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.

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

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 affects older dogs of many different breeds but seems more common in Shetland sheepdogs,3,10 Cocker spaniels,3,10 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 prescreened 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

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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 pri-

**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 noted in the medical record and the owner questionnaire was not returned for verification of 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 ≤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 ≤10. A backward modeling algorithm was proposed to test interaction terms using the Wald’s test at P value ≤0.05. 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 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 (χ², 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 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)               | χ²   | 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; χ², 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 osteoarthritis (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.

| Category of Drug Use                  | All Dogs                                      | Shetland Sheepdogs                              |
|--------------------------------------|-----------------------------------------------|------------------------------------------------|
|                                      | GBM (N = 81) | Control (N = 162) | GBM (N = 23) | Control (N = 46) |
|                                      | 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 \( \geq 2 \) 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 \( \geq 2 \) 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 \( \geq 2 \) 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).
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 = .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 3. Descriptive summary of documented use a of selected categories of drugs based on active ingredient in dogs with histologic diagnosis of gallbladder mucocoele and breed, age, and admission date-matched control dogs.

| Category of Documented Drug Use | All Dogs GBM (N = 81) | Control GBM (N = 162) | Shetland Sheepdogs GBM (N = 23) | Control (N = 46) |
|--------------------------------|-----------------------|-----------------------|---------------------------------|-----------------|
| 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 c                 | 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         |
| Cerecosp b                     | 1/23 4               | 4/22 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                          | 12/3 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.

aTable does not show concordance or discordance of information between matched pairs of individual gallbladder mucocoele and control dogs (1:2 case-to-control ratio).

bNot included in univariate conditional logistic regression because of low number of observations in the total population.
The 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 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 |
|----------------------------------|----------------------------------------------------------|------------|-------------|----------------|
| 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 methylsulfonyl methane    | 133                                                      | 0.768      | 0.299–1.976 | .58            |
| Exogenous steroid administration | 243                                                      | 1.641      | 0.805–3.344 | .17            |
| Treatment for endocrinopathy     |                                                          |            |             |                |
| Hyperadrenocorticismo            | 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.

### Table 5.

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

| Association                  | Shetland Sheepdogs | All Other Breeds |
|------------------------------|--------------------|------------------|
| No. of Observations Used in Conditional Logistic Regression Model | Odds Ratio | 95% CI (OR) | Wald Chi-Square |
| Use of imidacloprid         | 54                 | 9.292            | 1.103–78.239 | .04 |
| Hyperadrenocorticismo\(^a\) | 69                 | —                | —             | —    |
| Hypothyroidism              | 69                 | 2.732            | 0.648–11.514 | .17 |

\(^a\)No Shetland sheepdogs were diagnosed with hyperadrenocorticismo.
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 in 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 sheepdog subgroup because no Shetland sheepdogs were diagnosed with GBM formation were more likely than control Shetland sheepdogs. Only 33% of the dogs diagnosed with GBM formation were more likely than control Shetland sheepdogs to have used 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 attributed to the non-Shetland sheepdog subgroup because no Shetland sheepdogs were diagnosed with GBM formation 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 diagnosed with GBM formation. However, 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 increased risk of hypothyroidism.

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 the biliary system) and glandular organs including the adrenal and thyroid glands. Moreover, a number of nonneuronal cells, including T cells, macrophages, and airway epithelial cells, express nAChRs and may synthesize acetylcholine. Although not yet examined for gallbladder epithelium, in the airway nAChRs are expressed by epithelial cells and nicotine has been demonstrated to cause relaxation of guinea pig gallbladder by a mechanism independent of nAChR. Nicotine has also been demonstrated to cause relaxation of guinea pig gallbladder by a mechanism independent of nAChR. 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 increased risk of 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 diagnosed with GBM formation. However, 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 increased risk of hypothyroidism. 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 diagnosed with GBM formation. However, 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 increased risk of hypothyroidism.

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 omits large numbers 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 \( \geq 1 \) criteria. Secondly, as with most retrospective studies, an accurate estimation of drug observable toxicologic effects of topical treatment with imidacloprid.
Careful efforts to identify control dogs that lack any evidence in Shetland sheepdogs, such a study will require because of the high prevalence of occult GBM formations can be generalized to a larger population of dogs. In addition, increased 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 (e.g. 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 sheepdog subset, additional Shetland sheepdogs who 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 distinct association between use of imidacloprid 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.

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

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

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Footnote

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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.