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Association of Gallbladder Mucocele Histologic Diagnosis with Selected Drug Use in Dogs: A Matched Case-Control Study

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Background: The cause of gallbladder mucocele (GBM) formation in dogs currently is unknown. Many available drugs represent a newer generation of xenobiotics that may predispose dogs to GBM formation.

Objective: To determine if there is an association between the histologic diagnosis of GBM in dogs and administration of selected drugs.

Animals: Eighty-one dogs with a histologic diagnosis of GBM and 162 breed, age, and admission date-matched control dogs from a single referral institution.

Methods: Medical records of dogs with GBM and control dogs from 2001 to 2011 were reviewed. Owner verification of drug history was sought by a standard questionnaire. Reported use of heartworm, flea, and tick preventatives as well as nonsteroidal anti-inflammatory drugs, analgesics, corticosteroids, or medications for treatment of osteoarthritis was recorded.

Results: Dogs with GBM were 2.2 times as likely to have had reported use of thyroxine (as a proxy for the diagnosis of hypothyroidism) as control dogs (95% confidence interval [CI], 0.949–5.051), 3.6 times as likely to have had reported treatment for Cushing's disease (95% CI, 1.228–10.612), and 2.3 times as likely to have had reported use of products containing imidacloprid (95% CI, 1.094–4.723). Analysis of a data subset containing only Shetland sheepdogs (23 GBM and 46 control) indicated that Shetland sheepdogs with GBM formation were 9.3 times as likely to have had reported use of imidacloprid as were control Shetland sheepdogs (95% CI, 1.103–78.239).

Conclusions and Clinical Importance: This study provides evidence for an association between selected drug use and GBM formation in dogs. A larger epidemiologic study of Shetland sheepdogs with GBM formation and exposure to imidacloprid is warranted.

Key words: Bile; Canine; Mucus; Xenobiotic.

Gallbladder mucocele formation is a unique and emergent disease syndrome of dogs characterized by an insidious accumulation of thick, immobile, and viscous bile and mucus within the gallbladder. The syndrome was reported as a rare postmortem finding before 10 years ago and has emerged as one of the most commonly recognized causes of gallbladder disease in the dog.[1–10] The extent to which diagnosis of GBM formation can be attributed to increased use of abdominal ultrasonography in dogs is unknown. The disease afflicts older dogs of many different breeds but seems more common in Shetland sheepdogs,[3,10] Cocker spaniels,[9,10] Pomeranians,[10] Miniature Schnauzers,[10] and Chihuahuas.[10] A GBM typically is diagnosed in symptomatic dogs at the time of abdominal ultrasonography to investigate clinical signs of gastrointestinal illness that often are secondary to presumable gallbladder pain, gallbladder rupture, or common bile duct obstruction. Surgical removal of the gallbladder carries a good long-term prognosis for some dogs. However, surgery often is associated with a high mortality rate, with a median 2-week mortality of 27% (range, 7–45%).[1–4,8,9,11]

Several predisposing factors for GBM formation in dogs have been identified or are suspected such as concurrent hypothyroidism or hyperadrenocorticism,[6] hyperlipidemia,[3,10] and poor gallbladder motility.[12] However, the underlying cause of GBM formation essentially is unknown. The purpose of this study was to investigate for any association between the histologic diagnosis of GBM formation and reported use of a preselected group of drugs using a matched case-control retrospective review of medical records. We focused on drugs used for flea and tick infestation, heartworm prophylaxis, and degenerative joint disease because many represent a newer generation of foreign substances (xenobiotics) the widespread use of which could coincide with the increased diagnosis of GBM formation. Moreover, pharmacogenomic differences in drug metabolism or

Abbreviations:

ABC	ATP-binding cassette
CI	confidence interval
GBM	gallbladder mucocele
nAChR	nicotinic acetylcholine receptor
NSAID	nonsteroidal anti-inflammatory drug
OR	odds ratio
PO	per os

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biliary excretion could plausibly explain species or anatomic (gallbladder) predilections for GBM formation.

Materials and Methods

Dogs with Gallbladder Mucocele

Dogs diagnosed with a GBM between December 7, 2001 and November 26, 2011 were identified by an electronic search of the medical records, radiology information system, and histopathology database of North Carolina State University Veterinary Hospital (NCSU-VH) using the keyword mucocele. From November 27, 2011 to August 14, 2015 additional Shetland sheepdogs were identified for inclusion in the study to increase sample size and statistical power for analysis of this specific breed. For each dog identified with the condition, the paper medical records were examined. Selection criteria for dogs with GBM formation were based on a gold standard of light microscopic histologic diagnosis. Diagnosis of GBM formation was based on the presence of a large viscous accumulation of mucus that filled and distended the gallbladder lumen with formation by the gallbladder mucosa of long, thin and branching fronds of well-differentiated gallbladder epithelial cells containing modestly distended clear cytoplasm filled with mucus and supported by a scant amount of submucosa that extended into the mucus. Our decision to include only dogs having a histologic diagnosis of GBM formation was made to remove any ambiguity in defining inclusion criteria based on gallbladder ultrasonographic appearance alone.

Control Dogs

To increase the precision of the study, 2 matched control dogs were selected for each dog diagnosed with a GBM considering breed, age, and date of admission. A search initially was performed to identify all hospital accessions of any dog corresponding in breed to a dog diagnosed with a GBM over the retrospective study interval. The identified accessions were further prioritized where possible to those electronically coded as having been charged for an abdominal ultrasound examination. Where available, the abdominal ultrasonographic report was reviewed to confirm lack of GBM formation based on the absence of written documentation identifying the presence of immobile, nongravity-dependent, echogenic gallbladder content with or without a finely striated or stellate pattern and a hypoechoic peripheral rim. Dogs were not excluded based on the presence of gravity-dependent gallbladder sludge or diagnosis of nonmucocele gallbladder disease (eg, cholelithiasis, cholecystitis). To select control dogs having a similar clinical presentation to dogs diagnosed with a GBM, accessions were prioritized where possible to those electronically coded for an abdominal or nonthoracic soft tissue surgical procedure. Control dogs then were categorized by presenting problem or primary diagnosis as surgical or nonsurgical patients followed by primary organ system involvement. Because GBM formation has a breed predilection for Shetland sheepdogs, is reportedly a disease of older dogs, and is suspected to be increasing in incidence, we chose to control for these variables by selecting control dogs on the basis of matching breed and corresponding in age and year of hospital accession as closely as was possible to the dog diagnosed with a GBM. Control dogs were not matched by sex based on the lack of evidence of an association with GBM formation.[1,3,4,6]

Data Collection through Record Review and Owner Questionnaire

Each medical record was reviewed for documentation identifying the use and brand name of heartworm, flea, and tick preventatives. Also recorded was any history of PO administration in the preceding year of a minimum 2-month duration of medication used for the management of chronic pain including any nonsteroidal anti-inflammatory (NSAID) drug, any PO non-NSAID analgesic drug, corticosteroid, or medication used for adjunctive treatment of osteoarthritis. To determine the ability of our study to confirm results of a previous study that identified increased odds of GBM diagnosis in dogs with hypothyroidism or hyperadrenocorticism,[6] we also recorded medication histories specific for treatment of hypothyroidism, hyperadrenocorticism, or diabetes mellitus as proxies for prior diagnosis of these endocrinopathies.

Administration of the aforementioned drugs and current treatment of hyperadrenocorticism, hypothyroidism, or diabetes mellitus were obtained (if missing from the medical record) or verified against the medical record where possible based on results obtained from a questionnaire (Appendix S1). The questionnaire was sent by standard mail to owners of all GBM and control dogs included in the study. The questionnaire was accompanied by an introductory letter to the owner explaining the purpose of the study and inviting the owner to complete the questionnaire using a prepaid return addressed envelope. Each letter was customized making reference to the dog's specific name, sex, and date of diagnosis of GBM or date of admission to the NCSU-VH for control dogs. Letters that were sent to owners of dogs that were known to have died specifically acknowledged their loss. Similarly, for control dogs letters provided the first name, age, and matching breed identity of the dog diagnosed with GBM. Owners of all dogs were specifically asked to provide information pertaining only to medications administered before diagnosis of GBM or the provided date of admission to the NCSU-VH in the case of the control dogs.

Data Analysis

Medication data were organized by drug application into 5 categories: (1) flea and tick preventatives, (2) heartworm prophylaxis, (3) NSAIDs, (4) PO non-NSAID analgesics, and (5) drugs for treatment of osteoarthritis. For each drug category, dogs were divided into 3 groups. The "no documentation of use" group included those dogs in which use of a drug in the category was not noted in the medical record and the owner questionnaire was not returned for verification of nonuse. The "documented nonuse" group included dogs for which use of a drug in the category was not noted in the medical record and the owner questionnaire verified nonuse. The "documented use" group included those dogs with positive documentation of a specific drug use in the medical record or via completed owner questionnaire. If the owner reported use of a drug that differed in identity from that recorded in the medical record, both drugs were recorded as being administered. For each drug category, individual medications were grouped by active ingredient in accordance with the product label and further subdivided by brand name.

Only dogs within the "documented nonuse" and "documented use" groups were used in further statistical analysis. Drugs for which there was information for $\leq$6 dogs were not included in the statistical analysis. First, we obtained basic descriptive statistics for all variables collected. The number of observations available for statistics varied because of "no documentation of use" or missing observations. Conditional logistic regression was used to identify univariate associations between GBM formation and use of any of the above-mentioned medications. Odds ratios (OR) and the 95% CI for the OR were estimated. Variables were selected for inclusion in possible multivariate models using the Wald's test with a liberal P value of $\leq$.10. A backward modeling algorithm was proposed to test interaction terms using the Wald's test at P value $\leq$.05. An OR is defined as a measure of likelihood with defined magnitude and direction of association. In this study, the

OR is used to compare the relative odds of occurrence of GBM formation given exposure to selected drugs while considering cases matched to controls on the basis of breed, age, and admission date. An OR equal to 1 indicates there is no association between the outcome of interest and putative variables (medications in this case). The 95% CI for the OR is expected not to cross 1. SAS software[a] was used for statistical analysis.

Results

Gallbladder Mucocele Dogs

Spanning the 10-year time interval from 2001 to 2011, 76 dogs were identified at the NCSU-VH as having a histologically confirmed diagnosis of GBM (Figure S1). The dogs represented 29 different breeds and ranged in age from 4.0 to 15.2 years (median, 10.3 years). There were 33 spayed females, 37 castrated males, 3 intact females, and 3 intact males. Relative to the general hospital population, Shetland sheepdogs, Cocker spaniels, and Chihuahuas were significantly overrepresented among all breeds of dog having a histologic diagnosis of gallbladder mucocele formation over the study interval ($P = .024$). The Labrador retriever was significantly underrepresented among breeds of dog for which a histologic diagnosis of GBM formation was observed ($P < .001$; Table 1). From November 27, 2011 to August 14, 2015, an additional 5 Shetland sheepdogs were identified for inclusion in the study. Histologic diagnosis of GBM formation was based on tissue obtained by means of surgical cholecystectomy in 64 dogs and at necropsy in 17 dogs. Seventy-eight (96%) dogs also had concurrent medical record documentation of an abdominal ultrasound diagnosis of a GBM within 24 hours before surgical cholecystectomy or necropsy.

Control Dogs

One-hundred and sixty-two breed-matched control dogs were identified using a 2-to-1 ratio (control-to-case). Control dogs ranged in age from 2.8 to 16.5 years (median, 10.4 years). There were 77 spayed females, 70 castrated males, 5 intact females, and 10 intact males. The age at hospital admission of control dogs compared to the age at diagnosis of GBM formation in case dogs differed by an average of 12.2 ± 10.4 months (range, 0–4 years). Only 21 control dogs (13%) differed in age by >2 years at hospital admission compared to their matching GBM dog. The date of hospital admission of control dogs compared to the date of diagnosis of GBM formation in case dogs differed by an average of 6.8 ± 8.0 months (range, 0 months to 6.3 years). Only 5 control dogs (3.1%) differed in date of hospital admission by >2 years compared to their matching GBM dog. The chief complaint or principal diagnosis of control dogs at the time of admission were categorized as surgical in 63 dogs (39%) and nonsurgical in the remainder (Table S1). All but 1 control dog were documented to have undergone a full abdominal ultrasound examination in which absence of a GBM was confirmed. The remaining dog was determined to not have a GBM at the time of necropsy. The time between control dog hospital admission and performance of the ultrasound examination in these dogs ranged from 0 to 45 days with a median of 0 days.

Response to Questionnaire

Forty-five (61%) owners of dogs in the GBM group and 63 (44%) owners of dogs in the control group completed and returned the written questionnaire. A larger number of owners of dogs with a GBM completed and returned the questionnaire compared to the owners of control dogs (χ^2 $P = .025$). Among the responders, additional information that was not recorded in the original medical record was obtained for 5 (11%) GBM dogs and 37 (59%) control dogs. Only 5 owners of dogs with histologic diagnosis of GBM formation and 11 control dog owners reported use of a medication that differed in identity to a medication recorded in the medical record. Six (7%) questionnaires to owners of GBM dogs and 18 (11%) control dog questionnaires were returned as undeliverable.

Reported Use of Selected Drugs

A broad range of brand name products were reported as administered to the dogs included in the study. Because many of the products were represented in scant numbers, each product was categorized on the basis of

Table 1. Breeds of dog significantly overrepresented among all breeds of dog having a histologic diagnosis of gallbladder mucocele (2001–2011).

Breed	# Dogs GBM Over Study Interval	# Total Dogs Over Study Interval	%	Odds Ratio	95% CI (OR)	χ^2 *P* Value
Shetland sheepdog	18	543	3.31	14.288	8.370–24.391	<.001
Cocker spaniel	10	1,366	0.73	2.615	1.342–5.093	.007
Chihuahua	7	915	0.76	2.666	1.222–5.814	.024
Labrador retriever	3	5,737	0.05	0.137	0.0432–0.435	<.001

Other breeds diagnosed with GBM during the study period: Bichon Frise, Golden retriever, Pomeranian (3 each); Beagle, Dachshund, Jack Russell Terrier, Miniature pinscher, mixed breed dog, Poodle, Pug, Schnauzer (2 each); American Eskimo, Australian cattle dog/heeler, Border Terrier, Brussels Griffon, Cairn Terrier, Collie, German Shepherd dog, Italian greyhound, Maltese, Scottish Terrier, Shih Tzu, terrier (1 each).

NCSU-VH, North Carolina State University Veterinary Hospital; GBM, gallbladder mucocele; OR, odds ratio; χ^2, Chi-square statistic.

active ingredient(s). Documented use of a specific flea/tick or heartworm preventative in the medical record or owner questionnaire response was reported for the majority of dogs in the study (Tables 2 and 3). No association was identified between GBM histologic diagnosis and reported use or documented nonuse of flea/tick or heartworm preventative (Table 4). However, among dogs with reported use of a flea/tick preventative, univariate conditional logistical regression results indicated that dogs with GBM histologic diagnosis were 2.274 times as likely to have a report of use of products containing imidacloprid (95% CI, 1.094–4.723; $P = .028$; Table 4). There was no association between GBM histologic diagnosis and reported use of heartworm preventatives containing either ivermectin or milbemycin.

Documented use of a specific NSAID in the medical record or owner questionnaire response was reported for approximately 25% of dogs in the study. Fewer numbers of dogs were reported to have received PO non-NSAID analgesic drugs (Tables 2 and 3). No association was identified between GBM histologic diagnosis and reported PO use or documented nonuse of NSAID or non-NSAID analgesic drugs (Table 4).

Documented use of a specific supplement for the treatment of osteoarthritis in the medical record or owner questionnaire response was reported for 27% of dogs with histologic diagnosis of GBM and for 22% of control dogs (Tables 2 and 3). No association was identified between GBM histologic diagnosis and reported use or documented nonuse of a supplement, or active ingredients contained therein, for treatment of osteoarthitis (Table 4).

Concurrent Endocrinopathy

Documented treatment for hyperadrenocorticism, hypothyroidism, or diabetes mellitus was reported in the medical record or owner questionnaire response in 14%, 17%, and 7% of dogs with histologic diagnosis of GBM, respectively (Table 3). Univariate conditional logistical regression results indicated that dogs with

Table 2. Descriptive summary of documented use or nonuse[a] of selected categories of drugs in dogs with histologic diagnosis of gallbladder mucocele and breed, age, and admission date-matched control dogs.

	All Dogs				Shetland Sheepdogs			
	GBM (N = 81)		Control (N = 162)		GBM (N = 23)		Control (N = 46)	
Category of Drug Use	N	%	N	%	N	%	N	%
Flea and tick preventative								
Documented use	60/81	74	104/162	64	18/23	78	29/46	63
Documented nonuse	5/81	6	11/162	7	2/23	9	5/46	11
Total dogs with documented data	65/81	80	115/162	71	20/23	87	34/46	74
Use of 1 preventative	49/65	75	85/115	74	13/20	65	24/34	71
Use of ≥2 preventatives	11/65	17	19/115	17	5/20	25	5/34	15
Heartworm preventative								
Documented use	64/81	79	121/162	75	17/23	74	35/46	76
Documented nonuse	2/81	2	0/162	0	2/23	9	0/46	0
Total dogs with documented data	66/81	81	121/162	75	19/23	83	35/46	76
Use of 1 preventative	58/66	88	101/121	83	15/19	79	24/35	69
Use of ≥2 preventatives	6/66	9	20/121	17	2/19	11	11/35	31
Nonsteroidal anti-inflammatory								
Documented use	23/81	28	42/162	26	6/23	26	12/46	26
Documented nonuse	29/81	36	41/162	25	10/23	43	15/46	33
Total dogs with documented data	52/81	64	83/162	51	16/23	70	27/46	59
Use of 1 NSAID	19/52	37	35/83	42	6/16	38	10/27	37
Use of ≥2 NSAIDs	4/52	8	7/83	8	0/16	0	2/27	7
Non-NSAID oral analgesic								
Documented use	12/81	15	20/162	12	4/23	17	5/46	11
Documented nonuse	35/81	43	54/162	33	10/23	43	20/46	43
Total dogs with documented data	47/81	58	74/162	46	14/23	61	25/46	54
Supplement(s) for osteoarthritis								
Documented use	22/81	27	35/162	22	6/23	26	13/46	28
Documented nonuse	31/81	38	45/162	28	9/23	39	13/46	28
Total dogs with documented data	53/81	65	80/162	49	15/23	65	26/46	57

NSAID, nonsteroidal anti-inflammatory drug.

[a]Table does not show concordance or discordance of information between matched pairs of individual gallbladder mucocele and control dogs (1:2 case-to-control ratio).

Table 3. Descriptive summary of documented use[a] of selected categories of drugs based on active ingredient in dogs with histologic diagnosis of gallbladder mucocele and breed, age, and admission date-matched control dogs.

	All Dogs				Shetland Sheepdogs			
	GBM (N = 81)		Control (N = 162)		GBM (N = 23)		Control (N = 46)	
Category of Documented Drug Use	N	%	N	%	N	%	N	%
Flea and tick preventative	60/81	74	104/162	64	18/23	78	29/46	63
Use of any imidacloprid	20/60	33	20/104	19	6/18	33	1/29	3
Use of any fipronil	47/60	78	81/104	78	14/18	78	24/29	83
Use of any (s)-methoprene	10/60	17	14/104	13	3/18	17	6/29	21
Use of any dinotefuran[b]	2/60	3	2/104	2	1/18	6	1/29	3
Use of other[b]	6/60	10	22/104	21	3/18	17	6/29	21
Heartworm preventative	64/81	79	121/162	75	17/23	74	35/46	76
Use of any ivermectin	29/64	45	68/121	56	8/17	47	14/35	40
Use of any milbemycin	40/64	63	64/121	53	11/17	65	29/35	83
Use of other[b]	1/64	2	9/121	7	0/17	0	1/35	3
Nonsteroidal anti-inflammatory	23/81	28	42/162	26	6/23	26	12/46	26
Carprofen	13/23	57	23/42	55	3/6	50	7/12	58
Meloxicam	6/23	26	5/42	12	1/6	17	1/12	8
Aspirin[b]	3/23	13	6/42	14	1/6	17	2/12	17
Piroxicam[b]	0/23	0	6/42	14	0/6	0	0/12	0
Deracoxib[b]	4/23	17	4/42	10	2/6	33	2/12	17
Cerecoxib[b]	1/23	4	2/42	5	1/6	17	0/12	0
Tepoxalin[b]	1/23	4	1/42	2	0/6	0	1/12	8
Firocoxib[b]	0/23	0	2/42	5	0/6	0	1/12	8
Etodolac[b]	0/23	0	1/42	2	0/6	0	0/12	0
Non-NSAID oral analgesic	12/81	15	20/162	12	4/23	17	5/46	11
Tramadol[b]	12/12	100	20/20	100	4/4	100	5/5	100
GABApentin[b]	1/12	8	2/20	10	0/4	0	1/5	20
Amantidine[b]	0/12	0	1/20	5	0/4	0	1/5	20
Supplement(s) for osteoarthritis	22/81	27	35/162	22	6/23	26	13/46	28
Use of any glucosamine	22/22	100	33/35	94	6/6	100	12/13	92
Use of any chondroitin sulfate	10/22	45	13/35	37	4/6	67	3/13	23
Use of any methylsulfonylmethane	14/22	64	20/35	57	4/6	67	5/13	38
Use of any hyaluronic acid[b]	2/22	9	1/35	3	1/6	17	1/13	8
Exogenous steroids	16/81	20	21/162	13	3/23	13	5/46	11
Treatment for endocrinopathy								
Hyperadrenocorticism	11/81	14	8/162	5	0/23	0	0/36	0
Hypothyroidism	14/81	17	15/162	9	6/23	26	6/36	17
Diabetes mellitus	6/81	7	4/162	2	1/23	4	0/36	0

NSAID, nonsteroidal anti-inflammatory drug.

[a]Table does not show concordance or discordance of information between matched pairs of individual gallbladder mucocele and control dogs (1:2 case-to-control ratio).

[b]Not included in univariate conditional logistic regression because of low number of observations in the total population.

histologic diagnosis of GBM were 2.2 times as likely to have reported treatment with thyroxine (as a proxy for a diagnosis of hypothyroidism) as control dogs (95% CI, 0.949–5.051; $P = .07$) and 3.6 times as likely to have a report of treatment for Cushing's disease (95% CI, 1.228–10.612; $P = .02$; Table 4).

Multivariate Conditional Logistic Regression

Imidacloprid, hypothyroidism, and hyperadrenocorticism were selected for inclusion in a multivariate conditional logistic regression model. Diabetes was not included in the models for lack of biologic evidence of an association with GBM formation and the low number of dogs identified with diabetes mellitus in the study. Interaction terms between imidacloprid and hypothyroidism (P value = .99) and imidacloprid and hyperadrenocorticism (P value = .11) were tested and were not statistically significant. The final main effects model results were as follows: imidacloprid OR = 2.2 (95% CI, 1.048–4.795; P value = .037), hypothyroidism OR = 2.5 (95% CI, 0.839–7.559; P value = .10), and hyperadrenocorticism OR = 2.1 (95% CI, 0.562–7.689; P value = .30).

Shetland Sheepdog-Specific Breed Associations

Among all dogs having a histologic diagnosis of GBM formation over the study interval, 23/81 (28.4%) were Shetland sheepdogs. We questioned whether the

Table 4. Univariate conditional logistic regression model associations (N = 243) representing the likelihood that dogs with histologic diagnosis of gallbladder mucocele formation received a specified drug compared to breed, age, and admission date-matched dogs that were demonstrated to not have a gallbladder mucocele[a].

Association	Observations Used in Conditional Logistic Regression Model	Odds Ratio	95% CI (OR)	Wald Chi-Square *P* Value
Use of flea or tick preventative	180	1.877	0.490–7.189	.36
Use of imidacloprid	180	2.274	1.094–4.723	.03
Use of fipronil	180	1.264	0.624–2.560	.52
Use of (s)-methoprene	180	1.144	0.476–2.749	.76
Use of heartworm preventative[b]	187	—	—	—
Use of ivermectin	187	0.593	0.298–1.178	.14
Use of milbemycin	187	1.215	0.621–2.380	.57
Use of NSAID anti-inflammatory	135	0.747	0.327–1.707	.49
Use of carprofen	135	1.037	0.413–2.604	.94
Use of meloxicam	135	3.312	0.662–16.571	.14
Use of non-NSAID analgesic	121	0.853	0.327–2.226	.75
Use of supplements for osteoarthritis	133	0.831	0.328–2.106	.70
Use of glucosamine	133	0.900	0.365–2.219	.82
Use of chondroitin sulfate	133	1.081	0.285–4.102	.91
Use of methylsulfonylmethane	133	0.768	0.299–1.976	.58
Exogenous steroid administration	243	1.641	0.805–3.344	.17
Treatment for endocrinopathy				
Hyperadrenocorticism	243	3.610	1.228–10.612	.02
Hypothyroidism	243	2.189	0.949–5.051	.07
Diabetes mellitus	243	3.562	0.875–14.493	.08

[a]Data represent a 1:2 case-to-control match between dogs with and without diagnosis of gallbladder mucocele formation.
[b]Too few dogs had confirmation of nonuse of heartworm prevention for statistical analysis.

association between histologic diagnosis of GBM formation and use of imidacloprid could be ascribed to the Shetland sheepdogs in the study. Because cases and controls were matched by breed they could not be unmatched to determine if breed had an effect on the association. Therefore, 2 data subsets were analyzed considering all Shetland sheepdogs in 1 group (23 cases and 46 controls) and the other breeds in a different group. Results indicated that Shetland sheepdogs with a histologic diagnosis of GBM formation were 9.3 times as likely to have a reported use of imidacloprid as were Shetland sheepdog control dogs (95% CI, 1.103–78.239; $P = .04$). In a separate analysis of the data subset containing the other 28 non-Shetland sheepdog breeds, there was no indication of an association between GBM histologic diagnosis and reported use of imidacloprid (Table 5). For the Shetland sheepdogs, there also was no association between histologic diagnosis of GBM formation and record of treatment for hypothyroidism or hyperadrenocorticism. For non-Shetland sheepdog breeds, diagnosis of GBM formation was 3.6 times as likely in dogs with reported treatment of hyperadrenocorticism (95% CI 1.228–10.612, $P = .02$). Given the association between chronic PO imidacloprid exposure and thyroid morphologic changes in rats, we also tested an interaction term between imidacloprid and treatment for hypothyroidism in both the Shetland sheepdog data subset and the total study population and found no statistically significant associations.

Table 5. Univariate conditional logistic regression model for two data subsets for Shetland sheepdogs and all other breeds combined.

	Shetland Sheepdogs				All Other Breeds			
Association	No. of Observations Used in Conditional Logistic Regression Model	Odds Ratio	95% CI (OR)	Wald Chi-Square *P* Value	No. of Observations Used in Conditional Logistic Regression Model	Odds Ratio	95% CI (OR)	Wald Chi-Square *P* Value
Use of imidacloprid	54	9.292	1.103–78.239	.04	129	1.663	0.740–3.739	.22
Hyperadrenocorticism	69[a]	—	—	—	177	3.610	1.228–10.612	.02
Hypothyroidism	69	2.732	0.648–11.514	.17	177	1.945	0.690–5.484	.21

Odds ratios represent the likelihood that dogs with histologic diagnosis of gallbladder mucocele formation received imidacloprid or treatment for hyperadrenocorticism or hypothyroidism compared to breed, age, and admission date-matched dogs that were demonstrated to not have a gallbladder mucocele (Data represent a 1:2 case-to-control match between dogs with and without diagnosis of gallbladder mucocele formation).
[a]No Shetland sheepdogs were diagnosed with hyperadrenocorticism.

Discussion

Results of this study identified that Shetland sheepdogs having a histologic diagnosis of GBM formation were 9.3 times as likely to have reported use of products containing imidacloprid (95% CI, 1.103–78.239; $P = .04$) when matched to control Shetland sheepdogs on the basis of age and admission date. This association was not observed for the subset of data representing all of the non-Shetland sheepdog breeds in the study. The results of this study do not suggest that imidacloprid could be a primary cause of GBM formation in dogs, but possibly a contributing or exacerbating factor in Shetland sheepdogs. Only 33% of the dogs diagnosed with a GBM had a history of treatment with imidacloprid and, after omission of the Shetland sheepdogs, there did not appear to be any collective association among the other breeds. How a history of use of imidacloprid might further increase the odds of GBM formation in Shetland sheepdogs is not clear.

Imidacloprid (1-[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine) was discovered in 1984 as a novel synthetic compound having a high affinity for insect nicotinic acetylcholine receptors (nAChR).[13] Imidacloprid was first registered in the Unites States by the Environmental Protection Agency in 1994 and has been available in a topical formulation for the control of fleas on dogs and cats since 1996. Imidacloprid currently is the most widely used agricultural insecticide in the world.[14] Imidacloprid has structural similarity and a common mode of action with nicotine. In insects, imidacloprid binds to cation selective ligand-gated neuronal nAChR's causing a prolonged influx of extracellular Na^+ and Ca^{++} leading to depolarization and insect paralysis.[15,16] A higher affinity for insect neuronal nAChRs compared with that of mammals accounts for the selective toxicity of imidacloprid to invertebrate species.[17] Systemic absorption of imidacloprid after dermal application is expected to be minimal, but there are no published data examining this in any vertebrate species. After parenteral administration in rats, imidacloprid undergoes biotransformation in the liver to metabolites that are eliminated along with the parent compound in both urine (70–80%) and feces (17–25%).[18] Most of the fecal metabolites are eliminated via the bile.[18,19] Some of these metabolites, in particular desnitro-imidacloprid, have increased specificity for mammalian nAChR subtypes.[20,21] Significant differences in hepatocyte metabolism of imidacloprid among species have been demonstrated.[22] Whether Shetland sheepdogs biotransform imidacloprid differently than other breeds of dog is unknown. Shetland sheepdogs are known to carry an ATP-binding cassette (ABC) transporter gene polymorphism in ABCB1 (MDR1) that predisposes to ivermectin-induced neurologic toxicosis.[23] It is unknown if imidacloprid serves as a substrate for ABCB1 or other MDR proteins. However, studies of imidacloprid absorption by the human intestinal epithelial Caco-2 cell line suggest lack of involvement of classical ABC transport systems,[24] and ivermectin-sensitive Collies do not demonstrate any observable toxicologic effects of topical treatment with imidacloprid.[25]

Although our study does not provide any conclusive evidence that imidacloprid directly promotes GBM formation in Shetland sheepdogs, prolonged activation or desensitization of nAChRs might intersect with mechanisms suspected to underlie GBM formation in dogs. Parenteral administration of ^{14}C-labeled imidacloprid to rats indicates access of the drug and its metabolites to the gallbladder (via biliary excretion) and glandular organs including the adrenal and thyroid glands.[26] Moreover, a number of nonneuronal cells, including T cells, macrophages, and airway epithelial cells, express nAChRs and may synthesize acetylcholine.[27] Although not yet examined for gallbladder epithelium, in the airway nAChRs are expressed by epithelial cells[28,29] and nicotine has been demonstrated to decrease mucus transport,[30] increase mucin expression and mucus secretion,[31,32] alter mucus hydration, and increase the viscosity of mucus.[33] Nicotine also has been described to cause relaxation of guinea pig gallbladder by a mechanism independent of nAChRs.[34] Whether or not environmental exposure of dogs to nicotine could increase odds of GBM formation in dogs was not investigated in our study but should be considered. The primary adverse effects of long-term, low-dose PO exposure to imidacloprid in dogs are inappetence leading to loss of body weight, atrophy of the thyroid gland, hypercholesterolemia, and an increase in liver mixed-function oxidases.[35] Despite the association of imidacloprid exposure with thyroid atrophy in experimental studies in dogs, we did not find any evidence in our study of an association between use of imidacloprid and treatment for hypothyroidism.

Results of this study confirm the findings of another retrospective study in demonstrating that dogs diagnosed with GBM formation were more likely than control dogs to have a diagnosis of hyperadrenocorticism. This association was attributed to the non-Shetland sheepdog subgroup because no Shetland sheepdogs were identified as being treated for hyperadrenocorticism in our study. Lower OR here than reported by others[6] likely reflect our decision to conservatively define these diagnoses only in dogs undergoing treatment for each disorder. As such, each diagnosis likely is underrepresented in our study because we did not attempt to ascertain if dogs had untreated or subsequent diagnosis of any of these endocrinopathies.

Our study had a number of important limitations. First, the study had highly conservative criteria that limited the number of dogs that ultimately could be included. As such, only those dogs undergoing cholecystectomy or necropsy (ie, histologic diagnosis) were included, which omitted a large number of dogs that had only an ultrasonographic diagnosis of GBM formation. Our desire to match cases with controls on the basis of breed, age, admission date, and lack of ultrasonographic evidence of GBM formation was challenging. This led in some cases to a failure to closely match control dogs on ≥ 1 criteria. Secondly, as with most retrospective studies, an accurate estimation of drug

exposure can be limited by failure to document a complete medication history in the medical record. To increase the completeness of data collection in this study, the owners of each dog were sent a questionnaire inquiring about the use of specific drugs. Including brand name examples in the questionnaire may have been associated with recall bias, but this approach was believed necessary to assist owners in recognizing the types of drugs commonly represented in each category. In addition, some dogs were diagnosed with GBM formation as many as 10 years before we conducted our study, which likely added inaccuracy to owner recollection of medication history. Finally, this study was designed to examine dogs in our hospital population for an association between GBM formation and history of use of drugs that we hypothesized could ultimately be identified as contributory for disease causation. We chose not to document medications commonly used for management of specific medical disorders (eg cardiac, neurologic, ophthalmic, or gastrointestinal disease) simply because our clinical experience did not support a strong hypothesis for predisposition to GBM formation in dogs having these diseases. Because cases were matched to controls on the basis of breed, the study was not designed to examine the influence of breed on the association between GBM formation and use of these drugs. Only upon recognition that Shetland sheepdogs represented 23% of the dogs in the study did we consider the possibility that Shetland sheepdogs might be the source of the observed association between GBM formation and use of imidacloprid. The only way to answer this question within the context of the study design was to reanalyze the data considering only the Shetland sheepdogs as 1 subset and all of the other 28 breeds of dog in a separate group. To increase the power and precision of the estimate of the OR for the Shetland sheepdog subset, additional Shetland sheepdogs that met inclusion criteria between 2011 and 2015 also were included in the study.

Based on the results of our study, additional examination of Shetland sheepdogs for a direct association between use of imidocloprid and GBM formation is warranted. A large epidemiologic study designed to consider the impact of both drug and environmental exposure(s) to imidacloprid in addition to drugs with overlapping mechanisms of action such as nicotine may provide insight into the underlying mechanism(s) of GBM formation and establish whether or not our findings can be generalized to a larger population of dogs. Because of the high prevalence of occult GBM formation in Shetland sheepdogs, such a study will require careful efforts to identify control dogs that lack any evidence of GBM formation.

Footnote

[a] SAS 9.4, Copyright©2002–2013, SAS Institute Inc., Cary, NC

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

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Supporting Information

Additional Supporting Information may be found online in Supporting Information:

Figure S1. Gallbladder mucocele histologic diagnosis by year in dogs at NCSU-VH.

Table S1. Presenting problem or primary diagnosis of 162 dogs serving as breed, age, and admission date-matched controls to dogs having a histologic diagnosis of a gallbladder mucocele.

Appendix S1. Example letter sent to owners of dogs serving as a matching control to a dog diagnosed with a gallbladder mucocele.