saeki K., Endo Y., Uchida K., Nishimura R., Sasaki N. and Nakagawa T. (2012): Significance of tumor-infiltrating immune cells in spontaneous canine mammary gland tumor: 140 cases. *Journal of Veterinary Medical Science* **74**: 227-230.
- Sakai H., Murakami M., Mishima H., Hoshino Y., Mori T., Maruo K., Yanai T. (2012): Cytologically atypical anal sac adenocarcinoma in a dog. *Veterinary clinical pathology* **41(2)**: 291-294.
- Sala A., Schuszler L., Sabau M., Luca C., Igna C., Dascalu R., Brezovan D. (2010): Horse Sarcoids: A morphological and Therapeutical experience on ten cases. *Lucrari stiintifice Medicina Veterinara* **43(2)**: 109-118.
- Sato T., Aoki K., Shibuya H., Machida T., Watari T. (2003): Leiomyosarcoma of the kidney in a dog. *Transboundary and Emerging Diseases* **50(7)**: 366-369.

- Sawh R. N., Lele S. M., Borkowski J., Ventura K. C., Zaharopoulos P., Logrono R. (2000): Fine–needle aspiration cytology of hemangiopericytoma: Report of two cases. *Diagnostic cytopathology* **23(3)**: 187-191.
- Schulman F. Y., Krafft A. E., Janczewski T. (2001): Feline cutaneous fibropapillomas: clinicopathologic findings and association with papillomavirus infection. *Veterinary pathology* **38(3)**: 291-296.
- Schulz K. L., Anderson D. E. (2010): Bovine enucleation: A retrospective study of 53 cases (1998–2006). *The Canadian Veterinary Journal* **51(2)**: 611.
- Scott D. W., Miller Jr W. H. (2003): Equine dermatology. *Journal of equine veterinary science* **23(2)**: 65-67.
- Seite S., Fourtanier A., Moyal D., Young A. R. (2010): Photodamage to human skin by suberythemal exposure to solar ultraviolet radiation can be attenuated by sunscreens: a review. *British Journal of Dermatology* **163(5)**: 903-914.
- Sendag S., Cetin Y., Alan M., Ilhan F., Eski F., Wehrend A. (2008): Cervical leiomyoma in a dairy cow during pregnancy. *Animal reproduction science* **103(3-4)**: 355-359.
- Sexton M., Jones D. B., Maloney M. E. (1990): Histologic pattern analysis of basal cell carcinoma: study of a series of 1039 consecutive neoplasms. *Journal of the American Academy of Dermatology* **23(6)**: 1118-1126.
- Shah D. N. (2015): A Histopathological and Immunohistochemical Study of Cutaneous Nodular Lesions and Other Surface Swellings of Kenyan Horses. [Master's Thesis], Nairobi. Pp. 1-132.
- Sharma S., Bansal R., Upreti S., Khare A., Sharma S., Agarwal D. (2013): Ovarian Fibrothecoma with Extensive Cystic Degeneration: Two Case Reports. *Indian journal of clinical practice* **23(12):840-841**.
- Shokry M., Naoum E., El-Husseiny E., Battour A. (2015): Clinical survey of equine sarcoids in Egypt. *Issues Biol Sci Pharm Res* **3(8)**: 86-93.
- Simon D., Schoenrock D., Nolte I., Baumgartner W., Barron R., Mischke R. (2009): Cytologic examination of fine–needle aspirates from mammary gland tumors in the dog: diagnostic accuracy with comparison to histopathology and association with postoperative outcome. *Veterinary clinical pathology* **38(4)**: 521-528.

- Skoric M., Fictum P., Jekl V., Hauptman K., Knotek Z., Hermanova M. (2010): Vaginal leiomyosarcoma in a degu (*Octodon degus*): a case report. *Veterinarni Medicina* **55(8)**: 409-412.
- Slovis N. M., McEntee M. C., Fairley R. A., Galuppo L. D., Theon A. P. (2001): Equine basal cell tumors: 6 cases (1985-1999). *Journal of veterinary internal medicine* **15(1)**: 43-46.
- Sontas B.H., Yuzbasioglu Ozturk G., Toydemir T.F.S., Arun S.S., Ekici, H. (2012): Fine-Needle Aspiration Biopsy of Canine Mammary Gland Tumours: A Comparison Between Cytology and Histopathology. *Reproduction in Domestic Animals* **47**: 125-130.
- Speltz M. C., Manivel J. C., Tobias A. H., Hayden D. W. (2007): Primary cardiac fibrosarcoma with pulmonary metastasis in a Labrador retriever. *Veterinary pathology* **44(3)**: 403-407.
- Stanoszek L. M., Wang G. Y., Harms P. W. (2017): Histologic Mimics of Basal Cell Carcinoma. *Archives of pathology & laboratory medicine* **141(11)**: 1490-1502.
- Stockmann D., Ferrari H. F., Andrade A. L., Lopes R. A., Cardoso T. C., Luvizotto M. C. (2011): Canine transmissible venereal tumors: aspects related to programmed cell death. *Brazilian Journal of Veterinary Pathology* **4(1)**: 67-75.
- Strakova A., Murchison E. P. (2014): The changing global distribution and prevalence of canine transmissible venereal tumour. *BMC veterinary research* **10(1)**: 168.
- Svara T., Gombac M., Pogorevc E., Plavec T., Zrimsek P., Pogacnik M. (2014): A retrospective study of canine testicular tumours in Slovenia. *Slov Vet Res* **51**: 81-88.
- Talkuder S. (2007): Histopathological techniques: Tissue processing and staining. Available at [www.talukderbd.com](http://www.talukderbd.com). Pp. 1-11.
- Taylor S., Halderson G. (2012): A review of equine sarcoid. *Equine Veterinary Education* **25(4)**: 210-216.
- Terziev G., Roydev R., Kalkanov I., Borissov I., Dinev I. (2015): Papillomatosis in heifers-comparative studies on surgical excision and autogenous vaccine therapies. *Trakia Journal of Sciences* **13(2)**: 274-279.

- Thangathurai R., Balasubramaniam G. A., Manohar B. M. (2008): Cytological diagnosis and its histological correlation in canine transmissible venereal tumour. *Veterinarski Arhiv* **78(5)**: 369-376.
- Timaran V., Antonio D., Chaves Velasquez C. A., Astaiza Martinez J. M., Quintero Parra J. M., Pastas C., Mauricio J. (2015): Bovine dermal fibrosarcoma: a case report. *CES Medicina Veterinaria y Zootecnia* **10(2)**: 214-223.
- Togni A., Ratten M., Bley C. R., Hurter K. (2015): Metastasized Leydig cell tumor in a dog. *Schweiz Arch Tierheilkd* **157(2)**: 111-115.
- Top J. v. d., Heer N. d., Klein W. R., Ensink J. M. (2008): Penile and preputial tumours in the horse: a retrospective study of 114 affected horses. *Equine veterinary journal* **40(6)**: 528-532.
- Tozato C. C., Lunardi M., Alfieri A. F., Otonel R. A., Di Santis G. W., Alcantara B. g. K., Headley S. A., Alfieri A. A. (2013): Teat papillomatosis associated with bovine papillomavirus types 6, 7, 9, and 10 in dairy cattle from Brazil. *Brazilian Journal of Microbiology* **44(3)**: 905-909.
- Tsujita H., Plummer C. E. (2010): Bovine ocular squamous cell carcinoma. *Veterinary Clinics: Food Animal Practice* **26(3)**: 511-529.
- Valente P. C. L. G., Couto R. M., Gamba C. O., Vasconcelos A. V., Leme F. O. P., Ecco R., Paes P. R. O. (2017): Bone marrow bi-hypoplasia in a dog with a sertoli cell tumor. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* **69(1)**: 95-100.
- Valentine B. A. (2006): Survey of equine cutaneous neoplasia in the Pacific Northwest. *Journal of Veterinary Diagnostic Investigation* **18(1)**: 123-126.
- Vantuchova Y., Curik R. (2006): Histological types of basal cell carcinoma. *Scripta Medica (Brno)* **79(5-6)**: 261-270.
- Vetorazzo Filho J. E., Bahia L. A. C., Esteves P. E., Maron P. E. G., Vedovato B. C. s., Fernandes R. d. C., Perez M. D. C. (2015): Renal hemangiopericytoma: case report and literature review. *Einstein (Sao Paulo)* **13(2)**: 269-272.
- Walaas L., Angervall L., Hagmar B. r., Save-Soderbergh J. (1986): A correlative cytologic and histologic study of malignant fibrous histiocytoma: An analysis of 40 cases examined by fine-needle aspiration cytology. *Diagnostic cytopathology* **2(1)**: 46-54.

- Webster J. D., Yuzbasiyan-Gurkan V., Miller R. A., Kaneene J. B., Kiupel M. (2007): Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. *Veterinary pathology* **44(3)**: 298-308.
- Welle M. M., Bley C. R., Howard J., Rufenacht S. (2008): Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. *Veterinary dermatology* **19(6)**: 321-339.
- Withrow, S. J., Page, R., and Vail, D. M. (2013): Withrow and MacEwen's Small Animal Clinical Oncology-E-Book. Elsevier Health Sciences.
- Yang G. C., Mesia A. F. (1999): Fine-needle aspiration cytology of malignant fibrothecoma of the ovary. *Diagnostic cytopathology* **21(4)**: 284-286.
- Young R. H. (2011): Sex cord-stromal, steroid cell, and other ovarian tumors with endocrine, paraendocrine, and paraneoplastic manifestations. In: Blaustein's Pathology of the female Genital Tract. Springer . Pp. 785-846.
- Yumusak N., Caliskan M., Kutsal O. (2016): Fine needle aspiration cytology (FNAC) in the diagnosis of canine hepatoid gland tumors-A comparative study with histopathology. *Ankara universitesi Veteriner Fakultesi Dergisi* **63(3)**: 259-266.

## **8. ANNEXES**

### **Annex 1: Fine needle aspirations technique**

Once the mass is stabilized between the operator's fingers, a 21-25 gauge needle attached to a 3-5 ml syringe is inserted into the mass. When the needle is seated comfortably in the mass, negative pressure is applied to the plunger/syringe. Try and avoid redirecting the needle or moving it back and forth within the mass whilst vacuum (negative pressure) is applied as this generally results in increased blood contamination of samples. This procedure should be repeated at least 3-4 times at different angles within the lesion to obtain a representative cell population from the lesion in question. Smaller syringes attached to the needle offer the operator better control during the aspiration process, particularly when aspirating smaller lesions. A minimal amount of material within the hub of the needle is adequate and generally this is sufficient for cytological interpretation. Attempted further aspiration often leads to unwanted blood contamination. If blood is encountered during aspiration attempts, then the exercise should be ceased and repeated a little further away from the initial puncture site. Negative pressure should be released before the needle is removed from the mass and skin. The needle is removed from the mass and skin and the needle contents are expressed onto a slide and a smear prepared.

### **Annex 2: Impression smears**

The cut surface of the excised sample is blotted a number of times to remove surface blood and serum, and the dried surface is applied to a clean, dry slide with gentle pressure. A number of areas can be prepared on a single slide. The preparations should be quickly air dried and then stained.

### **Annex 3: Squash preparation**

A portion of the aspirate is expelled onto a glass microscope slide and another slide is placed over the sample to spread it. If the sample does not spread well, gentle digital pressure can be applied to the top slide; however, care must be taken not to place excessive pressure on the slide causing the cells to rupture. The slides are smoothly slid apart. The preparations should be quickly air dried and then stained.

### **Annex 4: Romanowsky-type stains**

These are permanent stains which stain organisms and the cytoplasm of cells excellently. The stains tend to have a "smudging" effect on the nucleus, thus, nuclear and nucleolar detail cannot be perceived as well as with the Papanicolaou-type stains. However, the detail is usually sufficient for differentiating neoplasia and inflammation and for evaluating neoplastic cells for cytologic evidence of malignant potential. Smears to be stained with Romanowsky-type stains must first be air dried to fix the cells and prevent them from falling off the slide during the staining procedure. These stains tend to dissolve lipids from cells leaving vacuoles. RNA stains blue and DNA stains purple with variations to red or pink.

#### Methods of staining (staining procedure)

##### Giemsa stain staining procedure

- ✓ Place dried smear in a coplin jar containing absolute methylalcohol for about 3 minute to fix the smear
- ✓ Drain off the alcohol and allow the slide to dry
- ✓ Transfer the slide in diluted giemsa stain for 35-45 minute
- ✓ Wash the stain with running/tap water, dry and examine under oil immersion microscope

## **Annex 5: Microscopic evaluation**

Once the smear has been prepared, stained and dried, it is scanned at low magnification (**4-10X objective**) to determine if all areas of the smear are stained properly and if there is adequate cellularity for evaluation. When proper staining is assured and all areas of increased and/or unique cellularity are recognized, magnification is increased to the 10X or 20 X objectives. An impression of the cellularity and cellular composition of the smear and of cell size are ascertained. Using the **40X** objective, nucleoli and chromatin pattern are discerned. Cell morphology is evaluated in detail with the 100X (**oil-immersion**) objective.

## **Annex 6: Histopathological technique**

1. **Trimming tissue:** Tissues samples were trimmed and put in to plastic tissue cassettes and then processed using an automatic tissue processor.

2. **Fixation of tissue** by 10% formaldehyde (Formalin-I for 2 hours & Formalin-II for hours).

3. **Tissue processing:** dehydration, clearing and impregnating.

❖ Dehydrating tissue by using increasing strength of alcohol; e.g. 70%, 95% and 100%.

Dehydration	70% alcohol	1 hour
	95% alcohol	1 hour
	100% alcohol I	1 hour
	100% alcohol II	2 hour
	100% alcohol III	2 hours

❖ Clearing of tissue by Xylene

Xylene I	1.30 hours
Xylene II	1.30 hours
Xylene III.....	1.30 hours

❖ Impregnation tissue with Paraffin wax

Paraffin wax I	2 hours
Paraffin wax II	3 hours

**4. Embedding or Blocking:** Tissue specimens embedded with paraffin wax named as tissue blocks were removed from the machine

**5. Section:** sectioning of tissue in to 4- 5 micron thickness using a semi-automatic microtome machine, tissue ribbons were spread on warm water bath and tissues were attached to albumenized glass slides. Then the slides were incubated in incubator at 60 ° C to avoid paraffin wax. The sectioned tissues were then deparaffinised in three changes of xylene (Xylene-I, Xylene-II & Xylene-III), and then rehydrated in descending grades of alcohol (100% I, 100% II, 100% III, 95 %, &70 %).

**6. Staining:** Automatic or manual staining with Haematoxylin and Eosin to give colour for sectioned tissue.

***Staining procedure:***

- ❖ Put the sections fixed on slides in xylene (xylene for 5 minutes and xylene for 5 minutes).
- ❖ Then transfer to absolute alcohol (100% for 3 minutes, 100% for 3 minutes and 100% for 3 minutes).
- ❖ Transfer to 95% alcohol for 3 minutes.
- ❖ Place in 70% alcohol for 3 minutes.
- ❖ Rinse the slide in running tap water for 1 minute and put in Harris's *Haematoxylin* for 10-15 minutes.
- ❖ Rinse in running tap water
- ❖ Counter stain with *eosin* (3 dips).
- ❖ Rinse in running tap water.
- ❖ Dehydrated in ascending grades of alcohol (70 % 3 dips, 95 % 3 dips, 100% I for 3 minutes, 100% for 3 minutes & 100% III for 3 minutes)
- ❖ Cleared it in xylene (Xylene-I for 5 minutes , Xylene-II for 5 minutes & Xylene-III for 5 minutes and mounted with DPX or Canada balsam.

***Microscopic examination:*** stained slide is examined under microscope at 4x, 10x, 40x and 100 x magnification for the presence of microscopic lesions and finally photographs of the slides were taken.

