A review of mammary gland neoplasia in the bitch and queen
Donald H. Schlafer
Department of Biomedical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY

Abstract
Mammary gland tumors are amongst the most common tumors of bitches and queens. About half of all tumors of the bitch arise from mammary glands and from one third to one half of these are malignant, making mammary tumors the most common malignant neoplasm of the bitch. In the queen, tumors arising from mammary tissues are the third most common occurring neoplasms after skin and lympho-hematopoetic tumors. Nearly all mammary gland tumors in cats are malignant.

Masses involving the ventral abdomen are occasionally not of mammary gland origin. The differential diagnosis includes skin tumors, foreign body reactions, hernias, neoplasms of other tissues, etc. Mastitis should also be considered. Another primary proliferative, but non-neoplastic mammary gland disease that can present as sudden growth of one or more mammary glands in young cats is a benign condition referred to as fibroadenomatous hyperplasia.

The majority of canine and feline mammary gland tumors arise from epithelial cells of either the glands or ducts. These become either benign (adenomas) or malignant (adenocarcinomas or carcinomas) neoplasms. “Mixed” mammary gland tumors are unusual in that they contain both neoplastic epithelial and mesenchymal cells. These occur in the bitch, but rarely in the queen.

Histopathology is the gold standard for tumor identification and characterization. Unfortunately, the nomenclature used by diagnostic pathologists can be complicated. For example, most classification systems include even very rare mammary tumors, which can make them seem overwhelming.

Several different classification schemes have been published that categorize mammary tumors of cats and dogs based on microscopic features. These have proven to be very useful for clinicians. Histopathology findings provide important prognostic information and regardless of the classification system used, they divide tumors into those that are likely to behave in a benign manner from those that have features that identify them as malignant “cancers”. Additionally histopathology allows for accurate staging of the neoplasm in a given patient.

Features that significantly affect survival of affected bitches and queens include: anaplastic phenotype of the neoplastic cells, tumor size and evidence of metastasis.

Keywords: Mammary, pathology, canine, feline, neoplasia, histopathology

Introduction
The incidence rate for canine mammary cancers is much higher than that for feline mammary cancers (198 and 12.8 per 100,000 in bitches and queens, respectively). Of those surgically removed from the bitch, over 30 percent are malignant. The likelihood that a tumor arising from mammary tissue in a queen will be malignant is much higher and the ratio of malignant to benign feline mammary tumors ranges from four to nine malignant cases for each benign case. Mammary tumors usually occur in females, rarely occur in males, and in the bitch, early ovariecytectomy confers significant protection against future development of mammary tumors.

Mammary gland anatomy
Mammary tissues respond to hormones. Dramatic changes occur in the gross and microscopic appearance of these tissues during different stages of the estrous cycle (figures 1 and 2). Much of the mass of mammary tissues during the pre-pubertal, anestrual, and geriatric periods is composed of adipose tissue with glandular and ductal tissues being very scant (figure 1).

There are four pairs of mammary glands in the queen, designated T1 and T2 for the thoracic pair and A1 and A2 for the abdominal pair. "Rudimentary" glands are present in the inguinal area in some queens. Lymphatic drainage does not cross the midline and somewhat surprisingly runs in both directions. Lymph, and hence neoplastic emboli that travel via lymphatics, tend to drain cranially from
the thoracic glands to the axillary lymph node centers and caudally from the caudal abdominal glands to the inguinal lymph nodes.\textsuperscript{2} It is important to remove draining lymph nodes for histopathology at the time of surgical excision of any suspected mammary neoplasm.

**Hyperplastic conditions of mammary glands**

Mammary tissues are target tissues for steroid hormones, and as such, undergo hyperplastic and hypertrophic changes during the estrous cycle. Compare the subgross (1X) photographs in figures 1 and 2.

The degree to which changes in glands during various stages of the estrous cycle can be detected grossly varies between individual animals. The corresponding dramatic histological changes of these target tissues are readily apparent when one compares the images of glands from a Beagle in anestrus with that of hyperplastic tissues from a Beagle in the diestrus. Exuberant mammary gland development can also occur as a paraneoplastic condition if functional tumors of the pituitary or gonads produce stimulatory hormones. In the cat, both males and females can develop a condition called feline mammary fibroepithelial hyperplasia.

**Feline mammary fibroepithelial hyperplasia**

This benign growth of mammary glands in the cat characteristically occurs in young female cats, with one or individual mammary glands being involved. Enlargement of more than one gland is common. All glands are frequently involved and enlargement of gland pair across the midline also occurs. This is not a pre-neoplastic condition as secondary bacterial infections can lead to mastitis.

Histologically, the enlarged glands are composed of very uniform proliferation of ducts and alveoli and stromal tissues that support them. The uniformity of the proliferation readily differentiates this exuberant proliferation from neoplastic differentiation found in adenomas, adenocarcinomas, sarcomas, or mixed mammary gland tumors. The condition is seen in males and females that have been treated with progesterone or related therapeutic compounds, in young females during their first cycles, during pregnancy and in pseudopregnant females. It can occur in older animals, but uncommonly so.

If the enlarged glands are traumatized, or if the expansion is so large such that it causes impairment to blood flow, the hyperplastic glands and/or overlying skin may undergo necrosis. Such secondary changes can be misdiagnosed as features of aggressive neoplasia.

**Mammary gland neoplasia**

Because breast cancer is the second most leading cause of death of women in the US, major efforts have been extended to identify appropriate animal models of human mammary neoplasia. There are many similarities between human and animal mammary neoplastic conditions. Considerable attention is being given to mammary tumors of the cat as one of the most similar. Underlying genetics,\textsuperscript{3,4} early stage of cellular events associated with malignant transformation,\textsuperscript{5} identification of prognostic features\textsuperscript{6-8} and therapeutic responses to neoplastic\textsuperscript{2} or hyperplastic conditions of mammary tissues\textsuperscript{9} have been studied in cats with spontaneously developing mammary diseases. Mammary neoplasms of the dog have less common features to those of women.

In general terms, the most prominent feature of a neoplastic cell is that it has "unregulated cell growth". Some cells that have undergone neoplastic transformation will behave differently. Depending on the nature and degree to which genetic controls are altered, the biological behavior of the transformed cells can lead neoplastic cells to behave in either benign or an aggressive and uncontrolled way.

If cells are less well-differentiated, their phenotype (appearance) will change and features such as rapid replication and altered cellular activity occur. Histopathologists rely on such changes in cell morphology and cellular behavior to characterize, differentiate, name and classify different tumors. Many studies have shown that microscopic features allow pathologists to classify mammary gland tumors into different types, and that tumors in these different categories will behave in a somewhat predictable way enabling them to provide reliable prognoses. Some tumor types also have predictable responses to different types of therapy. Identification of cellular features that do have predictive value has expanded...
greatly in recent years and goes beyond interpretation of morphologic features based solely on classic hematoxylin and eosin stained tissue sections. Use of special staining, cell sorting, and other molecular techniques are employed to finely characterize individual tumors, which is becoming increasingly common as therapeutic modalities expand and specific treatment regimes require more detailed characterization of any given tumor's phenotype. Several references are included in the bibliography as examples.3-5

Histogenesis of mammary gland neoplasms

Mammary gland tumors arise most commonly from epithelial cells. They are either secretory epithelial cells that line the alveolar glands or from those that line the ductal system. Tumors arising purely from stromal cells, including contractile "strap" cells that cuff the alveolae, are very rare. In the bitch, a combination of epithelial and these myoepithelial cells occur quite commonly and are called mixed or complex mammary gland tumors. They are less likely to be malignant than solid or ductal carcinomas.

Neoplastic transformation of cells of epithelial origin can lead to growth of tumors that behave in a benign growth pattern and are called adenomas. Those that have a more aggressive growth pattern which results in metastases, rapid tumor growth with subsequent implantation and growth at other sites frequently resulting in associated tissue destruction and/or functional compromise are adenocarcinomas.

Pathology of mammary gland neoplasms

Neoplastic conditions are part of a spectrum of changes cells can undergo. In the extreme, cells that have mutations of critical genes die. Those that cause alterations in genes associated with control of cell cycle and growth, those that affect DNA repair mechanisms, those that cause altered tumor suppression, or change the number of receptors on the surface of cell can lead to the transformation of normal cells to neoplastic ones. From a diagnostic perspective, changes in cell proliferation, growth patterns or evidence of altered function frequently affect the morphologic appearance of cells and the gross appearance of tissues they produce.

It is these types of changes that allow histopathologists to identify neoplasms and to further classify them. Cells that have not undergone permanent changes and thus are not truly neoplastic can resemble tumor cells. Cells that have reactive, metaplastic changes or dysplastic can be difficult to differentiate from true neoplastic conditions. In some, but not all tissues, these changes are considered to be "pre-neoplastic", and under certain conditions, progress to development of neoplasms.

One example of the effects of chronic stimulation of reproductive tissues, recognized some time ago, is the contraceptive use of progestagens. The adverse sequella also extends beyond our domestic species. The use of the synthetic progestin melengesterol acetate (MGA) causes similar diseases associated with hyperplastic and neoplastic conditions in zoo canids10 and felids.11 In zoo felids, there is a link between MGA administration and clinically aggressive mammary gland carcinomas.10,11

Classification of types of mammary tumors of dogs and cats

Based on common usage, the term "cancer" has become synonymous with "malignant". The phrase "benign cancer" is in common usage, yet less correct. In descriptive pathology, when morphologic features for a given tumor are being assessed, diagnostic pathologists frequently add modifiers that reflect the prominent microscopic pattern or other features. For example, these modifiers may include: solid, papillary, tubulo-papillary, cystic, etc. The tissue patterns may reflect the tissue of origin within a gland. This too is commonly included in the morphological assessment. Tumors arising from the ductal system are described as “ductal” or those with a glandular pattern are considered “adeno...”. An example of a tumor that has arisen from the epithelial lining of a duct is presented in figure 4. Note how it extends from its attachment to the inner wall of the duct and has grown to nearly fill the duct lumen.

Two photographs taken at the same magnification as those used for figures 1-4 reveal some of the morphologic changes associated with aggressive mammary gland carcinomas that were removed from two different queens (figures 5 and 6). Areas of central necrosis and invasion into the superficial dermis
causing ulceration are illustrated in the case shown in figure 5. Cancers (malignant tumors) that grow rapidly commonly contain areas of necrosis caused by ischemia as the tissue literally outgrows its own blood supply. Contrast these features to the sections from the cat with mammary fibroepithelial hyperplasia shown in figure 3. As shown at higher magnification in figure 5, the cellular pattern is very uniform and highly organized. Now compare this to the photomicrograph prepared at the same magnification of neoplastic cells epithelial cells from the carcinoma shown in figure 6. Several lymphatics have been invaded by the neoplasm (figure 8). For those interested, a more extended discussion of mammary gland histopathology is presented in the work of Dr. Misdorf.

It should be noted that primary tumors arising from other cell types, normally found in mammary tissue or the overlying skin, also occur. These may include: hemangiomas, fibromas, mast cell tumors, sebaceous adenomas, etc. Tumors from other sites can metastasize to mammary tissue, but this is uncommon. Specific diagnosis of mammary masses requires histopathology.

Prognostic features
Neoplasms of feline mammary glands are likely to be malignant, metastasize rapidly, and are often fatal. Simple features, such as size of the mass have predictive value. Following removal, the survival time in affected cats is inversely proportional to tumor size with median survival for cats with tumors greater than 3 cm in diameter being 12 months vs. 24 months for cats with tumors less than 3 cm.

The gold standard remains histopathology. Although different classification schemes have been proposed, regardless of which one is used, tumors types will behave differently.

There are two studies that have assessed histological "grade" and subsequent survival in feline mammary carcinomas (FMC). The studies included tumors in 55 and 84 cats, respectively. In the first study done in 1998, three histologic features were assessed and scored based on degree of tubular pattern, nuclear and cellular pleomorphism, and mitotic index. It was determined that there was good predictive value based on degree of tumor anaplasia as it correlated with poor prognosis for survival.

The second study, published in 2011 employed the same histologic criteria used to grade human mammary gland neoplasms. The study also concluded that histologic grading can be used as a prognostic factor for FMC. The criteria were similar to those in the first study: degree of glandular differentiation assessed by prominence of tubular formation, nuclear pleomorphism, and mitotic index. Grade was significantly related to tumor size, clinical stage, lymphatic invasion, mitotic index and survival.

A large study of tumor classification and life span outcome in Beagle bitches was published in 1999. The study included full lifetime evaluations of 1,343 Beagles of which 671 were males and 672 females. All mammary nodules detected were removed as were any mammary nodules detected at necropsy. Tumors were classified based on histologic features using a system published by the World Health Organization. Based on preliminary studies, a modification of this classification was developed and used. This system is referred to as the CRHL (Colorado Radiological Health Laboratory) classification.

The complexity associated with assigning tumors to different categories can be a formidable task as addressed by the authors in this classic paper. They compared the WHO and a widely used system of classification referred to as the Moulton system (named after the author of veterinary pathology textbook on tumors in domestic animals published in 1990). In their study, of the 672 Beagle bitches studied throughout their lives, an astounding 71% developed at least one mammary tumor and 61% had more than one during their lives. Only two male dogs developed mammary tumors.

Based on morphologic features using the CRHL system, of the subset of tumors that arose from epithelial cells and that had caused the death of the bitch (n=73), 66% were classified as "ductular carcinomas". These arose from small interlobular or intralobular ductules (as shown in figure 4) compared to 27% that were classified as being "other" types of adenocarcinomas. More metastases were also detected in bitches with carcinomas identified as ductal carcinomas. At the time, the finding that carcinomas in the bitch have such different prognoses, was unexpected.

Diagnostic pathologists in this country still tend to use a classification system described by Dr. Misdorp. Illustrations of the many types of mammary tumors are included in the papers and book.
chapters cited in this paper.\textsuperscript{1,12,13} For completeness, each of the classification schemes will include types of tumors that are less common or even rare, which can add to the complexity of trying to understand the diagnostic nomenclature.

From the laboratory diagnostian’s perspective, critical questions such as what features of the neoplastic tissue, allows one to provide an accurate prognosis that a given tumor will metastasize or, if left untreated, lead to the death of the animal. From a clinical perspective, the most important question is whether tumor is benign or malignant.

The last 10 years have seen the development of many new powerful molecular investigative techniques and these have been employed to generate a great deal of information about genetics, cell receptors and other potential markers in the study of human mammary neoplastic conditions. Investigations using some of these same markers on tumors from dogs\textsuperscript{1} and cats\textsuperscript{3-5} have been published. As is so common, with time and additional research, many of the diagnostic tests now being run on samples from humans will make their way into the armamentarium of the veterinary profession. But for the time being, analysis of morphologic features by histopathology does provide critical prognostic information.

References
Figure 1. Normal "inactive" canine mammary gland from a bitch in anestrus. The mammary tissues are inactive. Glands and ducts are dispersed throughout normal adipose tissue, as seen in this photomicrograph as clear spaces above the nipple. Subgross photomicrograph (1X) H and E stain. (Note: Figures 1 through 6 were each taken at the 1X original magnification. The epidermal surface is at the bottom of each of the photomicrographs in figures 1, 2and 4-6.)

Figure 2. Normal hyperplastic canine mammary gland from a bitch during diestrus. The mammary tissues have undergone proliferation. The mammary gland will be enlarged grossly. Mammary tissues at this stage of the cycle are under progesterone influence and are composed of a much greater amount of glandular and ductular structures. Note the uniformity of the tissues compared to neoplastic mammary tissues shown in figures 4, 5 and 6.

Figure 3. Abnormally abundant and uniformly proliferative mammary tissue taken from one of two enlarged mammary glands in a three year old cat. This benign condition is referred to as feline mammary fibroadenomatous hyperplasia. It is a benign condition that can involve one or more glands, tends to occur in young queens, and is not a pre-neoplastic condition. The tissues that undergo hyperplasia and hypertrophy include glandular epithelium, ductal epithelium and myoepithelial stromal cells. These are demonstrated at higher magnification in figure 7.

Figure 4. Papillary ductal adenoma from the mammary gland of a 10 year old Golden Retriever. The large intraluminal mass is attached to the wall of the duct (arrows). A smaller mass is present in the teat sinus (star).

Figure 5. A subgross section of a rapidly growing mammary gland adenocarcinoma in an 11 year old cat. The central area of the mass is necrotic (star) and a circumferential dense rapidly growing rim of neoplastic cells appears as a basophilic band along the expanding and invading peripheral interface with unaffected mammary tissue. The arrow points to an area of epidermal ulceration which is a feature of rapid aggressive growth and, of itself, is evidence of aggressive tumor growth.

Figure 6. This photomicrograph (taken at same 1 X magnification as those in figures 1-5) shows an aggressive malignant adenocarcinoma in a three year old Siamese cat. Multiple smaller masses are evident in the adjacent tissues. These neoplastic metastases are shown at higher magnification in figure 8.

Figure 7. Photomicrograph taken from tissues of the enlarged mammary gland in the young cat with fibroepithelial hyperplasia shown in figure 3. Ductal structures are present in the center of the field and very uniformly hyperplastic alveolar glands radiate peripherally. The uniform stromal in which these tissues are embedded is composed of myoepithelial stromal cells. (100 X original)

Figure 8. Photomicrograph taken from the feline mammary carcinoma in image 6 showing invasion of a lymphatic vessel by proliferating neoplastic glandular epithelial cells (100 X original). Several nodules of neoplastic cells that likely represent complete filling and occlusion of other lymphatics are also present.

(Editor’s Note: The photographs in this manuscript are available in color in the online edition of Clinical Theriogenology.)