RETROSPECTIVE HISTOPATHOLOGICAL STUDY OF CANINE MAMMARY GLAND TUMOURS DIAGNOSED FROM 2006 – 2012 IN UNIVERSITY PUTRA MALAYSIA

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SUMMARY

Forty-eight canine mammary tumours (CMT) diagnosed at the Histopathology Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia, were reviewed retrospectively. Two control groups, one comprising all other cases involving female dogs and another group comprising only cases of neoplasia involving female dogs diagnosed in the laboratory over the same period of time were used for comparisons in this study. Fisher's exact test and logistic regression were used to determine association between the factors and the risk of CMT. Thirty-nine (81.3%) of the tumours were diagnosed as adenocarcinoma, 8.3% (n=4) each, were the diagnoses for squamous cell carcinoma and mixed cell tumour. Adenoma had 2.1% (n=1). The prevalence of canine mammary gland tumours in this study is 39%. When CMT cases were compared with all other cases, significant association was observed with adult dogs ($p = 0.032$, logistic regression 0.012) and intact dogs ($p = 0.009$, logistic regression, 0.003). When CMT cases were compared with neoplasia cases, significant association was observed with pure breeds ($p = 0.025$) and intact dogs ($p = 0.000034$, logistic regression 0.00042). This study found that pure breed dogs, intact dogs and older dogs (> 5years) have higher odds of having CMT in Malaysian dog population.

Keywords: Mammary gland tumours, prevalence, histopathology, neuter status, breed

INTRODUCTION

Canine mammary tumour (CMT) is the second most common neoplasm in dogs and mostly affects female dogs (Benjamin *et al*., 1999). High prevalence of up to 50-70% has reported in the United States of America (Kelsey *et al*., 1998; Benjamin *et al*., 1999; Zuccari *et al*., 2011). In Sweden, 111 dogs are affected per 10,000 dog-years at risk (Egenvall *et al*., 2005), while 205 cases per 100,000 dogs/year have been reported in the United Kingdom (Dobson *et al*., 2002). In India, 39.9% prevalence has been reported (Dhami *et al*., 2010), while in South Africa, tumours of the female reproductive system represent 10.2% of total tumours affecting female dogs, 80% of these are mammary gland tumours (Bastianello, 1983).

There are several risk factors that are associated with the development of this disease in dogs. Exposure to ovarian hormones; oestrogen and progesterone significantly increase the risk CMT development, as early spay in bitches has a protective effect against CMT development. Bitches that are spayed before the first oestrus have a 0.5% risk of CMT development, while those spayed after the first and second oestrus have 8% and 26% risk, respectively (Schneider *et al*., 1969). The peak age for occurrence of CMT is around 8 to 11 years (Taylor *et al*., 1976; Sontas *et al*., 2011). Mammary gland tumours are more common in miniature and toy breeds (Sorenmo *et al*., 2011) with reported highest representation in English Springer Spaniels (Dobson *et al*., 2002), Poodle (Mitchell *et al*., 1974) and Terriers (Sontas *et al*., 2011). Obesity at one year of age or at least a year prior to diagnosis has been reported with increased risk

for CMT development (Perez Alenza *et al*., 2000). In the same study, dogs fed with homemade food or red meat also were at higher risk compared to dogs fed on commercial diet.

At the time of diagnosis, 50% to 86% of the CMT are diagnosed malignant (Zuccari *et al*., 2011; Klopfleisch *et al*., 2011). Metastasis through the blood or lymphatic system (Rasotto *et al*., 2012) mainly to the lungs is one of the causes of death in dogs with this disease (Klopfleisch *et al*., 2011). Once the tumour has developed at the primary site, there are several factors that can affect prognosis and predict overall survival. These include, tumour histopathology sub-type, stage of the disease and size of the tumour (Philibert *et al*., 2003; Itoh *et al*., 2005); older age at diagnosis (Perez Alenza *et al*., 2000); presence of metastasis to lungs, lymph nodes or other internal organs (Zuccari *et al*., 2008); as well as the expression of selected molecular markers by the tumour cells (Macewen *et al*., 1982; Lavalle *et al*., 2009).

CMT is one of the most studied neoplasms of the dog in various veterinary institutions but to date, there have been limited information on the mammary tumour development among local dogs in Malaysia and how far they resemble the characteristics reported in other geographical regions. The objective of this study is to determine the prevalence and risk factors for CMT development among the dog population diagnosed at a veterinary histopathology laboratory in Malaysia.

MATERIALS AND METHODS

Data retrieval

Mammary gland tumour tissues that were submitted between January 2006 and December 2012 to the veterinary histopathology laboratory of the Faculty of Veterinary Medicine (FVM) in Universiti Putra Malaysia (UPM) were reviewed. Four µm sections from the

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respective tumour tissues embedded in paraffin were stained with Hematoxylin and Eosin for routine histopathology.

Clinical information of the dogs was reviewed retrospectively from the request forms that were submitted to the laboratory upon specimen (tumour biopsy) submission. The data include age, sex, breed, neuter status, tumour size, the affected gland and the total number of glands affected in each dog. The age of dogs was categorised into dogs that are less than 5 years and those that are 5 years or older due to rarity of CMT in young dogs. Dog breeds were categorised based on pedigree as pure breeds and local/mix breeds because genetic variability is known to affect the occurrence of several types of cancer, including CMT. Another breed categorisation was based on body size, where dogs are placed in large breed (large and giant) or small-sized (miniature, small and medium) categories based on specific breed characteristics. All female dogs diagnosed with any conditions, using biopsy or at post mortem, (including neoplasms, excluding mammary gland tumours) in the histopathology laboratory, FVM, UPM, between January 2006 to December 2012 were selected to serve as control group 1 (CG1) for the study. The dogs in CG1 were diagnosed with conditions such as neoplasm, gastrointestinal disease, parasitism, bone and musculoskeletal disorders; poisoning, inflammatory diseases and other conditions. Female dogs diagnosed with neoplasia were further sub-grouped as control group 2 (CG2) for the purpose of statistical analysis where risk factors for CMT development among female dogs were calculated.

Statistical analysis

Fisher's exact test was used to determine the association between the occurrence of mammary gland tumours with age, breed and neuter status of the dogs. Logistic regression was performed on the variables that were associated with occurrence of CMT in dogs to ascertain if their association is really an influential one. Odds ratio was computed to determine the distribution of odds between categories of age, breed and neuter status for the occurrence of canine mammary gland tumours. Association is considered significant if $p < 0.05$ within a 95% confidence interval. Statistical analysis was performed using SPSS, version 20 (IBM Corporation, U.S. 1989, 2011).

RESULTS

Distribution of Canine Mammary Gland Tumours

All female dog cases diagnosed at histopathology laboratory (n=233) were included in this study with the exception of few (n=14) missing data for the selected parameters of interest. The number of CMT cases and dogs in the control groups are presented in Figure 1.
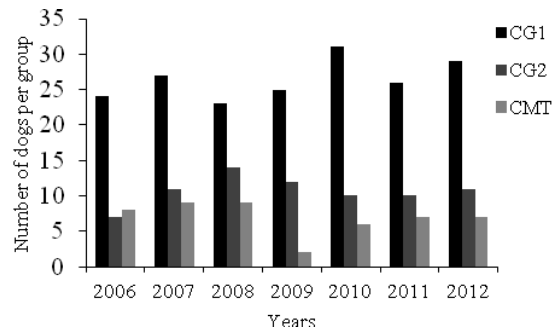

Figure 1. Canine mammary gland tumour cases and control groups 1 and 2 (CG1 and CG2) cases recorded per year over the period of study

Forty-eight CMT were available for evaluation. A total of 185 female dogs were included into CG1, out of which 75 female dogs diagnosed with neoplasia were used again as CG2. The years 2007 and 2011 have the highest number of CMT cases per year with 9 cases each, while 2009 has the lowest number of CMT cases (2) recorded. The average number of cases of CMT recorded per year is 6.8 cases per year.
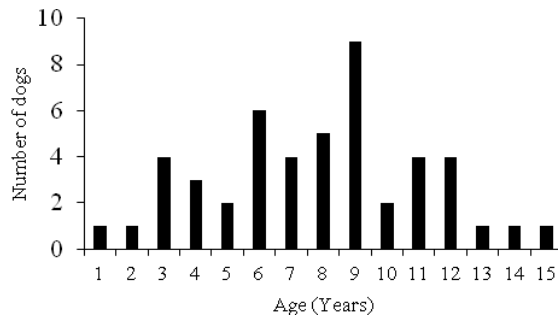

Figure 2. Age distribution of dogs diagnosed with mammary gland tumours

The very young dogs (less than 4 years of age) and very old dogs (more than 13 years of age) are the groups with least number of affected dogs, while dogs between 7 and 13 years of age have the highest number of dogs affected with CMT.

Forty-eight female dogs diagnosed with CMT qualified as cases, forty of which were submitted from the Universiti Veterinary Hospital (UVH) in UPM while eight were submitted by other local veterinary clinics in Malaysia through the Veterinary Laboratory Services Unit (VLSU). Biopsy samples were submitted for diagnosis in 46 of the cases, while 2 were diagnosed on post mortem.

The prevalence of canine mammary gland tumours in female dogs in this study is 39.0%. Overall, 89.6% (n=43) CMT were observed in intact females while the remaining 10.4% (n=5) were spayed. The mean age of occurrence of mammary gland tumour was 8.66 years (SD = ± 3.13), the median was 9 years. The age ranged from 2 to 16 years. A total of 12.5% of the dogs (n=6) aged less than 5 years and 87.5% (n=42) above five years (Figure 2).

Table 1. Summary of associations between selected parameters with the occurrence of CMT

Host factor	Comparison with all other female dogs diagnosed at the laboratory as control (CG1)			Comparison with female dogs diagnosed with other types of neoplasia as control (CG2)		
	Cases	Control	*p*-value	Cases	Control	*p*-value
Breed size						
Small	36	143	**0.568**	35	58	**0.517**
Large	12	42		13	17	
Breed pedigree						
Pure breed	40	133	**0.138**	40	48	**0.025**
Local/mix	8	52		8	27	
Age						
Young	5	48	**0.032**	5	11	**0.590**
Old	43	137	**Logistic regression: 0.012**	43	64	
Neuter Status						
Spayed	5	53	**0.009**	5	32	**0.00034**
Intact	43	132	**Logistic regression: 0.003**	43	43	**Logistic regression: 0.00042**

When cases were compared with control group 1, significant association (chi-square and logistic regression) was observed between old age (> 5 years) and spayed neuter status with occurrence of mammary tumours in the dogs. When cases were compared with control group 2, significant association was observed between breed pedigree (chi-square) and spayed neuter status (chi-square and logistic regression) with the occurrence of mammary gland tumours

Pure breed made up 83% (n=40) of the cases, comprising Terriers (n=6), Cocker spaniel (n=5), three each of Beagles, Doberman, Rottweiler and Shi Tzu. Two dogs each of German shepherd, Labrador, Spitz and Daschaund. Other breeds (Boxer, Pekingese, Chihuahua, Poodle, Pomeranian, Pit bull, Miniature Pinscher, Great dane and Tibetan spaniel) had a representative each. Sixteen percent of the dogs (n=8) were local/mixed breeds.

Other breeds (Boxer, Pekingese, Chihuahua, Poodle, Pomeranian, Pit Bull, Miniature pinscher, Great Dane and Tibetan Spaniel) had a representative each. Sixteen percent of the dogs (*n*=8) were local/mixed breeds.. A total of 25.0% of the dogs (*n*=12) were large-sized (dogs categorised as large and giant based on specific breed characteristics) while 75.0% (*n*=36) were small-sized (miniature, small and medium). Thirty-nine (81.3%) of the tumours were diagnosed as adenocarcinoma, 8.3% (*n*=4) each, were the diagnoses for squamous cell carcinoma and mixed cell tumour. Adenoma had 2.1% (*n*=1). Majority, whereby forty (83.3%) of the mammary gland tumours were equal to or greater than 3cm in diameter, while six (12.5%) were recorded less than 3cm. The sizes of two tumours (4.0%) were not available in the records. In 70.8% (*n*=34) of the tumours involve a single gland, while 29.2% (*n*=14) of them with multiple glands involvement. The most frequent gland affected is the inguinal mammary gland (49.3%, *n*=35) while the least frequent affected gland is cranial thoracic mammary gland (2.7%, *n*=2).

Influence of host factors on occurrence of CMT

For breed categorisation base on pedigree, this study found some level of protection against CMT for local/mix compared to pure breeds. Odds of 1.955 (CI 95%, 0.858 – 4.456) for CMT in pure breeds were seen for cases compared to all other cases as control (CG 1), and odds of 2.813 (CI 95%, 1.151 – 6.874) for CMT in pure breeds compared to local/mixed were seen for cases compared to other cancer cases as control (CG2) (Table 1). For breed categorization base on size, slightly higher odds for CMT were seen for small breeds. Odds of 1.265 (CI 95%, 0.613 – 2.607) for CMT were observed for small breeds compared to large breeds for cases compared to all other cases as control, (CG 1) and odds of 1.370 (CI 95%, 0.590 – 3.182) for CMT in small breeds for cases compared to other cancer cases as control (CG 2) (Table 1). The odds of developing CMT in intact bitches were 3.45 (CI 95%, 1.297 – 9.195) compared to spayed bitches, for cases compared to all other cases as control (CG1) and odds of 6.418 (CI 95%, 2.668 – 18.119) for CMT in intact bitches compared to spayed bitches in cases compared to other cancer cases as control (Table 1). Young age in dogs (less than 5 years) confers a significant level of protection from CMT, as older dogs (5 years or more than 5 years) had odds of 3.013 (CI 95%, 0.128 – 8.050) for having CMT compared to young dogs in cases compared to all other cases (CG1) and odds of 1.477 (CI 95%, 0.480 – 4.555) for CMT in older dogs compared to young dogs in cases compared to other cancer cases as control (CG 2) (Table 1).

DISCUSSION

This study reports for the first time a retrospective evaluation of signalment and histopathology diagnosis of CMT diagnosed at a veterinary histopathology laboratory in Malaysia. The prevalence of 39.0% in this study is low compared to the other reports (Andrade *et al*., 2010; Zuccari *et al*., 2011). This low prevalence could be due to the fact that some of the dogs brought to the UVH (where majority of the samples were submitted from) already had signs of distant metastasis and the owners will not allow for any form of intervention or euthanasia to be done. In

some other cases, the dogs are taken home to allow for natural death based on the dog owner's request. The prevalence reported here does not represent the true population in Malaysia but only pet dogs that most likely lived within the Klang Valley and small percentage from other states in Peninsular Malaysia. Besides that not all dogs diagnosed with mammary gland tumour on diagnostic cytology will undergo treatment intervention and few may be euthanized if tumours are extensive and for this the tumours from these dogs will not be submitted for histopathology diagnosis and can affect the true prevalence reported in this study.

The median age of 9 years observed in this study is consistent with other reports (Perez Alenza *et al*., 2000; Sarli *et al*., 2002; Rasotto *et al*., 2012). The development of mammary gland tumours in young dogs is not very common, as this study observed only 5 dogs below the age of 5 years. Tumour development in young female dogs (below 2 years) has been described as rare occurrence (Zatloukal *et al*., 2005). An increase in incidence from 3 cases among the 6 year old dogs, to 6 cases among the 7 year old dogs was observed. 10 years old dogs have the highest representation in our cases, with 17.6% (*n*=9) which is consistent with the peak age of occurrence reported by previous works (Zatloukal *et al*., 2005). A sharp decline in incidence was observed after 13 years, with a drop from 4 cases to 1 case each at 14, 15 and 16 years. The higher odds ratio of CMT for older dogs observed in this study is in accordance to published reports (Taylor *et al*., 1976; Sonnenschein *et al*., 1991; Zatloukal *et al*., 2005).

The prolonged diestrous phase in the canine reproductive cycle enhances the repeated and prolonged exposure of ovarian hormones (oestrogen) and especially the progesterone and hence pose a risk for mammary gland tumour development (Rehm *et al*., 2007). Spaying of bitches before 1 year of age has a protective effect on the dogs against mammary gland tumour development (Sonnenschein *et al*., 1991). In the results of this study, intact females have odds more than 6 times the odds of spayed bitches for developing mammary gland tumours. According to two other reports, spaying at less than 2 and the half years allows for advantage to prevent development of mammary gland tumour (Schneider *et al*., 1969; Sorenmo *et al*., 2000). Spaying of bitches after 4 years of age has no effect against CMT development or behaviour of the tumour (Taylor *et al*., 1976). Overall, exposure of female dogs to ovarian hormones early in life significantly contributes to CMT development in the dogs, and the risk is proportional to the duration of the exposure in the young dogs.

Canine mammary gland tumour studies comparing pure and mixed breed (Perez Alenza *et al*., 2000; Philibert *et al*., 2003) and breed sizes (Itoh *et al*., 2005) have been previously described. Predominance of small breed dogs for tumour development has already been reported (Itoh *et al*., 2005). Pure breed dogs are reported to have a higher incidence of CMT compared to mixed breeds. The results of this study showed a significant difference in the occurrence of CMT between pure breed dogs and local/mix breeds and that 84.3% of the dogs were pure breed with highest frequency in terriers (11.7%) the same

as reported by (Sontas *et al*., 2011), followed by cocker spaniels (9.8%). An over representation of pure breeds (75.7%) compared to mixed breeds was previously reported, although no significant associations was noted (Philibert *et al*., 2003). This could be explained to be due to genetic variability that is minimal in the pure breed dogs, allowing the inheritance of genetic mutations across generations, as opposed to the mixed/local dogs that have higher genetic variability. Furthermore, pure breed dogs such as German shepherd, Doberman, Daschaund and Boxer in this order were reported by the Malaysian Kennel Association as the commonest dog breeds that Malaysians keep as pets (Wong *et al*., 1985), and could explain why the dogs with CMT in this study were predominantly pure breeds. Large breeds have a higher incidence of CMT in other dog populations with over representation of German shepherd dogs (29.3%) (Srivastava *et al*., 2009) and (36.5%) (Dhami *et al*., 2010). In this study, there was a slight increase in the odds of CMT development in small breed dogs compared to large breed dogs, although the association was not significant.

In this study, 29.2% of the cases have multiple mammary gland involvement which is less than a report on 37.0% multiple gland involvement (Mitchell *et al*., 1974). Multiple glands involvement may indicate tumours that are highly invasive and likely to have lymph node or distant metastasis. Inguinal mammary gland has been involved in 50.7% of our cases. It has been reported to be the most affected gland in CMT (Mitchell *et al*., 1974; Taylor *et al*., 1976). It has been suggested that high involvement of the inguinal gland is due to its larger mammary tissue mass and it remains active after the other glands have stopped functioning (Mitchell *et al*., 1974). Majority of the tumours were large and this can be speculated that the awareness level for early detection of tumours when they are smaller in size is not there among Malaysians. Another reason could be that the tumours are malignant and grow rapidly upon first detection. Most of the tumours in this study were diagnosed malignant, hence it could be explained why majority of the tumours are large. In several other reports, the size was noted as one of the prognostic factor, where small sized tumours tend to be more benign than malignant and large tumours are associated with reduced survival time upon diagnosis (Perez Alenza *et al*., 2000; Sorenmo *et al*., 2011). According to the World Health Organization staging of tumours in domestic animals, tumours that are 3 cm or more are placed on tumour stage 2 (T_2) and tumour stage 3 (T_3) for clinical staging of CMT. Staging information is not entirely available for all the dogs in this study because most of the submission was only the primary tumour but not including the regional lymph nodes.

The most common histopathology subtype revealed in this study is adenocarcinoma. An incidence of adenocarcinoma ranging from 31.7 – 54.0% has been reported in several other studies (Philibert *et al*., 2003; Itoh *et al*., 2005). The incidence of 81.3% in our study is more than the reported incidence, and shows that adenocarcinoma is the most common CMT affecting dogs in this population. The possible explanation for this scenario is that most of the tumours that are ressected and presented to the histopathology laboratory are those truly

needing surgical intervention as compared to reduced possibility for local veterinarians to recommend resection of mammary masses diagnosed as hyperplasia on diagnostic cytology or masses that do not cause a significant problem to the dog in terms of cutaneous ulceration, varying degree of discharges from the affected ducts and pain to the dog. Having said that, one dog in this study was diagnosed with adenoma and although it is only a single case in this study, this histopathology variant is commonly reported in humans and studies on CMT elsewhere (Goldschmidt *et al.*, 2011).

There are several limitations to this study, including i) study population only represents the dogs that are diagnosed on histopathology at a veterinary based laboratory; ii) the dogs presented could be biased to a subpopulation of dogs with owners that are willing to subject their dogs for surgical intervention and histopathology and may have missed others where pet owners may decline further diagnosis; iii) the prevalence is not a true figure because the baseline control populations are biased for dogs that died or euthanised with severe diseases which may not ideally represent a good control population for comparison and further statistical analysis. Ideally, it is recommended that dogs that are presented to the veterinary practices and with confirmed diagnosis on histopathology and or diagnostic cytology could be useful for such epidemiological studies. However, this study has given a good overview of the histopathology subtypes and clinical characteristics of the dogs that developed mammary gland tumours in a subset of population of dogs in Malaysia, where most of the findings are consistent with previous reports in other geographical regions.

CONCLUSION

Overall, a conclusion can be assigned to this study where intact, small-sized and pure breed, with older than 5 years of age have independently increased odds of CMT development. The CMT in Malaysia may represent a comparable population for further studies on diagnostic or prognostic molecular markers and investigations on new therapeutic intervention which can be generalised to the canine populations worldwide.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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