

Comparative Pathology of Mammary Gland Cancers in Domestic and Wild Animals

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Abstract. Mammary cancer occurs among all taxonomic groups, and comparing the disease in animals with breast cancer in women could greatly improve our understanding of the relevant risk factors and genetic profiles for this disease. Differences in cancer prevalence between carnivores and herbivores and between captive and wild carnivores are striking and support the hypotheses that diet and reproductive history are major risk factors. Domestic dogs and cats have a high prevalence of mammary tumors, and the majority of tumors in cats are aggressive cancers. Many domestic dogs and cats are prevented from breeding, resulting in their being exposed to recurrent estrogen peaks followed by high persistent levels of progesterone. Therefore progesterone appears to be a significant risk factor for cancer development. Supporting this suspicion is the observation that most mammary cancers in zoo cats are in those treated with the potent synthetic progestin contraceptive, melengestrol acetate. The more common morphologic types of mammary cancer in canids and felids include tubulopapillary, solid, cribriform, comedo and anaplastic carcinomas. Dogs also develop complex carcinomas, which likely evolve from the complex adenomas or mixed tumors that are so common in this species and are promoted by exogenous progesterone treatment. Among zoo felids, jaguars are at higher risk for mammary cancer and also have a high prevalence of ovarian papillarycystadenocarcinomas, a profile similar to women with BRCA1 mutations. As for women, estrogen (ER) and progesterone receptor (PR) expression varies in canine and feline mammary cancers. In general, ER expression is low, but PR expression persists in most cancers. Alterations in molecular controls of cell proliferation or survival in breast cancer, such as cyclin A and p53 expression, have been identified in dog and cat mammary cancers. Overall, spontaneous mammary cancers in cats and dogs make excellent models for human breast cancer, and knowledge of mammary carcinogenesis would be greatly enhanced across all species by a “One Medicine” approach.

Keywords: Mammary cancer, dog, cat, progestin, carcinoma

INTRODUCTION

Breast cancer in women is a complex disease, for which the pathogenesis and risk factors are only partially understood. Similar mammary cancer occurs in many other mammalian species, and comparing cancers across these species could provide invaluable insight into key factors that are common to the pathogenesis of this important disease. Comparing the pathology of mammary cancers in domesticated and wild animals, including the hormonal, dietary, and geographic environments in which they evolved, could help distinguish critical from incidental risk factors for breast cancer in women. Domestic pets are particularly valuable mod-

els, as possible sentinels for human environmental and life-style risks.

Experimental models, principally genetically-engineered mice (GEMS), have been useful for investigating the role of steroid hormones, hormone receptors, and other growth factors in the pathogenesis of breast cancer. Yet rodents differ considerably in mammary gland development and types of breast cancer from women. Furthermore, many mammary gland cancers in mice are viral- or toxin- induced, so the validity of these models has been questioned. Old world primates are closer evolutionary models for women with similar breast development and endocrine profiles [20]; yet low prevalence of spontaneous breast cancer in macaques, as well as the high costs and humane issues of maintain-

ing them in the laboratory setting make them unsuitable for most studies.

The high prevalence of spontaneous mammary cancer in domestic dogs and cats closely mimics the disease in women, making these species more suitable comparative models. The occurrence of aggressive mammary cancer in zoo felids in association with progestin contraceptive exposure is particularly intriguing in light of the ongoing controversy regarding the contributions of progestins to breast cancer pathogenesis. The relative absence of mammary cancers in hoofed animals (ungulates/herbivores) is striking in comparison to cancer prevalence in carnivores. This review describes the spontaneous and progestin-associated mammary cancers in domestic and zoo mammals, highlighting evolutionary and comparative features that may shed light on breast cancer in women.

COMPARATIVE PREVALENCE OF MAMMARY CANCER

Mammary tumors are the most prevalent type of neoplasm in female domestic dogs and are the third most prevalent neoplasm in female domestic cats [111]. A cancer registry for dogs and cats determined that 41.7% of all cancers in 971 female dogs and 17.1% of all cancers in 205 female cats were of mammary gland origin [28]. In a lifetime study of 672 laboratory beagles, 70.8% developed at least one mammary tumor and 60.7% had two or more tumors within one or more of their 8–10 mammary glands [11]. Of the tumors that developed, 34% were cancer [11]. In hospital-based populations, the prevalence in dogs is estimated as 199/100,000 female dogs at risk [112]. Some breeds, such as boxers, English springer spaniels, and dachshunds, have a higher risk of developing cancer [88]. Hospital prevalence estimates in cats are 25.4/100,000 female cats at risk [29]. Cats (a species that also has 8–10 mammary glands but usually only 6 active glands) have a lower prevalence of mammary tumors than dogs, but the majority of feline tumors are cancer. The increasing popularity of pet health insurance, particularly in Europe (e.g. Swedish Agria Insurance database [32]), has provided claim databases from which more precise prevalence statistics on mammary cancers in these species can now be derived. It is important to note, however, that these databases will reflect regional cultural attitudes toward maintaining the sexual integrity of pets. For example, in some European countries, dogs and cats are not ovari-

ohysterectomized to control reproduction, a trend that will increase mammary cancer prevalence. In Norway, a country with almost entirely reproductively intact females, the prevalence of mammary cancer among 14,401 dogs was 53.3%, making it the most common tumor in Norwegian dogs [88].

The proportion of mammary tumors that are cancer notably differ between cats and dogs. In dogs, only approximately 30% of mammary tumors are malignant, whereas the proportion of tumors that are cancer in cats is more than 80% [46]. In both species, concurrent or sequential occurrence of more than one tumor is common. In fact, a range of hyperplastic to benign and malignant tumors are usually present throughout the mammary glands in middle-aged to older animals, particularly in dogs, most likely reflecting a field effect from lifelong hormonal exposure.

Accurate prevalence statistics are not available for zoo felid or canid populations because complete necropsies are not always performed and centralized databases are just now being developed. Most mammary tumors in zoo felids are aggressive cancers, mimicking their domestic counterparts [43,81]. Mammary cancers also occur in zoo canids (Munson, unpublished), mustelids [62], ursids (Munson, unpublished), viverrids [31], and carnivorous marsupials [15,31]. In the endangered black-footed ferret (*Mustela nigripes*) a species whose health has been carefully monitored, 10 of 81 captive female ferrets had mammary adenocarcinoma and 3 had mammary adenomas [62]. Data from most free-ranging carnivores is even more limited because deceased animals are only rarely found and necropsied. However, in two endangered carnivores, the cheetah (*Acinonyx jubatus*) and the island fox (*Urocyon littoralis*), whose free-ranging populations have been closely monitored for health, no cases of mammary cancer have been identified to date in over 500 necropsies performed (Munson, unpublished). Table 1 contains a list of mammary cancer reports in domestic and wild carnivores.

In contrast to domestic carnivores, mammary tumors only rarely occur in domestic mares [1,7,92,107,113], cows [5,10,73,103], ewes [4], goats [6], or sows [6]. Most notable is the low prevalence in the professional lactators (dairy cows and goats), species that have been selectively bred for mammary gland growth and milk production. Mammary cancer is also rare in zoo and free-ranging ungulates with the few published reports consisting principally of single cases, affirming the apparently low predilection for mammary cancer in these taxonomic groups [47,65,73,121,124]. Table 1 lists the few reports of mammary cancer in ungulates.

Table 1
Case reports or surveys for spontaneous mammary gland cancer in veterinary species

Taxonomic group	Latin name	N mammary cancer/N examined or surveyed	Reference
Carnivora			
Domestic Cat	<i>Felis domesticus</i>	21 of 256 cancers of all types in both sexes	[29]
		14 of 243 tumors of all types in both sexes	[8]
		4 of 59 tumors of all types in both sexes	[72]
		21 of 256 tumors of all types in both sexes	[111]
		71 of 97 cats with mammary lesions	[45]
Domestic Dog	<i>Canis familiaris</i>	1639 of 4755 mammary tumors in 672 females	[11]
		1187 of 9,602 mammary tumors in females	[105]
		252 of 915 tumors of all types in both sexes	[29]
		70 of 3,388 tumors of all types in both sexes	[9]
		86 of 899 tumors of all types in both sexes	[72]
Jungle cat	<i>Felis chaus</i>	2 case reports	[43]
Cougar	<i>Puma concolor</i>	2 case reports	[43]
Jaguar	<i>Panthera onca</i>	7 case reports	[43]
		10 of 62 females surveyed	[50]
Leopard	<i>Panthera pardus</i>	1 case report	[43]
Lion	<i>Panthera leo</i>	3 case reports	[43]
			[40]
Tiger	<i>Panthera tigris</i>	17 case reports	[43]
Black-footed ferret	<i>Mustela nigripes</i>	1 case report	[17]
		10 of 81 females surveyed	[62]
California sea lion	<i>Zalophus californianus</i>	1 case report	[80]
Binturong	<i>Arctictis binturong</i>	1 case report	[31]
Artiodactyla			
Domestic cow	<i>Bos taurus</i>	1 case report	[103]
		1 case report	[10]
		0 of 606 tumors of all types in both sexes	[5]
Domestic sheep	<i>Ovis aries</i>	0 of 673 tumors of all types in both sexes	[4]
Domestic goat	<i>Capra hircus</i>	0 of 21 tumors of all types in both sexes	[6]
Domestic pig	<i>Sus domesticus</i>	0 of 24 tumors of all types in both sexes	[6]
Dromedary camel	<i>Camelus bactrianus</i>	1 case report	[14]
		1 of 98 animals surveyed	[47]
Pere David deer	<i>Elaphurus davidianus</i>	1 case report	[124]
Water buffalo	<i>Bubalus bubalis</i>	1 of 300 tumors of all types in both sexes	[73]
Llama	<i>Lama glama</i>	2 of 180 animals surveyed	[121]
		1 case report	[65]
Alpaca	<i>Lama pacos</i>	0 of 368 tumors of all types in both sexes	[121]
Perissodactyla			
Domestic Horse	<i>Equus caballus</i>	1 case report	[1]
		1 case report	[92]
		4 case reports	[113]
		2 case reports	[107]
		0 of 378 tumors of all types in both sexes	[7]
Cetacea			
Beluga	<i>Delphinapterus leucas</i>	3of 80 animals surveyed	[79]
Marsupialia			
Red kangaroo	<i>Macropus rufus</i>	2 of 24 animals surveyed	[120]
		1 of 3 animals surveyed	[15]
Parma wallaby	<i>Macropus parma</i>	1 case report	[15]
Koala	<i>Phascolarctos cinereus</i>	0 of 26 animals surveyed	[15]
Squirrel glider	<i>Petaurus norfolcensis</i>	1 of 3 animals surveyed	[15]
Tasmanian devil	<i>Sarcophilus harrisii</i>	1 report	[31]
		0 of 16 animals surveyed	[16]

An interesting exception to the apparent low prevalence of mammary cancer in wildlife is the occurrence of three cases of mammary adenocarcinoma among 80 beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary in Quebec, Canada [79]. The polycyclic aromatic hydrocarbon pollution in this estuary is suspected to contribute to the estimated annual rate of 163/100,000 of cancer in this species.

COMPARATIVE MORPHOLOGY OF MAMMARY CANCER IN DOMESTIC AND WILD ANIMALS

The historical lack of consensus on morphological classification of domestic animal mammary tumors reflects controversies over histogenesis. Current classification separates carcinomas into *in situ*, simple, and complex carcinomas, and simple carcinomas are classified as tubulopapillary (Fig. 1), solid (Fig. 2), cribriform (Fig. 3), and anaplastic [85,86]. Complex carcinomas consisting of malignant epithelium and myoepithelium occur predominantly in the dog (Fig. 4). Other categories of carcinoma include spindle cell carcinoma, carcinoma with squamous differentiation, mucinous carcinoma and lipid-rich carcinoma. Sarcomas include fibrosarcoma, osteosarcoma, and carcinosaroma [85,86]. Inflammatory carcinomas also occur in dogs and cats (Fig. 5) [100,101]. Affected animals have widespread metastases to dermal lymphatics with overlying ulcerated and inflamed cutaneous lesions in the absence of a discrete mammary mass.

In domestic and zoo cats, morphologic patterns of mammary cancer are similar to breast cancer in women. Most cancers have some areas of tubulopapillary growth with formation of solid, comedone and cribriform patterns and both intraductal and infiltrating components (Fig. 6). Mucinous carcinomas are rare. When early lesions are examined histologically, they appear to originate from either intralobular or major lactiferous ducts. In domestic and zoo felids, most cancers are high grade and portend a poor prognosis (Fig. 7) [43, 81,85]. This poor prognosis may be due in part to the fact that diagnosis is often made at advanced stages of disease as a result of zoo felids requiring anesthesia for mammary examination and domestic felids rarely having their mammary glands examined by their owners. Insufficient data are available to link specific morphologic patterns of mammary carcinomas in cats with prognosis at this time.

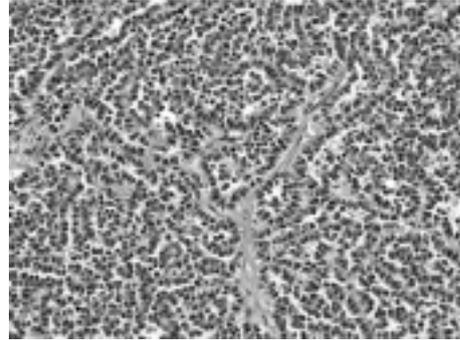


Fig. 1. Mammary cancer with a predominant tubulopapillary pattern in a domestic dog.

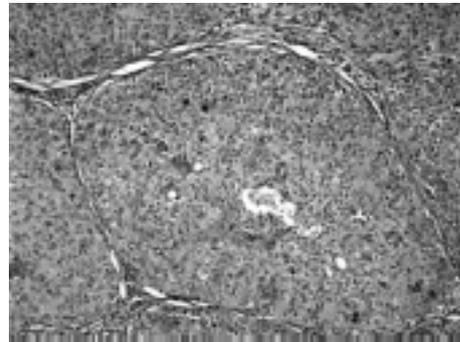


Fig. 2. Mammary cancer with a solid pattern and early comedone formation in a domestic dog.

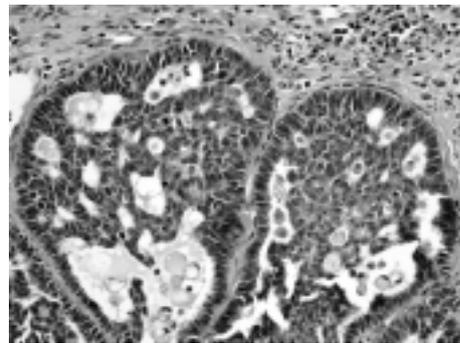


Fig. 3. Mammary carcinoma with a cribriform pattern in a domestic cat.

Mammary cancers in dogs are more diverse due to the occurrence of complex carcinomas, in addition to the simple carcinomas that occur in most mammals. The most common mammary tumors in dogs are the complex adenoma and benign mixed tumor (tumors of epithelial and myoepithelial origin with differentiation toward cartilage and bone). Malignant complex carcinomas and carcinomas or sarcomas arising from be-

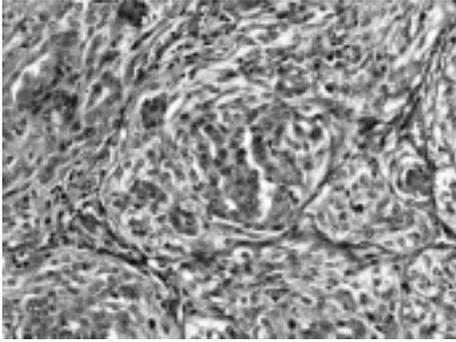


Fig. 4. A complex mammary carcinoma in a domestic dog. Malignant mammary epithelial cells form acini within nests of myoepithelial cells.

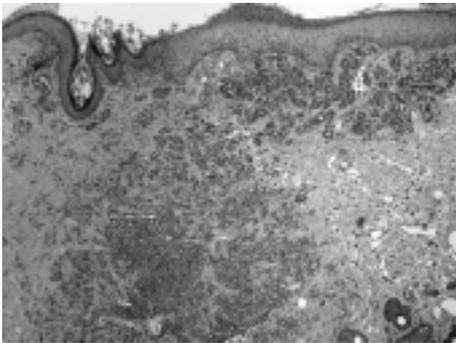


Fig. 5. Inflammatory carcinoma with widespread involvement of dermal lymphatics in the absence of a discrete mammary mass in a domestic dog.

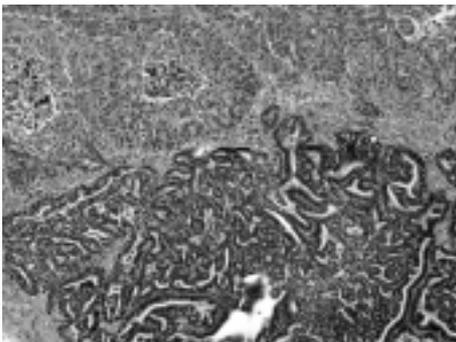


Fig. 6. Mammary carcinoma with both tubulopapillary and solid/comedone patterns in a domestic cat.

nign mixed mammary tumors therefore are also common. Local invasion, nuclear size, necrosis, and mitotic index distinguish complex carcinomas from benign complex adenomas. Occurrences of complex tumors make the dog a less suitable model for human breast cancer than cats. Simple carcinomas in dogs include the tubulopapillary and solid patterns seen in cats, but

anaplastic tumors also are common [85]. As in the cat, clinical outcome of dogs with specific morphologic patterns of mammary cancer have not been followed in sufficient numbers to make meaningful predictions of prognosis. An exception is the life-span study of 672 laboratory beagles conducted by Benjamin et al. [11] in which a new tumor classification was proposed based on clinical outcome. In this study, 18.7% of 1,639 carcinomas appeared to arise from small interlobular or intralobular ducts and were proposed to be classified as ductal carcinomas, whereas 80.7% were adenocarcinomas of other origin. The ductal carcinomas had a higher rate of metastasis and accounted for 65.8% of the cancer-associated fatalities [11].

Misdorp [85] reviews tumor and host factors of prognostic significance for canine mammary cancers seen in the hospital setting. In this context, tumor type, histological grade, degree of infiltration, and evidence of vascular invasion were the most useful criteria for predicting outcome. Tumor grade (based on the methods of Elston and Ellis [34] that include the percentage of tubular formation, mitotic count, and nuclear morphology) was highly predictive of outcome in zoo felids [81] and domestic cats [19]. In dogs, tumor grade was less predictive of outcome, because grade II simple carcinomas were as aggressive as grade III carcinomas [55]. In cats and dogs, tumors with extensive myoepithelial differentiation (complex tumors) tend to be lower grade and less aggressive [19,55,85]. In other carnivores and ungulates, too few mammary carcinomas have occurred to determine common patterns, apply grading criteria, or predict prognosis.

COULD DIETARY FACTORS EXPLAIN SPECIES DIFFERENCES

Lactation is critical to the survival of all mammalian species, so regulation of mammary growth and lactation are likely to be highly conserved. There are few evolutionary differences in lactation among species, other than in volume and duration; therefore, notable species differences in predilection for mammary cancer may reflect differences in diets or reproductive strategies. Diets and reproductive strategies differ widely among species, but tend to be similar within a taxonomic group, as is also true for the epidemiology of mammary cancer.

Evolutionary selection toward mammary growth alone does not appear to influence the occurrence of cancer, as evidenced by the very rare occurrence of

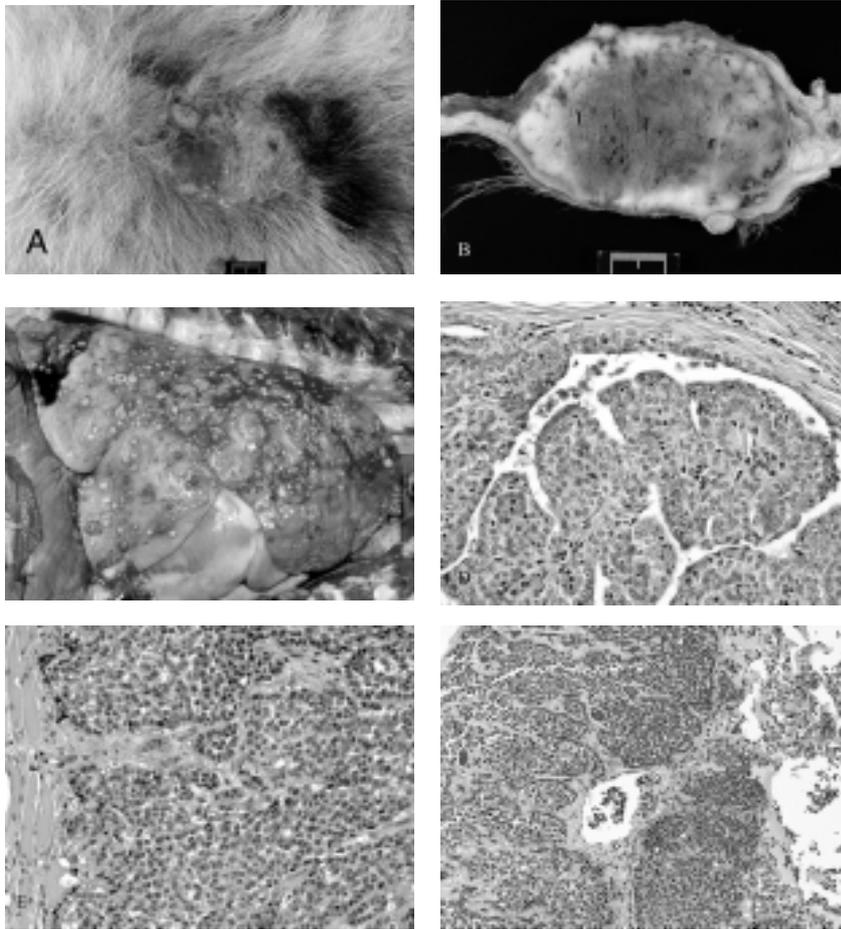


Fig. 7. A high grade mammary cancer in a tiger (*Panthera tigris*) from a zoological park. A) The mammary cancer adjacent to the nipple was concealed by the dense hair coat and detected clinically by the overlying epidermal ulceration. B) Cross section of the cancer at the level of the nipple revealing the extensive central necrosis and local invasion. C) Widespread mammary carcinoma metastases in the lung. D) The majority of the mass had a tubulopapillary pattern formed by densely packed epithelial cells with anisokaryosis. H&E 20X E) The cancer invades the musculature of abdominal wall and has lost the more differentiated tubulopapillary pattern. H&E 20X F) Pulmonary metastases have papillary and solid patterns, and tumor emboli are present in a vessel.

cancer in professional lactators despite selective breeding to increase lobular alveolar growth for milk production. Terminal differentiation necessary for lactation may well be protective across all species, but lactation alone cannot account for the striking differences in mammary cancer prevalence between carnivores and ungulates. Many horses, companion pot-bellied pigs, goats, and zoo ungulates are not bred and do not lactate, so other factors must account for the low prevalence in ungulates. While not advocating a diet of hay for women, one is tempted to consider the low prevalence in herbivores as evidence that a diet low in fat and high in vegetable fiber is beneficial, despite recent studies failing to support these benefits in women [104]. In contrast, dogs have diets that are principally meat-

based, and felids are obligate carnivores. These high fat, low fiber diets may contribute to the propensity of these species to develop mammary cancer [18]. Additionally, some domestic pets and zoo animals are over-nourished, and obesity is a risk factor for mammary tumors in dogs [102]. As carnivores at the top of the food chain, these species may also have diets that biomagnify exposures to environmental carcinogens or contain high levels of harmful hormones [53].

While diet may be a contributing factor, diet alone cannot account for the notably high prevalence of mammary cancer in domestic and zoo carnivores in contrast to their free ranging counterparts. Greater longevity in captivity may be one risk factor, but many wild felids live well beyond 8 years, the age when mammary can-

cer is first seen in zoo felids [43]. A more probable explanation for the difference between free-ranging and captive carnivores is the postponement of pregnancy in domestic and zoo felids and canids, a factor paralleling the situation in contemporary women [58]. Over-nutrition of captive wild animals may also lead to earlier sexual maturity, similar to the risk of early menarche in women from Western societies.

LEVELS AND PATTERNS OF STEROID HORMONES CONSTITUTE A SIGNIFICANT RISK

Across all species studied so far, mammary gland development and growth are under the influence of ovarian estrogens and progesterone, and the influence of these hormones is seen in numerous physiologic and pathologic contexts. The hormonal influence is constant and cyclic, with levels of estrogens and progesterone repeatedly rising and falling throughout the reproductive life of females.

In the classic study of Schneider et al. [112], a clear association between mammary cancer and ovarian hormones was established for dogs. Females ovariectomized before their first estrous cycle had a significantly lower risk (0.5%) of developing mammary cancer than females ovariectomized after one estrous cycle (8%) or after two or more estrous cycles (26%). Ovariectomy at any age may be beneficial to overall survival in dogs that develop mammary cancers [117]. Sexually intact cats are also at a much higher risk of developing mammary carcinoma [46,71]. One study calculated a seven-fold increase in risk for intact female cats [29]. What remains unclear is which hormone(s), at what level, and for what length of exposure constitutes the principal risk.

The sites where estrogen and progesterone may promote cancer could be predicted through their normal physiologic actions on mammary development. In mice, estrogens are necessary for duct development [60] whereas progesterone is necessary for side-branching and lobular-alveolar development [67]. In the cat as in the mouse, mammary duct growth is stimulated by estrogen, but growth of the lobulo-alveolar system also requires progesterone [96]. The hyperplastic and neoplastic lesions that arise in carnivores may reflect excessive stimulation by these hormones.

RECURRENT ESTROUS CYCLES IN DOGS AND CATS MAY PREDISPOSE TO CANCER

Females of all species experience a rise in estrogen as ovarian follicles mature (follicular phase), and a rise in progesterone after ovulation occurs and the corpus luteum (CL) develops (luteal phase). However, the pattern in which the follicles grow and ovulate and the length of the luteal phase vary markedly among species.

Dogs and cats have unique reproductive strategies that have led to the evolution of distinctive estrous cycles. Canids, whether wild or domesticated, reach puberty between 6–18 months and have only one or two estrous cycles per year followed by a prolonged luteal phase with progesterone levels and duration similar to pregnancy (63 days), whether or not the dog is pregnant [35]. In free-ranging canids, most estrous cycles would result in pregnancy with subsequent lactation. Felids on the other hand are seasonally polyestrous with an anestrus period between October and late December in temperate climates of the Northern hemisphere [35] and usually only ovulate if bred (“induced ovulators”). Most estrous cycles in free-ranging felids therefore would culminate in breeding with subsequent ovulation, pregnancy and lactation.

Reproduction is prevented in many domestic dogs and cats, as well as zoo canids and felids. Canids that are not pregnant following an estrous cycle are thus exposed to prolonged high levels of progesterone without subsequent lactation. In contrast, felids that are not bred are exposed to recurrent high peaks of estrogen, as waves of follicles develop and then undergo atresia. Furthermore, some domestic females housed in groups without a male are known to ovulate spontaneously [63]. So some domestic cats may also have recurrent prolonged progesterone exposure in addition to the estrogen peaks. Consequently, human interference with breeding of domestic pets and zoo animals results in these animals being exposed repeatedly to high levels of ovarian steroids without the benefit of lactation. In contrast to the carnivores, estrous cycles of most ungulates occur year round and are characterized by a follicular phase followed by a luteal phase that is shorter than the luteal phase in dogs and cats. This difference in hormonal exposure coupled with more regular occurrence of pregnancies may contribute to the very low prevalence of mammary cancer in ungulates.

The hormonal milieu that promotes mammary development in dogs and cats (estrogen promoting growth of interlobular ducts and progesterone promoting lobular-alveolar growth) may also contribute to the develop-

ment of cancer in the absence of lactation. The common hyperplastic lesions, that include intraductal proliferations (epitheliosis and duct hyperplasia/ectasia) as well as lobular hyperplasia (adenosis) [86], are manifestations of this influence of recurrent estrogen and progesterone. The high prevalence of high grade mammary cancers in domestic cats that only rarely spontaneously ovulate, implicate estrogens as a major risk factor. In contrast, domestic dogs are only transiently exposed to estrogen during their estrous cycle, but have a prolonged diestrual exposure to progesterone. In this species, progesterone must be a contributing factor to explain the high prevalence of mammary cancer in this species. The association of progestin contraceptives with the development of mammary cancer in zoo felids provides more convincing evidence that progestins are a major risk factor for mammary cancer in both canids and felids.

PROGESTIN CONTRACEPTIVES ARE A SIGNIFICANT RISK FACTOR FOR MAMMARY CANCER IN ZOO FELIDS

The progestin, melengesterol acetate (MGA), has been widely used in zoo felids as a contraceptive implant for more than 30 years to prevent unwanted pregnancies and plan breeding of endangered species. Felids treated with MGA-impregnated silastic implants, placed subcutaneously or intramuscularly, are continuously exposed to this potent synthetic progestin for two or more years. In the early 1990's, concerns were raised by zoo veterinarians that mammary cancers were arising in felids with MGA implants. A retrospective survey was then conducted to determine if there was a basis for this concern [43]. Fifty three zoo felids with mammary cancer were identified, and complete medical records were available for 31 of those cases. The potential risk factors of MGA exposure, parity, and species were assessed by cohort comparison. All cancers were aggressive with a rapid clinical course. Twenty-nine of 31 felids with mammary cancer (94%) had been treated with MGA, and this association was highly significant ($p < 0.002$). Jaguars (*Panthera onca*) and tigers (*Panthera tigris*) were over-represented in the cancer group. Neither MGA exposure (dose and duration) nor parity influenced the age of onset of cancer.

The cancers from this survey were subsequently evaluated for histological patterns and metastatic behavior [81], as well as graded by the method of Elston

and Ellis [81]. Twenty two of 31 tumors (70.9%) had multiple histological patterns, 29 (93.5%) were high grade and 28 (90.3%) had metastasized. A predominant tubulopapillary pattern was most common (87.1%, $n = 27$), although solid (61.3%, $n = 19$), cribriform (38.7%, $n = 12$), and comedone (25.8%, $n = 8$) patterns also occurred. Of the 17 cases from which tissue was available for steroid receptor immunohistochemistry, five carcinomas expressed progesterone receptors and one of those carcinomas also expressed estrogen receptors [81].

The persistent expression of progesterone receptors in the cancers of many felids continuously treated with a potent progestin was notable and similar to that reported for MPA-induced carcinomas in mice [48]. Folliculogenesis and ovulation are not suppressed in felids [57] or in canids (Moresco, unpublished) treated with MGA. Although serum steroid concentrations are not available, these observations suggest that MGA-treated carnivores are exposed to recurrent high levels of estrogen during folliculogenesis and continuous high levels of a potent synthetic progestin. Together, these hormones may create an environment highly conducive to developing cancer.

EXOGENOUS PROGESTINS ARE ASSOCIATED WITH MAMMARY TUMORIGENESIS IN DOMESTIC DOGS AND CATS

Although historically the emphasis has been placed on estrogen as the principal hormonal risk factor for breast cancer development, more recent evidence supports a role for progesterone in mammary carcinogenesis [12]. In veterinary species, the effects of progestins in mammary disease have been well documented (Table 2), supporting the reassessment of progestins as a significant promoter of breast cancer. Exposure to synthetic progestins, such as medroxy-progesterone acetate (MPA), megestrol acetate (MA), and MGA has been strongly linked with mammary cancer in domestic cats ($RR = 2.81$) [49,87]. Administration of MPA and MA is also associated with mammary hyperplasia (particularly fibroadenomatous hyperplasia) in female and male domestic cats [27,45,66,70] Anti-progestin therapy reverses the hyperplasia, providing further evidence for the role of progestins in proliferative lesions in cats [41,125].

In dogs, long term exposure to progesterone or synthetic progestins, such as MPA, MA, or MGA, result-

Table 2
Mammary tumors arising in progestin-treated carnivores

Species	Number of animals	Progestin Treatment	No. benign tumors	No. malignant tumors	Reference
Zoo felids	29	MGA	0	29(100%)	[43]
	2	Control	0	2	
	1	MPA	0	1	[51]
Cats	201	MPA, MA or Progesterone	16	59(29%)	[87]
	539	Control	19	95(18%)	
	5	MPA	0	2(40%)	[49]
Dogs	17	MA	1	2(12%)	[45]
	1019	Control	0	71(7%)	
	154	Ethinerone, WY-4355, or Anagestone acetate*	51	33(21%)	[39]
	18	Control	2	0(0%)	
	36	MA, MPA	15	11(31%)	[122]
	32	Control	10	8(25%)	
	120	MPA or Progesterone	37	2(2%)	[37]
	40	Control	0	0	
	70	MPA or Progesterone	134	0	[21]
	24	Control	2	0	

*When given concurrently with mestranol.
MA = Megestrol acetate.
MPA = Medroxyprogesterone acetate.
MGA = Melengestrol acetate.

ed in mammary hyperplasia and benign mammary tumors, but not cancers [18,21,33,37,39]. When high dose progestins are administered concurrently with estrogens, mammary cancers do arise in dogs [39,61,84]. One factor to be considered in comparing the results of these studies is that progestins differ in relative potencies; MGA is approximately 100X [30,119] and MPA approximately 50X the potency of progesterone [118]. Overall, cancers appear to occur when progestins are combined with estrogen or when high doses or potent progestins are used [43,85].

ARE JAGUARS GENETICALLY PREDISPOSED TO DEVELOP GYNECOLOGICAL CANCERS?

Jaguars were over-represented in the mammary cancer group relative to their overall representation in the Association of Zoos and Aquariums Contraceptive Advisory Group (AZACAG) Health Surveillance Program [43]. A survey of causes of morbidity and mortality in jaguars also demonstrated that mammary can-

cer was common (10 of 62 adult females; 16%) [50], and the actual prevalence is likely higher because the mammary glands of all animals may not have been examined. In a survey for endometrial cancer, MGA-treated jaguars were over five times more likely to develop endometrial carcinoma than other MGA-treated felids (OR = 5.5, 95% CI = 1.9–16.2) (M. Smith and Munson, unpublished). Furthermore, jaguars were the only species in the AZACAG surveillance program in which ovarian papillary cystadenocarcinomas were noted [93]. This apparent predisposition to develop mammary and ovarian cancer is remarkably similar to humans with BRCA1 gene mutations [64]. The addition of exogenous progestins to a genetically unstable environment may account for these patterns of disease in the jaguar. BRCA 1 regulates progesterone receptor signaling in mammary epithelial cells [69] and has an inhibitory effect on the proliferative action of progesterone in mice [68]. So, altered BRCA 1 function could result in increased progestin activity in the mammary gland. We have identified and partially sequenced the BRCA1 gene in the jaguar and plan to determine if ani-

mals with cancer carry mutations that match any linked to breast and ovarian cancer predilection in humans (A. Hughes and L. Munson, unpublished).

STEROID RECEPTOR PROFILES IN MAMMARY CANCERS OF DOMESTIC ANIMALS

The presence or absence of hormone receptors has been correlated with prognosis in human breast cancer and may provide useful parameters for predicting prognosis in veterinary species. Some studies have been performed with feline and canine mammary tumors, although routine analysis is not yet part of the standard of care for veterinary patients.

Estrogen Receptors (ER)

In the dog, normal mammary tissue, hyperplastic lesions and benign tumors are high in ER [82]. ER- α levels are lower in canine mammary carcinomas than in benign lesions [74,82,97,126]. Canine ER- β expression was also higher in normal and hyperplastic mammary glands and benign tumors (60%) than in mammary carcinomas (27%) [75]. Another study found most canine cancers to be ER- α negative, but ER- β was highly expressed in inflammatory carcinomas [52]. Although the presence of ER and/or progesterone receptor (PR) is associated with a longer survival time, neither ER or PR are reliable independent prognosticators of disease-free period in the dog after tumor size and stage have been taken into account [74].

In the cat, ER are present in normal mammary tissue, although in lower concentrations than in the dog, and ER positivity decreases with increasing malignancy [77,107]. Loss of ER- α levels in cat carcinomas is greater than in the dog [107]. Among domestic cats only approximately 10% of invasive carcinomas are ER positive [42,82]. In zoo felids, only one of 16 mammary carcinoma were ER positive [81]. The difference in the proportion of ER positive cases between human breast cancer and feline mammary cancer is one feature that makes the cat a less suitable model, except for recurrent breast cancers that tend to be ER negative.

Progesterone Receptors

The normal dog mammary tissue has abundant PR, and PR levels decrease as the tissue becomes more dysplastic/neoplastic [82]. Sixty-five percent of mammary

cancers in dogs were PR+; however, PR status was not a predictor of outcome [74]. In another study, 42% of canine mammary cancers were PR+ [36]. Neither phase of the estrous cycle nor exogenous progestins seem to affect the levels of PR in dog mammary carcinomas.

In contrast, the domestic cat has low PR numbers in normal tissue, higher PR numbers in hyperplastic lesions and *in situ* carcinomas, but lower PR numbers in cancers than hyperplastic lesions [107]. Immunoreactive PR was identified in 38.5% of feline mammary cancers and 66.7% of benign tumors and dysplasias [78]. Most cancers in this study were ER+PR+ or ER-PR+. In another study, 61% of invasive carcinomas were PR+ [82]. In zoo felids, 5 of 17 carcinomas were positive for PR, and three of those five cases occurred in cats treated with MGA [81]. Only one of these felid cancers was ER+ PR+.

Androgen Receptors (AR)

Androgen receptors are present in most breast cancers [26], and endogenous androgens or exogenous androgenic progestins [22] may bind to these receptors thereby stimulating cell growth. Studies have disclosed high expression of AR in 64% of canine inflammatory mammary, while 41% of non-inflammatory malignant mammary tumors were positive [52]. Among cats, two of nine mammary cancers [109] and three of three cases of inflammatory mammary carcinoma were positive for AR [101].

UNIQUE HORMONAL PROFILES OF DOMESTIC ANIMAL TUMORS

Normal mammary glands and mammary tumors in dogs produce growth hormone (GH) [116]. Mammary tumors and mammary hyperplasia of some progestin-treated dogs produce sufficient GH to result in signs of acromegaly [91,114,115,122]. GH receptors are also expressed in mammary epithelial cells and tumors, but are down regulated in terminally differentiated normal alveolar tissue [123]. The potential autocrine-paracrine effects of GH and secondary effect of stimulating insulin-like growth factor-1 (IGF-1) production may contribute to the proliferative environment in the canine mammary gland manifested by the occurrence of numerous hyperplastic and neoplastic lesions [90, 122]. In mice, IGF-1 appears to promote cell cycle progression or inhibit apoptosis [44], supporting a role for GH and IGF-1 production in carcinogenesis. Low lev-

els of GH expression have been demonstrated in some feline mammary tumors, but without clinical signs of GH excess [99]. In cats, IGF-I has also been documented in association with the expression of GH [99].

Prolactin is produced by canine tumors and has been associated with increased incidence of benign, but not malignant tumors [106]. Other unusual proteins expressed by canine mammary tumors are bone morphogenic protein-6 and its receptor in myoepithelial cells of canine mixed mammary tumors [3]. Whether these hormones contribute to carcinogenesis is unknown. In dogs with mammary cancer and hypercalcemia, mammary tumor production of parathyroid hormone-related protein (PTHrp) has been documented [59]. Hypercalcemia was also found in many MGA-treated zoo felids with cancer [43], although immunostains for PTHrp were not successful (Munson, unpublished).

ALTERED GROWTH CONTROL IN MAMMARY CANCERS OF DOMESTIC ANIMALS PARALLEL MECHANISMS OF CARCINOGENESIS IN HUMANS

Cancers arise from alterations in growth control across all species. Whether caused by genetic mutations and/or promoted by endogenous or exogenous hormones, cancers in women and domestic dogs and cats have similar expression profiles. These genetic alterations include factors promoting progression through the cell cycle, inactivation of tumor suppressor genes, inhibition of apoptosis, or prolongation of cell life span.

In cats, cyclin A was found to be over-expressed in mammary cancers (49%), but not in benign tumors (0%) [95]. In a small study of eight feline mammary carcinomas, *cyclin A* gene amplification was found in seven carcinomas [94]. Similarly in dogs, over expression of the cyclin A protein was seen only in malignant (50%) but not in benign (0%) tumors [95]. However, in dogs only 27% of mammary gland tumors showed an amplification of the *cyclin A* gene, with no difference in occurrence between malignant and benign cases. Cyclin D1 was expressed only in 5% of 38 canine mammary carcinomas, but was not amplified or over-expressed in any feline cancers [95].

Increased expression of vascular endothelial growth factor (VEGF) in human breast cancer is associated with shorter overall survival compared to cases where VEGF expression is normal [89]. In cats, increased

VEGF expression was also associated with decreased survival [83]. In dogs, higher levels of circulating VEGF were found post-operatively in animals with metastases than in those without metastases, conceivably associating these higher levels with shorter survival in dogs as well [56].

The expression of E-cadherin in feline mammary cancers was found to be reduced or absent, and aberrant expression was found in the cytoplasm in some cases [25]. In canine mammary cancer, E-cadherin expression is often cytoplasmic and reduced as well, with lowest expression in solid tumors [24,110]. Reduction in E-cadherin and beta-catenin expression was associated with more invasive cancers in dogs [13]. Aberrant expression of P-cadherin by epithelial cells has been observed in canine complex tumors with epithelial expression greater in malignant tumors [38].

HER-2/neu (c-erb-B2) mRNA was found to be elevated in three of three feline mammary gland carcinoma cell lines and in six of 11 carcinomas, and HER-2/neu protein immunostaining was prominent in 13 of 36 carcinomas [23]. In another study, HER-2/neu over expression was detected in 59.6% of 47 feline mammary carcinomas, and over expression correlated with decreased overall survival [82]. In dogs, c-erb-B2 over-expression occurred in 17 of 23 mammary cancers, but not in benign tumors [2]. In another study, only 17% of canine mammary cancers over-expressed HER-2/neu protein, detected with immunohistochemistry [76].

Alternations in other regulators of the cell cycle, apoptosis, and invasion are being assessed in dog and cat mammary cancers to determine if mechanisms of carcinogenesis are similar to those of breast cancer and to assess the potential application of new therapies to veterinary species. For example, aberrant expression and distribution of BRCA1 has been significantly associated with malignant characteristics in domestic dogs [98] and four of eight canine mammary cancers had significant loss of PTEN expression [54]. Abnormalities in p53 expression which is commonly found in breast cancer, are also present in dog and cat mammary cancer. In cats, 19% of 37 carcinomas, but none of six benign tumors expressed p53 [95], whereas in dogs, both benign and malignant mammary tumors were p53 positive (16% and 30% respectively) [108]. We can expect a wealth of comparative information to emerge over the next decade as molecular tools are applied to spontaneous mammary cancers in animals.

CONCLUSIONS

Differences in prevalence of mammary cancer among taxonomic groups are notable and may provide valuable insight into human risk factors. Spontaneous mammary cancer in domestic and zoo carnivores is strikingly similar in type and behavior to breast cancer in women, making them excellent candidates for studying comparative carcinogenesis and the efficacy of new therapies. In dogs and cats, as in women, life-long cumulative steroid hormone exposure (endogenous and exogenous) appears to be a major risk factor for mammary cancer development. The association of progestin contraceptive use with mammary cancer in pets and zoo carnivores is a cause for concern and may be relevant to women's health. Many of the same molecular abnormalities associated with transformation to malignancy in humans are present in mammary cancer of pets, further enhancing their value as a model. Major advances in the understanding and treatment of breast cancer can be accomplished by sharing information from experimental and epidemiological studies between veterinary and human medical fields.

ACKNOWLEDGEMENTS

The authors thank Dr. V. McElliott for photographs of the tiger mammary gland and lungs. We also thank Dr. Robert Cardiff for promoting the one medicine philosophy that made this special issue possible.

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