

Studies on Prevalence of Mammary Gland Tumors of Female dogs in Ludhiana (Punjab)

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Abstract

The female dogs with mammary gland tumors (n=106) presented at the small animal clinical complex, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab from different locations of Ludhiana city were studied for prevalence of the disease. Body temperature and respiration rate of tumor bearing bitches were comparable to that of values of healthy dogs but the pulse rate was observed to be significantly ($p<0.05$) increased in the tumor bearing group of bitches. 69.81% of cases presented were observed to be obese, 58.49% of were not regularly exercised and diet of 78.3% bitches consisted of red meat. Spaying was done in most (62.1%) of the tumor bearing bitches after 3 years of age. Most of the bitches (62.22%) presented gave birth to their young ones after 3 years of age. Adult female dogs (5-10 years old) were most frequently affected (54.71%) followed by old aged dogs (>10 years old). Spitz breed accounted for 39.62% of cases presented followed by German shepherd (31.13%), non-descript (16.98%) and Labrador (12.26%). Bitches from industrial areas are most susceptible to mammary tumors (79.24%). Gross pathological investigation reported that most of the developed tumors (45.28%) affecting first two glands. 79.24% of tumors were palpated to be hard and 20.75% are soft. Nodular type tumors consisted of 74.52% of cases and 49.05% of tumors were observed to be recurrent. Histopathological analysis revealed that 66.03% of tumors were malignant and 33.96% belong to benign group. Most of the tumor affected bitches were in stage III (41.50%). The present study observed that mammary tumor in female dogs is an emerging animal health problem and special attention is needed for its prevention and treatment.

Keywords: Female Dogs, Mammary Tumors, Prevalence

Introduction

Mammary gland tumors are the common form of neoplasm diagnosed in female dogs (Salas *et al.*, 2015). Nearly 50% of mammary tumors in bitches are malignant. Risks of developing malignant tumors are highest if these animals are spayed at a later age (Kumaraguruparan *et al.*, 2005). Size of Canine mammary tumors are variable ranging from a few millimeters to over a few centimeters (Wey *et al.*, 2000). Mammary tumor in bitches is a heterogeneous group of diseases. It varies according to morphology and biological behavior comparable to that of breast cancer in humans (Salas *et al.*, 2015). Histopathologically, canine mammary tumors are classified as benign and malignant. Malignant tumors can be sub classified as carcinoma (simple carcinoma, complex carcinoma, adenosquamous carcinoma and lipid rich carcinoma), sarcoma (osteosarcoma and fibrosarcoma) and carcinosarcoma. Mammary tumors in female dogs share some similarities with human breast cancer. Hence, canine mammary tumors can become a good model for investigating human breast cancer in relation to diagnosis, prognosis, prevention and treatment (Vascellari *et al.*, 2016). Genetic instability is usually observed in metastatic canine mammary tumors. Uncontrolled cell division, abnormal DNA replication, epigenetic modifications and mutations can be a reason for genetic instability (Klopfleisch *et al.*, 2010). Mutation inactivates the tumor suppressor gene and activates the proto-oncogenes to induce replication of damaged DNA (Halazonetis *et al.*, 2008).

Canine population is continuously increasing in Ludhiana city of Punjab state with increase in human population. Keeping into consideration, the high incidence of mammary tumors in female dogs, it is considered as an important health problem in canines. Better infrastructure, preventive, diagnostic, therapeutic approach is required for solving this problem. For this, studies on health status and prevalence of the disease provide the information for investigating the behavior of the disease, identification of risk factors, diagnostic and prognostic measures. Information drawn from these studies can set the stage for further experimental study on canine mammary tumors. Hence, we conducted this epidemiological study in female dogs diagnosed for mammary tumors at the small animal clinical complex (SACC), Guru Angad Dev Veterinary and Animal Sciences University (GADVASU).

Materials and Methods

This study included female dogs (n=106) clinically examined and diagnosed for mammary tumors at the SACC, GADVASU. These cases were presented from different locations of Ludhiana city, Punjab, India. Mammary tumors were confirmed on the basis of histopathology, ultrasonography and radiography investigatory reports. On the day of presentation, detail health parameters of tumor bearing bitches were studied. Information about vital signs (Body temperature, Pulse rate, Respiration rate), health parameters (obesity, exercise, diet), reproductive histories (spaying, parturition, estrous length etc.) age, sex, breed, environment and hormonal status was collected. For comparison, the healthy adult female dogs (control) were selected from the clinics presented for daily routine checkup and vaccination. Gross pathological characteristics of the mammary tumors (teat involvement, consistency, growth type) were investigated. The tumor samples were processed at the Department of Pathology, College of Veterinary Science, GADVASU (August 2011 to July 2013) for obtaining information regarding histopathological types and clinical stages. For histopathological analysis small portions of the mammary tumor tissues were fixed in 10% neutral buffered formalin (NBF), embedded in paraffin and tissue sections were prepared. Then the tissue sections were stained with hematoxylin and eosin (H & E) for examination. The clinical staging of mammary gland tumors was done by TNM (Tumor-Node-Metastasis) system as recommended by the world health organization (WHO). The female dogs were taken and their health files were investigated regularly for obtaining information regarding the recurrent status of the tumor.

Statistical Analysis

The data were subjected to the Student's t-test using statistical package for social sciences (SPSS), version 16.0.

Results and Discussion

The study covered two years of duration (August 2011 to July 2013) and 106 numbers of tumor bearing female dogs along with the mammary tumor samples were examined. Most of the studied female dogs were adult and old. Body temperature and Respiration rate recorded in tumor bearing bitches were comparable to that of healthy/ control dogs but the Pulse rate was found to be significantly ($p < 0.05$) increased in the tumor bearing group of bitches as compared to the control group of bitches (Table 1).

Table 1: Vital signs (mean $\pm$ S.E) in female dogs diagnosed for mammary gland tumors

Vital Signs	Healthy Dogs	Mammary Tumor Bearing Bitches	Range in Canine Mammary Tumors
Body Temperature ($^{\circ}$ F)	100.2 $\pm$ 0.81	100.8 $\pm$ 0.92	100.4-101.2
Resting Pulse Rate (bpm)	94.5 $\pm$ 0.64	132.4 $\pm$ 1.43*	92.3-135.4
Respiration Rate (rpm)	20.2 $\pm$ 0.28	25.11 $\pm$ 0.32	19-28

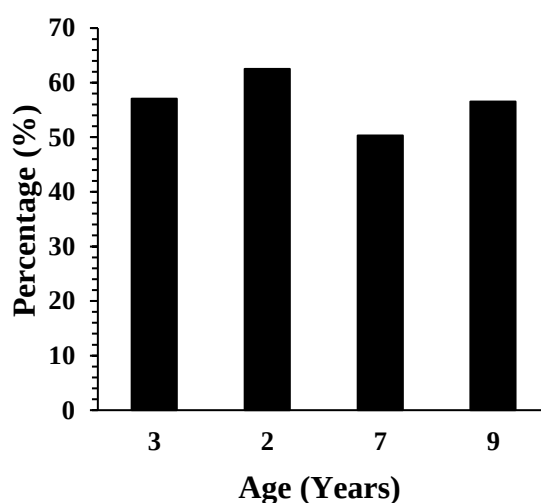
*Indicates significant ($p < 0.05$) difference in tumor bearing bitches as compared to the control group.

Investigation on the health characteristics of the female dogs (Table 2) also revealed that 69.81% of bitches were obese and 30.18% belong to non-obese group. 41.5% of bitches were exercised regularly by their owners where as 58.49% were not regularly exercised. Diet of most of the bitches (78.3%) consists of red meat followed by vegetarian diet (21.69%). Reproductive histories of female dogs (Table 3) diagnosed for mammary tumors revealed that the bitches experienced their first estrous between 5-8 months of age. Most of the female dogs (58.49%) were spayed after three years of age and around 31.13% of bitches were spayed before three of age. However, 10.37% of bitches were never spayed. Most of the tumor bearing bitches (52.83%) gave birth to their young ones after three years of age. 31.13% of bitches were parturiated before three years of age and 15.09% of bitches were not parturiated.

Table 2: Diet and exercise status in female dogs diagnosed for mammary gland tumors

Characteristics	Number of Female Dogs	Percentage (%)
A. Obesity		
Obese	74	69.81
Non-obese	32	30.18
B. Exercise		
Normal exercise	44	41.5
No exercise	62	58.49
C. Diet		
Non-veg. (Red meat)	83	78.3
Veg.	23	21.69
Total Number		106

Age and breed of the female dogs and their relation with mammary tumors were presented in Figure 1 and 2 respectively. Adult female dogs (5-10 years old) were most frequently affected (54.71%) followed by old aged dogs (>10 years old) with a frequency of 27.35%.

**Figure 1:** Age wise prevalence of mammary gland tumors in female dogs

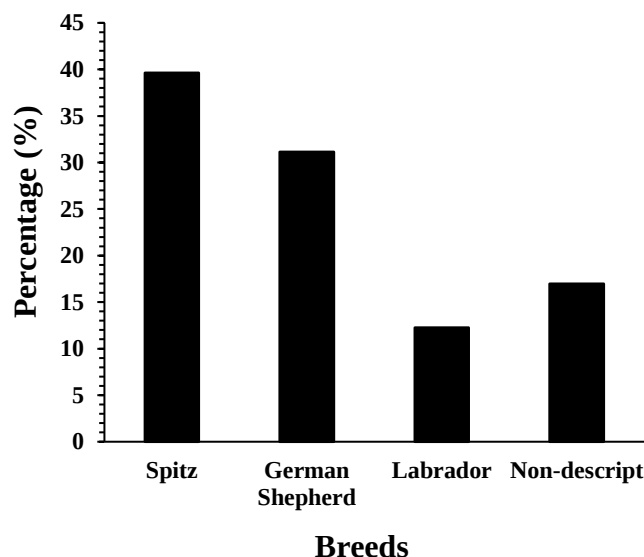


Figure 2: Breed wise prevalence of mammary gland tumors in female dogs

Female dogs aged between 3-5 years showed a frequency of 14.15% whereas very young bitches (<3 years) were least affected by mammary tumors. Spitz breeds showed the highest frequency of occurrence (39.62%) followed by German shepherd (31.13%) and non-descript (16.98) bitches. Labrador breed showed the least frequency of occurrence (12.26%). Female dogs domesticated in industrial areas were more susceptible for developing mammary tumors (79.24%) than bitches domesticated in non-industrial (20.75%) areas (Figure 3). Gross pathological characteristics of studied mammary tumors were presented in Table 3.

Table 3: Reproductive histories of female dogs diagnosed for mammary gland tumors

Reproductive History	Number of Female Dogs	Percentage (%)
A. Age range (Years) = 2.5 – 20		
B. Ovariohysterectomy		
< 3 years	33	31.13
> 3 years	62	58.49
None	11	10.37
C. Age at first oestrous (months) [mean±SD] = 6.5±1.4		
D. Age at first parturition		
< 3 years	34	32.07
> 3 years	56	52.83
None	16	15.09
Total Number		106

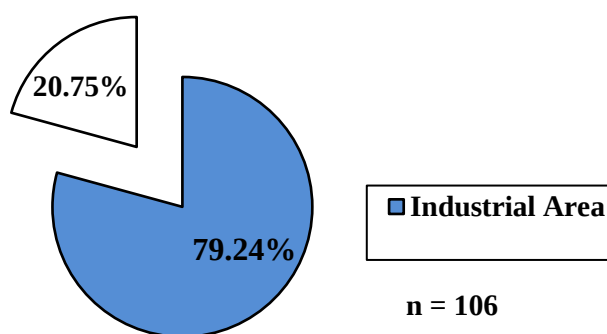


Figure 3: Area wise prevalence of mammary gland tumors in female dogs

Most of tumors developed affecting first two glands followed by one gland. Least percentage (1.88%) of tumors were developed affecting fourth or fifth gland. Consistency of most of the mammary gland tumors was observed to be hard (79.24%) with soft types (20.75%) encountered in some cases. Frequency of nodular type growth was observed to be higher among canine mammary tumors with single nodule dominating the multiple ones. Non nodular type tumors occur less frequently (Table 4). Out of the total numbers of tumor studied (Table 5), most of them belonged to malignant group (66.03%) and 33.96% were benign type.

Table 4: Gross pathological characteristics of studied mammary gland tumors in female dogs

Characteristics	Number of Female Dogs	Percentage (%)
A. Glands/teats involved		
First	31	29.24
Second	48	45.28
Third	19	17.92
Fourth	6	5.66
Fifth	2	1.88
B. Tumor consistency		
Hard	84	79.24
Soft	22	20.75
C. Growth type		
Nodular type	79	74.52
Single nodule	51	48.11
Multiple nodule	28	35.44
Non-nodular type	27	25.47
Total Number	106	

Table 5: Prevalence of mammary tumors in female dogs according to biological behavior and tissue of origin

Histological Types	Number of Female Dogs	Percentage (%)
A. Carcinoma		
Simple carcinoma	8	7.54
Complex carcinoma	11	10.37
Adenosquamous carcinoma	26	24.52
Lipid rich carcinoma	3	2.83
B. Sarcoma		
Osteosarcoma	6	5.66
Fibrosarcoma	4	3.77
C. Carcinosarcoma		
D. Adenoma	36	33.96
E. Recurrence of tumor		
Recurrent	52	49.05
Non-recurrent	54	50.94
Total Number	106	

Among the malignant tumors, carcinoma (Simple carcinoma > complex carcinoma > adenosquamous carcinoma > lipid rich carcinoma) occurred most frequently followed by sarcoma (osteosarcoma > fibrosarcoma) and carcinosarcoma. Almost half of the mammary tumors followed were observed to be recurrent. Clinical staging of mammary gland tumors (Table 6) in the present study revealed that most of the female dogs were in third stage (41.5%) of the tumor followed by stage IV (26.41%), stage II (19.81%) and stage I (12.26%). A recent study has compared cytology and histopathology which revealed a highly positive correlation in detecting malignancy and

tumor sub-type. A non-significant correlation was observed between cytology and histopathology reported in predicting the grade and stage of malignancy (Kaur *et al.*, 2019).

Table 6: Clinical stages of studied cases of mammary gland tumors in female dogs

Clinical Stages	Number of Female Dogs	Percentage (%)
Stage I (T ₁ N ₀ M ₀)	13	12.26
Stage II (T ₂ N ₀ M ₀)	21	19.81
Stage III (T ₃ N ₀ M ₀)	44	41.5
Stage IV (T ₂ N ₁ M ₁)	28	26.41
Total Number	106	

T: Primary tumor size (maximum diameter): T₁: <3 cm; T₂: 3-5 cm; T₃: >5 cm; N: Regional lymph node metastasis; N₀: No evidence of regional lymph nodes involvement; N₁: Ipsilateral regional lymph nodes involved; N₂: Bilateral regional lymph nodes involved; M: Distant metastasis; M₀: No evidence of distant metastasis; M₁: Distant metastasis involved

Female dogs were mostly affected by mammary tumors because intake of natural estrogens present in food, water etc. act tropically on the mammary gland stimulating them causing carcinogenesis. Previous studies suggested that 17-beta-estradiol was a risk factor for the development of breast cancer (Yu, 2002). Prevalence frequency of canine mammary tumors varies according to the place where the investigation was conducted. The present study was in accordance with the previous study conducted in 2005 on bitches of Ludhiana district of Punjab (Gupta *et al.*, 2012). The occurrence of mammary tumors in bitches has reduced in developed countries due to implementation of health programs in relation to the canine reproduction. It enables the spaying of bitches at an early age reducing the chances of mammary tumor development. However, in developing countries these programs are not implemented till now (Salas *et al.*, 2015). Hence chances of mammary tumor occurrence are increasing year by year in developing countries. In the present study the increased heart rate observed in the tumor bearing bitches indicated that they might be under stressful condition (Vascellari *et al.*, 2016). Age ranges of female dogs vary widely for presenting mammary tumors. It was previously reported that canine mammary tumors develop between 3 to 22 years of age with an average of 9 years (Sontasa *et al.*, 2009). In this study we reported a range of 2.5 to 20 years old with an average of approximately 11 years of female dogs. However, in some breeds of dogs mammary tumors may develop at younger age also. Previous studies on canine mammary tumor suggested that old age, obesity, feeding of more red meat and fat in diet of bitches, lack of exercise, spaying and parturition at a late age and polluted environment predisposes the female dogs for developing tumors of mammary gland (Kumaraguruparan *et al.*, 2005) which was in congruent with our results. Small breeds of bitches were mostly affected by mammary tumors (Salas *et al.*, 2015) and it varies according to the location of the study.

However, it is a problem in large-sized breeds also. In the present study the breeds commonly affected were Spitz, German shepherd and Labrador retriever. Other studies also reported that Poodle, Chihuahua, Cocker Spaniel and German shepherd breeds are affected by mammary tumors (Rivera *et al.*, 2009 and Bronden *et al.*, 2010). Mammary tumors were previously reported in crossbreed dogs, German shepherds and Labrador retrievers in India (Gupta *et al.*, 2012). The results of our study were in agreement with the previous studies. In the present study we observed that the most of tumors were nodular, developed mostly affecting first two glands and very hard in consistency. Malignant tumors are usually irregular in shape containing nodules; grow rapidly and firmly attached to the skin. The hardness is attributed to the deposition of fibrous tissue in the tumors (Vascellari *et al.*, 2016). Previous study conducted on canine mammary tumors in Ludhiana district of Punjab, India (Gupta *et al.*, 2012) was in line with the present study. Mammary tumors represent a heterogeneous group of disease in the female dogs in terms of biological behavior and tissue of origin (Goldschmidt *et al.*, 2011). More than 50% of mammary tumors in bitches are malignant (Oliveira *et al.*, 2003). Previous study on female dogs reported 28 malignant and 57 benign tumors (Rivera *et al.*, 2009). These reports were in line with the observations of our study. In the recent years, the numbers of malignant cases are increasing and this may be due to the exposure of bitches to cancer causing agents. Previous epidemiological studies suggested that, the environmental pollutants and chemical exposure can cause mammary gland tumors (Vascellari *et al.*, 2016). Pollutants released to the environment from different industries are claimed to be toxic and carcinogenic. These can generate reactive oxygen species in the mammary cells causing oxidative damage to biomolecules such as lipids, proteins and DNA resulting into carcinogenesis (Bertini and Cavallaro 2008). Type of diet, obesity and estrogens may result into development of mammary tumors in bitches. Diet, fat and hormones can promote development of mammary gland tumors by damaging the DNA (Salas *et al.*, 2015). The different histological types of tumors observed in this study were simple carcinoma, complex carcinoma,

adenosquamous carcinoma, lipid rich carcinoma, osteosarcoma, fibrosarcoma and adenoma. Adenocarcinoma was observed to be the most common among the carcinoma groups. This was followed by carcinosarcoma, complex carcinoma, simple carcinoma, osteosarcoma, fibrosarcoma and lipid rich carcinoma. In the present study, it was observed that almost 50% of mammary tumors are recurrent. Irreversible genomic damage, activation of oncogenes, inactivation of tumor suppressor genes and deranged signal transduction in cancer tissue results into their uncontrolled proliferation (Kumaraguruparan *et al.*, 2005). These might be the reasons for recurrent behavior of the tumors. The results of the present study were in accordance with the reports of previous epidemiological studies. Most of the mammary tumors in the present study were in later stages i.e., stage III and stage IV followed by stage II and stage I. This was attributed to the tumor development, progression, size of the tumors and metastasis. Myoepithelial cells exert their effects on tumor cell behaviour, separating them from stromal angiogenesis and causing abnormal growth and proliferation of tumor cells (Barsky and Karlin 2006). Evasion of apoptosis, a hallmark of malignant cells also enhances the proliferation of tumors (Kumaraguruparan *et al.*, 2005).

Conclusion

This study is important, because of large growth of mammary tumors in canine population within the last few years in Ludhiana city, Punjab, India. Most of the residents of Ludhiana city have pet dogs and neither India nor Punjab has following the laws related to canine reproduction. Hence, people of Ludhiana city are not regularly spaying their bitches also. Female dogs reach the age of maturity without being spayed and are susceptible for the development of mammary tumors. The present study concluded that, the mammary gland tumors in female dogs are an important health problem and it is increasing. More interdisciplinary experimental studies will be required for development of preventive, diagnostic, prognostic and therapeutic strategies for canine mammary tumors. Keeping this in view the similarity between mammary cancers in female dogs and human subjects, canines can be used as useful models for research on human breast cancer.

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Conflict of Interests

There is no conflict of interest.

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