Proteinuria in dogs with gallbladder mucocele formation: A retrospective case control study

Crystal Lindaberry1 | Shelly Vaden1 | Kathleen M. Aicher1,4,5 | Gabriela Seiler2 | James Robertson3 | Rachel Cianciolo4 | Ching Yang4 | Jody L. Gookin1

1Department of Clinical Sciences, North Carolina State University, Raleigh, North Carolina
2Department of Molecular Biomedical Sciences, North Carolina State University, Raleigh, North Carolina
3College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina
4Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio
5Veterinary Specialty Hospital of the Carolinas, Cary, North Carolina

Correspondence
Jody L. Gookin, North Carolina State University, 1060 William Moore Drive, Raleigh, NC 27607.
Email: jody_gookin@ncsu.edu

Funding Information
American Kennel Club Canine Health Foundation, Grant/Award Number: 01986; American Shetland Sheepdog Association; Collie Health Foundation

Abstract
Background: Proteinuria is an independent risk factor for morbidity and mortality in dogs. An association between proteinuria and gallbladder mucocele formation in dogs is unknown.

Objective: Determine if gallbladder mucocele formation or clinicopathologic comorbidities are associated with proteinuria.

Animals: Twenty-five dogs with mucocele formation and 25 breed and age-matched control dogs from a prior study.

Methods: Retrospective case control study. Proteinuria defined by calculated urine dipstick protein concentration (mg/mL) to urine specific gravity (USG) ratio. Clinicopathologic findings, postcosyntropin cortisol concentration, thyroid function profile, and illness severity score were recorded.

Results: Median urine dipstick protein concentration to USG ratio and number of dogs having a ratio \( \geq 1.5 \) were significantly higher for dogs with mucocele formation compared to control dogs. Proteinuria was not significantly associated with CBC or serum biochemistry profile abnormalities but increased in relation to severity of illness.

Conclusions and Clinical Importance: Gallbladder mucocele formation is significantly associated with proteinuria in dogs. Diagnosis and treatment of proteinuria in dogs with mucocele formation might minimize long term kidney morbidity in these patients.

KEYWORDS
Canine, dipstick, glomerular disease, urinalysis

INTRODUCTION

Proteinuria can be an important clinical sign of kidney disease in dogs. Persistent proteinuria promotes progression of kidney disease, and control of proteinuria may prolong maintenance of patient kidney
function. International Renal Interest Society (IRIS) and American College of Veterinary Internal Medicine (ACVM) guidelines suggest that proteinuria measured as a urine protein-to-creatinine ratio (UPC) of ≥0.5 in nonazotemic dogs warrants diagnostic investigation for underlying causes of kidney injury. Factors identified to predispose to kidney injury and proteinuria include acute extrarenal factors that affect perfusion and oxygenation, chronic processes including inflammatory conditions, remodeling and fibrosis, and systemic endocrine status, any of which may have breed associations.

Gallbladder disease currently is not recognized as a predisposing cause of proteinuria in dogs. Several studies describe associations between hyperadrenocorticism, hyperlipidemia, and pancreatitis with proteinuria in populations of dogs. Other studies separately have associated these diseases with gallbladder mucocoele formation. A direct association between gallbladder mucocoele formation and proteinuria has not been investigated previously. Gallbladder mucocoele formation is characterized by excessive secretion of abnormal mucus by the gallbladder epithelium that may lead to gallbladder rupture or bile duct obstruction. The disease affects older dogs and is observed in some breeds with genetic or metabolic predisposition to proteinuria such as the Shetland Sheepdog, Miniature Schnauzer, and Cocker Spaniel.

Our primary aim was to determine, while controlling for the influence of age and breed, if gallbladder mucocoele formation or concurrent clinicopathologic comorbidities are associated with increased incidence of proteinuria in dogs.

2 | MATERIALS AND METHODS

Medical records of dogs that were included in a prior case-control study of gallbladder mucocoele formation were retrospectively reviewed. Case and control dogs were selected for inclusion in the present study if both dogs had results of CBC, serum biochemical profile, and urinalysis reported within 1 month of gallbladder ultrasound examination. With regard to recruitment for the prior study, client-owned dogs with diagnosis of gallbladder mucocoele formation at the North Carolina State University Veterinary Hospital (NCSU-VH) were prospectively identified over the time period from February 2014 to January 2017. In each case, diagnosis was based on ultrasound findings of an enlarged gallbladder containing nongravity dependent, immobile bile having hypoechogenic extensions of mucus into the lumen, resulting in a stellate or finely striated bile pattern. In the event that the dog was euthanized or underwent surgery for removal of the gallbladder, the gross pathology and histopathology reports were reviewed to confirm the diagnosis of mucocoele formation. For inclusion as matched controls, apparently healthy, age- to within 2 years) and breed-matched client-owned dogs were recruited by the Clinical Studies Core facility at North Carolina State University over the same time period. Control dogs were determined to be healthy based on history, CBC, serum biochemistry profile, urinalysis and focal hepatobiliary ultrasound examination showing a normal-appearing gallbladder with normal wall structure and thickness. Sludge, if present, was gravity-dependent, occupied <50% of the gallbladder lumen, and was not attached to the wall.

Dogs were excluded from the prior study if they had a recent (within 2 months) history of treatment with ursodeoxycholic acid or drugs recognized or suspected to interfere with endocrine function testing (eg, topical or systemic corticosteroids, nonsteroidal anti-inflammatory drugs, anticonvulsants, furosemide, sulfa-containing drugs, fatty acid supplements), had history or physical examination findings suggestive of endocrinopathy, or were reproductively intact. Owners of the dogs signed informed consent forms for participation in the study. All study protocols were approved by the Institutional Animal Care and Use Committee of North Carolina State University (ID#14-049-O).

2.1 | Clinical pathology and endocrine testing

Each dog underwent a complete physical examination by the attending clinician. Blood was collected by venipuncture and urine by ultrasound-guided cystocentesis after a minimum fasting period of 12 hours. For diagnosis of hyperadrenocorticism, blood was drawn 1-hour after IV injection of synthetic cosyntropin for measurement of serum cortisol concentration as previously described. Postcosyntropin serum cortisol concentrations ≥200 ng/mL were considered consistent with a presumptive diagnosis of hyperadrenocorticism. For diagnosis of hypothyroidism, a serum sample from each dog was stored at –80°C and collectively submitted on dry ice to the Michigan State University Veterinary Diagnostic Laboratory for measurement of serum total thyroxine (T4), free thyroxine by equilibrium dialysis (FT4), and thyrotrpin (TSH) concentrations, and for antibodies against thyroxine (T4AA), triiodothyronine (T3AA), and thyroglobulin (TgAA). A laboratory-based diagnosis of hypothyroidism was defined by low serum total T4 and increased serum TSH concentrations or low serum FT4 concentration as previously proposed.

2.2 | Illness severity scoring

To evaluate the impact of overall systemic illness on proteinuria, all dogs were stratified by disease severity into 4 groups based on a previously described scoring system as follows: absent (0) for patients that had no clinical signs of systemic illness, mild (1) for patients with signs of clinical disease but suitable for outpatient care, moderate (2) for patients sick enough to require hospitalization and aggressive treatment, and severe (3) for patients with severe illness requiring intensive care and advanced treatment (including all dogs requiring emergency cholecystectomy).

2.3 | Assessment of proteinuria

Urinalyses were performed in the North Carolina State University Clinical Pathology Laboratory by an experienced laboratory technician. Urine specific gravity was measured using a digital refractometer (Palm Abbe Digital Refractometer #PA202, MISCO Cleveland, Ohio). Urine chemical analysis was performed using commercially-available
3 | RESULTS

3.1 | Case-control study population

A total of 30 case-control pairs of dogs from the previously published study\(^{12}\) had medical records screened for inclusion in the present study. Five case-control pairs were excluded because of lack of urinalysis in 1 or both dogs. Therefore, 25 case-control pairs of dogs met the criteria for inclusion in the study. Dogs were represented by 15 different breeds including 16 Shetland Sheepdogs, 4 American Cocker Spaniels, 4 Bichon Frise, 4 Chihuahuas, and 2 each of the following breeds: American Staffordshire Terrier, Beagle, Border Collie, Cavalier King Charles Spaniel, Cockapoo, Kerry Blue Terrier, Labrador Retriever, Miniature Poodle, Miniature Schnauzer, Pug, and Shih Tzu. There were 27 castrated males and 23 spayed females. The median age was 10 years (range, 6-16 years) and median body weight 9.4 kg (range, 2.9-35.6 kg). There was no statistically significant difference in sex (P = 1), age (P = .17), or body weight (P = .59) between control dogs and dogs with mucocele formation. Illness severity scores of dogs at the time of participation in the study were: 0 (absent) in 12 (48%) dogs, 1 (mild) in 5 (20%) dogs, 2 (moderate) in 6 (24%) dogs, and 3 (severe) in 2 (8%) dogs with mucocele formation. All control dogs had an illness severity score of 0 (absent). In addition to ultrasonographic diagnosis, gallbladder mucocele formation was confirmed by gross examination and histopathology of gallbladder tissue in 8 (32%) dogs, obtained at the time of surgery (5 dogs) or at necropsy (3 dogs).

3.2 | Proteinuria, urinalysis, and urinary tract ultrasound examination

A significantly larger number of dogs with gallbladder mucocele formation had dipstick-measured urine protein concentration ≥30 mg/dL compared to control dogs (P = .003). The median urine dipstick protein concentration to USG ratio (P = .008) and number of dogs having a urine dipstick protein concentration to USG ratio ≥1.5 (P = .005) was significantly higher for dogs with gallbladder mucocele formation compared with control dogs (Table 1 and Figure 1). One dog was not included in the analysis because of lack of recorded USG. A quantitative urine protein-to-creatinine (UPC) ratio was measured in 3 dogs with mucocele formation and ranged from 0.82 to 6.7 (concurrent urine dipstick protein concentration to USG ratio range, 1.76-11.11). A UPC was measured in 4 control dogs and ranged from 0.1 to 0.14 (concurrent urine dipstick protein concentration to USG ratio range, 0-0.7).

Prevalence of microscopic hematuria (P = .10) and presence of casts (P = .34) in the urine was not significantly different between dogs with gallbladder mucocele formation compared to control dogs. No dogs in the study were observed to have gross hematuria or pyuria. The number of casts of any given type did not exceed 2 per high power field in any dog (Table 1). Nine dogs (36%) with gallbladder mucocele formation had cylindruria with casts of the following types: hyaline (7 dogs), fine granular (4 dogs), waxy (2 dogs), coarse granular...
Among mucocele dogs with cylindruria, 2 dogs had a single cast type, 6 dogs had 2 cast types, and 1 dog had 3 cast types. Five (25%) of the control group dogs had cylindruria with casts of the following types: hyaline (3 dogs), fine granular (3 dogs), and coarse granular (1 dog). Among control dogs with cylindruria, 3 dogs had a single type and 2 dogs had 2 types of casts. Thirteen dogs had urine pH ≥ 8 (8 dogs with gallbladder mucocele formation and 5 control dogs). No association was found between presence of urine pH ≥ 8 and urine dipstick protein concentration to USG ratio ≥ 1.5 among dogs in the study (P = .30).

Quantitative urine cultures were performed in 9 gallbladder mucocele dogs with 1 positive for Escherichia coli on a catheterized sample with an inactive sediment and urine dipstick protein concentration to USG ratio of 0.5. One control dog had an active sediment on a voided sample with urine dipstick protein concentration to USG ratio of 0.8. No urine cultures were performed on control dogs.

Results of abdominal ultrasound examination that included the urinary tract were available for 12 dogs with gallbladder mucocele formation and 3 control dogs. Regarding the lower urinary tract, 1 control dog had small suspected uroliths and a urine dipstick protein concentration to USG ratio of 0.4. One dog with a gallbladder mucocele had mild irregularity and thickening of the apical urinary bladder, urine dipstick protein concentration to USG ratio of 11.1, an inactive urine sediment and negative urine culture. All remaining dogs had no ultrasonographic evidence of lower urinary tract disease. Abnormal ultrasound findings related to the kidneys were described in 9/12 (75%) dogs with gallbladder mucocele formation and included decreased corticomedullary distinction (6 dogs), peridiverticular mineralization (6 dogs), hyperechoic kidney cortices (3 dogs), chronic kidney infarcts (2 dogs), and cortical cysts (1 dog). Changes also were described in the kidneys of 2/3 (67%) control dogs, including decreased corticomedullary distinction (2 dogs), peridiverticular mineralization (2 dogs), and cortical rim sign (1 dog).

### 3.3 Clinicopathologic comorbidities

After correction for the impact of multiple testing on false discovery, results of CBC and serum biochemical profiles identified significantly higher results for the number of polymorphonuclear leukocytes...
# TABLE 2  Results of clinical pathological testing in control dogs and dogs with gallbladder mucocele formation

| Clinical variable | No gallbladder mucocele (25 dogs) | Gallbladder mucocele (25 dogs) | Mann-Whitney P-value (comparison of median values) | Fisher exact P-value (comparison of % abnormal values) |
|-------------------|----------------------------------|--------------------------------|---------------------------------------------------|-----------------------------------------------------|
|                   | Median | Range | Number (%) of dogs with abnormal value | Median | Range | Number (%) of dogs with abnormal value | Direction of abnormality | Reference range | |
| Complete blood cell count |         |       |                                      |         |       |                                      |                      |                | |
| Packed cell volume (%) | 44     | 34-52 | 1 (4) | 42     | 25-50 | 8 (32) | Low | 39.58 | .14 .02 |
| Total white blood cells ($\times 10^3$/mL) | 7.47   | 4.490-13.370 | 3 (12) | 9.820* | 4.670-34.570 | 9 (36) | High | 4.39-11.61 | .02 .09 |
| Polymorphonuclear leukocytes ($\times 10^9$/mL) | 5.358  | 2.371-10.450 | 3 (12) | 7.286* | 3.468-28.926 | 8 (32) | High | 2.841-9.112 | .01 .17 |
| Bands ($\times 10^9$/mL) | 0 | 0.0-0.190 | 8 (32) | 0.098** | 0-6.568 | 16 (64) | High | 0.0-0.0 | .004 .05 |
| Platelets ($\times 10^9$/mL) | 359 | 189-616 | 1 (4) | 394 | 143-695 | 2 (8) | Low | 191-468 | .48 1 |
| Serum biochemical analysis |          |       |                                      |         |       |                                      |                      |                | |
| Alkaline phosphatase (IU/L) | 52 | 6-251 | 4 (16) | 205*** | 21-3188 | 17 (68)*** | High | 16-140 | .001 <.001 |
| ALT (IU/L) | 48 | 11-215 | 8 (32) | 108** | 11-5393 | 16 (64) | High | 12-54 | .008 .046 |
| GGT (IU/L) | 3 | 3-6 | 0 (0) | 6** | 3-78 | 11 (44)*** | High | 0-6 | .002 .02 |
| Total bilirubin (mg/dL) | 0.1 | 0.1-0.1 | 0 (0) | 0.1** | 0-1.15 | 6 (24) | High | 0-0.2 | .005 <.001 |
| Cholesterol (mg/dL) | 249 | 165-452 | 4 (16) | 301 | 107-754 | 9 (36) | High | 124-344 | .03 2 |
| Blood urea nitrogen (mg/dL) | 16 | 9-26 | 0 (0) | 17 | 5-170 | 6 (24) | High | 8-26 | .93 .02 |
| Creatinine (mg/dL) | 0.8 | 0.5-1.1 | 0 (0) | 0.7 | 0.2-4.1 | 2 (8) | High | 0.7-1.5 | .31 .49 |
| Total protein (g/dL) | 6.2 | 5.1-7.0 | 1 (4) | 5.8 | 3-2.7 | 4 (16) | Low | 5.2-7.3 | .18 .35 |
| Albumin (g/dL) | 3.6 | 2.9-4.6 | 1 (4) | 3.4 | 1.6-4.2 | 4 (16) | Low | 3-3.9 | .07 .35 |
| Globulin (g/dL) | 2.4 | 1.9-4.0 | 1 (4) | 2.5 | 1.6-3.4 | 0 (0) | High | 1.7-3.8 | .78 1.00 |
| Lipase (IU/L) | 75 | 24-1032 | 2 (8) | 124** | 41-3920 | 12 (48)** | High | 12-147 | .003 .004 |
| Postcosyntropin cortisol (ng/mL) | 92 | 30-135 | 0 (0) | 110 | 37-699 | 3 (12) | High | <200 | .06 .23 |

Notes: Mann-Whitney rank sum test P-values compare the median value of each clinical pathology variable between control dogs and dogs with gallbladder mucocele formation. Fisher exact test P-values compare the % of dogs with an abnormal value for each clinical pathology variable between control dogs and dogs with gallbladder mucocele formation. All asterisked values shown have Benjamini-Hochberg corrected P-values <.05.

*P < .05. **P < .01. ***P < .001.
| Clinical variable | Urine dipstick protein to USG ratio < 1.5 (n = 12) | Urine dipstick protein to USG ratio ≥ 1.5 (n = 12) | Number (%) of dogs with abnormal value | Number (%) of dogs with abnormal value | Direction of abnormality | Reference range | Mann-Whitney P-value (comparison of median values) | Fisher exact P-value (comparison of % abnormal values) |
|-------------------|-------------------------------------------------|-------------------------------------------------|----------------------------------------|----------------------------------------|-------------------------|----------------|-----------------------------------------------|-----------------------------------------------|
| Urine dipstick protein to USG ratio | 0.37 | 0-1.30 | 0(0) | 3.25*** | 1.76-25 | 12(100)*** | High | <1.5 | <.001 | <.001 |
| Complete blood cell count | | | | | | | | | | | |
| Packed cell volume (%) | 43 | 38-50 | 2(17) | 40 | 25-46 | 5(42) | Low | 39-58 | .05 | .37 |
| Total white blood cells (<10^9/mL) | 8.130 | 4.670-34.570 | 3(25) | 10.460 | 6.390-34.030 | 5(42) | High | 4.39-11.61 | .12 | .67 |
| Polymorphonuclear leukocytes (<10^9/mL) | 5.585 | 3.468-24.545 | 2(17) | 8.573 | 4.556-28.926 | 5(42) | High | 2.841-9.112 | .09 | .37 |
| Bands (<10^9/mL) | 0.025 | 0.0-6.568 | 6(50) | 0.203 | 0-1.475 | 9(75) | High | 0.0-0.0 | .3 | .4 |
| Platelets (<10^9/mL) | 393 | 193-695 | 0(0) | 409 | 143-564 | 2(17) | Low | 191-468 | .83 | .48 |
| Serum biochemical analysis | | | | | | | | | | | |
| Alkaline phosphatase (IU/L) | 140 | 21-2817 | 4(50) | 261 | 66-3188 | 10(83) | High | 16-140 | .1 | .19 |
| ALT (IU/L) | 45 | 11-5393 | 5(42) | 172 | 30-3877 | 10(83) | High | 12-54 | .09 | .09 |
| GGT (IU/L) | 4 | 3-78 | 4(33) | 12 | 3-77 | 6(50) | High | 0-6 | .69 | .68 |
| Total bilirubin (mg/dL) | 0.1 | 0.1-11.5 | 1(8) | 0.1 | 0.1-3.5 | 4(33) | High | 0-0.2 | .38 | .32 |
| Cholesterol (mg/dL) | 342 | 107-711 | 6(50) | 283 | 206-754 | 2(17) | High | 124-344 | .54 | .19 |
| Blood urea nitrogen (mg/dL) | 16 | 6-32 | 2(17) | 15 | 5-170 | 3(25) | High | 8-26 | .98 | 1 |
| Creatinine (mg/dL) | 0.8 | 0.2-1.2 | 0(0) | 0.6 | 0.4-4.1 | 2(17) | High | 0.7-1.5 | .88 | .48 |
| Total protein (g/dL) | 6.0 | 3.2-6.8 | 2(17) | 5.8 | 4.9-7.2 | 2(17) | Low | 5.2-7.3 | .66 | 1 |
| Albumin (g/dL) | 3.5 | 1.6-4.2 | 2(17) | 3.2 | 2.3-4.2 | 2(17) | Low | 3-3.9 | .31 | 1 |
| Globulin (g/dL) | 2.3 | 1.6-3.2 | 0(0) | 2.6 | 2.0-3.4 | 0(0) | High | 1.7-3.8 | .52 | - |
| Lipase (IU/L) | 111 | 41-3920 | 4(33) | 167 | 43-1558 | 7(58) | High | 12-147 | .37 | .41 |
| Postcosyntropin cortisol (ng/mL) | 108 | 37.8-143 | 0(0) | 113 | 67-699 | 2(17) | High | <200 | .51 | .48 |

Notes: Mann-Whitney rank sum test P-values compare the median value of each clinical pathology variable between control dogs and dogs with gallbladder mucocele formation. Fisher exact test P-values compare the % of dogs with an abnormal value for each clinical pathology variable between control dogs and dogs with gallbladder mucocele formation. One dog excluded from analysis due to unavailable USG measurement.

***P < .001. All asterisked values shown have Benjamini-Hochberg corrected P-values < .05.
(P = .01) and bands (P = .004), serum activities of alkaline phosphatase (ALP; P = .001), alanine aminotransferase (ALT; P = .008), γ-glutamyl transferase (GGT; P = .002) and lipase (P = .003), and concentration of total bilirubin (P = .005) in dogs with gallbladder mucocele formation compared to control dogs. A significantly higher number of dogs with mucocele formation had results out of reference range limits for serum activities of ALP (P < .001), GGT (P < .001), and lipase (P = .004; Table 2).

Among dogs with gallbladder mucocele formation, no significant differences were found between median results of any CBC or serum biochemistry variables between dogs having a urine dipstick protein concentration to USG ratio ≥1.5 compared to those having a ratio <1.5. As categorized based on whether or not results were outside of reference range limits, no CBC or serum biochemistry variables were significantly associated with urine dipstick protein concentration to USG ratio ≥1.5 vs <1.5 in dogs with gallbladder mucocele formation (Table 3).

### 3.4 Endocrinopathy

Among dogs with gallbladder mucocele formation, test results consistent with diagnoses of hyperadrenocorticism or hypothyroidism were obtained for 3 and 5 dogs, respectively. No control dogs met criteria for diagnosis of either endocrinopathy. Postcosyntropin serum concentrations of cortisol in dogs with gallbladder mucocele formation (median, 110 ng/mL; interquartile range, 93-140 ng/mL) did not differ significantly from concentrations measured in control dogs (median, 92 ng/mL; interquartile range, 80-116 ng/mL; P = .06; Table 2). Among dogs with gallbladder mucocele formation, no significant association was found between urine dipstick protein concentration to USG ratio ≥1.5 vs <1.5 and postcosyntropin serum cortisol concentration (P = .51), diagnosis of hyperadrenocorticism (P = .48), or diagnosis of hypothyroidism (P = 1; Table 3).

### 3.5 Illness severity

Illness severity score was significantly associated with urine dipstick protein concentration to USG ratio. Each unit increase in the severity of illness was associated with a 173% increase in odds of a urine dipstick protein concentration to USG ratio ≥1.5 (P = .04; Figure 2).

### 4 DISCUSSION

Our case-control study documents a significant association between ultrasonographic diagnosis of gallbladder mucocele formation and incidence of proteinuria in dogs. Important features of the study were breed and age-matched design, ultrasound documentation of absent gallbladder mucocele formation in control dogs, and concurrent serum biochemistry and endocrine testing of all dogs. This design enabled the examination of potentially confounding influences of concurrent disease on presence of proteinuria in this population. As defined by a urine dipstick protein concentration to USG ratio ≥1.5, proteinuria in dogs with gallbladder mucocele formation was not significantly associated with any CBC or serum biochemistry profile abnormalities in our study. Proteinuria significantly increased in relation to severity of systemic illness. Although a causal relationship between illness severity and proteinuria in dogs with mucocele formation could not be further examined by our retrospective study, this finding suggests that systemic mechanisms such as underlying metabolic, vascular, inflammatory, or xenobiotic exposures should be considered.

No association was observed between proteinuria and postcosyntropin serum cortisol concentration or diagnosis of hyperadrenocorticism among dogs with gallbladder mucocele formation. Hyperadrenocorticism is known to be associated with proteinuria in dogs and is a common comorbidity in dogs with mucocele formation. Lack of association between proteinuria and hyperadrenocorticism in our study likely is related to selection criteria that decreased the number of dogs ultimately diagnosed with hyperadrenocorticism. However, hypercortisolemia was not responsible for proteinuria in these dogs.

Dogs with gallbladder mucocele formation are at increased risk for concurrent diagnoses of hypertriglyceridemia, hypercholesterolemia, and hypothyroidism. Dyslipidemia also is associated with proteinuria in people and dogs. We did not identify an association between proteinuria and median serum cholesterol concentration or number of dogs having high serum cholesterol concentrations. Dogs in our study did not have serum triglyceride concentrations measured. Also, no association was found between proteinuria and diagnosis of hypothyroidism.

Important limitations of our study include the retrospective nature of the case-control cohort, in which the UPC ratio was not systematically measured. This resulted in the need to approximate...
proteinuria using a ratio between the urine dipstick-measured protein concentration and USG. Accordingly, these results should be validated with future prospective studies. Our study was not designed to comprehensively investigate underlying causes of proteinuria in these dogs. Only a subset of dogs had urinary tract imaging or urine culture performed, but explanatory reasons for proteinuria were not identified in those dogs that did. Dogs did not have fasting triglyceride concentrations or systolic blood pressure measurements recorded, and these should be included in future studies. Dogs did not undergo an exhaustive search for underlying immune-mediated, neoplastic or infectious diseases that could have contributed to proteinuria, but these conditions were not reported in accessions and considered systematically unlikely within the study population. Although an association between serum lipase activity and proteinuria was not observed in our study, pancreatitis can be associated with proteinuria in dogs and is a common comorbidity in dogs with mucocele formation. Accordingly, future studies utilizing specific tests for pancreatitis in dogs with gallbladder mucocele formation and proteinuria may be warranted. Finally, we used a generic scoring system to stratify the severity of systemic illness among dogs. The system initially was described for examining the influence of systemic illness on thyroid function. Its use in our study served a similar purpose in capturing levels of illness severity without introducing biasing variables with respect to proteinuria.

In conclusion, assessment of proteinuria is warranted in dogs diagnosed with gallbladder mucocele formation. Moreover, occult gallbladder mucocele formation should be considered as a possible cause for proteinuria in dogs of predisposed age and breed. Prospective studies are warranted to more closely examine contributing factors to proteinuria in dogs with gallbladder mucocele formation with suggested emphasis on gaps left by our study such as measurement of systemic blood pressure, serum fasting triglyceride concentrations and markers of systemic inflammation, more specific testing for pancreatitis, and characterization of associated kidney pathology. Whether proteinuria can be ameliorated by surgical removal of the gallbladder in dogs with mucocele formation also is worthy of additional study.

ACKNOWLEDGMENT
Funding provided by the American Kennel Club Canine Health Foundation, American Shetland Sheepdog Association, and Collie Health Foundation (grant #01986; http://www.akcchf.org/).

CONFLICT OF INTEREST DECLARATION
Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION
Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION
Approved by the IACUC of North Carolina State University (ID#14-049-O).

HUMAN ETHICS APPROVAL DECLARATION
Authors declare human ethics approval was not needed for this study.

ORCID
Shelly Vaden https://orcid.org/0000-0003-4402-7830
Gabriela Seiler https://orcid.org/0000-0003-0643-8436
Jody L. Gookin https://orcid.org/0000-0002-2911-0874

REFERENCES
1. Lees GE, Brown SA, Elliott J, Grauer GE, Vaden SL. American College of Veterinary Internal Medicine. Assessment and management of proteinuria in dogs and cats: 2004 ACVIM Forum Consensus Statement (small animal). J Vet Intern Med. 2005;19:377-385.
2. Subgroup ICGSGD, Littman MP, Daminet S, et al. Consensus recommendations for the diagnostic investigation of dogs with suspected glomerular disease. J Vet Intern Med. 2013;27(suppl 1):S19-S26.
3. Hurley KJ, Vaden SL. Evaluation of urine protein content in dogs with pituitary-dependent hyperadrenocorticism. J Am Vet Med Assoc. 1998;212:369-373.
4. Smith RE, Granick JL, Stauthammer CD, Polzin DJ, Heinrich DA, Furrow E. Clinical consequences of hypertriglyceridaemia-associated proteinuria in miniature schnauzers. J Vet Intern Med. 2017;31:1740-1748.
5. Furrow E, Jaeger JQ, Parker VJ, et al. Proteinuria and lipoprotein lipase activity in miniature schnauzer dogs with and without hypertriglyceridaemia. Vet J. 2016;212:83-89.
6. Gori E, Pierini A, Lippi I, Boffa N, Perondi F, Marchetti V. Urinalysis and urinary GGT-to-urinary creatinine ratio in dogs with acute pancreatitis. Vet Sci. 2019;6:27.
7. Kutsunai M, Kanemoto H, Fukushima K, Fujino Y, Ohno K, Tsujimoto H. The association between gall bladder mucocoeles and hyperlipidaemia in dogs: a retrospective case control study. Vet J. 2014;199:76-79.
8. Kim KH, Han SM, Jeon KO, et al. Clinical relationship between cholestatic disease and pituitary-dependent hyperadrenocorticism in dogs: a retrospective case control study. J Vet Intern Med. 2017;31:335-342.
9. Mesich ML, Mayhew PD, Paek M, et al. Gall bladder mucocoeles and their association with endocrinopathies in dogs: a retrospective case-control study. J Small Anim Pract. 2009;50:630-635.
10. Allerton F, Swinbourne F, Barker L, et al. Gall bladder mucocoeles in Border terriers. J Vet Intern Med. 2018;32:1618-1628.
11. Aguirre AL, Center SA, Randolph JF, et al. Gallbladder disease in Shetland sheepdogs: 38 cases (1995-2005). J Am Vet Med Assoc. 2007;231:79-88.
12. Aicher KM, Cullen JM, Seiler GS, Lunn KF, Mathews KG, Gookin JL. Investigation of adrenal and thyroid gland dysfunction in dogs with ultrasonographic diagnosis of gallbladder mucocoele formation. PloS One. 2019;14:e0212638.
13. Kesimer M, Cullen J, Cao R, et al. Excess secretion of gel-forming mucins and associated innate defense proteins with defective mucin un-packaging underpin gallbladder mucocoele formation in dogs. PloS One. 2015;10:e0138988.
14. Pike FS, Berg J, King NW, Penninck DG, Webster CRL. Gallbladder mucocoele in dogs: 30 cases (2000-2002). J Am Vet Med Assoc. 2004;224:1615-1622.
15. Crews LJ, Feeney DA, Jessen CR, Rose ND, Matise I. Clinical, ultrasonographic, and laboratory findings associated with gallbladder disease and rupture in dogs: 45 cases (1997-2007). J Am Vet Med Assoc. 2009;234:359-366.
16. Worley DR, Hottinger HA, Lawrence HJ. Surgical management of gallbladder mucocoeles in dogs: 22 cases (1999-2003). J Am Vet Med Assoc. 2004;225:1418-1422.
17. Malek S, Sinclair E, Hosgood G, Moens NMM, Baily T, Boston SE. Clinical findings and prognostic factors for dogs undergoing cholecystectomy for gall bladder mucocele. Vet Surg. 2013;42:418-426.
18. Jaffey JA, Graham A, VanEerde E, et al. Gallbladder mucocele: variables associated with outcome and the utility of ultrasonography to identify gallbladder rupture in 219 dogs (2007-2016). J Vet Intern Med. 2018;32:195-200.
19. Furrow E, Lees GE, Brown CA, Cianciolo RE. Glomerular lesions in proteinuric Miniature Schnauzer dogs. Vet Pathol. 2017;54:484-489.
20. Davidson AG, Bell RJ, Lees GE, Kashtan CE, Davidson GS, Murphy KE. Genetic cause of autosomal recessive hereditary nephropathy in the English cocker spaniel. J Vet Intern Med. 2007;21:394-401.
21. Besso JG, Wrigley RH, Gliatto JM, Webster CRL. Ultrasonographic appearance and clinical findings in 14 dogs with gallbladder mucocele. Vet Radiol Ultrasound. 2000;41:261-271.
22. Peterson ME, Melian C, Nichols R. Measurement of serum total thyroxine, triiodothyronine, free thyroxine, and thyrotropin concentrations for diagnosis of hypothyroidism in dogs. J Am Vet Med Assoc. 1997;211:1396-1402.
23. Dixon RM, Mooney CT. Evaluation of serum free thyroxine and thyrotropin concentrations in the diagnosis of canine hypothyroidism. J Small Anim Pract. 1999;40:72-78.
24. Kantrowitz LB, Peterson ME, Melian C, Nichols R. Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in dogs with nonthyroidal disease. J Am Vet Med Assoc. 2001;219:765-769.
25. Vaden SL, Pressler BM, Lappin MR, Jensen WA. Effects of urinary tract inflammation and sample blood contamination on urine albumin and total protein concentrations in canine urine samples. Vet Clin Pathol. 2004;33:14-19.
26. Wilson DM, Anderson RL. Protein-osmolality ratio for the quantitative assessment of proteinuria from a random urinalysis sample. Am J Clin Pathol. 1993;100:419-424.
27. Steven LS, Michael AS. Fundamentals of Veterinary Clinical Pathology. Ames, Iowa: Wiley-Blackwell; 2008.
28. Benjamin Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Series B (Methodol). 1995;57:289-300.
29. Gibson KL, Gipson DS, Massengill SF. Renal manifestations of systemic illness in children. Semin Nephrol. 2009;29:360-369.
30. Jaffey JA, Pavlick M, Webster CR, et al. Effect of clinical signs, endocrinopathies, timing of surgery, hyperlipidemia, and hyperbilirubinemia on outcome in dogs with gallbladder mucocele. Vet J. 2019;251:105350.
31. Kohnken RA, Amerman H, Brown CA, Furrow E, Lees GE, Cianciolo RE. Glomerular lipidosis in Dogs. Vet Pathol. 2017;54:795-801.
32. Sato H, Takahashi N, Sato E, Kisu K, Ito S, Saito T. Pathology of glomerular lipidosis. Clin Exp Nephrol. 2014;18:194-196.
33. Zatelli A, Paltrinieri S, Nizi F, Roura X, Zini E. Evaluation of a urine dipstick test for confirmation or exclusion of proteinuria in dogs. Am J Vet Res. 2010;71:235-240.

How to cite this article: Lindaberry C, Vaden S, Aicher KM, et al. Proteinuria in dogs with gallbladder mucocele formation: A retrospective case control study. J Vet Intern Med. 2021;35:878–886. https://doi.org/10.1111/jvim.16051