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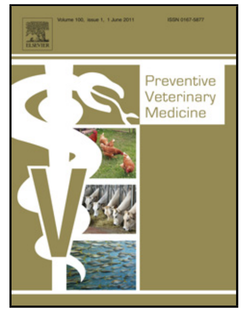

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Evaluation of intrinsic and extrinsic risk factors for dog visceral hemangiosarcoma: a retrospective case-control study register-based in Lazio region, Italy

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Highlights

- Individual and environmental risk factors were investigated for dog hemangiosarcoma
- This case-control study is based on data from Animal Tumour Registry, Italy
- The main risk factors for canine hemangiosarcoma were age and breed
- Neutered male and female dogs exhibited higher risk than the intact females

Abstract

The genetic and breed susceptibility of visceral hemangiosarcoma in dogs has been studied, but there is no evidence of environmental risk factors as reported in human medicine. We conducted a case-control study in which the sampling population was the list of canine oncology cases of the Animal Tumour Registry of Lazio region, Italy (2009-2017). We defined cases as dogs with visceral hemangiosarcoma and controls as dogs affected by another neoplasm. The ratio between controls and cases was 3:1. Analysed variables were: age, weight, sex, reproductive status, size, breed, nutrition habit, living environment and location of the house. We performed a preliminary univariate analysis to select potential risk factors (p-value < 0.2) then entered in a forward stepwise logistic regression model. Ninety-three cases enrolled in the study were compared with 279 controls. The multivariable logistic regression identified age, reproductive status and breed as significant risk factors. Results showed an increasing risk with increasing age for age classes 6-10 and > 10 years old (OR= 9.69, 95% CI: 1.21 – 77.62; OR= 14.01, 95% CI: 1.65 – 119.03). Neutered animals (male and female) were at greater risk compared to intact ones. The breeds at greatest risk were German shepherd (OR= 4.17, 95% CI: 1.25 – 13.86) and mixed breed (OR= 3.50, 95% CI: 1.44 – 8.51). The last finding could be explained by the genetic origin of the animals, which may include German shepherd or another possible breed at risk. No other individual or environmental variables were identified as risk factors. The findings of this work indicate that genetic predisposition is the key element in HSA development.

Keywords: Hemangiosarcoma; Cancer registry; Dog; Case-control study; One Health;

Abbreviations: HSA, hemangiosarcoma; IZSLT, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M.Aleandri; ICD-O, International Classification of Diseases for Oncology; ENCI, Ente Nazionale Cinofilia

Italiana –(Italian Kennel Club); GRA, Grande Raccordo Anulare; ROC, Receiver operating characteristic;

Introduction

The study of cancer epidemiology in canine populations may help in understanding determinants that contribute to tumour development. Better understanding of behavioural and environmental risk factors that exhibit correlation with spontaneous tumours will contribute to the improvement of oncology prevention strategies oriented to human and animal health, in the framework of the "*One health*" concept. Numerous tumours are similar in humans and animals and, through advancements in comparative oncology, it has been established that the dog is a suitable model for investigations on the aetiology and the pathogenesis of several neoplastic diseases (Rowell, et al., 2011; Pinho, et al., 2012). Hemangiosarcoma (HSA) is a malignant and aggressive vascular tumour that occurs spontaneously as a primary tumour in both animals and humans. In dogs, the most common primary sites for HSA are the visceral organs, most notably the spleen and the heart (usually involving the right atrium and auricle), but also the liver and other organs (Hammond and Pesillo-Crosby, 2008; Yamamoto, et al., 2013; Schiffman and Breen, 2015), including the subcutaneous tissue (Dobson, 2013). Visceral HSA represents approximately 5% of all non-cutaneous primary malignant neoplasms and from 12% to 21% of all mesenchymal neoplasms in dogs (Thamm, 2013). In other species, such as cats, horses, and large animals, visceral HSA is rarely described (Brown, et al., 1985; Linder, 2017). In human medicine, this neoplasm is one of the most rarely reported sarcoma subtypes, representing less than 1% of all sarcomas (Fayette, et al., 2007; AIRTUM, 2017). Clinical and histopathological characteristics of human cases are similar to those reported in dogs and the diagnosis, like in veterinary medicine, is often an incidental finding. In dogs, visceral HSA is commonly only detected during the post-mortem examination because of its unspecific clinical signs. In fact, these tumours can be clinically silent and can remain undetected until the vascular masses rupture. The consequent internal bleeding can lead the animal to death (Schiffman and Breen, 2015). The genetic and breed susceptibilities are reported in the literature as risk factors (Schultheiss, 2004; Hammond and Pesillo-Crosby, 2008; Dobson, 2013; Schiffman and Breen, 2015). There is no evidence of specific environmental factors that contribute to development of visceral HSA in veterinary medicine (Linder, 2017). Conversely, in human medicine there is a documented association between HSA and exposure to environmental and iatrogenic factors such as thorium dioxide (Th232), polyvinyl chloride and arsenic (Cogliano, et al., 2011; AIRTUM, 2017; Mundt, et al., 2017). During the period 2009-2017 there was a slight increase in the number of visceral HSA cases diagnosed by the pathology laboratory of Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M.Aleandri" (IZSLT), accounting for 20% of all malignant tumour cases detected at necropsy. The purpose of this study was to investigate the possible risk factors, both intrinsic and extrinsic, involved in visceral HSA development in dogs living in the Lazio region (Italy).

Material and methods

Study design

We conducted a retrospective registry-based case–control study. The sampling population was the list of canine oncology cases recorded at the

Animal Tumour Registry of the Lazio region (Carnio, et al., 2019). During the nine-year study period of 2009-2017, 2882 histopathological oncological diagnoses from 2607 dogs were found, including a wide interpatient and tumour variability. Diagnosis and classification of samples, derived either from tissues submitted as biopsy to the laboratory for histopathological examination or from carcasses sent to IZSLT for generic post-mortem examination, was performed by the IZSLT histopathological laboratory. In the registry, a record number is assigned to each dog (oncological case). From all the records, cases and controls were enrolled. The following criteria were used to select cases:

- Dog having a diagnosis of visceral HSA (ICD-O 3 9120) verified by histopathological examination and classified according to the International Classification of Diseases for Oncology (ICD-O 3) (Fritz, et al., 2013),
- Residence in Lazio region,
- Registration form for suspected neoplasm (Figure 1) collecting the following information: dog ID number (microchip or tattoo), breed, sex, reproductive status, age, size, weight, general condition, nutrition habit, living environment and habitat (described forward).

Inclusion criteria for controls were the same of the cases selection except for the diagnosis, which was a neoplastic condition other than visceral or cutaneous HSA (Table 1). All the records that fit the above criteria were sorted by date and then selected by systematic sampling. The first record was selected by drawing a random number between 0 and 10. The following controls were chosen systematically, using an interval calculated by dividing the total amount of records for the total number of controls to select, resulting in 10-record intervals.

Statistical analysis

The size of the overall sample (cases plus controls) was based on a control/case ratio of 3:1 and allowed to achieve a 90% statistical power. The analysis was performed using WINPEPI statistical package (Abramson, 2004). Nine variables were analysed and considered as potential risk factors: age, weight and a set of categorical variables: sex, reproductive status (intact or spayed/neutered), size (small, medium and large), breed (based on the Italian Kennel Club, known as ENCI), nutrition habit, living environment (indoor, if sharing the family home; outdoor, if spending the majority of the time outside in the yard; indoor and outdoor, if spending about the same time inside and outside; shelter) and location of the house (named "habitat": rural and urban). All the information listed above were reported by the veterinary practitioner in the registration form at the time of the sample submission to the laboratory. In order to avoid misinterpretation, we verified the correspondence of the size declared on the form with the breed standard according to the ENCI. The breed was individually reported when it occurred more than once in the case group, while the other breeds were grouped into a single category defined "Other breeds" and mixed breed dogs were in a separate category. Age and weight were described as continuous variables and then categorized in three classes for the statistical analysis. An additional risk factor analysed in this paper was the residency inside or outside the "Grande Raccordo Anulare" (GRA). The GRA is a 68 km long ring-shaped motorway that encircles the city of Rome and it potentially contains the most polluted territory area of the city. The place of residence was gathered by plotting the coordinates of the recorded address in a GIS project.

Data were summarised for each group using mean and standard deviation for continuous variables and using frequency tables for all other categorical variables. A preliminary univariate analysis of all factors was performed by two-way t test and chi-square test. Factors with a p-value of < 0.2 in univariate analysis were entered into a forward stepwise logistic regression model in order to evaluate the strength of associations between the occurrence of visceral HSA and each potential risk factor, controlling for confounding. After verification of the nonlinearity of the variable age and the logit of the outcome, we decided to classify this variable in three classes, corresponding to young (≤ 5 years old), adult (6-10 years old) and old (> 10 years old) animals. Because of collinearity between age and weight, we excluded the latter variable from the model. ORs with 95% confidence intervals (CIs) that did not include 1.00 and p values <0.05 were considered significant in the logistic model. The model fitness was assessed using the Hosmer-Lemeshow test and by calculating the area under the Receiver operating characteristic (ROC) curve (Hosmer and Lemeshow, 2000). The analyses were performed using software Stata 12.0 (StataCorp, College Station, TX).

Results

A total of 372 dogs met the inclusion criteria and were enrolled in the study, comprising 93 cases and 279 controls. Characteristics of the two groups are summarized in Table 2. The most frequent anatomical localisations of visceral HSA were spleen (45/93) and heart (30/93), frequently affected simultaneously. The mean age was 10 years for the case group (min 4; max 17) and 9 years for control group (min <1; max 19). The age (p = 0.004) and weight (p = 0.025) were significantly associated with the outcome. The reproductive status was also significantly associated with the presence of visceral HSA ($p = 0.005$). In the univariate analysis, in addition to age, weight and reproductive status, the breed and the size were identified as risk factors. The German shepherd (OR= 3.68, 95% CI: 1.27-10.70) and the big/medium sizes were the highest risk categories (OR= 2.47, 95% CI: 1.44 – 4.22). The neutered females showed a higher risk than the intact ones (OR= 5.89, 95% CI: 1.68 – 20.68). In the multivariable logistic regression analysis, the increasing class of age was associated with increasing risk. Dogs of 6-10 and >10 years old were at greater risk than younger ones (OR= 9.69, 95% CI: 1.21-77.62; OR= 14.01, 95% CI: 1.65-119.03). The reproductive status, the size, and the breed were confirmed as risk factors. German shepherd breed (OR= 4.17, 95% CI: 1.25 – 13.86) was strongly associated with the outcome, but also mixed breed showed an increase in risk (OR= 3.50, 95% CI: 1.44 – 8.51). The size, intended as genetic feature, was confirmed as a risk factor and the medium size was the category at greatest risk (OR= 4.18, 95% CI: 1.57 – 11.10).

No other individual or environmental variables resulted in increased risk. The ORs, 95% confidence intervals and p-values generated in the analysis are reported in Table 3. The Hosmer-Lemeshow test evidenced a fitness with a probability value of $p = 0.5493$ and the area under ROC curve (0.8027) indicated a good predictive ability.

Discussion

This is the first comprehensive analysis that has focused on the risk factors for canine visceral HSA in Italy. The influence of several individual and environmental variables on the risk for HSA were investigated. Since the controls were chosen among the other oncological cases (benign and malignant neoplasms) diagnosed by the same laboratory, we are confident that the two groups (cases and controls) belong to the same source population. Moreover, there are many different diagnoses of tumour in the control group, signifying a large variety of possible causes. In the present study, four significant risk factors stood out: age, reproductive status, size, and breed. We found no differences between cases and controls due to other studied factors such as nutrition habits and environmental characteristics.

The mean age in the case group was 10 years, which is similar to the age observed in other HSA studies (Schultheiss, 2004; Yamamoto, et al., 2013; Kim, et al., 2015), and the mean weight was 27.3 kg which is similar to that observed by Sherwoods et al. (2016), in which dogs over 27.8 kg were significantly more likely to be diagnosed with HSA. The body condition score was not recorded, so no information related to obesity was available. We found a higher risk for medium size dogs in agreement with O'Byrne and Hosgood (2019) study. The breed susceptibility for HSA has already been demonstrated, in particular for German shepherd, Golden retriever, Labrador retriever and Boxer (Schultheiss, 2004; Dobson, 2013; Yamamoto, et al., 2013; Kim, et al., 2015). In the present study, high susceptibility was observed for the breed of German shepherd only. Boxer and Labrador did not result at higher risk compared to the other breeds, however this could be due to their low frequency in our sample. We also observed an apparent higher risk in mixed breed, as was previously reported by Ware and Hoper (1999). This ambiguous finding may be explained by their genetic origin. In the cases group, many mixed breed dogs could be related to German shepherd dog, as this breed and its crosses are common in the region. Heritable factors could mould gene expression phenotypes and consequently biological behaviour of the tumour. The higher incidence of

HSA in Golden retrievers reported in the USA by other Authors (Schultheiss, 2004; Dobson, 2013), not yet reported from Europe, could be explained by the genetic variability among the same breed in far geographic areas (Quignon, et al., 2007). However, the low frequency of Golden retriever breed in this study did not allow a definitive conclusion about the risk of this breed. Regarding the variables of sex and reproductive status, males and neutered females showed a higher risk than intact females. These results agreed with previous studies in which a higher prevalence of HSA is reported in males (Clifford, et al., 2000; Schultheiss, 2004; Yamamoto, et al., 2013) and in spayed female dogs (Ware and Hoper, 1999; Gruntzig, et al., 2016). One possible explanation is that neutering is supposed to increase life expectancy and thus also the probability of developing HSA.

Overweight and obesity, determined by diet and nutrition habits, have been associated with several kinds of tumours in humans and dogs. In the present study, however, no differences related to nutrition habits were observed. Possible reasons for this are that the diet categories are not sufficiently representative because of the numerous possible combinations and lack of other details about food quantity and quality. Exposure to certain chemical elements has been associated with HSA in humans (Cogliano, et al., 2011; AIRTUM, 2017; Mundt, et al., 2017), while in dogs the influence of environmental factors on HSA development has been hypothesized but is still not well documented (Linder, 2017). We found no influence of the living habitat on HSA occurrence. A possible explanation of this could be the difference in etiopathogenic mechanisms between dogs and humans or the influence of specific environmental factors that were not investigated in detail in this work. In humans, angiosarcoma may be an occupational disease while canine HSA occurs in absence of exposure to those substances and the incidence is much higher. Other tumours in dogs are recognized to be related to the exposure to living environmental factors, for example polluted air causing primary lung cancer (Bettini, et al., 2010), environmental pollution increasing the risk for non-Hodgkin's lymphoma (Zanini, et al., 2013) or dioxin-like PCB family pollutants for breast cancer (Severe, et al., 2015). In the present study, the variable residence "inside or outside the GRA" was not significant, this could suggest no influence of environmental pollution on the HSA incidence. This result should be interpreted with caution, as there are also densely populated and industrialised areas, as well as 2 airports, outside the GRA.

The present study could be affected by a potential selection bias due to a heterogenous distribution of dog breeds among socioeconomic groups, and possible differences in propensity of their owners to seek laboratory diagnoses. However, since dogs were selected from the same source population, this bias could have affected both case and control groups in the same way, so it may not have any impact on the risk estimation. Moreover, breed distribution in the region is influenced by fashion trends and dog use (shepherds, watchdogs) so many breeds could be underrepresented. Therefore, we were only able to study breed susceptibility risk for a few breeds. Another potential source of bias is recall bias in the documentation of variables such as breed and size by veterinary staff.

Conclusions

In this study, age, breed, and neutered status indicated the greatest predisposition for visceral HSA in dogs, with older dogs, German shepherd and mixed breed, and male and female neutered dogs exhibiting a greater susceptibility. These results agree with what was previously described by several authors. Animal variables addressed in this study may contribute to a better understanding of the risk profile for developing visceral HSA according to dog characteristics and living conditions, which in turn can contribute to disease prevention and interventions. The findings of this work indicate that genetic predisposition is the key element in HSA development.

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Declarations of interest: none

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ANIMAL CANCER REGISTRY **Registration number: ___________**

Referring veterinarian: Dr.________________ **ASL** **Date at the sampling: __/__/____**

Animal Holder information

First name **Last name** **Tax id.number (fiscal code)**

Street address **N°** **Phone**

District **Locality** **City** **Postcode** **Province**

Animal information

Species **Breed** **Sex** O male O female O intact O neutered/ spayed

Date of birth **Name** **Microchip/Tattoo**

Size O small O medium O large **Weight**

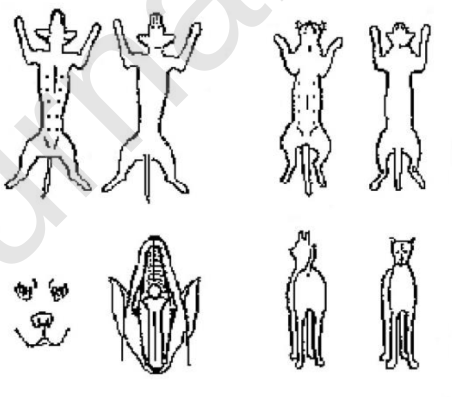
Body condition O optimal O normal O poor **Nutrition** O home-made O canned food O dry food

Living environment O indoor O outdoor O shelter O stray **Habitat** O urban O rural

Sample O Biopsy O surgery O necropsy Tumor O primary tumor O relapse O metastasis

Previous tumor O yes O not **Previous tumor info (data and diagnosis)** ______________________

Organ	Topography	First detection (date)	Dimensions (cm)	Submitted
--------------------	--	--------------------------	--------------------------	O total O portion
--------------------	--	--------------------------	--------------------------	O total O portion
--------------------	--	--------------------------	--------------------------	O total O portion



Vet's medical observation (i.e. past/recent disease; therapy)

Histological diagnosis (if performed by another laboratory)

Figure 1 - Registration form for the submission of biopsy or carcass with suspected of neoplasm

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Table 1 – Frequency of the control group diagnosis grouped according to the ICD-O 3rd edition.

Diagnosis group	Benign	Malignant	Total

Epithelial tumours ICD-O 8010-8587, ICD-O 9050-9058	38	102	140
Gonadal tumours ICD-O 8610-8670	9	8	17
Lymphoid tumours ICD-O 9590-9960	1	44	45
Melanoma ICD-O 8720-8730	5	7	12
Mesenchymal tumours ICD-O 8680-8711; ICD-O 8800-9040; ICD-O 9120-9150; ICD-O 9321; ICD-O 9587	34	26	60
Skeletal tumours ICD-O 9180-9262	0	5	5
Total tumour diagnosis	*87*	*192*	*279*

Table 2 – Descriptive and association analysis of cases (dogs with visceral HSA) and controls (dog with other tumours)

Factor	Cases (n=93)	Control (n=279)	p-value
Age (years)			**0.004**
Mean	10	9	
Std Dev	2.65	3.45	
Weight (Kg)			**0.025**
Mean	27.3	22.2	
Std Dev	12.27	13.84	
Sex (female, male)			0.218
Female	47	163	
Male	45	116	
Not reported	1	0	
Sex, reproductive status			**0.005**
Female			
Sexually intact	3	68	
Spayed	20	77	
Male			
Sexually intact	20	73	
Neutered	10	26	
Not reported	40	35	
Size			**0.000**
Small	23	126	
Medium	53	89	
Large	16	64	
Not reported	1	0	
Breed			**0.000**
Boxer	8	12	
German shepherd	20	24	
Labrador retriever	6	9	
Mixed breed	44	110	
Other breeds	14	124	
Not reported	1	0	
Nutrition habit			0.765
Homemade food	8	29	
Canned food	2	27	
Dry food	25	115	
Homemade and dry food	4	21	

Homemade, canned, and dry food	3	14	
Homemade and canned food	1	7	
Canned and dry food	4	28	
Not reported	46	38	
Living environment			0.161
Indoor	43	151	
Outdoor	29	70	
Shelter	3	24	
Indoor and outdoor	14	33	
Not reported	4	1	
Habitat			0.817
Rural	15	43	
Urban	76	235	
Not reported	2	1	
GRA			0.902
Inside	36	110	
Outside	57	169	

Std Dev: standard deviation; p-value calculated with t test (age and weight) and chi-square test (all other variables)

Table 3 – Multivariable logistic regression model analysis comparing cases group and controls group

Factor	Odds Ratio (OR)	p-value	95% CIs
Age			
≤ 5 years old (Ref)			
6-10 years old	**9.69**	0.032	**1.21 – 77.62**
> 10 years old	**14.01**	0.016	**1.65 – 119.03**
Sex, reproductive status			
Female, Sexually intact (Ref)			
Spayed	**3.91**	0.043	**1.04 – 14.68**
Male, Sexually intact	**3.95**	0.041	**1.05 – 14.79**
Neutered	**5.88**	0.016	**1.38 – 25.01**
Size			
Small (Ref)			
Medium	**4.18**	0.004	**1.57 – 11.10**
Large	2.01	0.149	0.77 – 5.20
Breed			
Other breeds (Ref)			
Boxer	4.00	0.068	0.90 – 17.78
German shepherd	**4.17**	0.020	**1.25 – 13.86**
Labrador retriever	1.82	0.533	0.27 – 12.03
Mixed breed	**3.50**	0.006	**1.44 – 8.51**

Ref: reference category