

Risk Factors Associated with Development of Histiocytic Sarcoma in Bernese Mountain Dogs

A. Ruple and P.S. Morley

Background: Histiocytic sarcoma (HS) is a rare but aggressive malignancy in humans that is poorly responsive to existing treatments. Although rare in most breeds of dogs, HS is common in Bernese mountain dogs (BMDs).

Objective: Determine risk factors associated with development of HS in BMD.

Animals: A total of 216 BMD were registered with the Berner-Garde Foundation.

Methods: An internet-based cross-sectional survey was used to collect information from owners of BMD diagnosed with HS and owners of disease-free littermates of dogs with HS. Mixed-effects logistic regression (MELR) and conditional logistic regression (CLR) were used in parallel to examine associations between potential risk factors and the occurrence of HS.

Results: When controlling for litter as a marker of relatedness, dogs diagnosed with orthopedic conditions were found to be more likely to develop HS (MELR, OR: 2.5, 95% CI: 1.5, 5.2; CLR, OR: 2.81, 95% CI: 1.1, 7.3), whereas dogs receiving prescription anti-inflammatory medications were found to be at considerably lower risk of developing HS (MELR, OR: 0.42, 95% CI: 0.2, 0.8; CLR, OR: 0.32, 95% CI: 0.1, 0.8).

Conclusions and Clinical Importance: These results suggest inflammation may be a modifiable risk factor for the development of HS in BMD.

Key words: Canine spontaneous tumor model; Comparative oncology; Epidemiology; Malignant histiocytosis.

Histiocytic sarcoma (HS) is a rare but aggressive cancer associated with high mortality in both dogs and humans.^{1–11} Tumors are comprised of mononuclear phagocytic cells; the pathology, histology, and immunodiagnostic features of histiocytic disorders of dogs recently have been reviewed.¹² In humans, the rarity of HS coupled with a high case fatality rate results in few opportunities to study this disease.^{2,13} Although uncommonly diagnosed in the general dog population, there is a strong predisposition for HS to occur in a few breeds of dogs.^{3–5,8,11,14} One breed in particular, the Bernese Mountain Dog (BMD), has been shown to be considerably more likely to be affected than dogs of other breeds, and reports have suggested that the lifetime risk may be up to 25% of the breed population.^{11,13} It has been estimated that BMD are 225 times more likely to develop HS than other breeds of dogs⁸ and are 17 times more likely to die as a consequence of the tumor than other breeds of dogs.¹⁵ The heritable predisposition of BMD to development of HS originally was thought to

Abbreviations:

BGF	Berner-Garde Foundation
BMD	Bernese mountain dog
CI	confidence interval
CLR	conditional logistic regression
HS	histiocytic sarcoma
MELR	mixed-effects logistic regression
OR	odds ratio
SD	standard deviation

be a result of polygenic effects,¹⁶ but more recently has been linked to abnormalities associated with the CDKN2A/B gene region.^{11,13,14} Abnormalities in the same gene region in humans encoding for p16 have been associated with several cancers, including HS.^{17,18}

The Berner-Garde Foundation (BGF) was established with the goal of decreasing the burden of HS and other genetic diseases in the BMD population. To aid in these efforts, the BGF maintains a breed-specific database comprised of data submitted by BMD owners or collected from public sources of information such as the Canine Eye Registration Foundation and the Orthopedic Foundation for Animals.¹⁹ The BGF database tracks lineage information, and all dogs included in this database are assigned a litter number, which is used to identify siblings. In addition, the BGF database is used to collect information on the health of individual dogs. Health information reported by owners must be verified by submission of supporting documentation from veterinarians and other health experts, such as histopathology reports, before the diagnosis is included in the database.²⁰ The database is freely accessible to the public, and BMD breeders are encouraged to investigate health status of dogs and their ancestors when making breeding decisions. Despite high awareness among owners and breeders and the availability of data regarding the occurrence of HS, the number of HS cases diagnosed in BMD appears to have increased steadily

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during the past 2 decades.¹¹ Over this same time period, almost all research regarding the occurrence of HS in BMD has focused on the heritability of the disease and the search for genes that might be responsible for the breed predisposition. This information clearly is valuable for owners of BMD and may be used to decrease the risk through genetic selection, but it cannot be used to alter the risk of HS in existing dogs. No published research is available regarding the influence of environmental or health-related factors on the occurrence of HS in BMD. Therefore, the purpose of our study was to investigate risk factors for the development of HS in BMD while accounting for the familial (genetic) effects within the study population.

Materials and Methods

Overview

Owners of BMD were recruited by means of the BGF breed registry. Participating owners were asked to provide information using a web-based survey on signalment, diet and other exposures, and medical history, including the occurrence of HS. Logistic regression was used to investigate potential risk factors for HS using 2 different statistical methods.

Study Population

Owners of BMD were invited to participate in this study indirectly through articles and advertisements placed in breed-specific newsletters and directly (via phone or email) when contact information was available in the BGF database. Study participation was voluntary, and no incentives for participation were offered. To be considered eligible for study participation, dogs were required to be registered in the BGF database. Cases were defined as histologically confirmed diagnoses of HS (supporting documentation was submitted to BGF) that had been made within 5 years before completion of the survey. The comparison group consisted of disease-free littermates of cases. The BGF litter number was used as a unique identifier of siblings born in the same litter.

Owner Survey

A survey instrument was designed to elicit data necessary to evaluate associations between potential risk factors and the outcome of HS. The survey was divided into 3 sections: demographics, health history, and environmental exposures. Demographic questions collected information on sex and neuter status, geographic location, date of birth, and BGF litter number. Health history questions collected information on diagnoses of medical conditions (orthopedic conditions, tick-borne diseases, and chronic conditions), prior surgeries, preventive treatments for fleas, ticks, or heartworm, vaccination status, body condition score, and treatment with prescription medications for ≥ 6 months, nutritional supplements (nonprescription supplements given by the owner with the intent to prevent illness or injury), or nonprescription medications given by the owner with the intent to treat an illness or injury for a period of >6 months. Questions about environmental exposures collected information on the type of food most often consumed by the dog (eg, commercial dry food, commercial canned food, or homemade diet), the feeding frequency for dogs, exposure to lawn or other chemicals, exposure to cigarette smoke, rural environments, and frequency of exposure to outdoor sources of water (irrigation ditches or canals, lakes, or streams). The final survey instrument^a consisted of 35 questions and was administered

by use of online survey software.^b The recruitment period was open for 18 consecutive months during which time the survey was accessible to owners interested in participating.

Data Analysis

The survey responses were transferred into a computer database;^c incomplete and duplicate responses were removed. Study eligibility was verified by ensuring that documentation of histopathologic diagnosis of HS was available for at least 1 member of each litter in the BGF database. Study results were summarized by calculating descriptive statistics. Frequency distributions of categorical variables were evaluated. Continuous variables were analyzed by calculating means, medians, standard deviation (SD), and ranges and were categorized to facilitate regression analysis.

Mixed-effects logistic regression (MELR) and conditional logistic regression (CLR) were used in parallel to examine associations between potential risk factors (exposure variables) and the occurrence of HS by statistical software.^d The mixed-effects logistic model used data from all dogs for which owners had submitted surveys. The study included all dogs for which surveys were completed, but both HS cases and noncases were not available for all litters. The BGF litter number was modeled as a random effect to account for potential clustering among litters in the mixed-effects models. CLR models only included dogs from litters that had data for both an HS case and a noncase within the same litter (ie, cases modeled as matched pairs with noncase siblings).

Exposure variables that were included in both of the modeling approaches (Table 1) were age, sex, neuter status, geographic location, diagnosis of any orthopedic condition (y/n), any reported surgical procedure other than spay or neuter (y/n), reported diagnosis of a tick-borne disease (y/n), long-term (≥ 6 months) treatment with veterinarian-prescribed medications, long-term (≥ 6 months) treatment with nonprescription medications given by the owner as a treatment for a disease or condition, long-term (≥ 6 months) treatment with nutritional supplements given by the owner to prevent occurrence of a disease or condition, diagnosis of any diseases other than HS, treatment with flea, tick, or heartworm preventive treatments (frequency was recorded as never, monthly for the entire year, monthly for part of the year, or sporadically), primary type of food (commercially prepared canned or dry, or home prepared), feeding frequency (free choice, once, twice, or 3 or more times per day), exposure to lawn chemicals used either to prevent weed growth or exposure to other chemicals (paints, solvents, lubricants, or other), amount of time spent in rural environments, exposure to cigarette smoke (never, occasionally, monthly, or daily), and exposure to irrigation ditches or canals (y/n), or lakes or streams (y/n). For each modeling method, univariable models were used to screen individual exposures. Variables that were statistically associated with the outcomes in initial screening ($P \leq .25$) were included in multivariable model building. Final multivariable models were identified by use of a backward selection procedure with a critical α for retention of 0.05. Previously excluded variables were reintroduced to the final model to ensure that the exclusion was appropriate and to evaluate confounding effects (identified by $\geq 20\%$ change in variable estimates). First-order interaction terms for main-effects variables included in final models were evaluated. Subject-specific odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by the results of the logistic regression models. As a sensitivity analysis, stratified analyses were conducted based on the presence or absence of diagnosis with an orthopedic condition, and Pearson's Chi-squared tests were utilized to better understand the relationship between long-term treatment with medications and the outcome of HS. In addition, a mixed-effects logistic regression model (outcome = HS) was constructed using data only from dogs within the study

Table 1. Characteristics of the 216 Bernese Mountain Dogs enrolled in the study (n [%]).

Characteristic	Category	All Dogs (n = 216)	Dogs with HS (n = 135)	Dogs Without HS (n = 81)
Demographics				
Age	<5	7 (3.2)	3 (2.2)	4 (4.9)
	5–7	86 (39.8)	60 (44.4)	26 (32.1)
	8–10	97 (44.9)	58 (43.0)	39 (48.1)
	>10	19 (4.6)	10 (7.4)	9 (11.1)
Gender	Male	28 (13.0)	21 (15.6)	7 (8.6)
	Male castrated	77 (35.7)	44 (32.6)	33 (40.7)
	Female	25 (11.6)	14 (10.4)	11 (13.6)
	Female spayed	84 (38.9)	55 (40.7)	29 (35.8)
Geographic region	Northeast US	50 (23.2)	30 (22.2)	20 (24.7)
	Midwestern US	30 (13.9)	21 (15.6)	9 (11.1)
	Southern US	25 (11.6)	16 (11.9)	9 (11.1)
	Western US	77 (35.7)	48 (35.6)	29 (35.8)
	Canada	30 (13.9)	18 (13.3)	12 (14.8)
	Europe	4 (1.9)	2 (1.5)	2 (2.5)
Medical history				
Orthopedic condition	Yes	57 (26.4)	43 (31.9)	14 (17.3)
	No	158 (73.2)	92 (68.2)	66 (81.5)
Any surgical procedure	Yes	126 (58.3)	79 (58.5)	47 (58.0)
	No	88 (40.7)	56 (41.5)	32 (39.5)
Tick-borne disease	Yes	26 (12.0)	15 (11.1)	11 (13.6)
	No	189 (87.5)	119 (88.2)	70 (86.4)
Flea preventative	Yes	143 (66.2)	93 (68.9)	50 (61.7)
	No	65 (30.1)	39 (28.9)	26 (32.1)
Tick preventative	Yes	129 (59.7)	83 (61.5)	46 (56.8)
	No	83 (38.4)	50 (37.0)	33 (40.7)
Heartworm preventative	Yes	157 (72.7)	105 (77.8)	52 (64.2)
	No	57 (26.4)	29 (21.5)	28 (34.6)
Vaccinated	Yes	213 (98.6)	134 (99.3)	79 (97.5)
	No	3 (1.4)	1 (0.7)	2 (2.5)
Other serious illness	Yes	72 (33.3)	40 (29.6)	32 (39.5)
	No	142 (65.7)	93 (68.9)	49 (60.5)
Long-term medications	Yes	73 (33.8)	40 (29.6)	33 (40.7)
	No	143 (66.2)	95 (70.2)	48 (59.3)
Homeopathic treatments	Yes	67 (31.0)	48 (35.6)	19 (23.5)
	No	148 (68.5)	86 (63.7)	62 (76.5)
Weight within normal range	Yes	190 (88.0)	114 (84.4)	76 (93.8)
	No	26 (12.0)	21 (15.6)	5 (6.2)
Environmental exposures				
Type of food	Commercial dry	160 (74.1)	101 (74.8)	59 (72.8)
	Cooked meat	9 (4.2)	6 (4.4)	3 (3.7)
	Raw meat	36 (16.7)	24 (17.8)	12 (14.8)
	Other	11 (5.1)	4 (3.0)	7 (8.6)
Feeding frequency	Once a day	11 (5.1)	5 (3.7)	6 (7.4)
	Twice a day	189 (87.5)	122 (90.4)	67 (82.7)
	Three times a day	8 (3.7)	5 (3.7)	3 (3.7)
	Free choice	7 (3.2)	2 (1.5)	5 (6.2)
Lawn chemicals	Yes	122 (56.5)	57 (42.2)	37 (45.7)
	No	94 (43.5)	78 (57.8)	44 (54.3)
Other chemicals	Yes	74 (34.3)	41 (30.4)	33 (40.7)
	No	137 (63.4)	92 (68.2)	45 (55.6)
Rural	Yes	188 (87.0)	114 (84.4)	74 (91.4)
	No	27 (12.5)	20 (14.8)	7 (8.6)
Cigarette smoke	Yes	44 (20.4)	26 (19.3)	18 (22.2)
	No	172 (79.6)	109 (80.7)	63 (77.8)
Irrigation ditches/canals	Yes	11 (5.1)	5 (3.7)	6 (7.4)
	No	204 (94.4)	130 (96.3)	74 (91.4)
Lakes or streams	Yes	99 (45.8)	62 (45.9)	37 (45.7)
	No	116 (53.7)	73 (54.1)	43 (53.1)

population that were free from diagnosis with an orthopedic disease. The independent variable included in this model was long-term treatment with prescription medications, and the BGF litter

number was modeled as a random effect to account for potential clustering among litters. A subject-specific OR and 95% CI were calculated using the results of this logistic regression model.

Results

Characteristics of Study Population

The majority of owners who elected to participate responded to direct contact by phone or email rather than indirect methods (eg, articles and advertisements placed in breed-specific newsletters). Four-hundred-ninety surveys were initiated by the online survey software, but 274 (55.9%) of those surveys were excluded from the analyses (Fig 1). Data were collected for a total of 216 eligible BMD representing 140 different litters (Table 1). Mean \pm SD age of dogs was 7.7 ± 2.0 years (median, 8 years; range, 2–13 years). The population was evenly distributed between males and females and the majority of the population, regardless of sex, was neutered (76%). Owners classified most dogs enrolled in the study as being of normal weight (88.0%) and without clinically relevant illness (65.7%). However, more than half of the dogs (58.3%) included in the study had undergone some type of surgical procedure other than neutering. One-third (73/216) of the study population had used medications for ≥ 6 months, and the most frequently reported (81.6%) types were anti-inflammatory drugs (eg, prednisone, carprofen, meloxicam) and supplements given for treatment and prevention of joint disease (eg, polysulfated glycosaminoglycan, glucosamine/chondroitin combination). Environmental exposure variables reported with the highest frequency were exposure to rural environments (87.0%) and exposure to lawn chemicals (56.5%).

Risk Factors for HS—Mixed-Effects Logistic Model

Exposure variables that were included in multivariable modeling in mixed-effects models were diagnosis of an orthopedic condition, whether heartworm preventative had been used, vaccination for rabies, history of other clinically relevant illnesses, long-term treatment with prescribed medications, treatment with nutritional supplements, treatment with nonprescription medications, weight outside of the normal range (high or low), exposure to rural environments, exposure to chemicals

other than lawn chemicals, and exposure to irrigation ditches or canals. Variables retained in the final multivariable model were diagnosis of an orthopedic condition, treatment with heartworm preventatives, long-term treatment with prescription medications, and treatment with nonprescription medications (Table 2). Interaction terms for main effects were not significant when included in the model. Individual dogs were 2.5 times more likely (OR: 2.49; 95% CI: 1.21, 5.14) to develop HS if they were diagnosed with an orthopedic condition than if they had not received such a diagnosis. Dogs also were at >2 times increased risk of developing HS if they were given nonprescription medications (OR: 2.10; 95% CI: 1.05, 4.18) or heartworm preventatives (OR: 2.11; 95% CI: 1.10, 4.05). Dogs also had >2 -fold decrease (OR: 0.43; 95% CI: 0.22, 0.81) in risk of developing HS when they were given prescription medications long term (≥ 6 months) as compared with their risk when they had not received prescription medications.

Risk Factors for HS—Conditional Logistic Model

Exposure variables included in multivariable modeling by CLR were diagnosis of an orthopedic condition, history of other clinically relevant illnesses, long-term treatment of prescribed medications, treatment with nutritional supplements, treatment with nonprescription medications, weight being outside of the normal range (high or low), age of the dog, and frequency with which the dog was fed. Variables retained in the final multivariable model were diagnosis of an orthopedic condition, history of other clinically relevant illnesses, long-term treatment with prescription medications, and treatment with nonprescription medication (Table 2). Interaction terms for main effects were not significant when included in the model. In this model, dogs diagnosed with an orthopedic condition were nearly 3 times more likely (OR: 2.81; 95% CI: 1.08, 7.26) to develop HS than they would have been had they not developed an orthopedic condition. Treatment with nonprescription medications also was associated with an increased

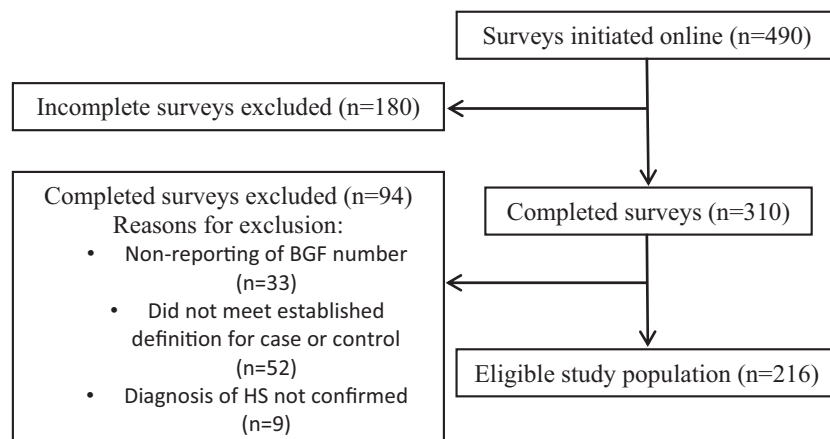


Fig 1. Survey responses from time of initiation online to determination of eligibility.

Table 2. Results of logistic regression models examining risk factors associated with development of histiocytic sarcoma in Bernese Mountain Dogs.

Risk Factor	Category	All Dogs, n = 216 (Mixed-Effects Model)			Matched Siblings, n = 118 (Conditional Logistic Model)		
		OR	95% CI	P-value	OR	95% CI	P-value
Orthopedic condition	Yes	2.49	1.21, 5.14	0.014	2.81	1.08, 7.26	.034
	No		Reference			Reference	
Nonprescription medications	Yes	2.10	1.05, 4.18	0.036	2.88	1.04, 7.90	.041
	No		Reference			Reference	
Treatment with heartworm preventative	Yes	2.11	1.10, 4.05	0.024		Not Significant	
	No		Reference				
Long-term prescription medication treatment	Yes	0.43	0.22, 0.81	0.010	0.32	0.12, 0.83	.020
	No		Reference			Reference	
Diagnosis with another illness	Yes		Not Significant		0.38	0.15, 0.93	0.035
	No					Reference	

risk of developing HS (OR: 2.88; 95% CI: 1.04, 7.90) in individual dogs. However, dogs receiving long-term (≥ 6 months) medications had a >3 -fold reduction in risk (OR: 0.32; 95% CI: 0.12, 0.83) and dogs diagnosed with an illness other than HS had a greater than >2 -fold reduction in risk (OR: 0.38; 95% CI: 0.15, 0.93) associated with diagnosis of HS as compared to their risk had they not received medications.

Risk Factors for HS—Stratified Analyses

When the study population was stratified based on presence or absence of diagnosis with an orthopedic disease, long-term treatment with medications only remained significantly associated (P -value = .038) with the outcome of HS in the population of dogs not diagnosed with an orthopedic condition. Among dogs without an orthopedic condition, dogs receiving long-term (≥ 6 months) medications had a > 2 -fold reduction in risk (OR: 0.48; 95% CI: 0.24, 0.96) associated with diagnosis of HS as compared to their risk had they not received medications.

Discussion

The concurrent findings of an increased risk of developing HS in association with the occurrence of an orthopedic disease and decreased risk of HS in dogs that had a history of long-term treatments that were predominantly anti-inflammatory and joint support medications suggest there may be an association between chronic inflammatory conditions and the occurrence of HS in BMD. Both modeling approaches identified similar relationships with the outcome of HS in BMD, which strengthens conclusions that might be drawn from our study. The relationship between orthopedic conditions and HS has been examined previously and it was reported that BMD were >5 times more likely to develop peri-articular HS in a previously diseased joint than a dog with no prior diagnosis of joint disease.²¹ To the authors' knowledge, however, associations between treatment with prescription anti-inflammatory medications and a decreased risk of developing

HS have not been reported previously in either dogs or humans.

The association between inflammation and cancer is well documented in humans and it is estimated that approximately 1 in 4 human cancers worldwide is associated with chronic inflammation.^{22–27} Associations also have been made between inflammation and the occurrence of multiple cancers in dogs including osteosarcoma, lymphoma, transitional cell carcinoma, mesothelioma, squamous cell carcinoma, and myxosarcoma.^{26–31} Both exposure variables reported here, diagnosis with an orthopedic condition and treatment with anti-inflammatory medications, could be considered surrogate indicators for inflammation, but direct measurement of inflammatory processes would not have been possible given the study design and retrospective nature of data collection. However, the findings of our study are consistent with associations that have been reported between inflammatory disorders and cancer in both humans and dogs,^{20–29} and thus support the hypothesis that chronic inflammation is associated with the occurrence of HS in BMD.

The use of a cross-sectional survey instrument was beneficial in that we were able to examine several exposure variables simultaneously, but this type of study design is not very useful for detecting associations between rare exposures and the outcome of disease. It is possible therefore that an exposure variable that was not statistically associated with the outcome of HS in BMD in this study is, in fact, biologically related to disease occurrence. Also, because information pertaining to both exposure and disease status was collected simultaneously, a temporal sequence was not established and it is possible that exposures associated with the outcome of HS actually may have occurred after the disease was detected. Another potential concern is that data collected in this study were reported by individual dog owners and interpretation regarding exposures could be biased (eg, recall bias, misclassification of frequency of exposures). There is no way to assess the effects of these potential biases within this study and, if present, they may have resulted in biased study results. However, we are confident that dogs classified as cases were

accurately diagnosed as a consequence of the pathologic confirmation of all HS diagnoses through BGF. Although it is possible misclassification of noncases could have occurred, we believe this is unlikely because of the severity of the disease when present and the fact that death caused by other causes also was confirmed by pathology reports submitted to BGF.

According to the Oxford Centre for Evidence-Based Medicine, results from studies such as ours are considered mid-level in the hierarchy of the likely best evidence produced by different study designs.³² Consequently, additional research is needed to substantiate our findings. Ideally, a large-scale prospective study design similar to the design utilized by the Morris Animal Foundation's Golden Retriever Lifetime Study³³ would be employed to examine a large population of BMD over time while collecting data on both exposures and outcomes throughout the study period. This design would allow for a temporal sequence to be established and, if the study population was large enough, may allow for distinctions to be made among different types and dosages of anti-inflammatory and joint support medications.

Footnotes

^a Copies of the survey are available from the corresponding author on request.

^b Surveygizmo 2.6, Widgix, LLC, Boulder, CO

^c Microsoft Excel, 2007 Redmond, WA, USA

^d STATA, release 11, College Station, TX

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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